Epidemiological transmission patterns of *Taenia solium* cysticercosis in endemic areas: The case of Ecuador

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“Ecuadoreans are strange and unique beings: they sleep peacefully surrounded by roaring volcanoes, they live poor among incomparable riches and they become happy listening to sad music.”

— Alexander von Humboldt
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Abbreviations

Ab  Antibody
Ag  Antigen
BOD  Burden of disease
CSF  Cerebrospinal fluid
CI  Confidence Interval
CLTS  Community led total sanitation
CNS  Central nervous system
CR  Confidence Region
CrI  Credibility Interval
CT-Scan  Computer tomography scan
DALY  Disability-Adjusted Life Year
EITB  Enzyme-linked Electroimmunotransfer blot assay
ELISA  Enzyme-linked immunosorbent assay
GBD  Global Burden of Disease
HCC  Human cysticercosis
HIV  Human immunodeficiency virus
IHC  Incidence of hospitalized cases
MDA  Mass drug administration
MRI  Magnetic resonance imaging
NCC  Neurocysticercosis
NTD  Neglected tropical disease
PCC  Porcine cysticercosis
PCR  Polymerase chain reaction
USD  United States of America Dollars
WHO  World Health Organization
YLD  Years Lived with Disability
YLL  Years of Life Lost
Chapter 1

1 General introduction & literature review
General Introduction & Literature Review

“Science is a way of thinking much more than it is a body of knowledge.”

— Carl Sagan

1.1 General Introduction

*Taenia solium* is a cestode that is transmitted between humans, the definite host in which it causes intestinal tapeworm infection (taeniasis), and pigs, the natural intermediate host in which it causes cysticercosis. Humans can also act as accidental dead-end intermediate hosts. Human cysticercosis (HCC) is a neglected zoonotic parasitic disease caused by the development of the metacestode larval stage of *T. solium* in different tissues. In humans the disease is responsible for different clinical manifestations that can be very severe and even cause death [1]. The parasite can establish in the central nervous system (CNS), called neurocysticercosis (NCC), which is the most important cause of acquired epilepsy in developing countries [2;3].

Other impacts of this disease include economic burden caused by the costs of medical care and disability, as well as economic losses for vulnerable traditional pig raisers in endemic countries caused by the devaluation and/or condemnation of infected pig carcasses [4]. Endemic areas of HCC are located in Latin America, Asia and Africa [5-7]. The presence of the parasite is related to poor sanitary conditions, inadequate hygiene, open defecation, presence of free roaming pigs and poverty [8;9]. However, HCC occurs in all the 6 WHO (World Health Organization) regions [10]. The disease was eliminated in the past century in most developed countries, but imported cases of HCC and taeniasis have been reported; new data suggests that immigration together with specific farming practices could lead to the re-introduction of the disease in these non-endemic areas [11-13].

While the life cycle of *T. solium* is well known and different approaches to interrupt transmission are available, there is no scientific evidence that supports elimination of HCC in endemic countries in the near future [14]. Better efforts are needed to understand the factors that affect the transmission dynamics of the parasite in order to design adequate interventions that can be integrated in national control programs.

Ecuador is a South American country considered as endemic for cysticercosis with some areas considered as hyperendemic such as the Andean region of the country [15]. Human and porcine cysticercosis cases are regularly reported in Ecuador and all the conditions related to the presence of the parasite are met within its borders. Studies conducted in the country, demonstrated the dynamic nature of the transmission of the parasite [16;17]. However, to date no national control program has been conducted in Ecuador.
The present chapter aims to present an overview of the current knowledge on *T. solium* infections, emphasizing its impact on public health and economy, and focusing on diagnosis, epidemiology and control programs with an special attention to Ecuador. The chapters 2, 3 and 4 present the analysis of data from the global to the local epidemiology of HCC in Ecuador. In chapter 5 the results from this research are discussed and conclusions and recommendations are presented.

1.2 *Taenia solium* life cycle

The natural life cycle of *T. solium* includes humans as the definitive hosts harboring the intestinal adult tapeworm, which causes taeniasis, and pigs as the intermediate hosts infected with the metacestode larval stage (cysticercus), generally in the muscular tissue [18;19].

Humans acquire the tapeworm through consumption of improperly cooked infected pork containing viable cysticerci. The cysticerci will evaginate in the small intestine liberating the scolex composed of suckers and hooks, will use these organs to effectively attach to the intestinal walls, and subsequently develop into an adult tapeworm.

The intermediate pig host gets infected by ingestion of parasite eggs containing the oncosphere larval stage, passed in the stool of a tapeworm carrier. In the intestine, the eggs hatch, liberating the embryos, which cross the intestinal mucosa to reach the bloodstream and are transported to different tissues.

Once established in the tissue of the intermediate host, the cysticercus develops into the viable stage, which is composed of a scolex visible through vesicular fluid and an opaline membrane inside a cyst [20]. The metacestode larval stage establishes in the pig’s muscles, brain and other tissues causing porcine cysticercosis (PCC) [19]. Unfortunately, humans can also serve as dead-end intermediate hosts by accidentally ingesting parasite eggs and develop the metacestode larval stage in their body tissues [21] (Figure 1).
1.3 Importance

The zoonotic nature of *T. solium* taeniasis/cysticercosis and the involvement of a productive animal species make this disease both a public health and a social agro-economic problem.

1.3.1 Clinical importance

While human taeniasis presents almost no clinical signs or mild and non-specific symptoms, HCC can cause severe clinical manifestations and in severe cases even death [1]. In humans the parasite tends to locate in the central nervous system (CNS) (neurocysticercosis (NCC)) causing a variety of neurological disorders [24].

HCC can also involve skeletal muscular tissue, myocardium, the eye and subcutaneous tissue [25-27], but these types of manifestations are considered less common or pathogenic compared to NCC [28]. The most frequent clinical disorders described for NCC include epilepsy and seizures, followed by headaches, focal neurological deficits and disorders related to increased intracranial pressure such as,
General introduction & literature review

hydrocephalus or papilledema. Other not so frequent manifestations are: meningitis, visual disorders, cognitive disorders, and other psychiatric manifestations related to altered mental states [29].

NCC is the most severe presentation of the infection and is considered the most important parasitic disease of the neural system and the main cause of acquired epilepsy in *T. solium* endemic areas, where NCC is associated with 14.2 to 50% of the acquired epilepsy cases [30;31].

Most of the disorders related to HCC appear after several months or even years after infection due to the ability of the cyst to evade the immune response of the host, thus preventing the inflammatory reaction, associated with the presence of the viable parasite in an asymptomatic phase [32]. When the cysticercus starts degenerating, which can be a few months to years after infection, the vesicular fluid becomes dense and opaque, the cyst loses its regular shape and becomes smaller. Finally, the cysticercus undergoes the stage of calcification in which it ends as a round white calcified nodule [28]. During this degeneration process, an inflammatory response is generated by the hosts’ immune system, resulting in a symptomatic phase [33]. Other elements influencing the gravity of these lesions and the appearance of symptoms are the localization of the cyst, number of larvae, type of cysticerci (racemose or vesicular) and the implication of the mechanical pressure caused by the cysts [34;35].

In pigs, the evolution of the infection is similar to HCC, however, the most frequent tissues infested are the skeletal muscles and the myocardium, and less frequently, the brain and other organs. In most cases no clinical manifestations are present but it is worth to mention that this may be due to the short life period of the pigs until they are slaughtered and the long period needed to develop symptoms [36;37]. However, some clinical manifestations such as, excessive salivation, tearing, fever, ataxia, excessive blinking and/or a subconjunctival nodule were observed after experimental infections in pigs [38]. In other studies, it has been observed that when the CNS is compromised in infected pigs, seizures and/or stereotypic walk in circles can occur [39]. Unfortunately, these manifestations are inconclusive on their own to diagnose PCC and must be accompanied by other diagnostic tests to confirm the infection [38;40].

1.3.2 Public Health and Socio-Economic importance

The first attempts to make a global estimation of deaths caused by HCC reported 50,000 fatalities per year [41;42], but more recent estimations in the Global Burden of Disease studies (GBD) for 2010 and 2013 calculated that between 1,200 and 700 yearly deaths, respectively are due to HCC [43;44]. However, accounting only for mortality data does not describe the real impact of a disease in the health status of a population. In fact, the most important impact caused by HCC is represented by the disability that affects the symptomatic patients and contributes to the burden of the disease (BOD) in a population.
1.3.2.1 The non-monetary burden of HCC

To quantify the non-monetary BOD, the DALY (Disability-Adjusted Life Year) metric was introduced in the early 1990's and it has been widely accepted and used by the WHO and other organizations involved in the local and global estimation of the BOD. The DALY metric combines the morbidity and mortality components of a disease [45-47]. The DALYs are the result of the addition of the YLD (Years lost due to disability) and the YLL (Years of life lost due to a premature death). This data is derived from the demographic data, epidemiological data and the information available about the severity of a disease [48], which makes the use of DALYs a better representation of BOD, making it less abstract, more manageable and helps to make comparisons of BOD among geographical locations or among diseases. In other words, each DALY represents a year of full health lost due to a disease.

In the GBD 2010 study, it was calculated that HCC was responsible for 503,000 DALYs or 0.07 DALYs per 1,000 individuals worldwide every year [49], but the number of DALYs associated with epilepsy that should derive from NCC and should be included in this calculation is unknown, which suggests an underestimation of the burden associated with HCC [50]. In the most recent GBD study (2013), HCC was estimated to be responsible for 310,400 YLDs (95%CI [212,200-409,500]) only for that year, YLLs and DALYs estimations from the 2013 GBD study are not yet published [51]. For the 2010 GBD study HCC ranked as the 13th neglected tropical disease (NTD) responsible of the DALYs attributed to all the NTDs for that year; HCC also accounted as the 9th NTD responsible for the YLDs and 8th for the YLLs attributed to all the NTDs for that year [52]. For the 2013 GBD study the estimated number of YLDs due to HCC was higher than the sum of the YLDs attributed to Chagas disease, leishmaniasis (visceral, cutaneous and mucocutaneous), African trypanosomiasis, and cystic echinococcosis together [51]. These estimations reveal the great contribution of HCC to the GBD, but also show that a global assessment of the GBD of HCC is challenged because of underestimations due to the characteristics of the clinical expressions of the disease, and because of the lack of quality data. Torgerson and McPherson (2011) gave a crude estimation of between 2 and 5 million DALYs associated with HCC yearly [53].

Only few studies estimated the BOD related to HCC in individual endemic countries. In 2009 in West Cameroon (Africa), it was estimated that 9 DALYs per 1,000 person-years (95% CrI [2.8–20.4]) are caused by HCC and the major monetary burden was related to the professional inactivity caused by the clinical manifestation of the disease [4]. In 2012 in Mexico (Latin America), the estimated BOD of HCC was 0.25 DALYs per 1,000 person-years (95% CR [0.12–0.46]) [54] and one year later, in Nepal (Asia) the BOD associated with HCC was estimated to be 0.543 DALYs per 1,000 persons per year (95% CrI [0.207–1.054]) [55]. It is worth mentioning that most of these estimations used
different methodologies; for example, the study conducted in Cameroon only took into account NCC-related epilepsy cases while the Mexican study incorporated chronic headaches and NCC-related epilepsy cases. However, despite of the variation in the methodologies, it can be observed that the BOD estimated for HCC in endemic countries is much higher than the BOD estimated by the GBD studies. In addition, the variability of the estimates between the studies suggests a variation on how the disease impacts the BOD in different populations [4;54].

The impact of the stigma that epilepsy carries in certain populations should be added to the non-monetary BOD as it increases the societal cost of the disease. The stigmatization of epilepsy patients leads to discrimination when applying for work, isolation and psychological pressure [4;56;57]. In children, this stigma leads to their exclusion from school and social activities. Sometimes, epileptic children are locked in their houses and because of the stigmatization have an increased risk of being malnourished. The mothers of epileptic children are also not well seen in many cultures and may be victims of stigmatization, all of it compromising in many ways the future of these children and their mothers [58-60].

1.3.2.2 The monetary burden of cysticercosis

The financial impact of HCC is difficult to measure at a global level. On the one hand there are the costs of health care and treatment. Depending on the health care system in a country, these costs are subsidized or not. Therefore, the financial impact can go directly to the patient or to the public system. On the other hand, there are monetary losses due to losses in productivity, for which estimations are relative, depending on the salaries and the cost per hour lost in every country [61].

To date, the global financial cost of porcine cysticercosis is unknown [53]. Porcine cysticercosis in most cases does not present clinical manifestations or presents symptoms that do not substantially affect the productivity of the pig [38;39]. The financial losses due to porcine cysticercosis are caused by the total or partial condemnation of carcasses, also, in endemic countries where pigs are slaughtered without meat inspection, pork showing physical lesions which include the presence of cysts are sold at a lower price. However, clandestine markets and abattoirs plus the backyard slaughtering of pigs for local consumption are regular practices in endemic countries, which limits the availability of accurate data on this matter [5;62;63].

Another consequence of the informal slaughtering of pigs is that while veterinary inspection is avoided, the infected meat is still sold, thus the risk of taeniasis infections continues [5;62;64;65]. In other cases, pig traders use the tongue inspection before buying pigs; infected pigs are not bought which implies economic loss for the pig owner [66]. However, infected pigs that are not sold will be slaughtered at the village and consumed anyway [67].
In Cameroon the economic burden of cysticercosis was estimated to be 10.3 million Euros per year with 95% of this burden attributable to HCC only in 2009 and it was estimated that treating a NCC patient costs 194 € yearly [4]. In the Eastern Cape Province in South Africa it was estimated that between 15 and 27.5 million € are lost per year (18.4-34.2 millions of USD (2004 currency exchange adjusted)) due to cysticercosis depending on the methods used for the estimation. HCC was responsible for 73-85% of these costs [68]. In Peru the cost estimated for the first two years of treatment for one NCC case was estimated at 996 USD (2007 currency exchange adjusted), the costs in the first year of treatment represented 54% of the minimum wage [69].

As these studies detail, it is clear that the monetary impact is more important on the public health level than on the agricultural level. It has to be taken into account that the population that is affected by T. solium infections usually belongs to the poorer strata. Because poorer people have less resources to access proper health care, the impact of NCC can be higher than in the wealthier part of the population [53;69].

Also, even if the agricultural economic impact seems to be lower when compared to public health costs, it has to be taken into consideration that this impact goes directly to the economy of poor traditional pig farmers who keep free roaming pigs and have very limited resources, thus the impact of a carcass condemned or sold at a lower price can greatly affect their household economy aggravating the socio-economic problem of NCC [70].

1.4 Diagnosis

Different tools are available for the diagnosis of taeniasis and cysticercosis. To date there is not a single test that can correctly identify all the cases of cysticercosis in a simple and practical way. There are many diagnostic techniques, which differ in the type of sample analyzed, test format, sensitivity, specificity, cost, availability and ease of use.

1.4.1 Clinical diagnostic tools

Human cysticercosis has multiple clinical manifestations depending on the organ affected, with the majority of the manifestations related to the infection of the CNS. However, there are no specific pathognomonic signs or symptoms that could guide to a specific NCC diagnosis [22]. Therefore, diagnosis is based on the clinical presentation, supported by neuroimaging, immunodiagnosis and epidemiological information. Neuroimaging tools such as, CT-Scan (Computer tomography) and MRI (Magnetic resonance imaging), are essential tools when symptomatology is compatible with neural lesions; but images on their own are inconclusive if specific parasite structures are not present [34]. Immunodetection methods on CSF (Cerebrospinal fluid) or serum can contribute to the clinical diagnosis of NCC but they are generally not sufficient on their own to allow for an accurate diagnosis.
For instance, detection of specific antibodies or circulating parasite antigens does not indicate the location and the number of the cysticerci [71;72]. Antibody detection can overestimate the presence of the parasite because anti-\textit{T. solium} antibodies can be detected in serum from patients that have been exposed to the parasite and not necessarily carry the cysts [73]. Detection of circulating antigens can efficiently diagnose active infection (presence of viable cysts) but its efficacy is limited in the case of calcified or highly degenerated cysts, which can pass undetected with this technique. Direct collection of cysticerci from the host' tissues for morphological identification and/or molecular analysis is the most accurate way to diagnose cysticercosis, however, the proper extraction of the cyst involves the risks of any invasive method, except for subcutaneous cysticercosis [34;74;75].

In order to correctly diagnose NCC, the use of multiple diagnostic tools and criteria has been proposed. Del Brutto et al. introduced in 1996 a set of diagnostic criteria, which has been revised and updated until recent years [76-78]. Their diagnostic criteria prioritize the results from the different diagnostic tests/tools available; four categories are distinguished: absolute, major, minor and epidemiological criteria, depending on the power of each test to correctly identify the presence of the parasite [78]. These criteria are based on clinical, imaging, immunological and epidemiological data [79].

An absolute criterion corresponds to the evidence of the undeniable presence of the parasite in the CNS such as, a cystic lesion showing the parasite scolex or the demonstration of the parasite from a biopsy. Major and minor criteria are applied when there is evidence suggesting the presence of the parasite, the difference between the two will depend on how powerful the evidence is of the presence of the parasite to confirm NCC. Epidemiological criteria are applied when there is evidence that the patient was or lives in an environment where \textit{T. solium} is present such as, an endemic area or a household with a history of a tapeworm carrier. The presence/absence of the criteria leads to two degrees of diagnostic certainty: Definitive and probable, which depend on the number, type and combination of the other diagnostic criteria present in a patient [78].

The main disadvantage when applying the diagnostic criteria proposed by del Brutto et al., is that to achieve a satisfactory result the use of multiple tools is requested [79]. In endemic countries, these are often either unavailable or involve high costs, are complex to perform, need trained personnel and specific equipment, which limits their use in field conditions. For these reasons, some authors suggest the inclusion of the detection of circulating antigens as a major criterion in order to add more confident elements to the diagnosis of NCC and a revision of the diagnostic criteria proposed by del Brutto et al. for use in endemic countries [71].
1.4.2 Epidemiological diagnostic tools

Serology has the big advantage of being relatively accessible, not difficult to use in large numbers of samples and at a relatively low cost when compared to other techniques. For these reasons, serology for immunodiagnosis has become an important tool in epidemiological studies, notwithstanding its limitations [75].

Immunodiagnosis can be performed either on serum samples, CSF, urine and saliva. However, for epidemiological surveys the diagnosis on CSF is impractical, while tests on urine and saliva tests still need to be better validated. The available serological tools can be classified into antibody detection methods and circulating antigen detection methods. For the former, several tools have been developed over the years (complement fixation test, haemagglutination, radioimmunoassay, ELISA, dipstick ELISA, latex agglutination and immunoblot) but the most used and accepted test for epidemiological studies is the Enzyme-linked Electroimmunotransfer blot assay (EITB), which is an immunoblot using seven lentil-lectin-purified glycoproteins isolated from cysticerci, with a reported sensitivity ranging from 97 to 98% and a specificity ranging from 97 to 100% to detect circulating antibodies to *T. solium* in human serum [72;75;80].

For the circulating antigen detection methods the monoclonal antibody-based ELISAs directed at specific *Taenia* antigens (Ag-ELISA) present the most accurate results [75;81-83]. The B158/B60 Ag-ELISA and the HP10 Ag-ELISA are the most representative tests for epidemiological studies for the detection of circulating antigens. The B158/B60 Ag-ELISA has a reported sensitivity of 90% (95% CI: [80-99%]) and a specificity of 98% (95% CI: [97-99%]) to detect circulating antigens released by *T. solium* metacestodes in humans, with no cross-reactions reported in humans to date [72;80;84]. For the HP10 Ag-ELISA the reported sensitivity ranges from 84.8% to 86% and the specificity is estimated at 94% to detect circulating antigens in human serum [85;86].

Circulating antigen detection and antibody detection differ in their interpretation. On the one hand, the detection of circulating antigens shows the presence of viable cysts or the active disease in an individual leaving calcified lesions undetected, while the detection of anti-*T. solium* antibodies indicates the exposure to the parasite, which means that viable cysts can be present or not, a positive detection can be the result of an aborted infection or even a past infection already healed, which can lead to overestimation of the prevalence of HCC [72;73;75]. A high seroprevalence of antibodies is indicative of a “hot spot” in a population, to which effective control measures should be directed. The information gathered from the use of these techniques provides valuable information on the potential risk factors for HCC [18;75;87]. The simultaneous use of both techniques provides a better understanding of the transmission of the parasite [72].
General introduction & literature review

Figure 2 describes the stages of the *T. solium* larval infection detected by an antibody test and antigen test.

![Timeline Diagram](image)

**Figure 1-2 circulating antigen and antibody detection in serological samples.**

It is worth mentioning that other techniques to detect HCC are under development such as the detection of cellular response in serum [88] as an alternative to the antibody and/or antigen detection; and the detection of antigens in urine, which can help to avoid invasive sampling methods [89-91].

### 1.4.3 PCC diagnostic tools

The diagnosis of PCC can be performed ante-mortem and post-mortem. Ante-mortem diagnosis can be useful for the purchase of live pigs and for epidemiological purposes such as prevalence and intervention surveys [81]. One of the most used diagnostic techniques is the tongue inspection, which consists in the examination of the pig tongue in search of cysticerci by visual inspection or by palpation. This technique is highly specific, quick to perform and does not involve any specific cost in reagent or laboratory usage. However, this technique has a low sensitivity since not all infected pigs will present lesions in the tongue. The sensitivity of this technique has been reported to vary from 21 to 70% and is greatly dependent on the intensity of the infection [92;93].

Immunological tools are also important ante-mortem techniques, which have been adapted in most of the cases from HCC or bovine cysticercosis serological techniques to detect both circulating antigens [82;94] and anti-*T. solium* antibodies [80;95]. Immunodiagnosis in pigs is widely used in epidemiological surveys and provides important information in live pigs at a relatively low cost [81]. However, the use of immunological tools for PCC present some disadvantages: they have similar drawbacks as for diagnosis of HCC [96], and have shown to be ineffective to detect light infections in
rural pigs [97]. Also, Ag-ELISAs are genus specific, thus false positives can arise because of cross reaction with infections caused by other *Taenia* species such as the non-zoonotic *Taenia hydatigena* [75]. The substitution of conventional antibodies by nanobodies for capturing antigens in these techniques seems promising for increasing species specificity but needs to be validated [98]. On the other hand, antibody detection techniques such as EITB do not cross react with *T. hydatigena* but the confidence of these tests to detect porcine cysticercosis in naturally infected pigs still needs to be fine-tuned [92;99]. Other ante-mortem techniques for PCC are 1) molecular diagnosis in serum and 2) the use of ProteinChip technology for detection of biomarkers in serum samples; however, both techniques are still under development and need to be validated [100;101].

Total dissection of the carcass can be considered as a “gold standard” test to detect PCC with a sensitivity close to 100% but only if meticulous manual slicing of all meat is conducted. However, this technique involves high costs because of the purchase of the pigs and the payment of trained personnel to perform the dissection. An alternative to this procedure is the partial or targeted dissection of the carcass, which reduces the time and cost of the test but has the disadvantage of lowering the sensitivity of the test [102].

Post-mortem diagnosis corresponds to the veterinary inspection of the carcasses and is routinely used in abattoirs. However, there exist different protocols of meat inspection that vary between countries and abattoirs. The sensitivity of meat inspection depends on the number of cuts, muscles targeted and the level of infection of the pigs; it has been estimated to be as low as 22.1% in abattoirs in Zambia and can be a cause of underestimation of the prevalence [81;93]. In endemic countries not all pigs are slaughtered in abattoirs, which contributes to an underestimation of the prevalence of PCC, thus limits its validity for epidemiological estimations [62;63].

The use of sentinel pigs in endemic areas combined with diagnostic techniques described for PCC including molecular tools, can give important information on the transmission dynamics of PCC, environmental contamination with *T. solium* eggs, human risk to infection and can help to assess the efficacy of control programs [103-105].

1.4.4 Taeniasis diagnosis

The questioning and self-detection/report technique is based on the auto-detection of expelled proglottids that can be found in feces. While it has the advantage of being low cost, this technique is not very efficient for the detection of *T. solium* since the expulsion of the proglottids is passive unlike *T. saginata* and the technique lacks specificity because the distinction between proglottids and other helminths and artifacts can be very difficult for the patient [81].
Conventional coprological examination can be applied at 1) macroscopic level if proglottids are found in feces or 2) microscopic level using concentration techniques such as, sedimentation or flotation methods to detect *T. solium* eggs in feces; or the use of the Graham test, which consists of the use of an adhesive tape to collect *T. solium* eggs from the perianal region. The advantages of these techniques are: the relatively low cost, and the fact that they are easy to perform. The drawbacks are the considerable lack of sensitivity, the need to apply these techniques several times for satisfactory results, and the fact that tests are genus specific, thus the differentiation of *T. solium*, *T. saginata* and *T. asiatica* is not possible [81;106-108].

More advanced diagnostic tests are the copro-antigen detection, molecular methods and serological antibody detection. Copro-antigen detection relies on the use of a polyclonal antibody-based sandwich ELISA to detect specific *Taenia* coproantigens. It is more sensitive than conventional coprological methods [109], it can detect early stages of infection, coproantigens disappear briefly after a successful treatment of taeniasis, and it can be used on formalin preserved stool samples, which is useful in field conditions [110]. The major drawback of this test is its genus specificity, thus in areas where *T. saginata* or *T. asiatica* are sympatric the prevalence of *T. solium* tapeworm carriers can be overestimated [81;107]. However, a novel *T. solium* species-specific coproantigen technique has been developed [111].

Molecular methods have been used for identification and differentiation of parasites when proglottids or other parts of the parasite can be recovered. They can also be used for taeniasis diagnosis on stool samples. Copro-PCR is highly sensitive and specific, but the costs involving DNA extraction from human feces for analysis is high, and PCR involves the use of a specialized lab, which limits its use for routine purposes [81;108;112]. Finally, an EITB on serum samples for taeniasis diagnosis using excretory-secretory antigens from adult *T. solium* has been developed. This technique is *T. solium* species-specific and has the advantage of avoiding handling of feces, which can put the personnel at risk. Its main drawback is the persistence of antibodies after treatment and elimination of the tapeworm [110;113;114].

### 1.5 Epidemiology

#### 1.5.1 Global epidemiology

HCC is a disease transmitted between humans. Infection occurs when a tapeworm carrier contaminates the direct environment, water or food with parasite eggs, which are subsequently ingested by a susceptible individual [115]. Based on the Multicriteria-based ranking for risk management of food-borne parasites, which measured criteria such as the number of food-borne illnesses, geographical distribution, acute and chronic morbidity and socio-economic impact amongst
others, HCC and *T. solium* taeniasis are ranked together as the most important food-borne parasitic diseases [116;117]. The 2013 GBD study estimated that there exist 1,030,800 (95% CI [901,400 – 1,185,600]) prevalent cases of HCC worldwide [51]. *T. solium* infections are distributed in all the 6 WHO regions [10]. Figure 1-3 describes the global distribution of HCC around the world.

![Figure 1-3 Areas at risk of Human cysticercosis in 2011 (WHO, 2012 [118])](image)

HCC can be found from the poorer rural settings to the more developed and industrialized countries, however, to maintain the full life cycle of the parasite, conditions such as, poor sanitation, lack of hygiene, open defecation and traditional pig rearing systems allowing free roaming of the animals are needed. Tapeworm carriers are the only source of HCC but the adult parasite can only be acquired by consumption of infected undercooked pork. These particular conditions divide the affected countries into three main categories, 1) endemic countries in which all conditions for completion of the life cycle exist and all the infections with *T. solium* are confirmed (HCC, PCC and taeniasis), 2) the non-endemic countries in which the conditions for the full life cycle are not met but which present cases of HCC and 3) the suspected endemic countries in which the existence of the full cycle of *T. solium* is probable but has not been proven consistently and some HCC cases have been reported. Endemic areas have been identified in Asia, Africa and Latin America [5;6;119;120].

Ito et al. describe HCC as a societal disease [115], they classified human societies in three groups: 1) societies where pork is not eaten because of cultural and religious beliefs, thus adult tapeworm free, 2) societies where pork is consumed but are PCC free, such as developed countries with improved
husbandry and rigorous sanitation measures and 3) societies where infected pork meat is present and consumed (endemic countries). Traditionally, societies in the third category are the societies carrying the higher burden of HCC. However, through the years the interaction between these three societies has increased and the transmission enhanced, thus increasing HCC infections in societies were pork is safe to consume or is not consumed at all. Globalization and (im)migration seem to be the greatest contributors to this evolution [12]. This historical evolution emphasizes the huge importance of mobility and contact with tapeworm carriers in the transmission of HCC and breaks the paradigms surrounding pig keeping and pork consumption as principal determinants for HCC [121]. In fact, in non-endemic countries two types of infections can be found, 1) imported cases of HCC when the infection was acquired in an endemic country, this could affect tourist travelers as well as immigrants from endemic countries [1;122-124] and 2) the infections in situ which are the result of imported cases of taeniasis and human-to-human transmission. HCC is a clustered mobile disease linked to the presence of a tapeworm carrier [12;125-127]. The infection in situ with adult tapeworms in non-endemic countries seems unlikely due to the strict restriction applied to pork trade [12]. However, new data suggests that Europe can return to an endemic state. Organic farming with outdoor access is becoming more common because of the increasing demand of organic products and animal welfare concerns. Another contribution to this matter is the increasing number of immigrants and travelers from endemic countries [12;13]. It has to be taken into account that until the first half of last century, Europe still had endemic cases within its borders. Cysticercosis was eradicated at that time due to improved meat inspection, sanitary conditions and pig farming [128]. In the USA, the number of HCC cases is increasing in the last decades. Between 2003 and 2012, 18,584 hospitalized cases were recorded nationwide, the costs of hospitalization of these cases surpassed USD 908 million. Most cases were reported in Latin American communities. From these figures, some authors consider that the disease should be a nationally reportable disease in the USA [129;130].

Despite the efforts to estimate a global prevalence of HCC, it is difficult to have a real estimate of the actual distribution of HCC. Most of the cases remain cryptic until appearance of clinical manifestations, or are diagnosed under research conditions as it happens in poor areas, thus the correct diagnosis of HCC is practically accidental [115]. These characteristics of the disease may imply that actual global figures are underestimating the real burden of HCC [50].

To date there is not a reliable estimate on the number of prevalent cases of taeniasis, an accurate detection of tapeworm carriers is almost impossible due to the main limitations of taeniasis diagnosis described in the section 1.4.4. [81;106]. For PCC there is a similar picture due to the diagnostic limitations described in the section 1.4.3. Furthermore, most of the pigs at risk to develop PCC are slaughtered in the backyard for local consumption, and serological tests available to date are confronted with problems of cost, non-adapted format for field use and cross-reactions to other
parasites; thus the prevalence of PCC at a global level is unknown [53;62;63;93]. However, in field studies in endemic areas using the B158/B60 Ag-ELISA, apparent prevalences ranged from 9.01-11.5% in Ecuador and Tanzania to 57.1% in Zambia [93;131;132].

The more reliable data for HCC are the figures obtained from epidemiological field studies and clinical data, though they only give a partial picture of the transmission of the disease. On the one hand, clinical data reflects the “tip of the iceberg” because they present only symptomatic patients [36]; on the other hand, epidemiological field studies rely on the sensitivity and specificity of the tests that are used. To date the best tools for epidemiological studies rely on immunodiagnosis provided by serology [75;133]. The prevalence of circulating antigens of *T. solium* metacestodes using the B158/B60 Ag-ELISA in endemic communities ranges from 0.57% in a rural community in Viet Nam to 21.63% in a rural community in the Democratic Republic of Congo [134;135]. The seroprevalence of anti-*T. solium* antibodies using the EITB technique in endemic communities ranges from 3.07% in Colombia to more than 30% in Ecuador, Peru and Zambia [17;73;136].

The variability of the prevalence and seroprevalence of HCC, PCC and taeniasis in endemic areas needs to be understood in order to obtain better estimates of the real impact of *T. solium* at a global level. Also, understanding the origin of this variability could help identifying important information that could be used to design different strategies to control *T. solium* in endemic areas.

### 1.5.2 *T. solium* taeniasis/cysticercosis in Latin America

Published data on HCC in Latin America show a significantly consistent association between cysticercosis and epilepsy with more than 12% of NCC cases among urban epileptic patients and more than 23% in rural settings [2]. Furthermore, it is estimated that there are 5 million people living with epilepsy in Latin America [137], suggesting that only in Latin America there are between 500,000 and more than 1 million cases of symptomatic HCC. These rough estimations contrast with the GBD 2013 estimations of around 1 million HCC cases worldwide [51]. Another study estimated that 75 million people in Latin America are at risk for HCC and that there are 400,000 cases of symptomatic NCC, based on the conditions observed in clinical and serological studies conducted in Peru [138]. The discrepancy among these estimates could be the reflection of a high variability of the epidemiology and the transmission dynamics within endemic regions [139].

Epidemiological studies using the EITB test for anti-*T. solium* antibodies, conducted in endemic areas in several countries in Latin America revealed seroprevalences varying from 1.59% in Brazil to 31.22% in Ecuador [17;140]. Studies using Ag-ELISAs found circulating *T. solium* metacestode antigens ranging from 0.94% in an endemic area in Ecuador (B158/B60 Ag-ELISA) to 9.12% in Venezuela (HP10 Ag-ELISA) [17;141]. These results also showed a significant difference between
anti-\textit{T. solium} antibodies seroprevalence and circulating \textit{T. solium} metacestode antigens seroprevalence, indicating a significantly higher exposure to the parasite and a relatively low prevalence of active infections [139].

The major risk factors studied for the presence of HCC in endemic areas of Latin America were mostly related to the lack of proper hygienic/sanitary conditions, the presence and proximity of tapeworm carriers, presence of infected pigs, pig farming/consumption and an increased risk of infection with increasing age [139].

The prevalences obtained for taeniasis in Latin America are very dissimilar and depend on the sensitivity and specificity of the tests; figures range from 0% to 17.5% in Ecuador and Guatemala, respectively. The techniques used were formalin-ether and magnesium sulphate flotation techniques for Ecuador and formalin-ether technique and copro Ag-ELISA for Guatemala [16;142].

Porcine cysticercosis has been found ranging from 0% to 33% in villages in a same endemic area in Mexico using a low sensitive test as tongue inspection. In Peru, serological studies using EITB report seroprevalences in pigs as high as 61% in small villages in endemic areas [143;144].

1.5.3 Epidemiology of \textit{T. solium} taeniasis/cysticercosis in Ecuador

Ecuador is a South American country, located at the Pacific Coast between the latitudes 2 °N and 5 °S. The country has a surface of 283,560 square kilometres; its borders are Colombia in the North, Peru in the East and South, and the Pacific Ocean in the West. It has a population of around 16 million. The country has 4 different geographical regions, the insular region of Galapagos, which is a group of islands declared national park and is a protected area, the coastal region with a typical tropical and sub-tropical weather and landscape, also known as Costa region, the Andean region or Highlands also known as the Sierra region with cooler temperatures and a mountainous landscape and the Amazonia region characterized by its hot and humid weather and dense rainforest (Fig. 1.4). The rural population of Ecuador accounts for 38% of the total population of the country and is composed in its majority by the indigenous communities dedicated to agricultural labours. The Sierra region and the coast region comprise most of the active agricultural zones [145-147].
Human and porcine cysticercosis occurrences are regularly reported to the OIE, the diseases are listed as present in the country [148]. Official reports from the Ministry of Public Health in Ecuador reported 67 new cases of cysticercosis in 2013 or 0.42 cases per 100,000 persons. Most of these cases correspond to symptomatic NCC hospitalized patients in public hospitals only, which suggests a large underestimation. The data show a decreasing trend since 1994 with 400 new cases or 3.6 cases per 100,000 habitants at that time. The Sierra region seems to carry the majority of the burden, with Pichincha and Loja provinces showing the highest numbers of reported cases and cases per 100,000 persons [149]. The decreasing trend over time is compatible with what has been observed by specialists in large hospitals in Ecuador and might be caused by the improvement of general life conditions, such as better sanitation and better access to health care [150;151]. However, this does not mean that these conditions have significantly changed in rural areas and that elimination or spontaneous regulation of the disease has occurred. In fact, the highlands of Ecuador have been considered hyper-endemic for HCC since a long time [15]. Possibly, the observed decreasing trend in part represents a decrease in the severity of symptomatic NCC cases. With the general improvements in the health care system in Ecuador, it is possible that symptomatic patients are properly diagnosed and treated in an early stage of the disease avoiding the progression of the disease and reducing the number of hospitalized cases, while the number of asymptomatic or non-hospitalized cases remains
stable [151;152]. However, the causes of this decreasing trend have to be further studied. On the other hand, the Ministry of Public Health reports a totally different picture for taeniasis: cases of taeniasis vary from 400 to 125 cases per year in 1994 and 2013, respectively, but there is not a clear trend during that period. For example, in 2002, 2006 and 2011 the number of reported cases was 94, 103 and 59, respectively, but in 2000, 2004, 2009 and 2012 there were 320, 475, 396 and 216 cases reported, respectively. In addition, there is no geographical trend in the reporting of taeniasis; the cases are reported from all regions of the country without a specific pattern [149]. Considering that the diagnosis of human taeniasis is difficult and the prevalence of tapeworm carriers recorded in most of the epidemiological studies in Ecuador is lower than the prevalence of HCC [16;106;131;153], either the reported cases of tapeworm infection are misdiagnosed and a proportion of the cases possibly corresponds to T. saginata infections, which is also present in Ecuador [131], or there is a serious underreporting of HCC cases.

Reports from epidemiological studies during the last decade demonstrated that HCC is still present in rural areas in the highlands and that the population is highly exposed to the disease. Indeed, epidemiological studies in rural areas of Loja province in the Southern Sierra geographical zone, reported antibody seroprevalences as high as 34.1% (95% CI [29.2–39.2]) [17]. Active HCC, measured by the detection of circulating antigens was found to be 4.99% in rural areas of the north of the country (95% CI [4.36-5.69]) [131]. T. solium taeniasis has been confirmed by different methods in epidemiological studies in different rural areas of the country ranging from 0% (95% CI [0-0.5]) to 1.63% (95% CI [0.89-2.72]) in two villages of the same province of Loja, 0.41% (95% CI [0.18-0.81]) in the northern provinces of Imbabura and Pichincha and 1.55% (95% CI [1.31-1.82]) in a large study conducted in several communities of the southern province of Loja and the coastal province El Oro, bordering with the province of Loja [15;16;131;153]. Similarly, for PCC, meat inspection detected metacestodes in 0.52% (95% CI [0.29-0.85]) of carcasses in the abattoir of Imbabura; the seroprevalence of T. solium circulating antigens for the same pigs was reported to be 9.01% (95% CI [7.33-10.92]) [131]. In a rural village in Loja, PCC diagnosed by tongue inspection was found in 3.56% of the pigs (95% CI [2.27-5.29]) while EITB demonstrated a seroprevalence of 73% (95% CI [63.20-81.39]) [153].

In the rural environment of Ecuador risk factors for taeniasis/cysticercosis such as open defecation, lack of latrines, poor hygiene, keeping free roaming pigs and eating under-cooked pork are commonly present [16;153]. Additionally, some studies found that the risk of infection with HCC in rural areas of Ecuador increases with age [16;17]. The presence of HCC has also been documented in urban environments of the country and it was found that in cities not only low income social classes are at risk of infection, but also middle to upper classes, which contrasts with the general statement that links cysticercosis mostly to the economically poorer social classes. Risk factors for infection in urban
areas were, working in manual labour, eating frequently outside and a history of other NCC patients in the same household [154;155].

Clinical manifestations of neurological disorders such as, epilepsy, migraine and chronic headache in Ecuador present a strong correlation with NCC [156-158]. For most of the NCC patients in Ecuador the cysticerci are located in the brain parenchyma and the single, small, enhancing CT lesion is the most common presentation of NCC, which is a common lesion found in patients with seizures in endemic countries [151;159-163]. Subcutaneous cysticercosis is practically absent (or not reported) in the country [156-158;160].

From the experiences gathered from these studies, it can be assumed that HCC is still an important public health problem in Ecuador that does not discriminate from poorer to richer social strata, neither from urban to rural settings. Also, a considerable gap between epidemiological studies and official data is present. To date no national control program for *T. solium* has been conducted in Ecuador.

While a decreasing trend is observed in the number of hospitalized cases of NCC in Ecuador, epidemiological studies show that there are no signs that the risk factors for *T. solium* life cycle maintenance in rural communities have disappeared. Neither there are signs that the transmission of HCC has been effectively interrupted in urban settings. The official reports of taeniiasis show fluctuations in the number of detected cases along the years. However, there are no clear patterns of the geographical location of the most affected areas for both conditions. PCC is still reported in the country but accurate data from traditional pig holders is unavailable. Data from epidemiological studies conducted in endemic areas of the country partially detail the situation of *T. solium* human infections. There is an urgent need to better understand the information gathered from these studies about how, when and where the transmission occurs in order to design appropriate control strategies.

### 1.5.4 Transmission dynamics

HCC is the result of human-to-human transmission. The level of the parasitic infection and the distribution of the parasite in an area are the result of the interaction between parasite, environmental and host-related factors [164]. To understand the transmission dynamics of HCC, it is important to have a complete view of the dynamics including also the adult tapeworm infection and PCC. Figure 1-5 represents the factors contributing to the transmission dynamics of the taeniid zoonoses.
Figure 1-5 Factors contributing to the transmission dynamics of the taeniid zoonoses. Adapted from Gemmell (1989) [165].

Adult tapeworms are capable of producing tens of thousands of eggs daily that are fecally shed by the tapeworm carriers. These carriers are the sole source of infection for pigs and other humans and they are capable of widely spreading eggs in the environment, depending on their behaviour. Egg dispersal from stools in the environment can be affected by different mechanisms such as; wind, rainfall, sewage systems, fertilizing systems, arthropods, and other warm-blooded animals. Eggs can rapidly be dispersed in a radius of 100 meters around a defecation site. This reflects the high biotic potential of the parasite [19;164-168]. The dispersion of eggs by arthropods is still debated. Flies have failed to prove to be mechanical vectors of *T. solium* eggs because the coprophagic behaviour of pigs does not allow enough time for flies to be impregnated with *T. solium* eggs [169]. On the other hand, dung beetles can play an important role in the transmission of *T. solium* eggs and subsequent infection in pigs [170;171].

Infection with *T. solium* may be regulated by the presence of other *Taenia* species in the same environment competing for both intermediate and definitive hosts. For adult tapeworms, the “crowding effect” when more than one adult parasite is present in a host reduces the fitness of the parasite which affects negatively the size of the parasite and subsequently the egg output. Also, the presence of an already established infection with an adult tapeworm from other *Taenia* species seems to be protective for the host preventing the infection with *T. solium* tapeworms. For infections with the metacestode, it seems that acquired immunity after exposure to *Taenia* spp. eggs or transferred
immunity from mother to the offspring may have a modulatory effect. The mechanisms of this regulation are not completely understood but it may be that cross-resistance plays a role; also, an interspecific competition in the host is plausible. This competition results in the limitation of the number of infections by limiting the number of susceptible hosts [168;172]. On the other hand, molecular studies have identified two main genotypes of *T. solium*, an Asian genotype and a Latin American/African genotype, the difference in their infective capability has to be further studied. For instance, the lack of subcutaneous HCC clinical manifestations in Latin America differ with commonly found subcutaneous HCC manifestations in Asia and some parts of Africa. Studying the infection patterns in epidemiological studies conducted in different geographical settings where each genotype is present could help characterizing possible differences between the infective capability of these types [115;173-175].

Field studies demonstrated that there is an increased seroprevalence of HCC surrounding tapeworm carriers. This seems to be moderated by the distance to the tapeworm carrier forming clusters, thus the level of infections in a population seems also to be regulated by the number of clusters present and the number of susceptible individuals living in proximity to tapeworm carriers. [18].

Climatic conditions affect the infectivity and the dispersal of the eggs in the environment. The effects of humidity, desiccation and temperature have an important impact on the survival and distribution of *Taenia* spp. eggs. Temperatures between 10° and room temperature and high humidity favour egg survival, while extreme temperatures and desiccation diminish the survival and infectivity of the eggs. The longevity of *Taenia* spp. eggs in the environment is variable depending on the species studied. It has been reported that in certain conditions of humidity and temperature *Taenia* spp. eggs can survive from 21 to 413 days (*T. saginata*) or from 0 to 300 days (*Taenia pisiformis*). Specifically for *T. solium*, information on the survival and viability of the eggs in the environment is not yet available. Most of the current knowledge comes from studies on other *Taenia* species. It has to be taken into account that extrapolating such information is hazardous because of the biological differences among *Taenia* spp. [164;176;177]. Yet, seasonal fluctuations of the prevalence of PCC suggest that environmental factors influence the transmission of the disease [178]. The maintenance of the infections in confined pigs also suggests that environmental contamination happens even when pigs have no direct access to infected feces. How environment influences PCC transmission still needs to be elucidated [178;179].

The presence of other pathogens in the same area seems to affect the hosts’ resistance to the parasite. In the case of HCC, debilitating infections such as tuberculosis and HIV, affecting the immune system of the host seem to increase the susceptibility to HCC [104;180-184]. In the case of PCC, the presence of other pathogens in the area indirectly affects the transmission of the disease. When African swine fever is present, farmers tend to keep their pigs penned to avoid infection. This change in the
production system may reduce the pigs’ exposure to human feces, thus negatively affecting the transmission of PCC [178]. The combination of these elements with a conditional exposure to eggs due to a lack of hygiene and behavioural, cooking and agricultural practices may greatly influence the pattern of infections [179;185].

The intrinsic factors of the human host are the final barrier that regulates the level of infection. Different immune profiles will allow or prevent infection. Age-related immunity plays a role in the susceptibility to infection in humans; older age groups appear to be more prone to HCC, and this phenomenon was attributed to immune senescence [16;17;136]. Hormonal profiles related to gender that have an effect on the immunity, nutritional status, ethnic susceptibility and innate or acquired immunity are also elements capable of influencing the susceptibility [104;186-188]. Similarly, for PCC the breed type, hormonal profile, acquired immunity, possible resistance through passively transferred maternal antibodies, pregnancy and castrated status influence the susceptibility to infection [189-193]. For human taeniasis there is no evidence suggesting development of immunity, however, transmission seems to be regulated by the competition with other Taenia species [167;172]. The regulatory capacity of these elements and their interaction need to be well understood to proficiently achieve the design of specific control strategies.

1.6 Control programs

In 1993 the International task Force for Disease Eradication (ITFDE) stated that HCC was a potentially eradicable disease. This statement was based on the fact that human tapeworm carriers are the only source of infection for HCC, pigs are the reservoir but have a rather short lifespan, there are no significant reservoirs in the wildlife, treatments for HCC and taeniasis are available and Europe was presented as an example where previously endemic T. solium was eradicated [194]. Following this approach, the preventive and control measures against T. solium infections that were the responsibility of the veterinary services alone, became also the responsibility of the medical services [195]. However, to date there is no indication that eradication of T. solium can be achieved in the near future in endemic areas [14].

The strategies proposed to control T. solium infections are based on interrupting the life cycle of the parasite, some of them are adapted from the experiences learned in developed countries where the disease was efficiently eradicated. Table 1-1 details the intervention strategies available for T. solium taeniasis with their advantages and disadvantages.
Table 1-1 Synopsis of intervention strategies available for *Taenia solium* taeniasis/cysticercosis, adapted from Allan et al. (2002) [196] and updated from Thys et al. (2015) [197]; Carabin et al. (2014) [14]; Braae et al. (2015) [179]; Gilman et al. (2012) [198]; Bulaya et al. (2015) [199]; Miwunda et al. (2015) [200].

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<th>Intervention strategy</th>
<th>Advantages</th>
<th>Disadvantages</th>
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| Elimination of infected pig carcasses (meat inspection) | • Known contribution to elimination of parasite from several developed countries  
• Relatively easy to integrate with meat inspection for several other important diseases  
• Low cost intervention | • Pigs in many endemic countries do not go to formal slaughter  
• Infected pigs can be diagnosed ante mortem (tongue inspection) and slaughtered outside regulated system to avoid condemnation of carcasses  
• Low sensitivity  
• Economical problem, especially for poor rural communities |
| Freezing meat | • Freezing meat can kill the parasite cysts | • Long periods of time are required to kill the cysts in frozen meat  
• Infrastructure to freeze the meat is not always available |
| Improvement of cooking practices | • Proper heat treatment of meat can kill the cysts | • Some cultures consume dishes containing raw or poorly cooked meat  
• Difficult to assess when large pieces of meat are cooked or boiled |
| Improved sanitation, hygiene, community led total sanitation (CLTS) | • Known contribution to elimination of parasite from several developed countries  
• Provides benefits beyond control of *T. solium* | • Economically difficult in many existing endemic areas  
• Resistance to use the latrines for cultural or practical reasons  
• CLTS requires monitoring over a long period of time  
• Results from current field works failed to prove a decrease in PCC  
• Require maintenance of latrines  
• CLTS must be accompanied by a health education program to increase awareness about the disease and the benefits of latrine use |
| Health education | • Provides benefits beyond control of *T. solium*  
• New media now widely available | • Improved knowledge does not always result in change of practices  
• Require long time follow-ups |

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<th>Intervention strategy</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Treatment of intestinal taeniasis (Targeted interventions)</td>
<td>• Highly efficacious drugs available now (some generically produced niclosamide may have low efficacy)</td>
<td>• Would require repeated interventions for long-term control</td>
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<td></td>
<td>• Demonstrated short-term benefits</td>
<td>• Requires specific infrastructure; not self-sustainable</td>
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<tr>
<td></td>
<td>• Removes known significant transmission risk</td>
<td>• Praziquantel should be used with caution in cases of NCC</td>
</tr>
<tr>
<td></td>
<td>• Reduced price when compared to MDA</td>
<td>• Targeted interventions are difficult to perform due to reluctance to collect stool samples for diagnosis and because of lack of sensitivity of diagnostic tests</td>
</tr>
<tr>
<td>Treatment of intestinal taeniasis (Mass drug administration (MDA))</td>
<td>• Highly efficacious drugs available now</td>
<td>• Some generically produced niclosamide may have low efficacy</td>
</tr>
<tr>
<td></td>
<td>• Demonstrated short-term benefits</td>
<td>• Transmission is recovered quickly after stopping the treatment</td>
</tr>
<tr>
<td></td>
<td>• Removes known significant transmission risk</td>
<td>• Environmental contamination with <em>Taenia</em> eggs</td>
</tr>
<tr>
<td></td>
<td>• Reduced logistic cost when compared to other strategies</td>
<td>• Niclosamide and praziquantel are not always available in endemic areas</td>
</tr>
<tr>
<td></td>
<td>• Increased feasibility when compared to targeted interventions</td>
<td>• Praziquantel should be used with caution in cases of NCC</td>
</tr>
<tr>
<td></td>
<td>• Could be paired with current treatments for geohelminths</td>
<td></td>
</tr>
<tr>
<td>Improved pig husbandry</td>
<td>• Known contribution to elimination of parasite from several developed countries</td>
<td>• Pigs are still infected even in confined environment</td>
</tr>
<tr>
<td></td>
<td>• Provides benefits beyond control of <em>T. solium</em></td>
<td>• Too expensive for poor farmers</td>
</tr>
<tr>
<td>Vaccination of pigs</td>
<td>• Long-term protection</td>
<td>• Free roaming is a practice to save money by avoiding having to feed the pigs</td>
</tr>
<tr>
<td></td>
<td>• Possible to integrate with existing veterinary and/or pig husbandry practices</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Provides economic benefit to end user (avoidance of carcass condemnation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Compliance monitoring possible (serological testing)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Many existing producers in endemic areas do not currently vaccinate against other diseases with high economic impact on pig production</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Vaccines not available now (other than at experimental level)</td>
<td>• Immunization schedule impractical in field conditions</td>
</tr>
<tr>
<td></td>
<td>• Not accessible for poor farmers who do not even have money to feed their pigs</td>
<td></td>
</tr>
</tbody>
</table>
### Intervention strategy

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy of infected pigs</td>
<td>• Effective drugs available</td>
<td>• Not all drugs can be found in endemic areas</td>
</tr>
<tr>
<td></td>
<td>• Highly effective</td>
<td>• Producers often do not treat for other economically important parasites despite economic benefits</td>
</tr>
<tr>
<td></td>
<td>• Producers have economic motivation (avoidance of carcass condemnation)</td>
<td>• Existing systems of avoiding meat inspection reduce economic advantages</td>
</tr>
<tr>
<td></td>
<td>• Other production benefits: can affect other economically important parasites of pigs</td>
<td>• Long withdrawal time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Drugs other than oxfendazole require more than one dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 3 months for complete disappearance of the cysts when using oxfendazole</td>
</tr>
</tbody>
</table>
Figure 1-6 shows a proposal for the integration of control strategies with the use of diagnostic tests according to the *T. solium* life cycle [201]. There is consensus among scientists that the best approach is a combination of different intervention strategies, in this case veterinary and medical services as well as other health-related scientists should join forces and follow the One Health approach [198;202-204]. An integrated control program should include the use of diagnostic tools, for correct diagnosis and for assessment of the control program [204].

![Figure 1-6 The cysticercosis cycle: intervention points [201]](image)

Studies conducted in China, Mexico, Peru, Tanzania, and Ecuador, demonstrated that a significant reduction of *T. solium* infections is achievable through the application of different intervention strategies. The interventions conducted in China, Mexico, Tanzania, and Ecuador aimed to control *T. solium* in specific areas, while the intervention in Peru aimed at eliminating *T. solium* from a province [15;198;205-207]. The strategies applied in China consisted of health education, health promotion, and chemotherapy of tapeworm carriers. These strategies significantly decreased infections with *T. solium* after 6 years of efforts [206]. In Mexico interventions consisted of either 1) health education only or 2) mass drug administration (MDA) in humans to treat taeniasis. Evaluation 6 and 42 months after intervention resulted in a significant reduction of the seroprevalence of cysticercosis in humans and pigs [205;207]. The intervention in Ecuador was based on the use of MDA. One year later PCC was
significantly reduced in pigs and no new tapeworm infections were recorded [15]. In Tanzania, the strategy applied was a health and pig management intervention. The results were measured one year later by using sentinel pigs. A considerable decrease in the seroprevalence of circulating antigens in these pigs was shown [208]. The elimination program in Peru was composed of combinations of different strategies. These included repeated chemotherapy for both humans and pigs and pig vaccination. This program achieved a focal elimination [198]. However, sustainability of elimination over time has yet to be evaluated. On the other hand, not all the experimental strategies have been successful in the field, in 2015 an intervention in Zambia using CLTS only, failed to reduce PCC [199].

A lesson learned from these intervention studies is that social and environmental differences in endemic areas have to be taken into account in order to adopt the best control program. Successful strategies may not work in all settings because of cultural taboos, occupational activities or behavioral practices [197-199]. Other concerns raised from these studies were the sustainability and the long-term results of these measures and the cost-efficiency of these.

The development and use of mathematical simulation models can guide the selection of the best control strategy, thus saving resources and time [209]. The use of simulation models can help to rank the control strategies depending on their relative effectiveness. To do so, the simulation models take into account the transmission dynamics of the disease and all known measurable parameters influencing it. In addition, simulation models can help to fill the gaps in the absence of convincing evidence from field trials [14]. However, to achieve these the model should be fed with correct parameters [14;165;209]. The current transmission dynamic model available for T. solium generates estimates for human taeniasis and porcine cysticercosis. From this study it was observed that the effective control of the parasite relies on the improvement of sanitation and the improvement of pig management practices [14;209]. To date there is no published transmission model for human cysticercosis.
Rationale & Objectives
Rationale

*T. solium* cysticercosis is considered a potentially eradicable disease. However, to date, there is no scientific evidence supporting eradication in the short term. Different interventions designed to reduce the number of infections had promising results in some endemic areas, but these results were not always reproducible in other areas. Gaps in the understanding of the transmission dynamics of the disease probably contributed to the limited success or to the failure of interventions implemented.

There is a need to create feasible and cost-effective control programs adapted to the current situation in *T. solium* endemic countries.

In Ecuador, *T. solium* cysticercosis/taeniasis are endemic and are responsible for public health, agricultural and socio-economic problems. Epidemiological studies and official reports show that *T. solium* infections are present both in rural and urban settings. An experimental intervention strategy conducted in the country achieved promising results showing that control of parasite transmission can be achieved. However, to date, no control program has been put in practice to control/eliminate the parasite in any part of the country and official reports keep on showing occurrence of new cases of HCC.

When designing control programs in endemic countries, it is critical to evaluate the general conditions that enhance the transmission, maintenance and negative health outcomes of a disease. This will help to identify and prioritize areas of intervention and consequently choose the adequate strategy for an effective control and elimination.

Additionally, in order to assess the success or failure of an applied strategy, it is important to understand the meaning of epidemiological patterns that can be obtained by field surveys or by hospital reports. It is crucial for policymakers and decision takers to have a correct interpretation of epidemiological data in dynamic conditions such as multiple affected communities located in different socio-economic, geographical and agro-ecologic settings within a region or country.

Objectives:

For these reasons, the main objective of this research is to contribute to the understanding of epidemiological patterns of the infection and exposure to *T. solium* in Ecuador and to contribute to the design of control programs.

To achieve this goal, 4 specific objectives were defined.

1) Characterize active infections and exposure to *T. solium* in endemic zones around the world.
2) Study the distribution in time and space of NCC hospitalized cases in Ecuador.
3) Identify potential indicators of NCC hospitalized cases in Ecuador.
4) Study the dynamics of active infections and exposure to *T. solium* in an endemic area in Ecuador.
Chapter 2

**Taenia solium** exposure and active infections around the world: Characterization and variability in endemic communities

Adapted from:

2.1 Chapter summary

Taenia solium cysticercosis is a zoonotic neglected disease responsible for severe health disorders such as seizures and death. T. solium cysticercosis is endemic in countries in Africa, Latin America and Asia where conditions such as inadequate hygiene, poor sanitary conditions, open defecation, free roaming pigs and poverty permit the transmission of the disease. Understanding the epidemiology of human cysticercosis (HCC) in endemic regions will help to expose critical information about the transmission of the disease, which could be used to design efficient control programs. Current serological diagnostic tools are capable of detecting exposure to eggs and infection levels in a population through antibody and antigen detection, respectively. This review gathered serological data on apparent prevalence of T. solium circulating antigens and/or seroprevalence of T. solium antibodies, apparent prevalence of human taeniasis and risk factors for HCC from endemic communities in order to understand the differences in exposure to the parasite and active infections with T. solium metacestodes patterns in endemic areas around the world. Three databases were used to search sero-epidemiological data from community-based studies conducted between 1989 and 2014 in cysticercosis endemic communities worldwide. The search focused on data obtained from T. solium circulating antigen detection by monoclonal antibody-based sandwich ELISA and/or T. solium antibody seroprevalence determined by Enzyme-linked Immunoelectrotransfer Blot (EITB). A meta-analysis was performed per continent. A total of 39,271 participants from 19 countries, described in 37 articles were studied. The estimates for the prevalence of circulating T. solium antigens for Africa, Latin America and Asia were: 7.30% (95% CI [4.23- 12.31]), 4.08% (95% CI [2.77-5.95]) and 3.98% (95% CI [2.81-5.61]), respectively. Seroprevalence estimates of T. solium antibodies were 17.37% (95% CI [3.33-56.20]), 13.03% (95% CI [9.95-16.88]) and 15.68% (95% CI [10.25-23.24]) respectively. Taeniasis reported prevalences ranged from 0 (95% CI [0.00-1.62]) to 17.25% (95% CI [14.55-20.23]). A significant variation in the sero-epidemiological data was observed within each continent with African countries reporting the highest apparent prevalences of active infections. Intrinsic factors in the human host such as age and immunity were main determinants for the occurrence of infections while exposure was mostly related to environmental factors, which varied from community to community. Understanding the epidemiology of cysticercosis in endemic regions will help exposing information on the transmission, which could in turn be used to design appropriate control programs.
2.2 Introduction

“The most dangerous worldview is the worldview of those who have not viewed the world”

— Alexander von Humboldt

*Taenia solium* human cysticercosis (HCC) is a zoonotic parasitic disease causing severe health and economic problems in endemic areas in Latin America, Africa and Asia [2;4;31;53]. Even though HCC is considered potentially eradicable, it is still highly prevalent in developing countries [210]. Different intervention measures have to be integrated to interrupt transmission of *T. solium*. Effective control programs will reduce the incidence and prevalence of the disease leading to acceptable and manageable levels, which could therefore become a first step towards elimination and eradication of the disease. Understanding the conditions for transmission is required for developing appropriate interventions [209]. This mainly involves accurate estimations of exposure and infection patterns in communities, which can be obtained by laboratory tests [17;209]. Current immunological tools used in HCC diagnosis can be classified into: 1) Tests detecting antibodies directed against *T. solium* cysticerci, identifying exposure to the parasite, and 2) Tests detecting circulating antigens produced by living cysticerci, identifying current infection with viable cysticerci [72]. Measuring the level of adult tapeworm infections in a population can be used as a support for results obtained from immunological tests to measure exposure. To understand exposure and infection patterns it is also important to have a correct interpretation of risk factors, information that can be provided by studying the correlation between host, environment and parasite factors and serological results for cysticercosis.

Similar sero-epidemiological studies conducted in two endemic communities in Africa and Latin America, presented comparable serological results for exposure to *T. solium* eggs with exposure levels of 34.55% and 31.22%, respectively but presented significant differences when reporting active infections with almost 12 times more infections in the African than in the Latin American community [17;136], suggesting that there were significant variations in the conditions for transmission and in the establishment of infection in each community. For this reason, extrapolating results from a single community to a regional or even global level can be a hazardous exercise.

The aim of this review is to systematically collect serological data on apparent prevalence of *T. solium* circulating antigens and/or seroprevalence of *T. solium* antibodies, apparent prevalence of human taeniasis and risk factors for HCC from endemic communities in order to understand the differences in exposure to the parasite and active infections with *T. solium* metacestodes in endemic areas around the world.
2.3 Methods

A systematic literature search on *T. solium* HCC seroprevalence in community-based studies performed in endemic countries in Africa, Asia, Latin America and the Caribbean was conducted on indexed literature published during the period from 1989 to 2014. In order to have comparable data, this search focused on the articles in which data was obtained using the following techniques and protocols: 1) Enzyme-linked Immunoelectrotransfer Blot (EITB) from Tsang et al. (1989) [80] for detection of antibodies directed against seven specific glycoproteins from *T. solium* metacestodes and/or 2) Enzyme Linked Immunosorbtent Assay detecting circulating antigens from the *T. solium* metacestode (B158/B60 Ag-ELISA or HP10 Ag-ELISA) from Brandt et al. (1992) [82], Van Kerckhoven et al. (1998) [211], Dorny et al. (2000) [94] and Harrison et al. (1989) [83]. Selection was restricted to both EITB and Ag-ELISA because of their performance, frequent use and acceptance as highly sensitive and specific tests in community-based studies when compared to other techniques. The EITB has a sensitivity ranging from 97 to 98% and a specificity ranging from 97 to 100% to detect circulating antibodies to *T. solium* in human serum. The EITB is considered positive when at least one of the seven specific glycoproteins from *T. solium* metacestodes is recognized by the serum. The B158/B60 Ag-ELISA has a sensitivity of 90% (95% CI: [80-99%]) and a specificity of 98% (95% CI: [97-99%]) to detect circulating antigens released by *T. solium* metacestodes in humans, with no cross-reactions reported to date [72;80;84]. For the HP10 Ag-ELISA the reported sensitivity ranges from 84.8% to 86% and the specificity is estimated at 94% to detect circulating antigens in human serum [85;86]. For the studies where the EITB was performed, either the Centers for Disease Control and Prevention (CDC) test or commercial kits were accepted for selection. Taeniasis apparent prevalence data was collected when available in the selected articles and no restrictions for the diagnostic method was made. It was anticipated that the taeniasis data would be influenced by the technique used because coprology, coproantigen ELISA and molecular methods have wide differences in their diagnostic performances. The estimated sensitivities of these tests were: 52.5% (95% CI: [11.1-96.5]) for coprology, 84.5% (95% CI: [61.9-98]) for coproantigen ELISA and 82.7% (95% CI: [57-97.6]) for real-time polymerase chain reaction assay (copro-PCR) and their specificities were: 99.9% (95% CI: [99.5-100]) for coprology, 92% (95% CI: [90-93.8]) for coproantigen ELISA and 99% (95% CI: [98.2-99.6]) for copro-PCR [108]. The wide confidence interval for the estimated value for the sensitivity of coprology is due to the low number of positive cases found by this method, which does not allow a more accurate estimation, however is in line with previous studies [81;107;212]. Language restriction was applied when the article was written in a language other than those spoken or understood by the authors of this review. The considered languages were English, Spanish, French, Portuguese and Dutch.
The selected databases for this study were: PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), LILACS (Latin American and Caribbean Health Sciences Literature, lilacs.bvsalud.org/en/) and Web Of Science (http://wok.mimas.ac.uk/). The search was performed from September 1st 2013 until October 31st 2014.

2.3.1 Search

The following search strategy was applied: In PubMed, using the Boolean operator AND, the terms “cysticercosis” AND “Taenia solium” AND “epidemiology” were introduced in the main search bar and the filters were activated for the period from 1988/12/31 to 2014/10/31. In Web of Science, the strategy applied was introducing in the basic search bar the topic “cysticercosis” adding fields with the correspondent Boolean operators: AND Topic=(Taenia solium) AND Topic=(epidemiology). In LILACS, the strategy adopted consisted in introducing in the main search bar the terms “cysticercosis Taenia solium”. In the latter case the term “epidemiology” was excluded to obtain the maximum return of articles since LILACS is a smaller targeted database when compared to PubMed and Web Of Science. Additionally, relevant articles were included that were not found with this search strategy but matched the selection criteria after manual search or expert recommendation.

2.3.2 Study selection

The articles were selected following three phases: The first phase consisted in the removal of all repeated studies from the title selection and all studies performed before 1989. The second phase consisted in the exclusion of articles from the title and abstract review as for the following exclusion criteria: 1) Wrong parasite species, 2) Studies performed in non-endemic countries, 3) Studies performed only in animals, 4) Clinical studies, 5) Studies which focused only on human taeniasis, 6) Studies carried out for assessing laboratory tests performance, 7) Studies that focused on NCC, 8) Studies conducted in specific targeted types of individuals (e.g. schoolchildren, refugees or soldiers), 9) Articles written in languages other than those spoken or understood by the authors of this review, 10) Interviews, letters, reviews or editorials not presenting original data and/or the techniques and protocols performed on their studies, 11) Studies not related to T. solium epidemiology. The third phase was applied when full texts were read and consisted in the study selection according to the following selection criteria: 1) Community-based studies, 2) Original HCC prevalence reports available, 3) Protocols applied for HCC diagnosis using the EITB and Ag-ELISA protocols mentioned previously in this document, 4) Random sampling method for selection of participants in the study or/and population representative voluntary participation. 5) Coverage of most age groups (young, adults and elderly).
2.3.3 Data collection

From every selected article the following items were collected and introduced in a data base: Author(s), year the article was published, country, number of participants for Ag-ELISA survey, number of Ag-ELISA positive detected cases, number of participants for EITB survey, number of EITB positive detected cases (when specified in the article, the EITB positive cases were considered when at least one band had a visible reaction [80]), estimated apparent prevalence expressed in percentage. When applicable, identified risk factors were also included in the database. For longitudinal studies where two or more sampling rounds were organized in a community, only data from the first round were taken into account in order to avoid any possible bias caused after contact with the teams collecting the samples. Additionally, if the study was conducted in parallel with a survey on human taeniasis, the prevalence and the method used to diagnose taeniasis were also gathered. All countries where the selected studies took place were visualized using QGIS software (version 2.8).

2.3.4 Statistical analysis

A meta-analysis was conducted using the “meta” package in R [213] to estimate the prevalence of circulating antigens for T. solium and the seroprevalence of antibodies in each continent based on a random effects model. A global prevalence estimation was not performed as it was not the intention of this study. Ninety five % exact binomial Confidence Intervals (95% CI) were calculated for every reported prevalence. The significant differences in estimated prevalences were evaluated using their 95% confidence intervals. The difference in prevalence between two regions was considered to be statistically significant if their 95% confidence intervals do not overlap.

2.4 Results

2.4.1 Study selection

Figure 2-1 describes the review process and the number of articles selected at each stage of the review. From an initial number of 696 articles, only 37 studies were included in this review; 9 studies were selected for the African region [134;136;186;214-219], 22 studies for Latin America [16-18;73;109;131;140-142;144;153;185;205;220-228] and 6 for the Asian region.
Figure 2-1. Flow diagram describing literature search and selection of studies (PRISMA 2009 Flow chart).
2.4.2 Human cysticercosis in Africa

From the 9 sero-epidemiological studies from 7 countries selected for the African region, 7 studies [134;186;214-216;218;219] used Ag-ELISA and two studies [136;217] used both Ag-ELISA and EITB. The total number of individuals sampled for serological testing in this region was 12,596. Prevalence of circulating antigens ranged from 0.68% to 21.63%, while seroprevalence of antibodies ranged from 7.69 to 34.55%. Detailed descriptions of each study are given in Table 2-1.
### Table 2-1. Sero-epidemiological studies using Ag-ELISA and/or EITB for human cysticercosis in Africa.

<table>
<thead>
<tr>
<th>Author</th>
<th>YOP</th>
<th>Country</th>
<th>Location</th>
<th>Circulating Ag positive cases/total participants</th>
<th>Circulating Ag prevalence (%) [95%CI]</th>
<th>Ab positive cases/total participants</th>
<th>Ab seroprevalence (%) [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nguekam et al.</td>
<td>2003</td>
<td>Cameroon</td>
<td>West province</td>
<td>347/4993</td>
<td>0.68 [0.47-0.95]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secka et al.</td>
<td>2011</td>
<td>Senegal</td>
<td>Ziguinchor</td>
<td>317/403</td>
<td>7.69 [5.28-10.74]</td>
<td>31/403</td>
<td>7.69 [5.28-10.74]</td>
</tr>
<tr>
<td>Mwape et al.</td>
<td>2013</td>
<td>Zambia</td>
<td>Eastern Province</td>
<td>141/1129</td>
<td>12.49 [10.61-14.56]</td>
<td>57/165</td>
<td>34.55 [27.32-47.33]</td>
</tr>
<tr>
<td>Mwanjali et al.</td>
<td>2013</td>
<td>Tanzania</td>
<td>Mbeya</td>
<td>139/830</td>
<td>16.75 [14.27-19.46]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas</td>
<td>2014</td>
<td>Kenya</td>
<td>Western and Nyanza</td>
<td>169/2094</td>
<td>8.07 [6.94-9.32]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td>852/12596</td>
<td>6.76 [6.33-7.22]</td>
<td>88/568</td>
<td>15.49 [12.61-18.73]</td>
</tr>
<tr>
<td><strong>REMEP:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.30 [4.23-12.31]</td>
<td></td>
<td>17.37 [3.33-56.20]</td>
</tr>
</tbody>
</table>

Legend: YOP: Year of publication; Location: Corresponds to the Province, State, Region or Department in which the communities where the studies took place are located; Ag: Antigen detection based on Ag-ELISA results; Ab: Antibody detection based on EITB results; 95% CI: 95% Confidence Intervals; REMEP: Random Effects Model Estimated Prevalence; a: Results obtained from B158/B60 Ag-ELISA; b: Results obtained from HP10 Ag-ELISA. Dem. Rep. of Congo: Democratic Republic of Congo.
2.4.3 Human cysticercosis in Latin America

Information on HCC in Latin America was obtained from 22 studies from 8 countries with two studies performing only Ag-ELISA [131;141], 17 studies performing EITB only [18;73;109;140;142;144;185;205;220-228] and three studies performing both Ag-ELISA and EITB [16;17;153]. The total number of individuals sampled was 21,911. Active *T. solium* infection ranged from 0.94 to 9.12% and antibody seroprevalence ranged from 1.82 to 31.22%. A detailed description of each study is given in Table 2-2.
Taenia solium exposure and active infections around the world: Characterization and variability in endemic communities

Table 2-2. Sero-epidemiological studies using Ag-ELISA and/or EITB for human cysticercosis in Latin America

<table>
<thead>
<tr>
<th>Author</th>
<th>YOP</th>
<th>Country</th>
<th>Location</th>
<th>Circulating Ag positive cases/Total participants</th>
<th>Circulating Ag Prevalence (%) [95%CI]</th>
<th>Ab positive cases/Total participants</th>
<th>Ab seroprevalence (%) [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Díaz et al.</td>
<td>1992</td>
<td>Peru</td>
<td>San Martin</td>
<td>30/371</td>
<td>8.09 [5.52-11.34]</td>
<td>30/371</td>
<td>8.09 [5.52-11.34]</td>
</tr>
<tr>
<td>García et al.</td>
<td>1998</td>
<td>Peru</td>
<td>Cusco</td>
<td>24/102</td>
<td>23.8 [15.69-32.96]</td>
<td>24/102</td>
<td>23.8 [15.69-32.96]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peru</td>
<td>Junin</td>
<td>140/398</td>
<td>35.18 [30.48-40.1]</td>
<td>140/398</td>
<td>35.18 [30.48-40.1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colombia</td>
<td>N.A.</td>
<td>23/750</td>
<td>3.07 [1.95-4.57]</td>
<td>23/750</td>
<td>3.07 [1.95-4.57]</td>
</tr>
<tr>
<td>Gomes et al.</td>
<td>2002</td>
<td>Brazil</td>
<td>Bahia</td>
<td>11/694</td>
<td>1.59 [0.79-2.82]</td>
<td>11/694</td>
<td>1.59 [0.79-2.82]</td>
</tr>
</tbody>
</table>

Table continues on the next page
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Location</th>
<th>District/Province</th>
<th>Prevalence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Venezuela</td>
<td>Lara</td>
<td>37/608</td>
<td>6.08 [4.32-8.29]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Venezuela</td>
<td>Lara</td>
<td>20/305</td>
<td>6.56 [4.05-9.95]</td>
</tr>
<tr>
<td>Agudelo-Flores &amp; Palacio</td>
<td>2003</td>
<td>Columbia</td>
<td>Antioquia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moro et al.</td>
<td>2003</td>
<td>Peru</td>
<td>Lima</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montano et al.</td>
<td>2005</td>
<td>Peru</td>
<td>Tumbes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodriguez et al.</td>
<td>2006</td>
<td>Ecuador</td>
<td>Loja</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agudelo-Flores et al.</td>
<td>2009</td>
<td>Colombia</td>
<td>Chocó</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lescano et al.</td>
<td>2009</td>
<td>Peru</td>
<td>Tumbes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Praet et al.</td>
<td>2010</td>
<td>Ecuador</td>
<td>Loja</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coral et al.</td>
<td>2014</td>
<td>Ecuador</td>
<td>Loja</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>347/7853</td>
<td>4.42 [3.97-4.90]</td>
</tr>
<tr>
<td>REMEP</td>
<td></td>
<td></td>
<td></td>
<td>2282/16369</td>
<td>13.94 [13.41-14.48]</td>
</tr>
</tbody>
</table>

Legend: YOP: Year of publication; Location: Corresponds to the Province, State, Region or Department in which the communities are located where the studies took place; Ag: Antigen detection based on Ag-ELISA results; Ab: Antibody detection based on EITB results; 95% CI: 95% Confidence Intervals; REMEP: Random Effects Model Estimated Prevalence; a: Results obtained from B158/B60 Ag-ELISA; b: Results obtained from HP10 Ag-ELISA; N.A.: Not available.
2.4.4 Human cysticercosis in Asia

The data obtained for HCC in Asia resulted from 6 studies from 4 countries organized in 4 studies performing Ag-ELISA only [84;135;172;229], one study performing EITB only [230] and one study performing both Ag-ELISA and EITB [231]. The total number of individuals sampled was 4,764. Seroprevalence varied from 0.57 to 5.71% for circulating *T. solium* metacestode antigens and from 12.60 to 19.17% for *T. solium* antibody seroprevalence. Detailed descriptions of each study are given in Table 2-3.
Table 2-3. Sero-epidemiological studies using Ag-ELISA and/or EITB for human cysticercosis in Asia

<table>
<thead>
<tr>
<th>Author</th>
<th>YOP</th>
<th>Country</th>
<th>Location</th>
<th>Circulating Ag positive cases/total participants</th>
<th>Circulating Ag prevalence (%) [95%CI]</th>
<th>Ab positive cases/total participants</th>
<th>Ab seroprevalence (%) [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theis et al.</td>
<td>1994</td>
<td>Indonesia</td>
<td>Bali</td>
<td>94/746</td>
<td>12.60 [10.3-15.2]</td>
<td>94/746</td>
<td>12.60 [10.3-15.2]</td>
</tr>
<tr>
<td>Somers et al.</td>
<td>2006</td>
<td>Viet Nam</td>
<td>Bac Kan</td>
<td>16/303</td>
<td>5.28 [3.05-8.43]</td>
<td>16/303</td>
<td>5.28 [3.05-8.43]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viet Nam</td>
<td>Ha Tinh</td>
<td>1/175</td>
<td>0.57 [0.01-3.14]</td>
<td>1/175</td>
<td>0.57 [0.01-3.14]</td>
</tr>
<tr>
<td>Raghava et al.</td>
<td>2010</td>
<td>India</td>
<td>Tamil Nadu</td>
<td>46/960</td>
<td>4.79 [3.53-6.34]</td>
<td>46/960</td>
<td>4.79 [3.53-6.34]</td>
</tr>
<tr>
<td>Jayaraman et al.</td>
<td>2011</td>
<td>India</td>
<td>Tamil Nadu</td>
<td>48/1064</td>
<td>4.51 [3.34-5.94]</td>
<td>48/1064</td>
<td>4.51 [3.34-5.94]</td>
</tr>
<tr>
<td><strong>REMEP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.98 [2.81-5.61]</td>
<td>15.68 [10.25-23.24]</td>
<td></td>
</tr>
</tbody>
</table>

Legend: YOP: Year of publication; Location: Corresponds to the Province, State, Region or Department in which the communities are located where the studies took place; Ag: Antigen detection based on Ag-ELISA results; Ab: Antibody detection based on EITB results; 95% CI: 95% Confidence Intervals; REMEP: Random Effects Model Estimated Prevalence; a : Results obtained from B158/B60 Ag-ELISA.
Globally, 39,271 participants from 19 countries were included in 37 studies, from which 24,467 were studied for circulating *T. solium* antigen with 5.52% (1,351/24,467) positive cases (95% CI [5.24-5.82]) and 18,643 were studied for anti *T. solium* antibodies with a crude seroprevalence of 14.20% (2,648/18,643) (95% CI [13.70-14.71]). Figure 2-2 represents the global distribution of the countries where the serological studies took place.

**Figure 2-2.** Global distribution of the endemic countries where Ag-ELISA and/or EITB based epidemiological studies were held.

Light yellow represents the countries confirmed as endemic by the World Health Organization (WHO) until 2012 [118]. Circles represent the countries where Ag-ELISA based studies took place. Each color represents the average prevalence per country found from the selected articles in this review classified in 0 to 5 percent; 5 to 10 percent and more than 10 percent. Triangles represent the countries where EITB based studies took place. Each color represents the average prevalence per country found from the selected articles in this review classified in 0 to 5 percent; 5 to 10 percent and more than 10 percent.

### 2.4.5 Taeniasis

Within the examined reports, twenty-four studies from 14 countries in Africa (6 studies), Latin America (13 studies) and Asia (5 studies) reported adult *T. solium* data, using different diagnostic techniques. In these studies, 4,682 African, 13,782 Latin American and 3,437 Asian subjects participated; with a global participation of 21,901 individuals. Apparent prevalence on taeniasis varied from 0 to 17.25%. Results are shown in table 2-4.
Table 2-4. Taeniasis reports from Africa, Latin America and Asia

<table>
<thead>
<tr>
<th>Continent</th>
<th>Author</th>
<th>Year of publication</th>
<th>Country</th>
<th>Diagnostic Technique</th>
<th>Positive cases/Total participants</th>
<th>Prevalence (%) [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>Kanobana et al.</td>
<td>2011</td>
<td>Dem. Rep. of Congo</td>
<td>Coprology†</td>
<td>3/816</td>
<td>0.37 [0.08-1.07]</td>
</tr>
<tr>
<td></td>
<td>Secka et al.</td>
<td>2011</td>
<td>Senegal</td>
<td>Direct fecal examination</td>
<td>2/43**</td>
<td>4.65 [0.57-15.81]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FECT</td>
<td>4/43**</td>
<td>9.30 [2.59-22.14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>worm expulsion and morphological identification</td>
<td>1/43**</td>
<td>2.33 [0.06-12.29]</td>
</tr>
<tr>
<td></td>
<td>Mwape et al.</td>
<td>2012</td>
<td>Zambia</td>
<td>FECT</td>
<td>2/718</td>
<td>0.28 [0.03-1.00]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Copro-Ag ELISA</td>
<td>45/712</td>
<td>6.32 [4.65-8.37]</td>
</tr>
<tr>
<td></td>
<td>Mwanjali et al.</td>
<td>2013</td>
<td>Tanzania</td>
<td>Copro-Ag ELISA</td>
<td>43/820</td>
<td>5.24 [3.82-7.00]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EITB (rES38)</td>
<td>34/820</td>
<td>4.15 [2.89-5.75]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FECT</td>
<td>9/820</td>
<td>1.10 [0.50-2.07]</td>
</tr>
<tr>
<td></td>
<td>Mwape et al.</td>
<td>2013</td>
<td>Zambia</td>
<td>FECT</td>
<td>0/226</td>
<td>0.00 [0.00-1.62*]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Copro-Ag ELISA</td>
<td>27/226</td>
<td>11.95 [8.02-16.90]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FECT</td>
<td>4/2059</td>
<td>0.19 [0.05-0.50]</td>
</tr>
</tbody>
</table>

Table continues on the next page
### Table continued from previous page

<table>
<thead>
<tr>
<th>Continent</th>
<th>Author</th>
<th>Year of publication</th>
<th>Country</th>
<th>Diagnostic Technique</th>
<th>Positive cases/ Total participants*</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin America</td>
<td>Diaz et al.</td>
<td>1992</td>
<td>Peru</td>
<td>microscopy, both directly and after FECT</td>
<td>1/305</td>
<td>0.33 [0.01-1.81]</td>
</tr>
<tr>
<td></td>
<td>Sarti et al.</td>
<td>1992</td>
<td>Mexico</td>
<td>FECT</td>
<td>4/1531</td>
<td>0.26 [0.07-0.67]</td>
</tr>
<tr>
<td></td>
<td>Sarti et al.</td>
<td>1994</td>
<td>Mexico</td>
<td>FECT</td>
<td>2/828</td>
<td>0.24 [0.03-0.87]</td>
</tr>
<tr>
<td></td>
<td>Garcia-Noval et al.</td>
<td>1996</td>
<td>Guatemala</td>
<td>FECT &amp; Copro-Ag ELISA</td>
<td>27/995</td>
<td>2.71 [1.80-3.92]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Guatemala</td>
<td>FECT &amp; Copro-Ag ELISA</td>
<td>123/713</td>
<td>17.25 [14.55-20.23]</td>
</tr>
<tr>
<td></td>
<td>Sanchez et al.</td>
<td>1998</td>
<td>Honduras</td>
<td>FECT</td>
<td>2/404</td>
<td>0.50 [0.06-1.78]</td>
</tr>
<tr>
<td></td>
<td>Sanchez et al.</td>
<td>1999</td>
<td>Honduras</td>
<td>FECT</td>
<td>12/480</td>
<td>2.50 [1.30-4.33]</td>
</tr>
<tr>
<td></td>
<td>Sarti et al.</td>
<td>2000</td>
<td>Mexico</td>
<td>Copro-Ag ELISA</td>
<td>16/1865</td>
<td>0.86 [0.49-1.39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FECT</td>
<td>11/1865</td>
<td>0.59 [0.29-1.05]</td>
</tr>
<tr>
<td></td>
<td>Gomes et al.</td>
<td>2002</td>
<td>Brazil</td>
<td>Copro-Ag ELISA</td>
<td>26/577</td>
<td>4.51 [2.96-6.53]</td>
</tr>
<tr>
<td></td>
<td>Rodriguez et al.</td>
<td>2003</td>
<td>Ecuador</td>
<td>FECT</td>
<td>30/1935</td>
<td>1.55 [1.05-2.21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PCR-RFLP (identification)</td>
<td>8/29</td>
<td>27.59 [12.73-47.24]</td>
</tr>
<tr>
<td></td>
<td>Garcia et al.</td>
<td>2003</td>
<td>Peru</td>
<td>microscopy, both directly and after FECT</td>
<td>8/1317</td>
<td>0.61 [0.26-1.19]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Copro-Ag ELISA</td>
<td>45/1619</td>
<td>2.78 [2.03-3.70]</td>
</tr>
<tr>
<td></td>
<td>Lescano et al.</td>
<td>2009</td>
<td>Peru</td>
<td>FECT</td>
<td>11/898</td>
<td>1.22 [0.61-2.18]</td>
</tr>
<tr>
<td></td>
<td>Praet et al.</td>
<td>2010</td>
<td>Ecuador</td>
<td>FECT</td>
<td>0/674</td>
<td>0.00 [0.00-0.55*]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MSFT</td>
<td>0/674</td>
<td>0.00 [0.00-0.55*]</td>
</tr>
<tr>
<td></td>
<td>Rodriguez et al.</td>
<td>2006</td>
<td>Ecuador</td>
<td>FECT</td>
<td>14/958</td>
<td>1.46 [0.80-2.44]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PCR-RFLP (identification)</td>
<td>12/12</td>
<td>100 [73.54-100*]</td>
</tr>
</tbody>
</table>

Table continues on the next page
<table>
<thead>
<tr>
<th>Continent</th>
<th>Author</th>
<th>Year of publication</th>
<th>Country</th>
<th>Diagnostic Technique</th>
<th>Positive cases/Total participants</th>
<th>Prevalence (%) [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>Erhart et al.</td>
<td>2002</td>
<td>Viet Nam</td>
<td>FECT</td>
<td>0/210</td>
<td>0.00 [0.00-1.74*]</td>
</tr>
<tr>
<td></td>
<td>Somers et al.</td>
<td>2006</td>
<td>Viet Nam</td>
<td>Copro-Ag ELISA</td>
<td>1/297</td>
<td>0.34 [0.01-1.86]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KATO</td>
<td>1/297</td>
<td>0.34 [0.01-1.86]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Worm expulsion &amp; PCR-RFLP for identification</td>
<td>1/297</td>
<td>0.34 [0.01-1.86]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Copro-Ag ELISA</td>
<td>3/166</td>
<td>1.81 [0.37-5.19]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KATO</td>
<td>2/166</td>
<td>1.20 [0.15-4.28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Worm expulsion and PCR-RFLP for identification</td>
<td>1/166</td>
<td>0.60 [0.01-3.31]</td>
</tr>
<tr>
<td></td>
<td>Raghava et al.</td>
<td>2010</td>
<td>India</td>
<td>Copro-Ag ELISA</td>
<td>22/729</td>
<td>3.02 [1.90-4.53]</td>
</tr>
<tr>
<td></td>
<td>Jayaraman et al.</td>
<td>2011</td>
<td>India</td>
<td>Copro-Ag ELISA</td>
<td>6/729</td>
<td>0.82 [0.30-1.78]</td>
</tr>
</tbody>
</table>

Legend: *: Fecal samples were provided voluntarily from participants when not specified otherwise; *: One sided 97.5% confidence interval; **: Technique applied in fecal samples from seropositive subjects for T. solium antibodies or circulating antigens; Coprology†: Technique not defined; FECT: Formalin-ether concentration technique; KATO: Kato-Katz technique; MSFT: Magnesium sulphate flotation technique; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; ELISA: Enzyme-Linked Immunosorbent Assay; Copro-Ag ELISA: Coproantigen ELISA; EITB: Enzyme-linked Immunoelectrotransfer Blot; Dem. Rep. of Congo: Democratic Republic of Congo.
2.4.6 Risk factors for human cysticercosis

Twenty-three articles out of the 37 selected articles reported statistically significant risk factors for the presence of *T. solium* circulating antigens or *T. solium* antibodies. The identified risk factors for the presence of *T. solium* circulating antigens in Africa were: insufficient latrines or sanitary toilets [217], washing hands by dipping method [219], history of taeniasis or proximity to tapeworm carriers [186;219], being male [134] and increased risk with age [134;136;186;214;217;219], while the identified risk factors for presence of anti *T. solium* antibodies were: insufficient latrines or sanitary toilets, and increased risk with age [217]. In Latin America the only identified risk factor for presence of *T. solium* circulating antigens was associated with increased age [16], while the identified risk factors for presence of *T. solium* antibodies were: insufficient latrines or sanitary toilets [222;225;228], lack of potable water [222], poor personal hygiene [185], deficient house hygiene [185], earthen floor [222], presence of infected pigs [109;144], pig owning [109;222;225;228], pork consumption [185;228], history of taeniasis/proximity to tapeworms carriers [18;109;140;142;185], being female [142;223;228], age (risk increased with age) [16;17;220;221;227], increased risk at younger age [226], low education level [222;226], presence of a sewage system [227]. In Asia the only risk factor for the presence of *T. solium* circulating antigens was the ownership of pigs [229].

2.4.7 Meta-analysis

The random effects model used in the meta-analysis gave an overall estimated prevalence for circulating *T. solium* antigens in Africa of 7.30% (95% CI [4.23-12.31]) from 9 studies. The overall estimated prevalence for circulating *T. solium* antigens in Latin America was 4.08% (95% CI [2.77-5.95]) from 5 studies and 3.98% (95% CI [2.81-5.61]) for Asia from 5 studies. For the anti *T. solium* antibodies seroprevalence, the overall estimated seroprevalence in Africa was 17.37% (95% CI [3.33-56.20]) from 2 studies, while in Latin America it was 13.03% (95% CI [9.95-16.88]) from 20 studies and in Asia 15.68% (95% CI [10.25-23.24]) from 2 studies. The prevalence of circulating *T. solium* antigens was not significantly different between continents and neither was the anti *T. solium* antibodies seroprevalence. The prevalence of circulating *T. solium* antigens was significantly lower than the *T. solium* antibodies seroprevalence in Latin America and Asia but not in Africa.

2.5 Discussion

The results of this review allowed us to characterize and compare estimates of active infections with *T. solium* metacestodes and exposure to the parasite in endemic communities of different countries in three continents. The estimated seroprevalence of anti *T. solium* antibodies in Africa (17.37%), Latin America (13.03%) and Asia (15.68%) was not significantly different, which could be interpreted as a
similar exposure to *T. solium* eggs in these regions, though, within each continent a visible heterogeneity was observed, with seroprevalence for antibodies ranging from 1.82% to 40% as shown in tables 2-1, 2-2 and 2-3.

The estimated prevalence of *T. solium* circulating antigens was higher in endemic areas of Africa (7.30%) compared to Latin America and Asia (4.08% and 3.98%, respectively), though this difference was not statistically significant. When studied in detail, all the African studies but one showed a higher prevalence of active infections than the studies in Latin America and Asia: the prevalence of circulating antigens reported in Latin America and Asia ranged from 0.57 to 9.12%, while the prevalence in Africa ranged from 6.13 to 21.63%, excluding one study from West Cameroon in 2003 reporting a prevalence of 0.68% [214]. The figures observed in tables 2-1, 2-2 and 2-3 demonstrate big variations in prevalence of active infection with *T. solium* cysticerci, similar as observed for seroprevalence of antibodies.

Studies from the Democratic Republic of Congo, Zambia and Tanzania [134;136;219] revealed very high prevalence figures of circulating antigens not registered in any other part of the world (21.63%, 12.49% and 16.75%, respectively). These figures suggest a higher occurrence of HCC in Africa when compared to Latin America and Asia. The estimated prevalence of *T. solium* circulating antigens in each continent was lower than the estimated seroprevalence for *T. solium* antibodies in the same continent, but this difference was only statistically significant for Latin America and Asia and not for Africa.

In studies that carried out both antigen and antibody detection tests [16;17;136;153;231] a significantly higher seroprevalence of *T. solium* antibodies was observed (Tables 2-1, 2-2 and 2-3) except for one study performed in Senegal [217] in which the number of positive cases for Ag-ELISA and the number of EITB positive cases were similar. However, in that study only a fraction of the positive cases was positive in both tests. This marked difference in the prevalence of circulating antigens and of antibodies has already been observed by Praet et al. (2010) [72]: exposure expressed by antibody seroprevalence could be interpreted as the result of a past infection, current infection or the result of a failed infection, while circulating antigens can only be detected if viable cysticerci are present.

Findings in this review from sero-epidemiological studies have shown that *T. solium* transmission varied from one geographical location to another, which raised several questions on the causes of these differences. It is reasonable to think that the variations in the figures observed in this study could be the result of a combination of more than one element capable of affecting the presence of *T. solium* cysticercosis in a zone, such as individual host characteristics, parasite singularities and environmental properties each of them prone to change depending on the geographical situation.
Table 2-5 summarizes the potential factors contributing to the variations observed in serology from endemic communities.
**Table 2-5. Factors affecting serological variations of human cysticercosis infection and exposure to *T. solium* eggs.***

<table>
<thead>
<tr>
<th>Factors affecting exposure in a population (Presence of detectable antibodies)</th>
<th>Human host (Accidental intermediate host)</th>
<th>Parasite (<em>Taenia solium</em>)</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (time exposed to the parasite) [214]</td>
<td></td>
<td></td>
<td>Number of adult parasites present (tapeworm carriers, hotspots) [18,188]</td>
</tr>
<tr>
<td>Gender (Role played in different cultures)</td>
<td></td>
<td></td>
<td>Number of eggs dispersed in the environment [164;177]</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td>Egg survival (egg viability) on climatic conditions: Temperature, Humidity, seasonality [164;177]</td>
</tr>
<tr>
<td>Factors affecting active infections in a population (Presence of circulating antigens)</td>
<td></td>
<td></td>
<td>Hygiene, sanitation, behavioral practices, agricultural &amp; cooking practices [185]</td>
</tr>
<tr>
<td>Age (Immunosenescence) [16]</td>
<td></td>
<td></td>
<td>Area (endemic)</td>
</tr>
<tr>
<td>Gender (Hormonal profile) [188]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity (Immune characteristics) [158;188;233]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional status [186]</td>
<td>Presence of other <em>Taenia</em> species (within host) [168;172]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquired immunity [187]</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Innate immunity [17;173;229]</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Immune profile [187;234]</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Level of exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of concurrent infections [183;184;186]</td>
<td>T. solium genotype: Asian or African/Latin American [173;174;232]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Presence of other <em>Taenia</em> species (cross-resistance) [168;172]:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Presence of other pathogens [183;184;186]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In this review, age was reported as a significant risk factor both for the presence of circulating antigens and/or antibodies, studied reports are compatible with what is described in table 2-5. The effect of gender on prevalence is less clear: the studies in Latin America [142;223;228] indicated female to be more likely to have antibodies, while in Africa, in one study, male were more likely to present circulating antigens [134]. The effect of gender on transmission should be further studied to determine whether there is a real difference in physiological gender susceptibility to *T. solium* infections [187], or rather a difference associated with the role gender plays in every culture, resulting in different exposure. It is clear that immunity plays a huge role in the host susceptibility/resistance to the parasite but the underlying mechanisms are not well understood. In some cases, the origin of the type of immune resistance/susceptibility present in a population is characterized by the surrounding environment more than of an intrinsic factor in the host. This is the case for low immunity related to the poor nutritive status, which could be a consequence of poverty [186].

Surprisingly, only for the studies from Latin America included in this review, the presence of pigs and the consumption of pork were identified as important risk factors for exposure. In one study from Asia this risk factor was associated with the presence of circulating antigens. In contrast, none on the African studies revealed an association between pig ownership and pork consumption and infection or exposure. These results are compatible with the results from Mwape et al. (2015) [121], reporting other risk factors as the number of inhabitants and age as determinants when studying and ranking *T. solium* infections determinants by classification trees. In fact, HCC is the result of the ingestion of eggs coming from tapeworm carriers that could have acquired the adult tapeworm from pork meat either locally produced or transported from other endemic regions [219], thus, presence of pigs in this case is not as relevant for HCC transmission than the local prevalence of tapeworm carriers. HCC is considered a “clustered” disease; some studies have pointed out the importance of the presence of tapeworm carriers in a household triggering HCC in endemic and non-endemic communities[125;235]. Twenty three out of 24 studies reporting taeniasis identified at least one tapeworm carrier, however the diversity of methods used to diagnose taeniasis did not allow to make adequate comparisons between studies, even more, parasitological tests are considered having a low sensitivity, suggesting underestimation of the prevalences. Nevertheless, tapeworm carrier identification can be an important tool to recognize endemicity, in a zone, to identify the sources of infection and to support serological results.

Other important risk factors reported from Africa and Latin America were related to poor sanitary conditions, the deficient handling of human feces, the presence or history of adult tapeworms near the positive cases and low educational level. In the latter case, educational level could be interpreted also as an indicator of rural environment and/or poor household income in endemic communities. An interesting finding is the association of the presence of sewage installations in the house and positivity
Taenia solium exposure and active infections around the world: Characterization and variability in endemic communities

to EITB in a village in northern Peru [227]. Usually, sewage systems could be interpreted as a sanitary improvement, but in the Peruvian study, sewage installations were present only in some houses, which could result in the concentrated use of hygienic systems, and if they are not handled properly, they can turn the house into a “hotspot” for HCC [18].

Little is known about *T. solium* eggs survival in the environment. Some studies have been done on related cestodes like *Taenia saginata* and *Echinococcus* spp. [164], however, extrapolating knowledge from those studies could result in inadequate interpretations because of biological differences between these species. In addition, flies may play a role in the transmission of eggs of some *Taenia* spp. [236;237]; in the case of *T. solium*, however, flies were found not to be important because of the coprophagic behavior of pigs that left not enough time after defecation to allow flies to get impregnated with *T. solium* eggs [169]. Dung beetles were also shown to carry infective *T. solium* eggs in their bodies making them capable of transmitting the parasite [170;171]; however, ingestion of the beetles is needed for effective transmission of the parasite, which makes this route of transmission more likely for porcine cysticercosis than for HCC. Nevertheless, from the experiences acquired in these studies [164;169-171;236;237], it is clear that climatic factors have a direct impact on the viability of taeniids eggs. Thus climatic factors can have a direct influence on the variations observed in the prevalence of active infections and exposure to *T. solium* in different climatic settings.

The results observed in the studies performing both Ag-ELISA and EITB could be interpreted as indicating a higher occurrence of active infection in African communities, under a similar exposure to the parasite, suggesting that in this continent there are factors enhancing the human infection regardless of the level of exposure. At a population level, variations and similarities in exposure could be the result of the environmental or behavioral conditions enhancing or disfavoring egg dispersal and survival. The presence of circulating antigens at a population level is the outcome of viable cysticerci, which reflects successful establishment of infection after exposure to *T. solium* eggs. This would indicate exposure to infectious eggs and human behavior ensuring the uptake of eggs. Another factor triggering infection is the contact with a large number of eggs, increasing the probability of breaking the immune barrier raising the chance of the cysticerci to establish. A high prevalence of active infections could be as well an indicator of weak immune responses due to multiple factors as described in table 2-5. Also, the level of active infections in a population could be inversely proportional to the level of intrinsic factors in the human hosts permitting the presence of innate or acquired resistance to the parasite.

To have a complete picture on the human-to-human transmission for *T. solium* cysticercosis, more longitudinal studies should be carried out following strict protocols in order to make them comparable. In the present review, only three studies were found that contributed incidence data, two from the Latin American region [17;73] and one from the African region [136]. The results from
Ecuador and Zambia reported at least one change between positivity and negativity in their EITB for *T. solium* antibodies in 20-32% of the population studied at one point during a year. For the circulating antigen detection, the percentage of subjects showing a similar phenomenon was about 11.5% of the studied population in Zambia. These results suggest that the number of subjects that are actually exposed to *T. solium* eggs or are infected with cysticerci every year is higher than what is reported in prevalence studies. Incidence data could help to explain the interactions of the different components for transmission and their evolution over time. Data obtained from these studies contribute enormous knowledge on how seroprevalence estimation is affected because of the dynamic nature of the infection and of the presence of transient antibodies and possibly transient antigens. Studying the fluctuations of the humoral response over time can also provide information about the level of individual susceptibility by measuring the establishment rate of cysticerci following infection. The information obtained from longitudinal studies can also help deciding on the duration of control programs to effectively have an impact in the reduction on the prevalence of the disease.

When designing control programs and strategies, the correct interpretation of exposure patterns can show which factors affect the egg dispersal in the environment and to locate the potential “hotspots”. By doing so, it will provide the necessary information to create targeted interventions to avoid the spread of the disease taking into account that these patterns vary from one location to another. Interpreting the different infection patterns observed in every community will help to prioritize which strategy should be better adapted to the demo-geographical setting in order to decrease the number of new negative health outcomes.

Variations in exposure to the parasite and active infection with *T. solium* cysticerci in endemic communities present a challenge when implementing control programs. For these reasons, more studies searching in parallel the presence of antibodies directed against *T. solium* metacestodes and the presence of circulating antigens need to be conducted in other endemic communities around the world with detailed characteristics of potential risk factors. It is also recommended that these studies be paired with the study of the adult *T. solium* prevalence that can provide another element of the transmission of the parasite.

The main limitation of this study was the lack of a standardized protocol in community-based studies, which made comparable data scarce. Moreover, the reliance on only one serological test (EITB or Ag-ELISA) in most of the current sero-epidemiological studies gives only a partial view of the current status of *T. solium* infection/exposure in the communities in which they were carried out. Another limitation is that B158/B60 Ag-ELISA and HP10 Ag-ELISA have not been compared in human samples, which could lead to different results given the fact that the monoclonal antibodies used are directed against different antigens in each test. On the other hand, the large time period that elapsed (22 years from 1989 to 2014) between the oldest and the most recent studies may also imply a
 Certain bias in the health, sanitary and educational conditions that may have changed and may have had a direct impact on the *T. solium* life cycle, thus affecting its transmission dynamics and prevalence. Another important drawback is the fact that not all the studies selected for this review reported the presence of adult tapeworms. Additionally, the methods used in the studies reporting the presence of tapeworms were diverse and considered with a low sensitivity and specificity, complicating comparisons between studies. Moreover, not all the studies presented details of the studied population such as representativeness of each age group, health status or the presence of other pathogens that could have interfered when characterizing and comparing prevalence figures.

In conclusion, this review demonstrated the variability on the occurrence of active *T. solium* infections and exposure to the parasite in endemic zones with some African communities reporting the highest prevalence levels for cysticercosis active infection around the world with results bordering 20%. Several significant risk factors were listed for both active *T. solium* infections and exposure to the parasite, some of them being determinant depending on the geographical location, climatic, economic and socio-cultural conditions. The findings in this review should be taken into account in order to help defining priority areas for intervention and control of *T. solium*. 
Chapter 3

3 Human neurocysticercosis and epilepsy in Ecuador: Distribution in space and time of hospitalized reported cases.

Adapted from:


*Joint first authorship
3.1 Chapter summary

*T. solium* neurocysticercosis (NCC) is considered the most important parasitic disease of the central nervous system in humans; it is estimated to be responsible for at least one third of acquired epilepsies in developing countries. In Ecuador, the relationship between acquired epilepsy and NCC remains unclear due to different factors such as, the lack of specialized health care personnel, appropriate diagnostic techniques and the fact that acquired epilepsy is characteristic of many other infectious and non-infectious diseases in the endemic zones of the country. In this chapter, spatial and temporal variations in the incidence of hospitalized cases of epilepsy and NCC in Ecuadorian municipalities were analyzed in order to locate and characterize important clusters in space and time. Additionally, potential socio-economic and landscape indicators were evaluated to understand in part the macro-epidemiology of the *Taenia solium* taeniasis/cysticercosis complex.

Data on the number of hospitalized epilepsy and NCC cases by municipality of residence were obtained from morbidity-hospital systems in Ecuador. SatScan software was used to determine whether variations in the incidence of hospitalized epilepsy and NCC cases were clustered in space and time. In addition, several socio-economic and landscape variables at municipality level were used to study factors intervening in the macro-epidemiology of these diseases. Negative Binomial regression models through stepwise selection and Bayesian Model Averaging (BMA) were used to explain the variations in the incidence of hospitalized epilepsy and NCC cases.

This study identified traditional endemic clusters in the highlands for both conditions as well as new clusters appearing in recent years in other zones not considered endemic. An increase in the implementation of systems for eliminating excrements would help to reduce the incidence of hospitalized cases of epilepsy by 1.00% (IC\(_{95}\%\); 0.2% - 1.8%) and by 5.12% (IC\(_{95}\%\); 3.63%-6.59%) for NCC. For epilepsy, the percentage of dwellings with piped water and the number of physicians per 100,000 inhabitants were associated with an increase in the number of cases hospitalized. Pig population was related to NCC emergence. Some variables were related to some development indicators in services which could have poor quality in developing countries. The presence of clusters of municipalities with high incidence of hospitalized epilepsy cases could be the result of a high incidence of NCC cases or other acquired epilepsy cases. Both disorders were related to the lack of an efficient system for eliminating excrements. Given the appearance of recent epilepsy clusters, these locations should be studied in depth to discriminate epilepsies due to NCC from epilepsies due to other causes. More specific studies are needed to evaluate the true prevalence of cysticercosis in humans and pigs in different zones of the country in order to better implement and manage prevention and/or control campaigns.
3.2 Introduction

“A hospital bed is a parked taxi with the meter running.”
— Groucho Marx

*T. solium* NCC has been found to be the leading cause of acquired epilepsy in endemic countries. In fact, NCC was found to be responsible for at least half or a third of acquired epilepsy cases [31;156]. In developing countries, NCC is often an underrecognized and neglected disease [4;239]. The large variety of clinical signs and symptoms, and the inaccessibility of highly sensitive tests, like Computerized Tomography (CT) or Magnetic Resonance Imaging (MRI) (due to high costs involved and the unavailability of neuroimaging facilities), have contributed to the underreporting of NCC.

In Ecuador, an average of 480 and 1670 patients are hospitalized each year because of NCC and epilepsy, respectively [147]. Notification of hospitalized cases for both disorders is mandatory in Ecuador. Tools for diagnosing causes of acquired epilepsy, including parasitic and infectious diseases are available in the country; however, these diagnostic tools are not regularly used because of their high costs, thus, as in other NCC endemic countries, in Ecuador more than 82% of epilepsies do not have definitive diagnosis and the cause remains unknown [30;147;240;241].

Different measures such as education, improvement of household sanitation, changes in meat inspection practices, identification and treatment of tapeworm carriers, mass drug administration and modifications in pig-rearing methods have proven, at least at short term, to be effective in lowering levels of transmission of NCC [143;205;242]. However, practices like free roaming pig management, clandestine marketing of living pigs and pork, open defecation, and the use of residual waters in irrigation [143;167], make the disease still prevalent in many regions.

Symptomatic and asymptomatic cases of NCC have been studied in urban [151;154;155] and rural [15-17;131;153;156] areas of Ecuador in different epidemiological studies. Endemic areas were found mostly in the highlands, where the population has a high exposure to the parasite as measured by specific antibody detection. Up to one third of the population is seropositive in some of these areas [17]. In some endemic communities in these highland areas, human cysticercosis active infections, as measured by antigen detection are present in up to five percent of the population [131]. However, in the past three decades, a decreasing trend in the incidence of hospitalized cases (IHC) of NCC has been observed in the country, apparently due to an improvement in sanitary conditions and the use of better diagnostic tools [151].
Epidemiological surveillance data in the Ecuadorian health status reports only include ambulatory cases from the public health care system. The public registers from the Ministry of Public Health are the only official health reports available. The consultations at any level attended in the public health system have been estimated to be around 30% of all the medical consultations in the country [243]. However, for the ambulatory cases the reporting is not always done properly, mainly due to the lack of synchronization among the different types of health care services in Ecuador [244]. Figure 3-1 explains the reporting system for NCC and epilepsy cases in Ecuador. Additionally, and in a parallel way, the National office of Statistics (INEC) collects all information on morbidity registered in hospitals and clinical centres (from public, private or social security systems) where patients are attended at the secondary health care level and also in case of emergencies irrespective of their underlying sources. In these registers, both NCC and epilepsy are frequent causes of hospitalization. Thus, epidemiological data generated by the Ecuadorian office of statistics offers the possibility of studying important variables related to the macro-epidemiology of NCC and epilepsy in Ecuador. Here, we use macro-epidemiology in terms of the determinants of disease, including economic, social, and climatological factors into national patterns in risk assessment [245].
In this study we aimed at identifying areas (municipalities) with a high IHC of NCC and epilepsy between 1996 and 2008. In addition, given the fact that the IHC of NCC and epilepsy have been related to several socio-economic, and landscape variables, we evaluated the macro-epidemiology of epilepsy and NCC in Ecuador at the municipality level. Finally, spatio-temporal analysis was implemented in order to investigate the distribution of the IHC of epilepsy and NCC in Ecuador.

3.3 Materials and methods

3.3.1 Ethics statement

Ethical approval was not required for this study. All information used in this study came from public sources freely available on the referenced websites. Reports of human cases belonged to the public health surveillance system, the anonymity of clinical histories is guaranteed by legal mandate.
3.3.2 Study region and data

The study unit was the municipality. The number of patients hospitalized in different institutions with diagnosis of epilepsy and NCC, from 1996 to 2008 was obtained from hospital morbidity and mortality databases managed by the National Office of Statistics (INEC) [147]. This time period was chosen because of the availability of digitized morbidity data (www.inec.gob.ec), and because data on agricultural and life conditions of the population were available for that time period [147;246]. Additionally, we did not consider the data of the period after 2008 because biases in the number of cases were expected given the fact that public health systems increased their coverage and became more accessible and free of charge, including the distribution of parasitic drugs [247]. The cases were identified through the ICD-10 codes for disease classification [248]. In Ecuador, the protocol to declare a patient with NCC is defined by the neurology department of hospitals. Briefly, the diagnosis of NCC follows the directions of the Del Brutto (2012) criteria. The diagnosis is based on patient’ clinical symptoms and signs (seizures, headache, dementia, hydrocephalus, among other neurological disorders), serology (detection of antibodies directed to T. solium metacestodes and/or circulating antigens of T. solium metacestodes in serum or cerebrospinal fluid) and imaging (CT, MRI) [78]. In the public sector there exist 39 neuroimaging facilities (CT-Scans) the majority of them are in the provincial capital cities (24 provinces). The private sector also has neuroimaging capacity but the number of scanners is unknown. The database has information about each hospitalized patient with cause-specific morbidity, hospital of attendance, and the place (parish or municipality) where the patient lives (access to official databases are included in S2).

All municipalities in the continental part of the country were included in this study (217 municipalities). The registers of the Galapagos Islands were excluded because they might distort the spatial analysis, although both disorders have also been reported there. Each record was designed to contain the total number of NCC and epilepsy cases, the total population, the year and the geographical coordinates of the centroid of each municipality. Time trends of hospitalized reported cases for both disorders were tested using Negative Binomial regression models. Furthermore, the relationship between the number of hospitalized cases of epilepsy and NCC was evaluated using correlation analysis on Log transformed data.

Additionally, data on several explanatory variables were gathered using governmental databases about several socio-economic indices, information from the agricultural census conducted in 2000, and climatological information from the National Hydrometeorological Service [249]. The information on explanatory variables possibly associated with NCC and epilepsy at municipality level were grouped into different classes. Climatic variables: (tropical or highlands) (ZONE), rainfall (RAIN) and number of days without precipitations (DRYD). For each municipality, values of RAIN and DRYD were inferred on the basis of 205 weather stations, and ordinary Kriging was used to interpolate values. The
similarity with rainfall maps published by the National Hydrometeorological Service (INAMHI) let us choose the proper model [249]. Population variables: population number (POPULATION), percentage of indigenous population (%INDG), and percentage of rural population (%RURAL); Educational level: mean number of years of schooling (SCHOOL), mean number of years of schooling for farmers in the municipality (FSCHOOL), and percentage of farms receiving technical assistance (TECHASSIS); Sanitary conditions: % of families with piped water (TUBWAT), % of dwellings with systems for eliminating excrements (EXCR), and physicians per 10,000 inhabitants (PHYS); Poverty indices such as: percentage of families with unsatisfied basic necessities (UBN) (Number of people or families that live under poverty conditions with respect to the population in a specific year, referring to the lack of dwelling, health, education and employment [246]), and percentage of people under extreme poverty (EXTPOOV); Livestock: percentage of agriculture land dedicated to pastures (%GLS) and pig population (PIG) [246]. In Ecuador, the majority (58.8%) of the pig population is raised under the traditional husbandry system if we consider smallholder producers in Ecuador as those farmers owning ≤10 pigs [145]. The agricultural office of geographical information systems provided the map showing the political division of the country at municipality level. A description of all the variables considered for this study is presented in Table 3-1.

Table 3-1 Socio-economic and demographic factors used to model the incidence risk of hospitalized human cases of epilepsy and neurocysticercosis in Ecuador from 1996 to 2008.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPULATION</td>
<td>Population</td>
</tr>
<tr>
<td>EPI</td>
<td>Number of epilepsy cases (1996-2008)</td>
</tr>
<tr>
<td>CYS</td>
<td>Number of NCC cases (1996-2008)</td>
</tr>
<tr>
<td>Zone</td>
<td>Tropical (1) or temperate zone(0)</td>
</tr>
<tr>
<td>RAIN</td>
<td>Rainfall during the year</td>
</tr>
<tr>
<td>DRYD</td>
<td>Number of days during a year with precipitations ≤ 1mm</td>
</tr>
<tr>
<td>%GLS</td>
<td>Percentage of agriculture land dedicate to pastures</td>
</tr>
<tr>
<td>PIG</td>
<td>Number of pigs (Criollo breed)</td>
</tr>
<tr>
<td>SCHOOL</td>
<td>Average number of years on formal education in people ≥24 years old</td>
</tr>
<tr>
<td>PHYS</td>
<td>Number of physicians per 10,000 inhabitants</td>
</tr>
<tr>
<td>TUBWAT</td>
<td>Percentage of dwellings with piped water</td>
</tr>
<tr>
<td>EXCR</td>
<td>Percentage of dwellings with some kind of system for eliminating excrements.</td>
</tr>
<tr>
<td>UBN</td>
<td>Percentage of families with unsatisfied basic necessities</td>
</tr>
<tr>
<td>EXTPOOV</td>
<td>Percentage of extreme poverty</td>
</tr>
<tr>
<td>%INDG</td>
<td>Percentage of indigenous population</td>
</tr>
<tr>
<td>%RURAL</td>
<td>Percentage of rural population.</td>
</tr>
<tr>
<td>TECHASSIS</td>
<td>Percentage of land with technical assistance</td>
</tr>
<tr>
<td>FSCHOOL</td>
<td>Years of education of farmers&gt; 24 years</td>
</tr>
<tr>
<td>N</td>
<td>217 municipalities</td>
</tr>
</tbody>
</table>

3.3.3 Space-time analysis

Space-time analysis was used to determine whether municipalities with high incidence of hospitalized cases (IHC) of epilepsy and NCC are clustered in space and in time [250;251]. The space-time scan
software [250] was used to search, and test for significance and identify approximate locations of areas with an increased risk for the occurrence of NCC and epilepsy cases. The analysis was run in SatScan Software v9.4, with case file as the number of hospitalized reported cases, population file as the estimated total number of individuals in each municipality per year and as the coordinate file, the latitudes and longitudes of the centroids of each municipality. The spatial dimension varied from 0 up to 25% of the total number of centroids in the study region. The temporal dimension was established with a maximum of up to 50% of the study period with a time precision of 1 year. Poisson-distribution was used to contrast the number of cases in the scanning of areas. Space-time clustering was assessed by comparing the iRR (incidence rate ratio) of epilepsy and NCC IHC within a specific area and time in contrast to an expected iRR of hospitalized NCC and epilepsy cases if their incidences were randomly distributed. The cylinder with the maximum likelihood ratio was selected as the most likely cluster (Primary cluster), and others no overlapping significant clusters were also selected. The significance of identified space-time clusters was tested using the likelihood ratio test statistic and p-values of the test were obtained through Monte Carlo simulations (999). The significance was arbitrated at the 5% level. All significant clusters were visualized using QGIS software (version 2.8).

3.3.4 Regression analysis

Multivariable-count regression models were used to assess the relative contribution of different socio-economic and demographic variables to the IHC of epilepsy and NCC from 1996 to 2008 across the country. For the case of hospitalized NCC cases, an excess of zero cases were observed. For this reason, Zero inflated negative binomial models (ZINB) were used to explain the over-abundance of zero cases [252].

A manual forward stepwise procedure was implemented to select the set of independent variables that describe the number of NCC hospitalized cases or the probability of not observing any hospitalized case of NCC using the zinb command in STATA, version 12 software (StataCorp LP, College Station, Texas). The procedure started with the null model (with no covariates), and subsequently, covariates were added and evaluated for their importance. The Akaike Information Criteria (AIC) was used as the calibrating parameter and models with lower AIC values and few parameters were preferred. Two models were considered to be significantly different whenever the difference in AIC was greater than 3 [253]. In the forward selection procedure, each covariate was added in either the linear predictor for the count part or in the logit function for the absence of the disease, and the covariate with the best explanation preserved for the second round. If the addition of a covariate improved the model explanation through the reduction in AIC, the variable was captured and the process was repeated until the AIC value could not be reduced further [254]. The significance level for the covariates in the model was set at 0.05. For the case of NCC Vuong’s test was applied in order to evaluate if zero-inflation was more appropriate as compared to the standard negative binomial model.
To assess the influence of the selected indicators on the IHC of epilepsy, a Poisson regression model was used, and due to the presence of over-dispersion, Negative Binomial models were also evaluated through a likelihood ratio test for over-dispersion using the function \texttt{odTest} (in \texttt{pscl} package, in the R software) to test the null hypothesis that the restriction implicit in the Poisson model is true [255]. The approach of bidirectional elimination of variables was applied for variable selection, which also used the AIC as calibrating parameter. The functions \texttt{glm.nb} and \texttt{stepAIC}, in the \texttt{MASS} package under the R environment were used [256].

In addition, for the study of epilepsy, Bayesian model averaging (BMA) was used in order to deal with the uncertainty about the “correct” model [257]. BMA chooses the better model according to the best posterior probabilities among the models using the Occam’s window principle [253]. The inference about the explanatory variables in the best model is expressed as posterior effect probabilities, which indicate evidence of the importance of the effects of each variable in the model. The function \texttt{bic.glm()} in the \texttt{BMA} package of the R software was used [258] with the specification that counts follow a quasi-Poisson distribution, and that variance increases with the square of the mean: an equivalent version of NB regression [259]. No explicit prior distributions for models and model parameters were assumed implying that all models were equally likely.

3.4 Results

3.4.1 Space-time analysis

Figures 3-2 and 3-3 display the histogram of the cumulative incidence of hospitalized epilepsy (Figure 3-2) and NCC (Figure 3-3) cases, during the study period (between 1996 and 2008) over all the municipalities. For epilepsy, the distribution is more uniform compared to that of NCC; however, for NCC there are high proportions of zero cases throughout the municipalities. Figure 3-4 presents the Incidence of hospitalized cases (IHC) with both health problems over time in Ecuador. In the case of NCC, from 1996 to 2008, there was an overall decreasing trend with around 5 cases per 100,000 inhabitants in 1996 to around 3 cases per 100,000 inhabitants in 2008. The decreasing trend was statistically significant (p<0.001); thus, annually a reduction of 5.68% (IC\textsubscript{95%}: 4.5%-6.4%) in hospital cases is expected. In contrast, for the case of epilepsy, the incidence appeared to be slowly and steadily increasing from 1996 to 2008. The increasing trend was statistically significant (p<0.001). Annually an increase of 4.7% (%(IC\textsubscript{95%}: 3.7%-5.7%) in reported cases is expected. It was also observed that as long as the incidence of epilepsy increased through time, the annual IHC of NCC appeared to decrease slowly; and the IHC for epilepsy was always higher than that of NCC.
Figure 3-2: Histogram of the incidence of hospitalized epilepsy cases per 100,000 inhabitants between 1996 and 2008 in Ecuadorian municipalities.

Figure 3-3: Histogram of the incidence of hospitalized neurocysticercosis cases per 100,000 inhabitants between 1996 and 2008 in Ecuadorian municipalities.
Table 3-2 presents a list of the top 15 municipalities with the highest IHC of epilepsy and NCC. It can be observed that municipalities with a high IHC of epilepsy did not necessarily have a high IHC of NCC. However, an overall (all years combined for each municipality), highly significant positive linear correlation ($r=0.78$, IC$_{95\%}(0.72$-1.00), $p<0.0001$) was found on log(x+1) transformed number of hospitalized cases of epilepsy and NCC reported in each municipality. Similar significant positive linear trends within municipalities were observed for each year evaluated separately. The IHC of epilepsy varied from 0 to 505 cases per 100,000 with an average number of 127.1 cases. On the other hand, for NCC, the IHC varied from 0 to 282.54, and the average number of cases was 35.1. Out of the top 15 municipalities with high IHC for epilepsy, six also featured amongst the top 15 for municipalities with high IHC for NCC. It should be highlighted that Quito (capital city) presented the highest number of patients (1829 and 3123) in hospitals for NCC and epilepsy during the study period, but the rates were diluted because of the city’s large population size.
Human neurocysticercosis and epilepsy in Ecuador: Distribution in space and time of hospitalized reported cases.

Table 3-2 Municipalities having the highest incidence of hospitalized cases (/100,000 inhabitants) of neurocysticercosis and of epilepsy from 1996 to 2008.

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Highest IHC for NCC</th>
<th>Municipality</th>
<th>Highest IHC for epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IHC NCC</td>
<td></td>
<td>IHC NCC</td>
</tr>
<tr>
<td>Chinchipe</td>
<td>282.5</td>
<td>Paulo VI</td>
<td>0.0</td>
</tr>
<tr>
<td>Loja</td>
<td>241.6</td>
<td>Chinchipe</td>
<td>*</td>
</tr>
<tr>
<td>Quitanga</td>
<td>218.2</td>
<td>Zamora</td>
<td>*</td>
</tr>
<tr>
<td>Espindola</td>
<td>215.8</td>
<td>Sta. Clara</td>
<td>0.0</td>
</tr>
<tr>
<td>El Tambo</td>
<td>206.0</td>
<td>El Chaco</td>
<td>130.4</td>
</tr>
<tr>
<td>Azogues</td>
<td>204.9</td>
<td>Sucúa</td>
<td>13.8</td>
</tr>
<tr>
<td>Zamora</td>
<td>183.6</td>
<td>Santiago</td>
<td>0.0</td>
</tr>
<tr>
<td>Cañar</td>
<td>171.7</td>
<td>Baños</td>
<td>0.0</td>
</tr>
<tr>
<td>Paltas</td>
<td>165.9</td>
<td>Azogues</td>
<td>*</td>
</tr>
<tr>
<td>Calvas</td>
<td>159.4</td>
<td>Sta. Rosa</td>
<td>28.1</td>
</tr>
<tr>
<td>Biblian</td>
<td>144.7</td>
<td>Biblian</td>
<td>*</td>
</tr>
<tr>
<td>Gonzanama</td>
<td>140.1</td>
<td>Loja</td>
<td>*</td>
</tr>
<tr>
<td>Cuenca</td>
<td>138.8</td>
<td>Morona</td>
<td>51.0</td>
</tr>
<tr>
<td>Riobamba</td>
<td>135.5</td>
<td>Riobamba</td>
<td>*</td>
</tr>
<tr>
<td>Ibarra</td>
<td>134.4</td>
<td>Penipe</td>
<td>61.0</td>
</tr>
</tbody>
</table>

Legend: NCC: Neurocysticercosis; IHC: Incidence of hospitalized cases *: Data reported in the previous column

3.4.2 Significant space-time clusters

Four clusters were detected for the iRR of epilepsy when up to a maximum of 25% of centroids were included in the scanning window (Figure 3-5). The most likely cluster extended from the center to the northern part of the country, involving municipalities from Tungurahua, Cotopaxi, Napo and Pichincha provinces. This cluster lasted between 2003 and 2008 with an iRR of 1.57 in contrast to centroids outside the cluster (p =0.001). A secondary cluster was located in the Guayaquil municipality in Guayas province in the Southern coast of the country with an iRR of 1.69 (p=0.001). This cluster existed between 2005 and 2008 with 1745 hospitalized cases when only 1070 cases were expected. A third secondary cluster was located in the southern part of the country in municipalities in the provinces of El Oro, Loja Zamora, Cañar, Azuay, Morona Santiago, and Chimborazo. This cluster was the biggest cluster found, and the iRR was 1.60 (p=0.001) compared to the municipalities outside the cluster. This cluster existed from 2002 to 2007. Finally, in the western coast of the country, a secondary cluster covering 11 municipalities of Manabí province was detected in 2008, which had an iRR of 1.62 (p=0.001).
Human neurocysticercosis and epilepsy in Ecuador: Distribution in space and time of hospitalized reported cases.

Figure 3-5 Significant space-time clusters of hospitalized epilepsy cases with up to a maximum of 25% of the total centroids included in the scanning window between 1996 and 2008.
Like for epilepsy, four significant clusters were identified for NCC. A main cluster was found in the southern part of the country, which existed from 1996 to 2001 with an iRR of 3.78 (p=0.001) (Figure 3-6). Municipalities of El Oro, Loja, Zamora, Azuay and Morona Santiago provinces were part of this cluster within the given time frame. Likewise, in the central-northern part of the country, some municipalities from Imbabura, and Pichincha provinces were part of an important secondary cluster that lasted from 1996 to 2001. The iRR for the municipalities within this cluster was 2.75 (p=0.001). Additionally, there was a secondary cluster in the municipality of Riobamba in Chimborazo Province in the middle of the country that lasted between 2000 and 2005. The iRR for this cluster was 3.15 (p=0.001). Finally, besides the last zone, some municipalities from Cotopaxi and Tungurahua provinces were part of an additional cluster that had an iRR of 2.09 (p=0.001) and had a relatively high incidence starting in 1996 until 1997.
3.4.3 Potential indicators associated with the incidence of hospitalized epilepsy and neurocysticercosis cases.

The analysis of several socio-economic and demographic variables affecting the IHC of epilepsy between 1996 and 2008 is presented in Tables 3-3 and 3-4. Table 3-3 presents the set of covariates selected by stepwise procedure and Table 3-4 presents the total set of variables included in this set of potential indicators and their partial contribution according to the BMA methodology. Posterior effect probabilities \( P(\beta \neq 0|D) \) were included to show the evidence of an effect for each covariate when model uncertainty was incorporated. According to the results, the variables that significantly increased the IHC of epilepsy in municipalities were: the number of physicians per 10,000 inhabitants (PHYS) iRR=1.045 (IC\(_{95\%}\): 1.031-1.059), and the percentage of families having piped water (TUBWAT, not necessarily drinking water) iRR=1.022 (IC\(_{95\%}\): 1.014-1.029). On the other hand, the only variable that apparently led to a significant reduction in the IHC of epilepsy in municipalities was the percentage of houses having any kind of system to eliminate excrements (EXCR) iRR=0.986 (IC\(_{95\%}\): 0.979-0.993). The climatic variable (Zone) showed that municipalities located in the highlands presented an increased risk of epilepsy in contrast to municipalities located in tropical zones (iRR=1.02 (IC\(_{95\%}\): 0.855-1.207)) even though it was not statistically significant at a 5% level. For the BMA variable selection, out of 15 variables evaluated, 3 had substantial evidence \( P(\beta \neq 0|D)>90\% \) of being different from zero (Table 5); PHYS, TUBWAT, and EXCR. Overall, the BMA and stepwise selection methods yielded similar results.

**Table 3-3: Indicator factors associated with the incidence of hospitalized epilepsy cases in Ecuador from 1996 to 2008 (negative binomial model chosen by AIC criterion).**

<table>
<thead>
<tr>
<th>Coefficients:</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>z value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-6.77</td>
<td>0.199</td>
<td>-33.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zone (Tropical)</td>
<td>-0.16</td>
<td>0.088</td>
<td>-1.82</td>
<td>0.069</td>
</tr>
<tr>
<td>PHYS</td>
<td>0.04</td>
<td>0.007</td>
<td>6.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TUBWAT</td>
<td>0.02</td>
<td>0.004</td>
<td>5.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EXCR</td>
<td>-0.01</td>
<td>0.004</td>
<td>-3.85</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Dispersion parameter for Negative Binomial (3.50)
AIC: 1769.8

Legend: PHYS: Number of physicians per 10,000 inhabitants; TUBWAT: Percentage of dwellings with piped water; EXCR: Percentage of dwellings with some kind of system for eliminating excrements; AIC: Akaike Information Criterion; Std. Error: Standard Error.
**Human neurocysticercosis and epilepsy in Ecuador: Distribution in space and time of hospitalized reported cases.**

Table 3-4: Indicator factors associated with the incidence of hospitalized epilepsy cases in Ecuador from 1996 to 2008 based on Bayesian model averaging (BMA).

| Coefficients: | \( P(\beta \neq 0 | D) \) | Mean | SD |
|---------------|----------------------------|------|----|
| Intercept     | 100                        | -6.84| 0.699 |
| PHYS          | 100                        | 0.05 | 0.008 |
| TUBWAT        | 96.7                       | 0.02 | 0.006 |
| EXCR          | 94.0                       | -0.01| 0.005 |

Legend: TUBWAT: Percentage of dwellings with piped water; PHYS: Number of physicians per 10,000 inhabitants; EXCR: Percentage of dwellings with some kind of system for eliminating excrements; SD: Standard deviation; \( P(\beta \neq 0 | D) \): Posterior effects probability.

Table 3-5 presents the results of the potential indicator factors associated with NCC in hospitals. In the binary part, three covariates were chosen (EXCR, PIG, %RURAL). According to this selection procedure, the covariates that positively influenced the odds of hospitalized NCC cases in the communities were the implementation of systems for eliminating excrements (EXCR) (OR=0.94; IC\(_{95\%}\): 0.89 - 1.0), and pig population (PIG) (OR=0.999; IC\(_{95\%}\): 0.999-1.0). In contrast, the higher the proportion of rural population in a community the lower the odds ratio of reporting NCC hospitalized cases (OR=1.073 (IC\(_{95\%}\): 1.01 - 1.14)).

Table 3-5: Factors involved in the incidence of hospitalized neurocysticercosis cases in Ecuador from 1996 to 2008 based on a zero-inflated negative binomial regression model.

| Count model | Coef. | Std. Err. | Z   | P>|z| | [95% Conf. Interval] |
|-------------|-------|-----------|-----|----|----------------------|
| Intercept   | -4.32 | 1.29      | -3.35| 0.001| [-6.843 - -1.790] |
| ZONE (Tropical) | -1.72 | 0.170   | -9.69| <0.001| [-2.070 - -1.37] |
| SCHOOL      | 0.19  | 0.092     | 2.09 | 0.037| [0.012 - 0.372] |
| EXCR        | -0.05 | 0.008     | -6.62| <0.001| [-0.068 - -0.037] |
| TUBWAT      | 0.02  | 0.008     | 1.98 | 0.047| [0.0002 - 0.030] |
| TECHASSIS   | 0.05  | 0.015     | 3.46 | 0.001| [0.0222 - 0.080] |
| %GLS        | 0.01  | 0.004     | 2.52 | 0.012| [0.0024 - 0.019] |
| EXTPOOV     | -0.02 | 0.008     | -2.33| 0.020| [-0.0362 - -0.003] |
| %RURAL      | -0.01 | 0.005     | -2.03| 0.042| [-0.0192 - -0.0001] |

Zero inflation

| Intercept   | -1.97 | 3.099    | -0.64| 0.525| [-8.0451 - 4.102] |
| EXCR        | -0.06 | 0.028    | -2.09| 0.036| [-0.1134 - -0.003] |
| PIG         | -0.001| 0.0003   | -2.46| 0.014| [-0.0011 - -0.0001] |
| %RURAL      | 0.07  | 0.032    | 2.13 | 0.033| [0.0056 - 0.131] |
| log(alpha)  | -0.70 | 0.1453   | -4.74| <0.001| [-0.9734 - -0.403] |
| Alpha       | 0.50  | 0.0730   |       |      | [0.3777 - 0.667] |

Legend: SCHOOL: Average number of years on formal education in people \( \geq \) 24 years old; EXCR: Percentage of dwellings with some kind of system for eliminating excrements; TUBWAT: Percentage of dwellings with piped water; TECHASSIS: Percentage of land with technical assistance; %GLS: Percentage of agriculture land dedicated to pastures; EXTPOOV: Percentage of extreme poverty; %RURAL: Percentage of rural population; PIG: Number of pigs (Criollo breed); Coef: coefficient; Std. Err.: Standard Error.
On the other hand, for the count model, the variables that positively influenced the number of hospitalized cases were SCHOOL, TUBWAT, TECHASSIS, and %GLS, and the variables that were associated with a decrease in the number of hospitalized cases were Zone (Tropical), EXCR, EXTPOOV, and %RURAL. The temperate zone (highlands) had by far higher NCC cases compared to the tropical zones, so that the possibility of having a NCC diagnosis in Andean zone hospitals is higher (iRR=5.6 times (IC95%; 3.95-7.92)). Variables such as, the schooling in municipalities (SCHOOL) (iRR=1.212; IC95%; 1.012-1.451), the percentage of dwellings having piped water (TUBWAT), (iRR=1.020; IC95%; 1.001 - 1.031), the proportion of farms with technical assistance (TECHASSIST) (iRR= 1.051; IC95%; 1.022 - 1.084), and the percentage of land dedicated to pastures (%GLS) (iRR=1.010; IC95%; 1.002 - 1.019) were positively associated with an increase in the IHC of NCC. In contrast, covariates that negatively affected the number of NCC hospitalized cases in hospitals were: the percentage of dwellings having any system for eliminating excrements (EXCR) (iRR=0.951; 0.934 - 0.964), extreme poverty (EXTPOOV) (iRR=0.980; IC95%; 0.965 - 0.997); and %RURAL population (iRR=0.990; IC95%; 0.9812 - 0.999). The implementation of systems for eliminating excrements (EXCR) reduced the IHC of NCC in average by 4.87% (IC95%; 3.6% - 6.6%).

The model chosen for the iRR of epilepsy fitted better than the model without over-dispersion. The likelihood-ratio test for the over-dispersion parameter was significantly different from zero (p<0.001), meaning that Negative binomial regression was preferred over Poisson regression. For the case of NCC, when the zero-inflated negative binomial model was contrasted against the zero-inflated Poisson model, the likelihood-ratio test was significant in favour of taking into account the overdispersion (different from zero) (p<0.0001). In the same way, Vuong’s test confirmed that the assumption of zero inflation in the model for NCC was preferred over a model without this assumption (p=0.02).

3.5 Discussion

During the study period, NCC still had an impact on the general health status of the population in Ecuador. Our findings indicate that 6294 cases of NCC and 19821 cases of epilepsy were hospitalized between 1996 and 2008. Additionally, there was a significant increasing time-trend for IHC of epilepsy, but a decreasing time-trend for IHC of NCC overall. In contrast, within municipalities a positive linear relationship between both disorders was found. Also, the number of hospitalized cases (both for epilepsy and NCC) was related to some potential indicators evaluated. A general reduction in the IHC of both conditions was observed with an increasing percentage of systems to eliminate excrements. Moreover, the presence of pig production was related to the IHC of NCC.
3.5.1 Epilepsy

In the case of epilepsy, according to the spatio-temporal analysis, all significant clusters of municipalities with high incidence of epilepsy existed between 2002 and 2008. The incidence risk ratio (iRR) of the epilepsy clusters were not so high (they ranged from 1.57 to 1.69), meaning that epilepsy can be almost classified as an endemic disorder in Ecuador. A possible explanation for the epilepsy clusters since 2002 is that during these years, health facilities could have improved in municipalities leading to a better coverage of hospitalization.

Clustered epilepsy municipalities were located in the Sierra region, mainly in the southern and some in the central-northern part of the country. Some of those municipalities were known as endemic for epilepsy and as epilepsy-NCC related zones, with some new clusters in the Sierra region that have not been pointed out before as endemic areas in previous studies [15;131;153;160]. The appearance of this new epilepsy cluster could also be related to the presence of other infectious and non-infectious diseases present in coastal tropical zones [260], but the evaluation of the newer clusters become urgent as this could be a strong predictor for NCC [261].

Based on two selection methods, there was a positive association between number of physicians and number of hospitalized cases of epilepsy. This effect occurs mainly in provincial capital cities where more health services exist and consequently specialized physicians are available to people. The implementation of systems for eliminating excrements, such as latrines, septic tanks, or sewage systems, seemed to have an impact on the occurrence of epilepsy, which suggests that a part of hospitalized epilepsy cases might be due to NCC or other fecal-related causes of epilepsy [262].

In this study period, the majority of the epilepsy cases were classified as non-specific cases (ICD codes: G408 and G409), and they were between 81% and 92% every year. As in other places, the majority of epilepsy cases apparently do not have a recognized etiological origin well identified [30;241]. However, not all types of acquired epilepsy can be attributed to NCC [30;261;262]. Other causes related to poverty, such as poor nutrition, neurological sequels due to infectious agents, and head traumas related to occupational accidents and violence can also contribute to the number of hospitalized epileptic cases [231;262-264]. The other variable was the percentage of dwellings with piped water. Having piped water not necessarily refers to water that is drinkable. According to estimations, in Ecuador, 30% of the piped water in urban zones is not potable water. [265].

3.5.2 NCC

In Ecuador, the protocol to declare a patient with NCC depends on the diagnostic capacity of the neurology department, which in many cases, only exists in the main cities [77]. Based on national hospital data, 35.5% of the NCC cases were reported by the public sector, 39.4% were reported by the private sector and 23% by the systems of social security insurance. Furthermore, the appearance of
clinical signs of NCC in patients might occur several months or even years after infection, so that some etiological factors could not have been measured correctly at the beginning of this study. Likewise, it is worth to mention that only a part of the cases of human cysticercosis are symptomatic, and therefore the statistical relationships found in this study are valid only for the hospitalized cases reported. A wider spectrum might be found with the addition of asymptomatic cases and people suffering from chronic headaches who do not often consult physicians, which only can be found in field studies[14;54;261;266;267].

NCC clusters in Ecuador apparently appeared earlier compared to epilepsy clusters and the majority of them existed between 1996 and 2001. Only one of them was identified from 2000 to 2005 in Chimborazo province and in some surrounding municipalities in Bolívar Province. This area presents ideal conditions for the *T. solium* life cycle, as it is located in the highlands with a high percentage of rural population and lack of basic services. In this cluster, more than 90% of the pig producers are smallholders [147]. According to the last Agricultural National Census carried out in 2000, 80.5% of the smallholders (≤10 pigs) are located in the Sierra region and 18.6% in the Coastal region. Porcine Cysticercosis has not been reported in slaughterhouses by the National Veterinary Services (Agrocalidad) since 2001 [268]. However, only 30% of slaughtered pigs in these facilities is provided by smallholder. [269].

Based on a zero-inflated negative binomial model, the percentage of rural population in municipalities was associated with a reduction in the IHC of NCC; so that urban zones increased their incidence in contrast with previous studies published [270]. Nowadays, the rural population in Ecuador accounts for less than 38% of the total population, which is a significant reduction compared to previous decades when the rural population was over 50%. Translocated rural communities tend to settle in slum zones of big cities [271]. However, rural and sometimes poor communities might have not been well represented in the data, as they may refrain from hospitalization due to the high costs of diagnosis and treatments [272]. This concern is a limitation of hospital-based registers [231]. In our study only 7% of patients appeared to belong to rural communities, although, if we consider the patients not living in the provincial capital cities this percentage increases to 26.7%. The structure of the data makes it difficult to differentiate people from peri-urban zones or slums, or semi-rural towns and communities from urbanized areas. On the other hand, housemaids and food vendors coming from endemic rural zones have a higher chance to be tapeworm carriers and can be at the origin of spreading *T. solium* infection among the urban population [154;223;273]. Another possible explanation is that traditional livestock systems are still preserved on a small scale in urban slums, although this presumption has not been quantified.

The presence of pigs was the most important positively associated with the appearance of hospitalized-symptomatic NCC cases. Industrialization of pig production, in many cases is not
responsible for the increase in NCC cases. On the other hand, the presence of free roaming pigs has been associated with an increased risk for the occurrence of cysticercosis [153;274]. In Ecuador, despite 58.8% of pigs are raised in traditional production systems it has been estimated, that it only represents nearly 30% (50% in year 2000) of pork available in markets. [269].

In the negative binomial count model, eight variables were associated with the IHC of NCC. As in the case of epilepsy, the implementation of systems for eliminating excrements was involved in a reduction in the IHC of NCC. More in depth studies are needed to evaluate the real scale of those variables in the macro epidemiology of NCC, although some of them express the lack of quality in offering services.

### 3.5.3 NCC & epilepsy

It has been mentioned that the difficulties of identifying the etiology of epilepsy could play an important role in the sub-notification of NCC [15;275;276]. The condition most commonly associated with NCC is epilepsy, but many cases of NCC are asymptomatic or manifest chronic headaches or other neurological disorders [14;54;261;267]. In *T. solium* endemic communities in Ecuador an important proportion of acquired epilepsy cases were due to NCC [15;160]. Although extrapolating this quantity to the current reality in the country may be biased; zones with an apparent increase of epilepsy cases may elucidate the origin of new suspected NCC cases [19;261]. Additionally, epilepsy and NCC in developing countries have been reported to be clustered [18;231;235], but the presence of imported cases has also been mentioned as an important factor in urban zones.

NCC underreporting might be due to a misdiagnosis in the epilepsy etiology. However, in our case both disorders were linearly related. This positive relationship is an indicator of an apparent constant relationship between epilepsy and NCC. This relationship has to be further studied and the meaning of this pattern has to be elucidated.

Additionally, given that the lack of sewage systems was demonstrated to be associated with an increase in the incidence of both conditions. Increasing the sewage systems could be used as an important control tool to reduce the incidence of the hospitalized cases. The installation of these systems, at municipal level varied from 20.6% to 96.3% of coverage with a median value of 68.5%, so there are still many municipalities that lack basic services.

The zone where the municipality was located was one of the principal indicators affecting the IHC of NCC and epilepsy. Municipalities located in temperate zones (highlands) had a significantly higher number of hospitalized NCC cases. From the BMA procedure, in the case of epilepsy, the posterior effect had a small probability (11.8%), so we argue that in tropical zones lack of appropriate diagnostic tools and specialized knowledge of health staff might make it difficult to properly identify
NCC cases [263]. Likewise, the levels of coverage of basic services is lower in tropical zones of Ecuador [147]. Thus, the presence of the life cycle of T. solium in these zones traditionally considered to be NCC free cannot be ruled out.

A limitation of this study was that peri-urban zones where poverty belts of cities are frequently located could not be analyzed separately given that records do not use this residence category for patients. The conditions in peripheral zones can differ from city to city, thus the assumption that the origin of the hospitalized cases in big cities come from peripheral zones should not be extrapolated in all cases. As in other studies based on hospital data, rural communities might not be appropriately represented in the sample. This could be a major limitation of our study, and also because of the asymptomatic human cysticercosis cases, migraine-type and chronic headaches [54;261;267]. However, due to the fact that ambulatory cases do not offer reliable data, hospital data is a better attempt to represent the situation of the disease in a municipality. Another limitation in this study might be the presence of duplicated cases in the data base. These cases might be due to the fact that a patient was hospitalized more than once, or because some NCC cases were diagnosed as epilepsy before. But given the mentioned limitations, the present results are still reliable due to their apparent representation of the municipalities in Ecuador, and because they are based on the appropriate statistical tools. So given the data constraints, the methods used to identify risk indicators and/or areas based on available data presents valuable results for veterinary and public health sectors at no cost.

There is a need to re-evaluate the current situation for both disorders throughout the country as life conditions have been changing over time [243;247;277]. Given the recent changes in the organization of the public health sector, new trends need enough data collection-time to be evaluated again.

In conclusion, NCC might still have a relevant presence in Ecuador and might play an important role as a cause of acquired epilepsy in Ecuador [160]. Although the real burden of NCC is still unknown, we found that the hospitalization rate of patients with epilepsy has been increasing in recent years (Figure 3-4). Traditional NCC and epileptic endemic zones were recognized as high risk zones even though more recent clusters of both diseases seem to have appeared. Although the lack quality of basic services was related to the IHC in both disorders, one important finding of this study was that the implementation of systems for eliminating excrements helped to reduce the incidence of hospitalized cases of both epilepsy and NCC, which could be used as an indicator strategy for planning control programs. More specific studies linking human NCC with epilepsy and their respective factors in field conditions are needed to evaluate the prevalence of the disease in humans throughout the country and generate data that could be used for estimation of the burden disease.
Chapter 4

4 Serological dynamics of cysticercosis in an endemic area in Ecuador.

Adapted from:

4.1 Chapter summary

While human cysticercosis (HCC) prevalence data become available worldwide, incidence rate and cumulative incidence figures are lacking, which limits the understanding of the *Taenia solium* epidemiology. The present chapter describes the dynamic nature of human *Taenia solium* larval infections in an Ecuadorian endemic community. In this study it is reported for the first time incidence rate and cumulative incidence figures of human *T. solium* larval infections in Latin America. The simultaneous use of antibody and antigen serological detections allowed estimating both parasite exposure and infection rates, respectively. A sero-epidemiological cohort study was conducted in a south-Ecuadorian community to estimate the incidence rate of infection with and the incidence rate of exposure to *T. solium* based on antigen and antibody detections, respectively. The incidence rate of infection was 333.6 per 100,000 person-years (95% CI: [8.4-1,858] per 100,000 person-years) contrasting with a higher incidence rate of exposure 13,370 per 100,000 person-years (95% CI: [8,730-19,591] per 100,000 person-years). The proportion of infected individuals remained low and stable during the whole study year while more than 25% of the population showed at least one antibody seroconversion/seroreversion during the same time period. While about 13% of the inhabitants were exposed to *T. solium* eggs, less than 1% of the population became yearly infected with the parasite. This contrast between exposure and infection may be linked to an effective resistance to the parasite acquired through long-term exposure of the population and differs from the African situation, where much higher levels of infection have been observed. These estimates are of high importance to understand the epidemiology of *T. solium* in order to develop ad hoc cost-effective prevention and control programs. They are also essential to assess the burden of *T. solium* cysticercosis since longitudinal data are needed to make regional and global projections of morbidity and mortality related to cysticercosis. The estimates generated here may now be incorporated in epidemiological models to simulate the temporal transmission of the parasite and the effects of control interventions on its life cycle.
4.2 Introduction

“Art is not a handicraft, it is the transmission of feeling the artist has experienced.”

— Leo Tolstoy

In Latin America human cysticercosis has been reported in at least 18 countries and is considered a major public health problem, especially in poor rural areas [6;119]. The Andean region of Ecuador and neighboring countries is hyper-endemic for cysticercosis [15]. While reliable prevalence data become available worldwide, they may considerably vary depending on the diagnostic test used [72;81;113]. Serological antigen and antibody detections are valuable tools when conducting epidemiological studies, since they inform on infection with and exposure to the parasite, respectively. Taking the latter distinction into account, studies conducted in Ecuadorian endemic rural communities have shown an exposure to the parasite ranging from 25 to 40% and a proportion of infected individuals ranging from 2.25 to 4.99% [16;131;153]. However, prevalence figures do not inform on the evolution of the number of positive cases over time and estimates for human cysticercosis incidence rate and cumulative incidence are lacking, which limits the understanding of the transmission dynamics of T. solium and does not allow a precise estimation of its disease burden. García et al. (2001) [73] conducted longitudinal studies in endemic areas of Peru and Colombia and demonstrated the presence of transient antibody responses suggesting a high number of antibody seroconverted cases per year ranging from 8 to 25% of the population depending on the studied area. Through rule-based modeling, Praet et al. (2010) [16] simulated the annual antibody seroconversion rate in an endemic area of Ecuador. They estimated an annual incidence rate of exposure of people becoming seropositive of 14 per 100 person-years. On the other hand, studies estimating both incidence rate of infection and cumulative incidence are scarce [16;73;209]. Mwape et al. (2013) [136] reported an incidence rate of infection of 6,300 per 100,000 person-years in a rural community of eastern Zambia. Such estimates for Latin America are inexistent. For this reason, the present study aims at estimating the cumulative incidence and the incidence rate of human CC in an endemic area of Ecuador. A sero-epidemiological cohort study was conducted to investigate the transmission dynamics of T. solium among individuals living in a southern Ecuadorian rural community. This paper reports estimates of the incidence rates, cumulative incidences of active infection and exposure rates to T. solium and discusses the implications for the disease burden assessment and control.

4.3 Materials and methods

4.3.1 Ethical clearance

The protocol used in this study was approved by the Ethical Committee of the Central University of Ecuador (IRB 00002438) and by the Ethical Committee of The University Hospital of Antwerp,
Belgium. Written informed consent was obtained from each individual willing to participate in the study. For participants aged less than 18 years old written informed consent was also obtained from a parent or a legal adult representative. Individuals testing positive for *T. solium* cysticercosis antigens were referred to the local health center for follow-up.

### 4.3.2 Study area, population and design

The study was conducted in the rural parish of Sabanilla (4º 12’ S, 80º 8’ W) belonging to the Celica canton in the Southern Ecuadorian province of Loja. The parish has 1145 inhabitants; most of them are farmers involved in activities related to agriculture and animal husbandry. The climate is semi-arid, and the altitude is 700 meters above sea level. The region is endemic for *T. solium* cysticercosis and presents the risk factors for the transmission of the parasite [153;278]. A sero-epidemiological community-based cohort study was performed. Three blood sampling rounds were organized in Sabanilla in a period of 13 months: the first sampling round took place in June 2009 (SR1), the second in November 2009 (SR2) and the third one in July 2010 (SR3). Based on the three sampling rounds, three periods of time were defined as follows: a six-month period from June 2009 to November 2009 (P1), a seven-month period from November 2009 to July 2010 (P2), and a total 13-month period from June 2009 to July 2010 (P3).

First, an informative meeting inviting the population to participate took place at the beginning of the study in collaboration with the local authorities. Then, a census of the population was conducted based on a door-to-door survey, including collection of information on age and sex of the inhabitants. After informed consent, all individuals older than one year willing to participate and present at the time were blood sampled.

### 4.3.3 Samples collection and analyses

At each sampling round, 10 ml of blood was collected in dry tubes. After coagulation and centrifugation, serum was collected and stored at -20°C until analysis. Two serological diagnostic tests were performed. (1) The Enzyme Linked Immunosorbent Assay for the detection of circulating antigens of the metacestode of *T. solium* (Ag-ELISA) [84;93;133]. The sensitivity and specificity of the Ag-ELISA for detecting active infection in humans are 90% (95% CI: [80-99%]) and 98% (95% CI: [97-99%]), respectively. No cross-reaction with other parasites has been reported [72;84]. (2) The Enzyme-Linked Immuno-electrotransfer Blot (EITB) for the detection of antibodies directed against seven specific *T. solium* metacestode glycoproteins [80]. The sensitivity and specificity of the EITB for detecting exposure to the parasite range from 97% to 98% and from 97% to 100%, respectively [72;80].
4.3.4 Antigen and antibody seroprevalence

The antigen and antibody seroprevalence (Ag and Ab seroprevalence), as based on the results of the Ag-ELISA and of the EITB, respectively, were calculated for each sampling round for the whole population and by sex.

A multinominal Bayesian model adapted from Berkvens et al. (2006) [279] was used to estimate the true prevalence of *T. solium* larval infections for each sampling round based on the antigen seroprevalence data and on prior information on the test characteristics (sensitivity and specificity of the Ag-ELISA). Prior information was extracted from the available literature [72]. A uniform distribution with lower and upper limits of 0.80 and 1.00, and 0.97 and 1.00 were used to constrain the sensitivity and the specificity of the test, respectively. The analysis was conducted in WinBUGS and R [280;281]. Three chains, 20,000 iterations, following a burn-in of 5,000 were used to assess the convergence of the results. Criteria assessing the fit between prior information and the seroprevalence data were evaluated, i.e. the Bayesian p-value (Bayesp), the Deviance Information Criterion (DIC) and the number of parameter effectively estimated by the model (pD) [279;281].

4.3.5 Seroconversion, seroreversion and incidence rate

First, proportion of change to antigen seropositivity/seronegativity (change to Ag seropositivity/seronegativity) and proportion of antibody seroconversion and seroreversion (Ab seroconversion and seroreversion) were calculated to characterize the transmission dynamics of the disease.

Seroconversion is defined as the change from a negative to a positive serological test result between 2 sampling rounds; the opposite is defined as seroreversion [282]. The proportion of Ab seroconversion and the proportion of change to Ag seropositivity reflect the cumulative incidence for a defined time period. They were calculated by dividing the number of new cases by the number of susceptible individuals (having a negative test result at the previous sampling round) during a given time. The proportion of Ab seroreversion and the proportion of change to Ag seronegativity were calculated by dividing the number of positive tests that turned negative by the number of positive tests at the previous sampling round.

The incidence rate of infection with the larval stage of *T. solium* and the incidence rate of exposure to *T. solium* eggs were also calculated based on the results of the antigen and antibody detection tests, respectively.

The incidence rate was calculated as the number of new (change from seronegativity to seropositivity) cases in a defined time period divided by the number of person-time units at risk during the time-period. Yearly incidence rates were multiplied by 100,000 to be expressed by 100,000 person-years
The person-time unit represents one person for a defined period of time. The latter was calculated as described in Ngowi et al. (2008) assuming that the infection occurs uniformly over time and considering halfway the period between two sampling rounds [208]. For example, if a person is followed up for six months and does not seroconvert during this time, this person will contribute 0.5 person-year to the person-time at risk. If a person that is followed up for the same period but seroconverts during that period, this person will contribute 0.25 person-year to the person-time at risk. Yearly incidence rates were calculated based on this calculation method. Ninety-five % exact Poisson confidence intervals were calculated using the epitools package in R for all incidence rates [285].

4.3.6 Data management and statistical analyses

Data were entered in Excel 2010 (Microsoft Office 2010). Statistical analyses were performed in Stata (Stata Corp., College Station, TX) and in R:[213]

Fisher exact test was used to compare (1) Ag/Ab seroprevalence between sex within each sampling round and (2) Ag seroprevalence with Ab seroprevalence within each sampling rounds. Also, McNemar test was performed to compare the sero-Ag and sero-Ab prevalence between rounds. Multivariate logistic regression analysis was used to study the association between sero-Ag/Ab prevalence and age and sex, and this for the three samplings rounds. The significance level was set at 0.05. Fisher exact test was used to compare (1) the proportion of Ab seroconversion with the proportion of antibody seroreversion and the proportion of change to Ag seropositivity with the proportion of change to Ag seronegativity within periods, (2) the proportions of Ab seroconversion/seroreversion and the proportions of Ag change to seropositivity/seronegativity between sexes, (3) the proportions of Ab seroconversion/seroreversion and the proportions of change to Ag seropositivity/seronegativity between periods.

In addition, a change point analysis was used to compare the proportion of Ab seroconversion with the proportion of Ab seroreversion in function of age. The change point analysis classifies the population into 2 age groups at different age points (10, 20, 30, 40, 50, 60, 70, 80 years old). The Fisher exact test was then used on both age groups in order to identify any change of significance when comparing the proportions of Ab seroconversion and seroreversion [16;136;286]. The significance level was set at 0.05 for all statistical analyses.

4.4 Results

A total of 967 (84.45%) individuals from the 1145 inhabitants listed in the census participated in the study. Depending on the willingness of the individuals to participate, their presence at the time of sampling and on the quantity of serum available, EITB was performed on 743 (64.9%), 538 (47%) and 518 (45.2%) sera for June, November and July, respectively. Ag-ELISA was performed on 744
(65%), 538 (47%) and 514 (44.9%) sera for the same time periods. Figure 4-1 describes in detail sera availability and individual participation during the three sampling rounds.

Figure 4-1. Sera availability and individual participation during the three sampling rounds. (Format adapted from Mwape et al., 2013[136]). S1, S2 and S3 stand for first, second and third sampling rounds, Ag-ELISA: Enzyme Linked Immunosorbert Assay for the detection of circulating antigens of the metacestode of *T. solium*, EITB: Enzyme-Linked Immunoelectrotransfer Blot for the detection of antibodies directed against seven specific *T. solium* metacestode glycoproteins.

### 4.4.1 Antigen and antibody seroprevalence

The Ag and Ab seroprevalence for each sampling round for the whole population and by sex are presented in Table 4-1. The prevalence adjusted for misclassification error of *T. solium* larval infections was 0.7% (95% Credibility Interval (CI): [0.03-1.75]), 0.7% (95% CI: [0.03-2.00]) and 1.1% (95% CI: [0.05-2.84]) for SR1, SR2 and SR3, respectively. All except one Ag positive individuals were also Ab positive in the 3 sampling rounds. Fisher exact test did not reveal any significant difference of Ag and Ab seroprevalence between sexes, McNemar test did not reveal any significant difference of Ag and Ab seroprevalence between rounds. Ab seroprevalence was significantly higher than Ag seroprevalence within each sampling round. Multivariate logistic regression analysis showed a significant positive correlation between Ab seroprevalence and age.
### Table 4-1. Antigen and antibody seroprevalence figures (as based on the results of the Ag-ELISA and the EITB, respectively) by sex and by sampling round.

<table>
<thead>
<tr>
<th>Seroprevalence</th>
<th>Sampling round</th>
<th>Sex</th>
<th>Number of positive cases/Total number of individuals</th>
<th>Percentage of positive cases [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>SR 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Female</td>
<td>2/367</td>
<td>0.5 [0.1-2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>5/377</td>
<td>1.3 [0.4-3.1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>7/744</td>
<td>0.9 [0.4-1.9]</td>
</tr>
<tr>
<td></td>
<td>SR 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Female</td>
<td>1/293</td>
<td>0.3 [0.1-1.9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>4/245</td>
<td>1.6 [0.4-4.1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>5/538</td>
<td>0.9 [0.3-2.2]</td>
</tr>
<tr>
<td></td>
<td>SR 3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Female</td>
<td>3/265</td>
<td>1.1 [0.2-3.3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>5/249</td>
<td>2 [0.7-4.6]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>8/518</td>
<td>1.5 [0.7-3]</td>
</tr>
<tr>
<td>Antibody</td>
<td>SR 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Female</td>
<td>124/364</td>
<td>34.1 [29.2-39.2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>108/379</td>
<td>28.5 [24.3-33.3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>232/743</td>
<td>31.2 [27.9-34.7]</td>
</tr>
<tr>
<td></td>
<td>SR 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Female</td>
<td>100/293</td>
<td>34.1 [28.7-39.9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>83/245</td>
<td>33.9 [28.4-40.2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>183/538</td>
<td>34 [30.3-38.2]</td>
</tr>
<tr>
<td></td>
<td>SR 3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Female</td>
<td>80/270</td>
<td>29.6 [24.2-35.5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>77/248</td>
<td>31 [25.3-37.2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>157/518</td>
<td>30.3 [26.4-34.5]</td>
</tr>
</tbody>
</table>

<sup>a</sup>CI= Binomial exact 95% Confidence Intervals; <sup>b</sup>SR = sampling round; <sup>c</sup>SR 1= June 2009 sampling, <sup>d</sup>SR 2= November 2009 Sampling; <sup>d</sup>SR 3= July 2010 Sampling

Table 4-2 shows the proportions of antigen and antibody seropositive and/or seronegative individuals who participated in all 3 sampling rounds and whose sera were available for both tests (n = 277). Only one individual changed to antigen seropositivity status throughout the entire study period. Eighteen percent of this restricted population remained antibody positive throughout the entire study period while about 20% of the individuals showed at least 1 change of antibody positivity status.
Serological dynamics of cysticercosis in an endemic area in Ecuador.

Table 4-2. Proportions of antigen and antibody positive and/or negative individuals who participated in the 3 sampling rounds.

<table>
<thead>
<tr>
<th>Test result per sampling round</th>
<th>Number of individuals (antibody detection)</th>
<th>Percentage of individuals (antibody detection; [95%CI])</th>
<th>Number of individuals (antigen detection)</th>
<th>Percentage of individuals (antigen detection; [95%CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>3.24 [1.49-0.6]</td>
<td>1</td>
<td>0.36 [0.01-1.99]</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>2.89 [1.25-5.61]</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>3.24 [1.49-6.07]</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>4.69 [2.52-7.89]</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>1.44 [0.36-3.66]</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>5.05 [2.79-8.33]</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>277</td>
<td>277</td>
</tr>
</tbody>
</table>

a CI= Binomial exact 95% Confidence Intervals; SR=sampling round; b SR 1= June 2009 sampling, c SR 2= November 2009 Sampling; d SR 3= July 2010 Sampling.

4.4.2 Seroconversion, seroreversion and incidence rate

The overall incidence rate of human *T. solium* larval infection based on antigen detection was 333.6 per 100,000 person-years (95% exact Poisson CI: [8.4-1,858] per 100,000 person-years). The overall incidence rate of exposure to *T. solium* based on antibody detection was 13,370 per 100,000 person-years (95% exact Poisson CI: [8,730-19,591] per 100,000 person-years). Ag proportion of changes to seropositivity/seronegativity and proportion of Ab seroconversion/seroreversion by period are represented in Table 4-3. Incidence rates estimates for individuals who participated in at least two of the sampling rounds are given in Table 4-4.

Table 4-3. Antigen change to seropositivity/seronegativity test result, Antibody seroconversions/seroreversion figures (as based on the results of Ag-ELISA and EITB respectively) by period.

<table>
<thead>
<tr>
<th>Test</th>
<th>Period</th>
<th>Parameter</th>
<th>Number of individuals</th>
<th>Percentage of individuals ( [95%CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>P1</td>
<td>Change to seropositivity for 6 months</td>
<td>0/421</td>
<td>0 [0-0.9]a</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>Change to seronegativity for 6 months</td>
<td>0/3</td>
<td>0 [0-70.8]b</td>
</tr>
<tr>
<td>P2</td>
<td>P2</td>
<td>Change to seropositivity for 7 months</td>
<td>1/317</td>
<td>0.3 [0-1.7]</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>Change to seronegativity for 7 months</td>
<td>0/1</td>
<td>0 [0-97.5]a</td>
</tr>
<tr>
<td>P3</td>
<td>P3</td>
<td>Change to seropositivity for 13 months</td>
<td>2/373</td>
<td>0.5 [0-1.9]</td>
</tr>
<tr>
<td>Antibody</td>
<td></td>
<td>Change to seronegativity for 13 months</td>
<td>1/1</td>
<td>1 [2-100]a</td>
</tr>
<tr>
<td>P1</td>
<td>P1</td>
<td>Seroconversions</td>
<td>26/288</td>
<td>9.6 [6-12.9]</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>Seroconversions</td>
<td>25/135</td>
<td>18.5 [12.4-26.1]</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>Seroconversions</td>
<td>17/226</td>
<td>7.5 [4.4-11.8]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seroreversions</td>
<td>26/101</td>
<td>25.7 [17.6-35.4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seroreversions</td>
<td>24/264</td>
<td>9.1 [5.9-13.2]</td>
</tr>
</tbody>
</table>

a CI= Binomial exact 95% Confidence Intervals, b =one-sided, 97.5% confidence interval; c P1=June-November period; d P2=November-July period; e P3=June-July period.
Serological dynamics of cysticercosis in an endemic area in Ecuador.

Table 4-4. Yearly incidence rates for infection and exposure (as based on the results of Ag-ELISA and EITB, respectively) of individuals who participated in two sampling rounds by period.

<table>
<thead>
<tr>
<th>Test</th>
<th>Period between samplings</th>
<th>Number of individuals</th>
<th>Yearly incidence rate (95% Poisson CI) per 100,000 person-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>P1(^{a}) (6 months)</td>
<td>0/421</td>
<td>0[0-1790.7]</td>
</tr>
<tr>
<td></td>
<td>P2(^{b}) (7 months)</td>
<td>1/317</td>
<td>541.6[13.7-3018]</td>
</tr>
<tr>
<td></td>
<td>P3(^{c}) (13 months)</td>
<td>2/373</td>
<td>496.3 [60.1-1792.7]</td>
</tr>
<tr>
<td>Antibody</td>
<td>P1(^{d}) (6 months)</td>
<td>26/288</td>
<td>18,909.1[12,352-27,706.2]</td>
</tr>
<tr>
<td></td>
<td>P2(^{e}) (7 months)</td>
<td>17/226</td>
<td>13,399[7,805.4-21,453.1]</td>
</tr>
<tr>
<td></td>
<td>P3(^{f}) (13 months)</td>
<td>24/264</td>
<td>8,791.2[5,632.7-13,080.6]</td>
</tr>
</tbody>
</table>

\(^{a}\)CI= Poisson exact 95% Confidence Intervals; \(^{b}\)P1 = June-November period; \(^{c}\)P2 = November-July period; \(^{d}\)P3 = June-July period.

Fisher exact test did not show any difference of proportion of Ab seroconversion/seroreversion and proportions of change to Ag seropositivity/seronegativity between sexes, nor between periods. Proportion of Ab seroreversion was significantly higher than proportion of Ab seroconversion for each period (Figure 4-2). The change point analysis showed that the proportion of Ab seroreversion was significantly higher than proportion of Ab seroconversion until the age of 30 years. After this change point, the difference was not significant (Figure 4-3).

Figure 4-2. Proportions of Ab seroconversion and seroreversion by time period

Legend: white bars represent proportions of Ab seroconversion whereas grey bars represent proportions of Ab seroreversion; vertical error bars represent the upper and lower limits for the 95% binomial exact confidence interval; Period 1 stands for the period between Sampling round 1 and sampling round 2 (June-November 2009); Period 2 stands for the period between sampling round 2
and sampling round 3 (November 2009-July 2010) and Period 3 stands for the period between sampling round 1 and sampling round 3 (June 2009-July 2010).

Figure 4-3: Results of the change point analysis with a change point at 30 years old

Legend: The vertical dotted/lined line represents the change point at 30 years old; white bars represent proportions of Ab seroconversion whereas grey bars represent proportions of Ab seroreversion; vertical error bars represent the upper and lower limits for the 95% binomial exact confidence interval.

4.5 Discussion

This is the first study reporting cumulative incidence and incidence rate figures of human *T. solium* larval infection in Latin America. The overall incidence rate of infection in the endemic rural community of Sabanilla, was 333.6 per 100,000 person-years (95% exact Poisson CI: [8.4-1,858] per 100,000 person-years), which suggests that less than 1% of the population becomes infected yearly with the parasite. In contrast, the incidence rate of exposure to *T. solium* is much higher: about 14% of the population has a yearly contact with the parasite. The latter estimates are in line with observed and simulated antibody seroconversion rates ranging from 8 to 25% in Peru, Colombia and Ecuador [16;73]. Proportions of change to Ag seropositivity/seronegativity and Ab seroconversion and seroreversion were identical in males and females indicating that both genders get equally infected with cysticercosis and are equally exposed to the parasite. Moreover, these proportions did not significantly vary in time (one year period). On the other hand, proportion of Ab seroreversion was significantly higher than proportion of seroconversion Ab for each period and a change point analysis
showed that proportion of Ab seroreversion was significantly higher than proportion of Ab seroconversion until the age of 30 years. After this change point, the difference was not significant. These results corroborate the findings of Praet et al. (2010) [16] suggesting a higher proportion of seroreversion before the age of 40 years due to a higher number of primary immune responses before this age. In other words, individuals will serorevert more rapidly before the age of 30-40 years because primary humoral response is shorter and weaker than secondary response. Thus, the proportion of seroreversions depends on the immunological status of the individuals.

The dynamics of infection and exposure in the population, represented by the proportions of antigen and antibody results from the individuals who participated in all 3 sampling rounds, showed that the proportion of infected individuals remains low and stable during the whole study year, while the proportion of exposed individuals is remarkably higher. Of note is the high level of serological status variation with more than 20% of the population showing at least one antibody seroconversion/seroreversion during the year. Together with the prevalence estimates presented by period, these longitudinal data corroborates the findings of other studies conducted in Latin America highlighting a high prevalence of exposure to the parasite but a low prevalence of active infections. This contrast between exposure and infection may be linked to an effective resistance to the parasite acquired through long-term exposure of the population. In addition, these results confirm the occurrence of transient antibody responses in individuals living in T. solium endemic areas and suggest exposure to the parasite without infection or mild infections that are aborted by the natural immunity of the individual [136]. Mwape et al. (2013) [136] conducted a similar community-based longitudinal study in the Eastern Province of Zambia. While a much higher incidence rate was observed in the African endemic area, similar higher proportion of Ab seroreversion than Ab seroconversion and the presence of transient antibody responses were described. Further studies are needed to unravel the difference of parasite transmission patterns in different epidemiological settings. Specifically, research should focus on identifying the causes for differences in infection levels. In this context, the identification of tapeworm carriers in a community should be based on improved methods, because sensitivity and specificity of conventional coprological methods are low [108].

Our study has limitations that are mainly due to the inhabitant proportion of participation. Although 967 individuals from a total of 1045 inhabitants provided at least one blood sample, 396 provided only one sample and another 283 provided two blood samples. Compliance in participating in all 3 sampling rounds was of 288 volunteers despite extensive information sessions prior to the sampling procedure. The main reason for irregular participation was the absence of the individuals for professional duties. Reduced participation can have an impact on the precision of the estimation of the incidence rate, however, the participation on the three sampling rounds is still representative of the total population and all the participants selected for the incidence rate estimation match all the
Serological dynamics of cysticercosis in an endemic area in Ecuador.

selection criteria for this calculation (n=288 (27.6% (95% CI: [24.9-30.4]))). Another limitation of the study is the limited number of samplings and the relatively long sampling intervals depending on logistic, economic and ethical constraints. In other words, much more information could have been produced if more sampling had been organized at shorter time intervals, i.e. the positivity status of the participants would have been more accurately monitored over time. The incidence rate estimation for both infection and exposure is likely to be lower with increasing intervals between samplings: a proportion of the new infections may be undetected and the time of occurrence of a new infection overestimated. A quicker detection of new infections will result in a decrease of the number of person-years at risk and consequently in higher estimates of the incidence rate. Finally, even though the tests used in this study have shown high sensitivity and specificity, false positive and negative individuals may bias the prevalence and the incidence rate estimates. Bayesian estimation of infection with *T. solium* larva prevalence has been used to estimate the true prevalence of infection with an exposure to *T. solium*. The Bayesian estimation corrects the apparent prevalence at, but does not allow to know the true infection status at the individual level. Consequently, it does not allow to estimating the true incidence rate.

In conclusion, the present study underlines the importance of conducting longitudinal serological follow-up allowing generating incidence rather than prevalence data to fully understand the transmission dynamics of the infection and to avoid under/overestimation of the occurrence of the parasite. Similar cohort studies assessing the effect of risk factors such as development of immunity and behavioral factors should be conducted to identify all the parameters that may influence parasite transmission. Understanding the transmission dynamics of *T. solium* is essential to develop ad hoc cost-effective prevention and control programs. The estimates generated here may now be incorporated in epidemiological models to simulate the temporal transmission of the parasite and the effects of control interventions on its life cycle [209]. These estimates are also of high importance to assess the burden of *T. solium* cysticercosis since incidence data are needed to make regional and global projections of morbidity and mortality related to cysticercosis. To this end, the link between the incidence rate of infection and health outcomes related to human cysticercosis, such as epilepsy and chronic headache, as well as the case-fatality ratio still need to be estimated.
Chapter 5

Towards prioritization of intervention areas of *Taenia solium* taeniasis/cysticercosis in Ecuador.
Towards prioritization of intervention areas of *Taenia solium* taeniasis/cysticercosis in Ecuador.

“We may brave human laws, but we cannot resist natural ones.”

— Jules Verne

5.1 Introduction

The main objective of this dissertation was to better understand the epidemiological patterns that affect the exposure to and infection with *T. solium* in endemic areas, with special reference to Ecuador, in order to provide significant information that would assist in the formulation of appropriate control strategies.

In this chapter, the findings of this thesis will be discussed in a broader context and the limitations of the studies will be explored to give recommendations and conclusions from the knowledge acquired.

5.2 Characterizing *Taenia solium* human cysticercosis transmission in endemic areas.

Sero-epidemiological data from endemic communities show a high variability between and within regions. In endemic areas the epidemiological situation can be categorized in: (1) areas where high exposure and high numbers of active infections predominate; (2) areas with high exposure but rather low numbers of active infections; (3) areas with low exposure and low numbers of active infections. From the systematic review on epidemiological studies it appeared that the category 1 situation was significantly more prevalent in Africa than in Asia and Latin America. For instance, in Ecuadorian rural communities, seroprevalence of antibodies measured by EITB was very high, indicating high exposure to *T. solium* eggs, while the proportion of active cysticercosis cases in the population, measured by Ag-ELISA was low (category 2). In some African countries, like Zambia, the proportion of both EITB and Ag-ELISA was high (category 1). The reasons for these marked differences are not clear but are probably multifactorial, as discussed in chapter 4. It is evident that the causes of these epidemiological variations must be better understood before implementing interventions. The study of risk factors can greatly contribute to a better understanding of the local transmission factors. Indeed, different climatic, economic and socio-cultural conditions in endemic communities can significantly affect transmission. However, one must be cautious when interpreting data from risk factor studies. Sometimes, expected risk factors in endemic areas are not significant because they are masked by other factors. For example, latrine use has been found to be a risk factor for both PCC and HCC while in other studies it was identified as a protective factor [179;217;220;287]. These contradictory findings are probably related to the proper use and maintenance of such facilities, cultural taboos or the effect of environmental contamination. Additionally, in some studies the latrine use has not been found to be a significant risk factor due to the fact that most of the households did not have latrines; also, some households in rural settings often share their latrines with neighbors and other villagers,
therefore the relationship between latrine use and HCC cannot be determined [139;197;227;287-289]. Studies on exposure and infection heavily rely on serology and the performances of serological methods and on the way the results are interpreted have been contested [102]. However, in this thesis we only selected and used serological tests that have shown to be most reliable, such as the EITB and B158/B60 ELISA for measuring specific antibodies and circulating antigens, respectively.

The variability within each region and country should be better represented. The use of maps extrapolating the situation of HCC (e.g. the WHO world map of cysticercosis, page 20) from one area to the whole country might hide important elements that could wrongly influence decision takers, policy makers and the public in general. For example, in some countries there are areas where active transmission occurs but also areas in which transmission of *T. solium* is rare due to religious beliefs and the subsequent feeding habits (e.g. Burkina Faso that is considered endemic on the WHO map). In Ecuador we used hospital records to identify areas with higher numbers of HCC and these appeared to be unevenly distributed in the country. Thus, a global or regional characterization of an endemic area is likely to be inaccurate, the term “endemic area for cysticercosis” should be updated and the characteristics of the area should be taken into account for a better classification.

A more accurate classification would discriminate highly endemic countries from countries where the disease is present but only in specific locations. To properly do so, it would be recommended to categorize each country depending on the geographical distribution, number and level of prevalence of HCC of affected areas within each country. A first attempt to provide a more detailed description of the situation of cysticercosis in Africa was published by Braae et al. (2015) [290]; In their work, the authors presented maps in which the districts where the presence of *T. solium* is recorded are highlighted over the countries in which *T. solium* is considered as present based on literature or by reports from the OIE. Also they presented maps in which the approximate simple size and the prevalence of taeniasis and porcine cysticercosis are represented graphically and located geographically accordingly. Burden estimations and global projections of the prevalence of HCC would greatly benefit from this approach, avoiding overestimations.

Global estimates available for HCC seem to be affected by the large variability in the epidemiological data; indeed, depending on the source and the method, the local, regional and global projections for HCC seem to differ widely [41;42;44;50;51;138;291]. Future estimations should analyze more in detail the impact of this variability before making extrapolations of particular situations and correct accordingly in the calculations for better estimates with the appropriate methodology.

The characterization of endemic areas is challenged by different problems. There is a general lack of data. Official country reports rely on hospital-based symptomatic cases that are just the pinnacle of a bigger problem [36;115]. The more reliable data relies on epidemiological studies in the field.
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However, it has to be taken into account that: 1) The diagnostic arsenal available for human T. solium infections is limited as a result of the cost, availability, acceptance of communities to participate, low sensitivity and specificity, especially for tapeworm carrier detection, although new promising tools are being developed [110;111;113;114]; 2) diagnosis of PCC is difficult because of imperfect diagnostic methods and because many slaughtered pigs are escaping meat inspection [75;81;92;93]; 3) different study designs and diagnostic tools used do not allow accurate comparisons of results; 4) there is almost no knowledge on the environmental component, though new evidence highlights the importance of the environment in the transmission of T. solium infections [178].

To overcome these difficulties, we suggest to: 1) develop a standard protocol for epidemiological field-studies. This protocol should recommend the use of validated serological tests and questionnaires for identifying key risk factors; 2) develop cheap, sensitive and specific tests to detect taeniasis and PCC and include these in the previously suggested protocol; 3) use sentinel pigs to better characterize transmission [104;105;178]; 4) promote research to understand the influence of environment in the transmission of HCC and PCC; 5) declare HCC and PCC reportable diseases worldwide [41]. In order to develop an efficient protocol for epidemiological field-studies, it would be recommended to incorporate elements from other protocols already standardized that have been used for other helminths such as Schistosoma spp. and soil transmitted helminths. These protocols have been used to determine the prevalence of the infections and additionally helped to select the type of MDA to be applied, identifying the target population and the frequency of interventions [292].

5.3 Identifying priority areas of intervention within a country with special attention to Ecuador.

The identification of priority areas is important for focal interventions. HCC is a “clustered disease” and this means that interventions should focus on priority areas. In the epidemiological studies performed in Ecuador, high estimates of exposure to the parasite and non-negligible levels of active infections were found. However, these studies alone are not sufficient to represent the situation of the whole country, neither to identify all the priority areas for intervention. Official data in Ecuador reports the incidence of hospitalized cases of NCC as well as for other neurological disorders such as epilepsy, which are strong predictors of NCC in endemic areas [261]. These data are represented geographically by the municipality of residence of the patient and are available for each individual year. Additionally, data on different socio-economic and landscape variables are also available at municipality level and per year. Analyzing these data together allowed us: 1) to identify priority areas for intervention in space and time and 2) to fine-tune the knowledge about these areas.
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Traditional and new clusters were identified, as well as urban and rural clusters. These findings showed that despite the apparent decreasing trend of NCC cases [150;151], the disease is still present and is extending geographically. These clusters are priority areas of intervention. It is plausible that the presence of urban cases of NCC is the result of the import of tapeworm carriers from rural settings, highlighting the importance of the rural to urban migration in the transmission of NCC [273;293]. The problem of this event is that it becomes more difficult to detect and treat a tapeworm carrier in an urban setting. Studies conducted in urban settings in Ecuador failed to detect tapeworm carriers among NCC symptomatic patients, suggesting that most infections were acquired by contact with other individuals or by a contaminated environment [154]. In the cities, mobility is high and usually the distances between the workplace, house, markets and eating places are not comparable to the ones found in rural settings; also, public transportation and crowding in public places increases the contact with tapeworm carriers. The tapeworm can survive for several years and spread the disease when the tapeworm carrier remains untreated. All these elements added to the high cost of conducting a massive survey to detect specific antibodies in a city, makes that identifying “hot spots” in urban centers is complicated. Also, in Ecuador, both T. saginata and T. solium have been reported and these species cannot be differentiated by routine diagnostic tools [131]. In any case, identification of a tapeworm carrier in urban centers should be done when a NCC case is suspected. In those cases tapeworm diagnosis should be directed in the first place to the people surrounding the NCC patient such as his/her family living in the same house and house workers. In addition, when taeniasis is accidentally detected by a routine coprological examination, differentiation of the species should be requested and if T. solium is detected, people surrounding the tapeworm carrier should be examined for HCC.

Despite the many different causes of epilepsy [262], indicators of hospitalized cases for both NCC and epilepsy were similar, highlighting the relationship of these two conditions and indicating the priority factors that should be tackled or enforced to prevent these negative health outcomes. For example, one indicator for the increase of both epilepsy and NCC hospitalized cases was the lack of services for elimination of excrements. However, even if NCC is not the only fecal related cause of epilepsy, this suggest that controlling T. solium and other fecal related conditions through the improvement of these services is possible [294].

The presence of these clusters fall into the definition of “T. solium infection focus” for intervention purposes [261], indeed, some authors consider that the more appropriate term to be used for the control of T. solium should be “control of taeniasis/neurocysticercosis (T/NCC)” instead of the commonly used “control of taeniasis/cysticercosis (T/CC)” based on the fact that the clinical features that justify the control of T. solium are the ones involving neurological disorders [294]. However, neurological disorders derived from NCC are only the “tip of the iceberg” [36], many asymptomatic
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cases are not diagnosed until appearance of clinical evidence of NCC; also, the results of any control strategy on NCC will be observed in long-term due to the long period between the infection and development of clinical signs.

Thus, identifying and characterizing NCC clusters is a valid and powerful methodology to identify and characterize priority areas of intervention, but it should be complemented with the analysis of data from other areas where no NCC cases are reported but where risk factors such as, the lack of hygienic services, presence of PCC and/or free roaming pigs, among others, are present. Also, it has to be taken into account that the efficacy of the use of IHC to detect priority areas depends on the quality of data.

5.4 Considerations when designing control strategies

HCC was previously declared as potentially eradicable, however, despite the apparent feasibility, this statement has changed over the years. To date, elimination was achieved only focally in Peru as a result of an intensive integrated program. Other strategies applied in field trials and pilot projects do not provide enough scientific material to suggest that the disease can be eradicated in the foreseeable future, but suggest that control is feasible at a large scale [14;198;295]. Mixed results observed on the different strategies applied to control T. solium in different settings are probably derived from the variability in the factors affecting the transmission dynamics in every area [296]. Every control strategy aims to attack a certain point in the life cycle of T. solium in order to interrupt it, but the feasibility of these interventions or the impact that the strategy would have in the complete life cycle depend of the situation in every setting.

To implement a national control program for T. solium, the first step should be the identification of priority areas of intervention. Active and passive surveillance can provide most of the necessary indicators to identify priority areas. Passive surveillance would provide the first approach to identify the most critical areas to intervene at national level. For this, passive surveillance would be done at ministerial levels by both the ministry of public health and the ministry of agriculture through the routine reporting of HCC and PCC cases from health facilities, abattoirs and veterinary centers. Active surveillance would be required for the areas where information is not available but where the presence of the parasite is suspected. Active surveillance in this case would be done by the municipal public health and veterinary centers or by synchronized activities with research centers, such as universities and national institutes of public health. However, to achieve this the development of a coordination system is necessary to synchronize, update, process and ensure a routine analysis of all the obtained data, and a clear procedure on how this processed information should reach the relevant authorities who should act upon the reception of this information if required[195]. Also, the support of a legislation to approve the compulsory reporting of T. solium taeniasis/cysticercosis at any level is
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necessary. As such, interventions could be focused on priority areas only. However, synchronized actions are required in order to avoid the reintroduction of the disease in an intervened area.

Hospital data of *T. solium* infection cases would be the most obvious indicator to prioritize an area of intervention, however, as mentioned in the previous chapters, this information is not sufficient to characterize the whole situation of *T. solium* due to many factors such as the silent course of the disease in early stages of infection, asymptomatic cases, sensitivity of diagnostic tools and mild symptomatology (if any symptom is present) in the case of taeniasis, among other factors. For these reasons the indicators that could be paired with the analysis of hospital data of *T. solium* infection cases could be: 1) reports from PCC cases in abattoirs with their respective traceability; 2) independent veterinary reports of suspected PCC areas; 3) reports from epidemiological studies in rural or urban areas; 4) identification of areas combining high occurrence of epilepsy and other neurological disorders with risk factors such as, pigs kept in free ranging systems, lack of basic services such as sewage, potable water and latrines.

Once the priority areas of intervention are identified, classification of these areas should be done in order to apply the most suitable strategy. In this case the most evident step would be the discrimination between rural and urban zones. The approach for control interventions at urban and rural level should be adapted accordingly. In urban settings of Ecuador, tapeworm infections are not as common as in rural settings, because pork sold usually comes from official abattoirs where veterinary inspection is mandatory. Also, the slaughtered pigs usually come from improved pig farms where contact of pigs with human excrements is not possible. Therefore, most efforts in urban areas should target at the interruption of the human-to-human transmission. To interrupt this transmission in urban areas, the strategies should be directed to 1) raise awareness about the parasite, how it is transmitted (including self-detection) and the urgency to contact a physician for proper diagnosis and treatment in case of suspicion of infection; 2) highlight the importance of personal hygiene to avoid HCC; 3) identification and treatment of tapeworm carriers and HCC patients; 4) identification of possible pig rearing in slums or in peri-urban areas.

In urban centers, mass media, such as television, radio and newspapers are more available. These media can be used to transmit health education messages and awareness about the disease. In rural areas health education would be rather transmitted through conferences, meetings with the citizens, information campaigns and interventions in schools. A national campaign of health education to prevent *T. solium* infections has to include both approaches and it would be recommended to include the interventions in both rural and urban schools. These type of campaigns can be paired with other health campaigns, such as dengue prevention or vaccination campaigns.
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The social security system in urban centers could apply strategies, such as mandatory parasitological diagnosis and treatment as a requirement to access to the benefits of this system. This type of strategy would not be applicable in rural centers in Ecuador where only few people are in the social security system. Knowing that taeniasis is acquired more commonly in rural settings, targeted studies to detect and treat tapeworm carriers in urban settings, can be directed to groups where immigration from the rural areas is high, such as housemaids, traders of agricultural products, construction workers and refugees, among others [273;293]. In priority areas (urban and rural), it would also be recommended to include serological diagnosis for HCC and taeniasis in routine laboratory analysis.

The municipalities of each big city should create special units to periodically visit slums and peri-urban areas in search of pig rearing and other farm practices. The approach of these units to the peri-urban farmers should be initially as advisors on how to improve sanitary conditions, but in a next stage as a regulatory organism if sanitary conditions in these areas do not improve.

Interventions in priority endemic rural areas may have an impact on the macro-epidemiology of T. solium. Prioritizing the treatment of tapeworm carriers in rural settings and then interrupting the infection in pigs should decrease gradually the number of HCC infections in urban settings. A high proportion of infections occurring in urban centers are concentrated around tapeworm carriers migrating from rural endemic populations [12;125;293;297].

In rural settings, the strategies are directed to interrupt the life cycle of the parasite. These include: 1) improvement of sanitary conditions; 2) restriction of pigs to access human feces; 3) identification of PCC and sanitary sacrifice of infected pigs; 4) improvement of farming practices; 5) identification and treatment of tapeworm carriers and HCC patients; 6) health education.

The improvement of sanitary conditions was shown as an indicator of the reduction of NCC cases in Ecuador. This improvement was based on an increased coverage of systems to eliminate excrements. This strategy has to be financed and directed by the affected municipalities with the support of the government. This strategy has also to be paired with health education to avoid open defecation. However, in field conditions it has to be taken into account that latrines and toilets are often far from the work places. As a result, a fraction of the population that will practice open defecation will remain. For these cases the use of mobile latrines with a proper system of excrement disposal could be recommended.

Pig penning should be encouraged to avoid access of pigs to human feces, although, this practice does not always avoid PCC [179]. In rural areas, pigs are kept in a free roaming system, mainly because the owners do not need to invest in the construction of a pig pen and in animal feed. Veterinary services should encourage pig owners to join forces and to develop common pig pens and feeders with a sanitary maintenance system to reduce the costs of the pig keeping. Also, veterinary services could
introduce alternatives to pig farming, such as farming of original species like the south American camelid *Lama glama* (lama), the rodent *Cavia porcellus* (guinea pig or “cuy”), or even the capybara (*Hydrochoerus hydrochaeris*). The advantages of farming these species is that they could partially reduce pork consumption, therefore reducing taeniasis infections. This type of farming can also increase the economy of the farmers because these species can also provide fur, wool and leather and the productivity is high, mainly in the case of guinea pigs. This increase in the economy of farmers could probably lead to an increase of the sanitary conditions of their households. Also, farming these species has a low ecological impact due to the fact that these species were originally living in these areas before the introduction of pigs. These species are resistant to many diseases but it is advised to encourage the veterinary services to regularly visit and monitor these farms to reduce the risk of introduction of other zoonotic diseases. However, benefits of this proposed change in animal species can only be expected on a longer term.

Identification of PCC can be done at post-mortem inspection and in living pigs. Post mortem-inspection can lead to the total or partial condemnation of pigs, but if the sanctions are too high, farmers will avoid taking their animals to official abattoirs and escape the control. Therefore, it would be necessary to introduce a compensation system that will not discourage official inspection but at the same time will not encourage the intentionally infection of pigs. It has to be taken into account that in rural settings, slaughter of pigs is often done in a house or backyard in the village [62;63]. It would be interesting to explore the possibility of training some villagers for tongue inspection and inspection of the carcasses [298]. The inspection reports, both from abattoirs and villages, must be addressed to the local veterinary services in order to send an official report to the authorities of public health and agriculture ministries. These authorities must coordinate the interventions in the affected areas.

The identification of PCC in living pigs can be done through surveys in coordination with the public health and veterinary services. This kind of surveys has to be directed to identified priority areas in order to reduce costs. These surveys are limited mainly by the characteristics of the available diagnostic tools, such as low sensitivity (tongue inspection) and specificity (circulating antigens detection). Nevertheless, new improvements have been done in this field and it is anticipated that better diagnostic tools will become available in the near future. Another strategy that has to be explored is the use of sentinel pigs, taking into account all the complexities described by Devleesschauwer et al. (2013) [104;105]; however this strategy should be further studied in independent studies in order to assess its feasibility and usefulness in a control program.

Treatment with oxfendazole and vaccination of pigs as intervention methods to interrupt transmission should be further studied for applicability in Ecuadorian conditions. Both strategies have proven to be promising in Peru [198]; however, the success of these strategies depends on the combined efforts with the identification and treatment of tapeworm carriers [198;295].
Identification of tapeworm carriers and HCC patients in rural settings of Ecuador is only done by epidemiological research interventions or in the presence of symptomatic cases. To get over this situation, the inclusion of serological diagnosis for both HCC and taeniasis in the priority areas in routine laboratory analysis, is a strategy that should be explored. CDC Atlanta is currently developing a point of care test for simultaneous serological diagnosis of HCC and taeniasis that will be evaluated on a large scale in the field (P. Dorny, personal communication).

Health education in rural settings has to be much more elaborated than the message given in urban settings. In rural areas, messages on the improvement of pig farming and sanitary conditions, in addition to the awareness of the disease have to be clear and feasible. Also, in rural areas different groups can be targeted with different priority messages. Meetings with pig owners should focus on the importance of good disposal of human waste and the importance of impeding the access of pigs to these; meetings with school children have to highlight the importance of hygiene and the use and maintenance of latrines and toilets [200]; meetings with the general townspeople should highlight the awareness about the parasite, its consequences and what are the basic procedures to avoid the infections. Meetings with the authorities and representatives must highlight the importance of reporting *T. solium* taeniasis/cysticercosis cases.

Understanding the transmission dynamics in rural settings is a necessary step to implement an effective control measure in these areas. The longitudinal study conducted in an endemic area of Ecuador showed the dynamic nature of the HCC infection and exposure. Prevalence data on exposure showed that more than 30% of the population is exposed to the disease at a specific time, but the longitudinal analysis revealed that yearly, around 40% of the population is effectively producing antibodies against *T. solium*. Such figures suggest that there is a part of the population that is constantly exposed to the parasite, probably surrounding the tapeworm carrier(s) and another part of the population that has sporadic close contact with these individuals. Identifying the individuals permanently exposed could lead to detect the tapeworm carriers or “hot spots” [18;75], using this approach the targeted drug therapy of the suspected tapeworm carriers and the people surrounding him/her could be useful to save efforts and resources. Targeted interventions as suggested here have been tested in rural communities in Peru with promising results [299].

The low number of infections in this Ecuadorian rural community, contrasting to a high exposure suggests that exposed individuals are at high risk of infection but that there is an unknown factor avoiding this to happen. Some authors suggest that this presentation of high exposure/low infections is the result of acquired immunity in high prevalence areas [154;300]. Another hypothesis is the presence of cross-resistance due to the presence of other *Taenia* species in the same area [168;172]. However, the validity of these hypotheses still needs to be studied further.
In general, if the understanding of the epidemiological transmission patterns in epidemiological studies is well achieved, the control strategies derived from them could be extrapolated to similar endemic settings in priority areas of intervention.

The estimates generated from longitudinal data may be used to simulate the temporal transmission of the parasite and the effects of control interventions on its life cycle [209]. These type of simulations would be useful to make fine-tuned estimations of cost-effectiveness of the interventions/control programs, to help to fill the gaps in the absence of convincing evidence from field trials and to rank the control strategies depending on their relative efficacy [14]. Another advantage of such simulations is the estimation of the duration of the effect of an intervention. Estimating the duration of the effect of an intervention is important to assess the sustainability of a program, also, it will help to estimate how many interventions and when they should be applied for the efficient control of a disease.

To date, the only simulation models available are the model published by Kyvsgaard et al (2007), and the agent based model by Braae et al (2015) presented at the first CYSTINET (European network on taeniasis/cysticercosis) conference held in Belgrade [209;301]. However, these models do not include HCC in the simulations. The current data from longitudinal studies allows an approximate estimation of the human-to-human probability of transmission. This probability can be included in the cited models to add the HCC component in the simulations. As a result, the impact of the simulated control measures in the transmission of HCC could be represented.

This updated simulation model could be used to calculate the burden of the disease. However, there is still a gap between the infection rates (HCC), and the appearance of the clinical disease (mainly due to NCC) and the mortality that should be filled. Also, the influence of the environment in the transmission has not yet been well documented [178].

In conclusion, the implementation of a control program for *T. solium* in Ecuador should synchronize efforts from different sectors of the society. The government represented by the Ministries of Public Health and Agriculture and its Veterinary services should act in the first instance in the identification of priority areas of intervention. To achieve this, an appropriate legislation has to be created to support any effort on this matter such as mandatory reporting of any cases and routine diagnosis of *T. solium* infections in priority areas or suspected areas. Once the affected areas are identified, the control strategies to be applied should be designed accordingly depending on the type of area to be intervened. In urban areas, the more feasible strategies are the ones involving the social security system, education at schools and the mass media, also, these strategies could be part of other control strategies for other diseases or sanitary campaigns. These efforts could be managed at municipal level with the support of the government. In rural areas, the control strategies to be applied should be carried out joining forces of the Ministry of Agriculture and its Veterinary Services, the Ministry of
Public Health and the authorities of rural municipalities. Pig farming has to be improved, pig access to human waste has to be eliminated and the proper elimination of infected pigs has to be encouraged. Municipalities have to develop proper systems to eliminate excrements where it is possible. The Ministry of public Health, represented by the local health care centers should take action with targeted interventions of tapeworm carriers every time HCC and eventually PCC are detected and reported. Also, health education has to be promoted at any level. Research centers such as universities and health institutes, should also participate with the support of the concerned authorities. The inclusion of these centers would help in the fine-tuning of the strategies applied and would bring new information that could be incorporated in the design of control strategies. Finally, the results of this control program should be assessed at governmental level by the regular measuring of indicators such as prevalence of HCC, PCC and taeniasis.

5.5 Perspectives in Ecuador

Official data and clinical studies present a decreasing trend of hospitalized cases of NCC without any specific national program for control of HCC. However, this data reflects only partially the situation of the country. Sanitary and health conditions have improved in most of the country in the last decade, reducing the transmission of *T. solium* and lowering the incidence of HCC [150;151]. But the problem will persist if no control measures are taken in rural settings. The life cycle of *T. solium* continues to exist in rural areas despite the improvement of general conditions. The movement of tapeworm carriers between rural and urban settings is impossible to prevent, thus intervening at a rural level to interrupt the cysticercosis/taeniasis cycle is a must.

The monitoring and assessment at a country level of control programs for *T. solium* could be done through the continuous analysis of hospital data [295]. The hospital reports for NCC and neurological disorders is made continuously and is mandatory, but an improvement to the actual reporting system in Ecuador is necessary to generate more accurate figures. However, results of the intervention strategies would only be reflected on the long-term in this kind of reports. Reports from hospital cases are not immediately available and there is a long period between the infection and the appearance of symptoms. Monitoring and assessment of control programs for *T. solium* must be supported by other sources of data, such as reports from veterinary services, abattoirs and periodical coordinated epidemiological surveys in priority areas.

It would be necessary to enlarge the status of mandatory reporting of NCC and epilepsy ambulatory cases from private and social security hospitals, which are not included in the reports of the Ministry of public health. Also, monitoring of control programs for *T. solium* would benefit from a legislation that supports the compulsory diagnosis of HCC and taeniasis (with species identification) through the
social security system. Veterinary services should direct efforts in the traceability of PCC and increase the attention to informal pig rearing and slaughter.

Recently, Ecuador was capable to eradicate onchocerciasis being the second country in the world to achieve so [302]. In the animal health field, Ecuador was declared in 2014 free of Food and Mouth disease [303]. Educational campaigns have been successful in the country to control seasonal diseases such as dengue fever. Also, focused elimination of *T. solium* in Peru achieved promising results, giving some examples of strategies that could be applied in Ecuador given the similarities with this neighboring country [198]. Therefore, if Ecuadorian authorities dedicate enough attention to *T. solium* taeniasis/cysticercosis, control of the parasite is achievable.

If control measures are not taken in time, elimination of *T. solium* in Ecuador will not be achievable in a near future. The Ecuadorian authorities must conduct a control program integrating veterinary services, and public health agencies to act in rural areas to interrupt the life cycle of *T. solium*. The approach used in this thesis can guide the design of control programs involving simultaneous targeted interventions in priority communities. The application of such strategies at a large scale (provincial or country level) could have a measurable impact on the overall prevalence and incidence of the disease that if turned successful could be an example for other endemic countries.

5.6 Conclusions & recommendations

The existing variability in endemic areas affects the global estimates and control programs but studying the geographical distribution of high and low HCC prevalence areas can be used to identify priority areas of intervention. Ecuador appeared as one of the countries where HCC transmission dynamics could be further studied being a priority zone with high estimates for exposure to the parasite and non-negligible levels of active infections.

Spatio-temporal analysis with hospital data at country level is an important tool that should be incorporated when designing national/large scale control programs. The use of spatio-temporal tools with hospital data in Ecuador allowed to: 1) identify priority areas of intervention and 2) identify the indicators enhancing or preventing the appearance of negative health outcomes. Also, spatio-temporal tools could be used for the monitoring of the disease and for the assessment of control programs. A better reporting system should increase the power of the analysis of official databases. This kind of approach should be paired with reports from the veterinary services and epidemiological surveys to increase the coverage when identifying priority areas of intervention or monitoring control programs.

Studying longitudinal data in an endemic community in Ecuador permitted to obtain incidence estimates. These estimates can be incorporated in epidemiological simulation models to calculate the burden of the disease and the cost-effectiveness of an intervention. The results from this study also
revealed specific characteristics in this community that could be taken into account when designing control strategies.

To design an effective control program, the availability of quality epidemiological data is essential. Appropriate reporting should be encouraged at rural level and mandatory at health care centers at any level as well as in abattoirs. Sero-epidemiological monitoring of HCC and taeniasis should be incorporated in existing health campaigns. Better diagnostic tools that are more affordable, available and less invasive should be developed for both HCC and taeniasis diagnosis. An attractive compensation program should be developed for smallholders that should be enough to avoid underreporting but also not too high as to encourage the intentional infection of pigs. Pig husbandry in endemic communities has to be improved taking into account the economic issues for smallholders. Health education has to be continued at many levels in priority areas of intervention.

Given the experiences from neighboring countries when controlling *T. solium* taeniasis/cysticercosis, and given the recent success in Ecuador to control some other infectious and parasitic diseases, Ecuador can be one of the first countries to effectively achieve the control of *T. solium* taeniasis/cysticercosis if the respective authorities direct the necessary efforts to achieve this. To ensure the success in this integrated fight, it is necessary that veterinary and medical services as well as other health related scientists join forces following the One Health approach.

Global projections for the burden and the prevalence of HCC should take into account the variability in endemic areas and within. A correct methodology should be developed to correct this matter.
6 Summary

*Taenia solium* taeniasis/cysticercosis is a neglected zoonotic parasitic disease complex that causes public health and socio-economic problems in rural communities in many developing countries. It has become of increasing concern in developed countries due to globalization and human migration. *Taenia solium* is a cestode that is transmitted between humans, the definitive host in which it causes intestinal tapeworm infection (taeniasis), and pigs, the natural intermediate host in which it causes cysticercosis; but humans can also act as an accidental dead-end intermediate host. Human cysticercosis occurs when *T. solium* eggs that are fecally shed by tapeworm carriers are accidentally ingested. The disease is characterized by the development of the metacestode larval stage of *T. solium*, the cysticercus, in different tissues. Human cysticercosis (HCC) presents a wide variety of signs and symptoms that include several important neurological disorders (neurocysticercosis), in most severe cases cysticercosis can even cause death. Porcine cysticercosis is responsible for economic losses among vulnerable traditional pig farmers mainly due to the condemnation of carcasses and decreased carcass value.

Despite the wide knowledge about the biology and life cycle of the parasite, and the risk factors for infection, control of *T. solium* has received little attention from governments of endemic countries. Pilot control strategies have been tested in the field achieving varying results. Better efforts are needed to understand the factors that affect the transmission dynamics of the parasite in order to design adequate interventions that can be integrated in national control programs. To date, no national control program has been implemented in Ecuador although the country is considered endemic for HCC.

The objective of this thesis was to study the transmission patterns of *T. solium* taeniasis/cysticercosis in order to design appropriate and effective control strategies for this Neglected Zoonotic disease in endemic areas, with special reference to Ecuador. To achieve this goal, four specific objectives were identified: (1) to characterize active infections of and exposure to *T. solium* in endemic zones around the world; (2) to study the distribution in time and space of NCC hospitalized cases in Ecuador; (3) to identify potential indicators of NCC hospitalized cases in Ecuador; (4) to study the dynamics of active infections and exposure to *T. solium* in an endemic rural area in Ecuador.

The first chapter of this thesis presents an overview of the current knowledge of *T. solium* including the life cycle of the parasite, the implications in public health and the economic impact of the disease, its diagnosis, epidemiology with an emphasis on Latin America, with particular attention to the situation in Ecuador. In addition, the current control measures available for *T. solium* taeniasis/cysticercosis are described.
In the second chapter, serological data on apparent prevalence of *T. solium* circulating antigens and/or seroprevalence of *T. solium* antibodies, apparent prevalence of human taeniasis and risk factors for HCC from endemic communities were gathered in order to understand the differences in patterns of exposure to the parasite and active infections with *T. solium* metacestodes in endemic areas around the world. A total of 39,271 participants from 19 countries, described in 37 articles were studied. The estimates for the prevalence of circulating *T. solium* antigens for Africa, Latin America and Asia were: 7.30% (95% CI [4.23 - 12.31]), 4.08% (95% CI [2.77 - 5.95]) and 3.98% (95% CI [2.81 - 5.61]), respectively. Seroprevalence estimates of *T. solium* antibodies were 17.37% (95% CI [3.33 - 56.20]), 13.03% (95% CI [9.95 - 16.88]) and 15.68% (95% CI [10.25 - 23.24]), respectively. Taeniasis reported prevalences ranged from 0 (95% CI [0.00 - 1.62]) to 17.25% (95% CI [14.55 - 20.23]). A significant variation in the sero-epidemiological data was observed within each continent with African countries reporting the highest apparent prevalences of active infections. Intrinsic factors in the human host such as age and immunity were the main determinants for the occurrence of infections while exposure was mostly related to environmental factors, which varied from community to community.

In the third chapter, spatial and temporal variations in the incidence of hospitalized cases of epilepsy and NCC in Ecuadorian municipalities were analyzed in order to locate and characterize important clusters in space and time. Additionally, potential socio-economic and landscape indicators were evaluated to understand in part the macro-epidemiology of the *T. solium* taeniasis/cysticercosis complex. Data on the number of hospitalized epilepsy and NCC cases by municipality of residence were obtained from morbidity-hospital systems in Ecuador. This study identified traditional endemic clusters in the highlands for both conditions as well as new clusters appearing in recent years in other zones considered non-endemic. An increase in the implementation of systems for eliminating excrements would help to reduce the incidence of hospitalized cases of epilepsy by 1.00% (95% CI; 0.2% - 1.8%) and by 5.12% (95% CI; 3.63%-6.59%) for NCC. For epilepsy, the percentage of dwellings with piped water and the number of physicians per 100,000 inhabitants were associated with an increase in the number of hospitalized cases, whereas pig keeping was related to NCC emergence. The presence of clusters of municipalities with high incidence of hospitalized epilepsy cases could be the result of a high incidence of NCC cases or other acquired epilepsy cases. Given the appearance of recent epilepsy clusters, these locations should be studied in depth to discriminate epilepsies due to NCC from epilepsies due to other causes.

The fourth chapter describes the dynamic nature of human *T. solium* larval infections in an Ecuadorian endemic community. In this study incidence rate and cumulative incidence figures of human *T. solium* larval infections are reported for the first time in Latin America. A sero-epidemiological cohort study was conducted in a south-Ecuadorian community to estimate the
incidence rate of active cysticercosis and the incidence rate of exposure to *T. solium*, based on antigen and antibody detections, respectively. The incidence rate of infection was 333.6 per 100,000 person-years (95% CI: [8.4-1,858] per 100,000 person-years) contrasting with a higher incidence rate of exposure 13,370 per 100,000 person-years (95% CI: [8,730-19,591] per 100,000 person-years). The proportion of infected individuals remained low and stable during the whole study year while more than 25% of the population showed at least one antibody seroconversion/seroreversion during the same time period. While about 13% of the inhabitants were exposed to *T. solium* eggs, less than 1% of the population became infected yearly with the parasite. This contrast between exposure and infection may be linked to an effective resistance to the parasite acquired through long-term exposure of the population and differs from the situation in African countries, where much higher levels of infection have been observed. These estimates are of high importance to understand the epidemiology of *T. solium* in order to develop ad hoc cost-effective prevention and control programs. They are also essential to assess the burden of *T. solium* cysticercosis since longitudinal data are needed to make regional and global projections of morbidity and mortality related to cysticercosis. The estimates generated here may now be incorporated in epidemiological models to simulate the temporal transmission of the parasite and the effects of control interventions on its life cycle.

In the fifth and final chapter of this thesis, the findings of this research are discussed in the context of their contribution to the design of control strategies for *T. solium* with special attention to the case of Ecuador. This research showed the implication of the variability of epidemiological patterns of *T. solium* in different endemic areas in the design of control strategies. Also, some tools were provided that could be used to help in the identification of priority areas of intervention within a country. The transmission dynamics of human cysticercosis was analyzed in order to give further recommendations when applying control measures in endemic rural and in urban areas of Ecuador. The approach given in this research could be used to fine-tune the current control strategies of *T. solium*. However, further studies are needed to fill in the gaps in the epidemiology of *T. solium* such as understanding the role of the environment in the transmission of taeniasis/cysticercosis.
7 Samenvatting

*Taenia solium* taeniasis/cysticercosis is een verwaarloosd zoönotisch ziektecomplex met belangrijke gevolgen voor de volksgezondheid en met socio-economische impact op de landbouwsector in vele ontwikkelingslanden. Het is ook een toenemend probleem in industrielanden als gevolg van de globalisatie en menselijke migratie. *Taenia solium* is een cestode die wordt overgedragen tussen de mens, de eindgastheer bij de welke het een intestinale lintworm infectie veroorzaakt (taeniasis), en het varken, de natuurlijke tussengastheer waarbij de parasiet cysticercose veroorzaakt. De mens kan echter ook accidentele tussengastheer zijn waarbij de infectie dan blind eindigt. Humane cysticercose is het gevolg van de toevallige opname van *T. solium* eieren die door een lintwormdrager in de stoelgang worden uitgescheiden. De ziekte wordt gekenmerkt door de ontwikkeling van het metacestode larvaal stadium van *T. solium*, de cysticerc, in verschillende weefsels. Humane cysticercose kan zich in verschillende ziektekenen en symptomen uiten. De meest ernstige vorm van cysticercose ontstaat bij ontwikkeling van cysticercen in het centraal zenuwstelsel (neurocysticercose), die zelfs tot de dood kan leiden. Porciene cysticercose is verantwoordelijk voor economische verliezen als gevolg van het afkeuren van besmette karkassen. Vooral traditionele varkenshouders in endemische gebieden worden hierdoor getroffen.

Ondanks de goede kennis van de biologie en de levenscyclus van de parasiet, en van de risicofactoren voor besmetting, heeft de bestrijding van *T. solium* weinig aandacht gekregen in endemische landen. Piloot bestrijdingsstrategieën werden in het veld uitgetest met wisselende resultaten. Meer inspanningen zouden moeten gedaan worden om de factoren die de transmissiedynamiek van de parasiet bepalen beter te begrijpen, zodat aangepaste interventiestrategieën kunnen opgenomen worden in nationale bestrijdingsprogramma’s. Tot op heden werd geen nationaal controleprogramma geïmplementeerd in Ecuador, alhoewel dit land beschouwd wordt als endemisch voor humane cysticercose.

Het objectief van deze thesis was om de transmissiepatronen van het *T. solium* taeniasis/cysticercosis complex te bestuderen zodat aangepaste en efficiënte strategieën kunnen uitgewerkt worden ter bestrijding van deze verwaarloosde zoönotische ziekte in endemische gebieden, met focus op Ecuador. Om dit te realiseren werden vier specifieke objectieven geïdentificeerd: (1) het karakteriseren van actieve cysticercose en blootstelling aan *T. solium* in endemische gebieden van de wereld; (2) de studie van de spatio-temporale distributie van gehospitaliseerde gevallen van neurocysticercose in Ecuador; (3) het identificeren van potentiële indicatoren van gehospitaliseerde gevallen van neurocysticercose in Ecuador; (4) de studie van de dynamiek van actieve cysticercose en blootstelling aan *T. solium* in een endemisch ruraal gebied in Ecuador.
Samenvatting

In het eerste hoofdstuk van deze thesis wordt een overzicht gegeven van de huidige kennis van *T. solium*, namelijk, de levenscyclus van de parasiet, de implicaties voor de volksgezondheid en de economische impact van deze parasiet, de diagnosis en de epidemiologie met nadruk op Latijns Amerika, met daarbij de focus op de situatie in Ecuador. Tenslotte worden de beschikbare bestrijdingsmethodes van *T. solium* taeniasis/cysticercose beschreven.

In het tweede hoofdstuk werden gepubliceerde gegevens verzameld van endemische gebieden in de wereld, over serologische resultaten van de schijnbare prevalentie van circulerende antigenen van *T. solium* en/of de seroprevalentie van antistoffen gericht tegen *T. solium* bij de mens, de schijnbare prevalentie van humane taeniasis, en de risicofactoren van humane cysticercose. Het doel was om verschillen in patronen van blootstelling aan de parasiet en infectie met cysticercen bij de mens in verschillende gebieden beter te begrijpen. In totaal werden 37 wetenschappelijke publicaties weerhouden met daarin gegevens over 39271 studiedeelopers in 19 landen. De schattingen van de prevalentie van circulerende antigenen van *T. solium* voor Afrika, Latijns Amerika en Azië waren respectievelijk, 7,30% (95% CI [4,23 - 12,31]), 4,08% (95% CI [2,77 - 595]) en 3,98% (95% CI [2,81 - 5,61]). Schattingen van de seroprevalentie van antistoffen gericht tegen *T. solium* waren respectievelijk, 17,37% (95% CI [3,33 - 56,20]), 13,03% (95% CI [9,95 - 16,88]) en 15,68% (95% CI [10,25 - 23,24]). De gerapporteerde prevalentie van humane taeniasis varieerde van 0 (95% CI [0,00 - 1,62]) tot 17,25% (95% CI [14,55 - 20,23]). Significante verschillen in sero-epidemiologische data werden gemeten binnen elk continent. De hoogste schijnbare prevalentie van actieve infectie werd waargenomen in Afrikaanse landen. Intrinsieke factoren gebonden aan de menselijke gastheer, zoals leeftijd en immuniteit waren de voornaamste determinanten van infectie, terwijl blootstelling aan de parasiet eerder gerelateerd was aan omgevingsfactoren die varieerden tussen de studiegebieden.

In het derde hoofdstuk werden spatiale en temporale variaties in de incidentie van gehospitaliseerde gevallen van epilepsie en neurocysticercose in Ecuadoriaanse gemeentes geanalyseerd met als doel belangrijke clusters die zich geografisch en in tijd voordeden te karakteriseren. Bovendien werden potentiële socio-economische en landschapsindicatoren geëvalueerd om de macro-epidemiologie van het *T. solium* taeniasis/cysticercosis complex te begrijpen. Gegevens over het aantal ziekenhuisopnames van epilepsie en neurocysticercose gevallen per gemeente werden verkregen van hospitaal-morbiditeit systemen in Ecuador. De studie identificeerde naast de traditionele endemische clusters voor beide aandoeningen in bergachtige gebieden, ook recentere nieuwe clusters in zones die als niet-endemisch werden beschouwd. Een verbetering van de systemen voor de eliminatie van fecaliën zou de incidentie van ziekenhuisopnames voor epilepsie met 1,00% (95%CI; 0,2% - 1,8%) en voor neurocysticercose met 5,12% (95%CI; 3,63% - 6,59%) verminderen. Voor epilepsie waren, het percentage van woningen met aansluiting aan het drinkwaternet en het aantal dokters per 100000 inwoners, geassocieerd waren met een toename van het aantal ziekenhuisopnames, terwijl dit voor
neurocysticercose het houden van varkens was. De aanwezigheid van clusters van gemeentes met hoge incidentie van ziekenhuisopnames voor epilepsie kunnen veroorzaakt zijn door een hoge incidentie van neurocysticercose of van andere oorzaken van verworven epilepsie. Het ontstaan van nieuwe epilepsie clusters vereist een grondige studie naar de oorzaak ervan en van de potentiële rol van neurocysticercose.

In het vierde hoofdstuk wordt het dynamische karakter van humane cysticercose in een Ecuadoriaanse endemische gemeenschap beschreven. Voor het eerst in Latijns Amerika konden we in deze studie gegevens over incidentie en cumulatieve incidentie van humane cysticercose verzamelen. De seroepidemiologische cohortstudie werd uitgevoerd in een Zuid-Ecuadoriaanse gemeenschap waar we door middel van antistof en antigeen detectie gegevens bekwamen over respectievelijk, de incidentie van blootstelling aan de parasiet en actieve cysticercose. De incidentie van actieve infectie was 333,6 per 100000 personen-jaren (95% CI: [8,4 - 1858] per 100000 personen-jaren); dit contrasteerde met de hogere blootstelling aan de parasiet van 13370 per 100000 personen-jaren (95% CI: [8730-19591] per 100000 personen-jaren). De proportie van geïnfecteerde individuen bleef laag en stabiel gedurende de gehele studieperiode, terwijl meer dan 25% van de bevolking minstens één antistof seroconversie/seroreversie vertoonde tijdens die periode. Terwijl ongeveer 13% van de inwoners per jaar blootgesteld werd aan *T. solium* eieren, werd actieve infectie op jaarbasis slechts bij 1% van de bevolking vastgesteld. Het waargenomen contrast tussen blootstelling en infectie kan te wijten zijn aan de opbouw van beschermende immuniteit tegen de parasiet als gevolg van langdurige blootstelling. Deze observatie verschilt van de situatie in Afrikaanse landen, waar voor een vergelijkbare blootstelling veel meer actieve cysticercose wordt gemeten. Deze bevindingen zijn van groot belang voor het begrijpen van de epidemiologie van *T. solium* en voor het ontwikkelen van ad-hoc kosten-efficiënte preventie en controleprogramma’s. Ze zijn ook essentieel voor het bepalen van de ziektelest van *T. solium* cysticercose omdat longitudinale data vereist zijn om regionale en globale projecties van cysticercose-gerelateerde morbiditeit en mortaliteit te kunnen maken. De schattingen bekomen uit deze studies kunnen gebruikt worden in mathematische epidemiologische modellen om de temporale transmissie van de parasiet en de effecten van controlemaatregelen op de levenscyclus te genereren.

In het vijfde en laatste hoofdstuk van deze thesis worden de belangrijkste bevindingen van dit onderzoek besproken in de context van het ontwikkelen van strategieën voor de bestrijding van *T. solium*, met speciale aandacht voor het toepassen in Ecuador. Het onderzoek toonde de implicaties aan van de variabiliteit van epidemiologische situaties van *T. solium* in verschillende endemische gebieden op de ontwikkeling van bestrijdingsmethoden. We brachten ook werkmiddelen aan die kunnen helpen om gebieden in het land te identificeren waar interventies prioritair zijn. De transmissie dynamiek van humane cysticercose werd geanalyseerd om recommandaties te geven voor
interventie maatregelen in endemische rurale en in stedelijke gebieden in Ecuador. De aanpak beschreven in ons onderzoek kan ook gebruikt worden om bestaande controle strategieën in andere gebieden te verfijnen. Tenslotte identificeerden we ook lacunes in onze kennis van de epidemiologie van *T. solium*, zoals onder andere de rol van het milieu in de transmissie van taeniasis/cysticercose.
8 Publications


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9 Abstracts and Posters


10 Curriculum vitae

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2011 Master of Science in Tropical Animal Health (MSTAH), option: Epidemiology; Institute of Tropical Medicine, Antwerp, Belgium.

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2009 Quantitative Risk Assessment for Medical and Veterinary Public Health Officers and Researchers. Institute of Tropical Medicine, Antwerp, Belgium

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