PREVALENCE AND MOLECULAR STUDIES OF *TAENIA SOLIUM* AND ASSESSMENT OF SANITARY CONDITIONS OF SOME PIG SLAUGHTER SLABS IN KADUNA METROPOLIS, NIGERIA

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DECLARATION

I hereby declare that the work in this thesis entitled **"Prevalence and Molecular Studies of *Taenia solium* and Assessment of Sanitary Conditions of some Pig Slaughter slabs in Kaduna Metropolis, Nigeria"** has been performed by me in the Department of Microbiology under the supervisions of Prof. (Mrs.) H.I. Inabo, Prof. (Mrs.) V.J. Umoh and Prof. C.M.Z Whong. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at this or any other Institution. I take exclusive responsibility for its strength and weaknesses.

_____ _____

Uregwu Agnes Edia ASUKE Date

CERTIFICATION

This thesis entitled **"Prevalence and Molecular Studies of *Taenia solium* and Assessment of Sanitary Conditions of some Pig Slaughter slabs in Kaduna Metropolis, Nigeria"** by Uregwu Agnes Edia ASUKE, meets the regulations governing the award of the degree of Doctor of Philosophy (PhD) in the Department of Microbiology, Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the loving and fond memories of my late father; Barrister O.J. Edia, who gave me every encouragement needed to get me where I am today, and made me understand that hard work never kills.

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ABSTRACT

A community-based cross-sectional survey was undertaken to investigate the prevalence and risk factors of *Taenia solium* infections along with genotyping *T. solium* and assessment of sanitary conditions of some home-based pig slaughter slabs in Kaduna metropolis, Nigeria. The study was conducted in pig raising areas of Kaduna South and Chikun Local Government Areas in Kaduna metropolis. Three hundred (300) human blood and four hundred and fifty (450) human stool samples were obtained and analysed for cysticercosis and *T. solium* taeniasis from individuals who gave their consent to participate in the survey. Structured, self-administered questionnaires were used to obtain relevant information on sociodemography and risk factors that might be associated with cysticercosis and taeniasis in the study area. The blood samples were analysed for cysticercal antibodies by use of IgG antibody ELISA, while stool samples were analysed using microscopic examination for taeniid ova, in-house coproantigen ELISA for detection of coproantigens and by molecular assays for confirmation of *T. solium* present. *Taenia solium* DNA was extracted from corpoantigen positive stool samples by Qiagen stool mini kit and then amplified by polymerase chain reactions using species-specific primers. Genetic markers targeted were, nuclear internal transcribed spacer regions (*ITS I*) and mitochondrial cytochrome oxidase C subunit 1 (*mt cox 1*) genes. The amplified PCR products were partially sequenced, edited and aligned for phylogenetic analysis using BioEdit and CLUSTAL W computer programs. Evolutionary distances were computed by Kimura's two parameter method, and neighbor joining, bayesian and maximum likelihood trees were constructed using the PAUP vers-5.0 packages. The trees were evaluated using the bootstrap test based on 1000 resamplings. Phylogenetic trees were out group-rooted using nucleotide sequences from *Echinococcus*

granulosus. Sanitary conditions of some pig slaughter premises within some homes were assessed by critical observation of slaughtering procedures, on-site activities, available slaughter equipment and risky habits of butchers. Pig carcasses within the slaughter slab premises were examined for cysts by post mortem meat inspection. Cysticercosis and taeniasis prevalence of 14.3 and 8.8% were reported respectively. There were statistically significant associations ($p<0.05$) between pork preparation method, history of epileptic seizures and seropositivity of cysticercosis. Risk factors likely contributing ($p<0.05$) to the high taeniasis prevalence in the study areas include; hand washing practice, regular pork consumption, major source of ready-to-eat-pork, backyard pig rearing and level of knowledge of cysticercosis/taeniasis. The home slaughter slab conditions were very substandard, with bare concrete floors used as slaughter slabs. Butchers operating the slaughter slabs lacked adequate knowledge of *T. solium* and 30% of them were diagnosed with taeniasis at the time of survey. Porcine cysticercosis prevalence of 9.3% was detected among pigs slaughtered at the premises and meat inspection of slaughtered pigs by relevant authorities was lacking. Out of 15 *T. solium cox1* sequences analysed genetically, only 3 variant nucleotide positions were detected along the sequence length at positions 20, 53 and 415, thereby indicating a high level of similarity and minimal variation between the *mt cox1* genes of the *T. solium* population. Results revealed that *T. solium* in circulation in Kaduna metropolis, Nigeria is the African/Latin American type, therefore suggesting a common ancestral lineage despite the difference in geographical locations.

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Abbreviations

Ab- ELISA- Antibody ELISA

AgB- Antigen B

ADB- Agarose Dissolving Buffer

AL- Lysis Buffer 2

ASL- Stool Lysis Buffer 1

AW1- Wash Buffer 1

AW2- Wash Buffer 2

CE- Crude Extracts

CNS- Central Nervous System

COX 1- Cytochrome Oxidase C Subunit 1

CSF- Cerebro Spinal Fluid

DNA- Deoxyribonucleic Acid

DNAsp- Deoxyribonucleic Acid Sequence Polymorphism

ELISA- Enzyme Linked Immunosorbent Assay

GP- Glycoprotein

IEP- Immunoelectrophoresis

IgG- Immunoglobulin G

ITFDE- International Task Force for Disease Eradication

ITS rDNA- Internal Transcribed Spacer Region DNA

LL- Lenthil Lecithin

ML- Maximum Likelihood

Mt- Mitochondria

MtDNA- Mitochondrial Deoxyribonucleic Acid

NCC- Neurocysticercosis

NJ- Neighbour Joining

OD – Optical Density

OMS- Organizacion Mundial de la Salud

OPD – Ortho Phenylenediamine Substrate

OPS- Organizacion Panamericana de la Salud

PAUP- Phylogenetic Analysis Using Parsimony and Other Methods

PBS – Phosphate Buffered Saline

PBS – T20 - Phosphate Buffered Saline – Tween 20

PCR- Polymerase Chain Reaction

RAPD- Random Amplified Polymorphic DNA

RFLP- Restriction Fragment Length Polymorphism

SSCP- Single Strand Confirmation Polymorphism

SPSS- Statistical Package for Social Scientists

TBE- Tris Borate EDTA Buffer

TMB – Tetramethyl Benzidine

T20 – Tween 20

US- United States

UV- Ultraviolet Light

WHO- World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Re-emerging zoonotic diseases have recently raised new global concerns because of their disastrous impacts on human health; they have also become difficult to control because they are not only emerging in developing countries where most associated risk factors are found, but are now resurfacing in regions where they were previously eradicated. Cysticercosis and taeniasis are important zoonotic infections caused by different stages of the life cycle of same parasite *Taenia solium*, commonly called the pork tapeworm (Arora and Arora, 2012).

The pork tapeworm has a complex life cycle involving two animal hosts. Humans are known to be the definitive hosts, harbouring the adult tapeworm (taeniasis), while both pigs and humans are the intermediate hosts, harbouring the larvae or cysticerci (Garcia *et al*., 2003). Cysticercosis and taeniasis are neglected parasitic zoonoses which occur worldwide, primarily in poor rural communities in developing countries of Latin America, sub Saharan Africa and Asia where pigs are raised freely, pork is consumed, hygiene standards are low and there is lack of meat inspection (Xu *et al*., 2010; Da Silva *et al*., 2012; Mwape *et al*., 2013; Coarl-Almeida *et al*., 2014). The two diseases therefore are termed diseases of poverty because they are strongly associated with poverty and smallholder pig farming. Cysticercosis is a disease caused by infection with the larval stage of *T. solium*. Humans and pigs acquire cysticercosis by ingesting *T. solium* eggs shed in the faeces of humans with taeniasis (O'neal *et al*., 2012). Upon ingestion of eggs by individuals or pigs, larval worms result and migrate to different parts of the human body and pigs via the circulatory system and encyst in internal organs resulting in cysticercosis. A principal site for migration in humans is the central nervous system (CNS), resulting in human neurocysticercosis (NCC) characterized by such

neurologic dysfunctions as seizures, severe headaches, hydrocephalus, and incapacitation of the infected person. Neurocysticercosis is considered the most important parasitic disease affecting the nervous system and accounts for about 30% of all acquired epilepsy cases in endemic areas (Ndimubanzi *et al*., 2010). The International League against Epilepsy has cited NCC as the main cause of epilepsy in the world and the World Health Organization (WHO) estimates that throughout the world, at least 50 million people are infected with the parasite that causes annually more than 50,000 deaths (Schantz *et al*., 1993).

Taeniasis occurs only in the human hosts after ingestion of undercooked pork infected with cysticerci which later develops into the adult tapeworm in the intestine. "The adult tapeworm causes only mild inflammation at the implantation site, without substantial damage to the intestine" (Schantz *et al*., 1998). Most of the time, infected people neither notice the worm parts or segments expelled in their stools, nor seek medical attention, because they do not notice any life threatening symptoms. However, detection of taeniasis due to *T. solium* is important because of the risk of cysticercosis in the carrier or contamination of the immediate environment (Garcia *et al*., 2003; Nakao *et al*., 2010)).

In Nigeria, *T. solium* infections are a problem not only in rural areas where pigs are kept, but also in urban areas where infected pork can be consumed (Weka *et al*., 2013) and where small holder farming is now being embraced as an additional source of income. Small holder pig farming or otherwise back yard pig farming, home pig slaughter, pork sale and consumption have been identified as common practices in some parts of Kaduna metropolis, Nigeria and this raises concerns about the likely exacerbation of the taeniasis-cysticercosis complex within the metropolis, which until recently has not been investigated.

As is widely recognized for many parasites, knowledge of life cycles, mechanisms and dynamics of transmission and infection, all form the basis for effective control strategies. With the progresses in the area of molecular biology, molecular tools are being used to develop and strengthen these areas of knowledge.

Despite such achievement, not much is known about intra-specific variation of *T. solium* in contrast to inter-specific variations that have demonstrated deep separations among species (Kandil *et al*., 2010). Such information about the genetic structure of the parasite could prove useful in understanding its epidemiology and transmission dynamics, since genetic variants are known to differ in infectivity or pathogenicity to both man and other animals (Okamoto *et al*., 2001; Campbell *et al*., 2006). Mitochondrial DNA (mtDNA) is considered one of the best neutral markers for revealing phylogenetic relationships among closely related groups (Okamoto *et al*., 2001) and has been used to study intra–specific variation in *T. solium* in this survey. Regions of mtDNA have proven useful in biology, epidemiology and diagnosis of several parasitic infections of human and veterinary importance (Kandil *et al*., 2010).

1.1 Statement of the Problem

Previously, cysticercosis caused by *T. solium* was considered primarily a Latin American problem, however in recent times, the parasite is emerging as a serious problem across sub-Saharan Africa, South Asia (e.g. Nepal, India) and South-East Asia (e.g. Vietnam, Cambodia, Indonesia, South-western China) where pig keeping and pork consumption are increasing dramatically (Flisser *et al*., 2006). *Taenia solium* cysticercosis is said to be one of the most potentially lethal helminthic infections in humans and an important public health problem worldwide (Anantaphruti *et al*., 2007). It is particularly reported to pose serious public health consequences in developing countries (Allan *et al*., 2003; Deckers and Dorny, 2010).

Worldwide, about 20 million people are reported to be affected by cysticercosis with a resultant annual fatality rate of approximately 50,000 people (Berkvens *et al*., 2006). Neurocysticercosis is the most common and most fatal of human cysticercosis presentations and has equally been described as the most important parasitic disease and most frequently reported helminthic infection affecting the CNS (Berkvens *et al*., 2006; Ndimubanzi, 2010). Neurocysticercosis has been identified as a major cause of acquired epilepsy in cysticercosis endemic regions and is associated with considerable morbidity (Flisser, 2006). In actual fact, according to Dorny *et al.* (2004), it is responsible for about 20-50% of all late onset epilepsy cases; in essence accounting for about 30% of all acquired epilepsy cases and about 10% of all acute neurological admissions in such endemic areas (Carabin *et al*., 2006; Ndimubanzi, 2010).

Cysticercosis has a negative impact on health, agriculture and economy; it renders pork unsafe for consumption and immensely reduces the market value of pigs by rendering them unfit for consumption. A very conservative economic estimate collated by Zoli *et al*. (2003) reported the annual losses due to porcine cysticercosis in ten West African countries to be about 25 million Euros. In many African communities, the social impact of cysticercosis is believed to far outweigh the financial and economic losses incurred by the disease because of the particular perception of epilepsy (Zoli *et al*., 2003); such as ostracism, segregation and association of epileptics with the "supernatural". Human cysticercosis is a neglected disease which causes disabilities, interferes with work capacity, and results in stigmatization and ostracism (Xu *et al*., 2010; Mwang'onde *et al*., 2012). It also poses a great economic burden on countries where it is present; the minimum estimate of costs of admissions to hospitals and wage loss for NCC in the US which is considered to be non-endemic is $ 8.8 million

annually, while in Mexico and Brazil which are endemic countries, it amounts to $89 and $85 million annually, respectively (Garcia *et al*., 2003; Xu *et al*., 2010). Economic loss due to porcine cysticercosis in Nigeria as at 2003 was estimated at 17, 442, 000 Euros (Zoli *et al*., 2003).

Taeniasis may not be as life threatening as cysticercosis, but it puts individuals at risk of cysticercosis, particularly where poor personal hygiene and poor sanitation exist. It is therefore considered to be a risk factor for acquisition of cysticercosis.

In many developing countries where regulations concerning meat inspection and control are inadequate or non-existent, consumers are highly exposed to a host of pathogens including zoonotic parasites. Durga *et al*. (2003) revealed that due to the lack of implementation of the Meat Inspection Act and resultant absence of meat inspection, meat from sick or parasite-infected animals serve as a source of infection to people who are unaware of the disease; this may continue to be the case except feasible intervention measures are put in place.

1.2 Justification of the Study

Taenia solium is anticipated to be present in most of the pig-raising regions of West African countries (Zoli *et al*., 2003) where there is no available data on *T. solium* infections. Studies that report prevalence of human cysticercosis are very scarce and reportedly absent for sub-Saharan Africa (Mwape *et al*., 2013). In some regions of Nigeria, the reported prevalence of porcine cysticercosis and human taeniasis is quite high (20.5% and 8.6% respectively) as reported by Onah and Chiejina (1995) and 14.4% as reported by Gweba *et al.* (2010). Surprisingly, however, no cases of human cysticercosis beside the use of hospital records

have been reported despite this high prevalence of porcine cysticercosis, taeniasis and the high prevalence of epilepsy (37 per 1000) reported by Onah and Chiejina (1995).

Although in recent times, efforts have been made in documenting porcine cysticercosis in Nigeria, epidemiologic information on taeniasis and human cysticercosis is still very scanty even among risk groups. The cysticercosis-taeniasis complex is not clearly understood in many endemic areas in Nigeria and information on the transmission dynamics is lacking, making it difficult for effective control measures to be put in place.

There is also limited data on molecular identification and genetic variation of *T. solium* among risk populations in Kaduna State and in fact in Nigeria as a whole. Knowledge of genetic variation in *T. solium* is potentially important in order to improve on the understanding of host-parasite interaction. It is also an important tool in taxonomy and evaluation of effective drug treatments, diagnostics, vaccines and interpretation of epidemiology of taeniasis and cysticercosis.

Inadvertently, immunization or mass treatments alone as control measures have not shown adequate long term effectiveness (Pal *et al*., 2000). In addition to these population control measures, preventive studies such as the sanitary assessment of abattoirs is being advocated with a strong focus on identification of infected animal carcass and intervention measures targeted at reducing infection risk to pork consumers and raw meat handlers.

With this in view, this community based-epidemiologic survey was necessitated to obtain baseline information on the prevalence, risk factors, and genetic variation of *T. solium* present in the populace.

1.3 Aim of the Study

The aim of the research was to conduct prevalence and molecular studies of *T. solium* and assess sanitary conditions of some pig slaughter slabs in Kaduna metropolis, Nigeria.

1.4 Specific Objectives

The specific objectives were to:

1. Establish the existence of human cysticercosis and *T. solium* using IgG antibody ELISA, coprology and in-house coproantigen ELISA.
2. Determine the prevalence of human cysticercosis and *T. solium*-taeniasis in the study area.
3. Identify some socio demographic and risk factors associated with cysticercosis and taeniasis in the study area.
4. Assess sanitary conditions of some pig slaughter slabs within homesteads in the study area and conduct post-mortem meat inspection of meat parts.
5. Isolate and amplify *T. solium* DNA from human faeces by kit extraction and polymerase chain reaction.
6. Determine the genetic variation of *T. solium* within the different study locations by DNA sequencing and phylogenetic analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taeniid Tapeworms

Tapeworms as internal endoparasites infect a wide range of mammals and have a complex two- host life cycle. Tapeworms usually require a herbivorous or omnivorous intermediate host where the asexual stage occurs and a definitive host where sexual reproduction occurs. To complete its life cycle, the ova of the tapeworm hatch and transform into larvae in the intermediate host, and only reaches sexual maturity in the definitive host where sexual maturity and reproduction take place and the adult tapeworm lives. The genus *Taenia* comprises different species of tape worm infecting a host of animals. Most *Taenia s*pecies are very host-specific with regards to their animal host and so are therefore named in line with the hosts involved in their life cycles, for example, *T. saginata* is also known as beef tapeworm because the larvae reside in the muscle or meat of cattle. On the basis of host specificity, *Taenia spp* of human and animal importance include (O'meara, 2010):

1. Human tapeworms

   T. solium: referred to as the pork tapeworm

   T. saginata: referred to as beef tapeworm

   T. s. asiatica: Asian tapeworm

2. Zoonotic tapeworms

   T. pisiformis: Canine tapeworm whose larval stage is carried by rabbits and hares.

   T. ovis: canine tapeworm whose larval stage is found in muscles and heart of sheep.

   T. taeniaformis: cat tapeworm whose larval stage is carried by rodents.

T. hydatigena: canine tapeworm whose larval stage occurs in sheep, goats, pigs, cattle and wild livestock ungulates.

T. multiceps: a canid tapeworm with its larval form occurring in sheep brain, goats and occasionally horses, rabbits and cattle.

T. crassiceps: tapeworm of foxes or dogs whose larval stages are carried by rodents.

T. serialis: fox and dog tapeworm with its larval stages in rabbits, hares and rodents.

T. braunii: fox and dog tapeworms carried by gerbils.

2.2 Origin and History of Taeniid Tapeworms

For many decades, the exact time of association between taeniid tapeworms and humans remained a very controversial issue. Many scientists and researchers believed for a very long time that the long standing, interesting relationship between humans and taeniids came into existence about 10,000 years ago, coinciding with animal husbandry of potential tapeworm hosts such as; cattle, sheep, dogs, etc. Up until very recently, the source of the first tapeworm that colonized humans was linked back to the species circulating between dogs and ruminants which were in close contact with humans as a result of domestication (Cameron, 1956). This conventional belief asserts that the interesting association developed relatively recently.

This theory was however short lived as another contrasting theory (Baer, 1940) postulated that the association between humans and *T. solium* may have preceded swine domestication and that *T. solium* was probably not closely related to *T. saginata*. Researchers then started to carry out detailed studies of *T. solium, T. saginata* and *T. asiatica* which were the three known human tapeworm species, examining their characteristics and their evolutionary relationships with their hosts. Findings from one of such evolutionary studies (Hoberg, 2002) revealed that tapeworms became associated with humans when they were hominids,

speculating therefore that this relationship existed long before domestication began. Molecular studies and phylogenetic analysis carried out by these researchers (Eom and Rim, 1993; Hoberg *et al*., 2002) reason that the sharing of food by ancient hominids with large cats such as hyenas and lions on the savannas of Africa was the main source of tapeworm infections in humans.

The results from the estimated age for divergence of *T. saginata* and *T. asiatica* have got researchers thinking that the two *Taenia spp* diverged from a single ancestor species between 780,000 and 1.7 million years ago (Eom and Rim, 1993). This suggests that the ancestor tapeworm was already infecting humans and adapted to homosapiens for a long time. "*T. solium* even seemed to have an even earlier relationship with humans, having been infecting humans for perhaps 2.5 million years" (Whitehouse, 2014).

The adaptation of tapeworms to their human host is believed to have been favoured by the shift of human diet from herbivory to omnivory and then facultative carnivory, some 2 to 2.5million years ago (Eom and Rim, 1993). This report postulates that *T. solium* has been maintained among human populations successfully due to cannibalism which occurred in a "human-to-human" cycle and "human-to-wild suids" cycle through hunting, hence establishing the "human-to-pig" cycle (Hoberg *et al*., 2001; Nakao *et al*., 2002; Hidalgo, 2007).

About 500years ago, during the colonial era, European *T. solium* isolates were introduced into African and Latin American countries together with humans and pigs (Nakao *et al*., 2002; Ito *et al*., 2003).

2.3 Classification of *Taenia solium*

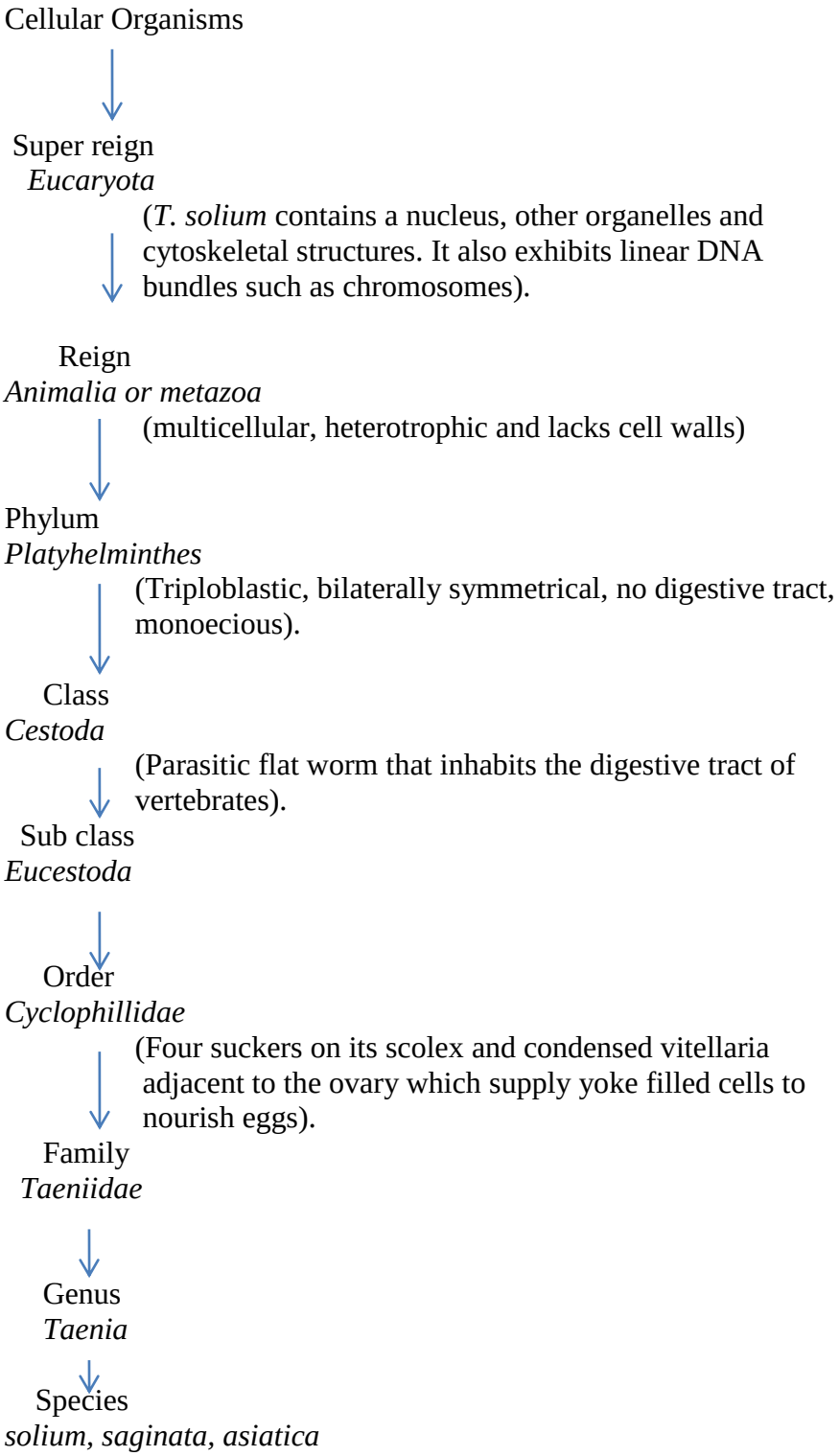

Fig. 2.1. Taxonomy of *Taenia spp.* according to the "GenBank" database of the NBCI. Adopted from: Hidalgo, 2007

2.4 *Taenia solium*

2.4.1 Description and morphology

T. solium is a white, flattened, ribbon-like tapeworm, measuring about 2 to 8cm (6.56 to 38.81ft). The adult *T. solium* morphology can be conveniently divided into three parts: The scolex, neck and strobila.

2.4.1.1 The scolex

The knob-like head of the tapeworm is referred to as the scolex and it is anteriorly positioned on the organism. It is the main organ of attachment as it possesses four radially arranged suckers (acetabula) and a rostellum with a double row of hooks which actually aids the scolex in attaching to the inside of the human's small intestine.

2.4.1.2 The neck

The adult tapeworm possesses an unsegmented, elongated region separating the scolex from the entire body. The body of the tapeworm actually extends from the neck.

2.4.1.3 The strobila

The flat ribbon-like body of the tapeworm that makes up the rest of the organism is called the strobila. The strobila is actually the formed body of the tapeworm which can grow to about 6mm in width and 2-7mm in length, with about 800 to 900 segments called the proglottids. These proglottids grow into maturity with the matured, gravid proglottids containing about 60,000 fertile eggs (Garcia *et al*., 2000) packed in a long and profusely branched central uterus. The proglottids therefore play a very important role in reproduction.

Fig, 2.2. *Taenia solium* (pork tapeworm).

Source: Odermatt, 2014

Fig. 2.3 *Taenia solium* scolex

Source: Odermatt, 2014

2.4.1.4 Eggs

Taenia solium eggs are difficult to differentiate from *T. saginata*, however, they have an outer shell which is fragile and usually shed once the eggs exit the body of the hosts. The eggs are fully embryonated and highly infective. They are "very resistant to adverse environments; they can remain viable for up to two months in water, soil and vegetation, particularly in humid and warm environments" (Chung, 2011). Once the outer shell is shed, the oncosphere larva becomes exposed to the external environment. The oncosphere larva is a solid mass of cells which measures about 30µm in diameter (Chung, 2011). *Taeni*a eggs are made of a chorionic membrane, a thick embryophore which constitutes embryophoric blocks of a keratin-like protein and an embryophoral membrane. The egg also comprises two oncospheral membranes surrounding the oncosphere (also named hexacanth embryo), bearing three pairs of hooks (Hidalgo, 2007).

2.4.2 Development and life cycle of *T. solium*

The primary host of the tapeworm which is the human being, acquires infection with the adult tapeworm by consumption of raw or inadequately cooked, measly pork infected with viable cysticerci. The gastric acid in the stomach aids in the digestion of the cysticercus wall. Following this, the released scolex evaginates, migrates to the small intestine and attaches itself to the mucosa (Garcia *et al*., 2000; Hidalgo, 2007). As the parasite feeds on the nutrients within the duodenum, it grows into segments called proglottids which progress posteriorly and within two to three months after infection, gravid segments are formed (Garcia *et al*., 2000; Hidalgo, 2007; Chung, 2011). The gravid segments containing about 50,000 to 60,000 fertile eggs (Garcia *et al*., 2000), break off the distal end of the organism and are excreted in the faeces of the infected individual. These eggs are capable of causing infection in both the

primary and intermediate hosts. In poor hygienic settings where people defaecate in the open, infective eggs passed with excrement unto the environment are eaten by pigs due to their coprohagic nature. Sometimes the pigs also ingest the eggs indirectly by consumption of contaminated food or water. Humans on the other hand acquire cysticercosis through faecal oral route when food or water contaminated with *T. solium* eggs is consumed; in this case, humans are said to be accidental intermediate hosts due to this aberration in the life cycle. Humans could also ingest eggs from the contaminated hands of a carrier who is also at risk of self-infection, therefore, cysticercosis could also be acquired by people who do not eat pork (Garcia *et al*., 2003).

In the stomach, the eggs hatch and become activated in the small intestine aided by enzymatic activity and the vigorous movement of the hooks. Eventually, the eggs migrate through the walls of the intestine and are carried via the circulatory system all over the body. Within three months, oncospheres develop to cysticerci which serve as the infective form.

2.4.3 Life span and longevity

The exact life span of *T. solium* has been a controversial issue to researchers; some believe the tapeworm can survive 20 to 25 years in its human host (Pawlowski and Schultz, 1972; Richards and Schantz, 1985), while other age-specific data suggest a shorter life span of less than 5 years (Allan *et al*., 1996; Garcia *et al*., 2003).

2.4.4 Reproduction in *T. solium*

The proglottids play a very important role in the reproduction of the pork tapeworm. *Taenia solium* possesses both male and female reproductive organs and so is considered a hermaphroditic or monoecious specie (Pawlowski, 2002; Chung, 2011). Both the male and

female reproductive systems are housed within a single proglottid. The proglottids located near the scolex are not involved in reproduction because they lack sexual organs and so the farther away they are from the scolex, the more sexually matured they are. The main reported form of fertilization in *T. solium* is self-fertilization, however reports of cross fertilization have also been documented (Scuitto *et al*., 2000; Pawlowski, 2002; Maravilla *et al*., 2003, Roberts and Janovy, 2009). Self-fertilization takes place in the oviduct where the sperm meets the unfertilized egg transported via the oviduct from the ovary. The female reproductive system consists of the vagina, ovary, oviduct, vitelline gland, mehlis glands, seminal receptacle and the uterus, all of which play specific and important reproductive roles. The male reproductive system has numerous testes in the proglottids and also possesses the vas deferens. The sperm is produced in the testes and gets to the unfertilized egg in the oviduct through its swimming action via the vas deferens, into the vagina which is connected to the genital pore. Once fertilization has taken place, the vitelline and mehlis glands produce secretory substances that surround the zygote and form the embryophore. The zygote continues its growth in the uterus which results in the gravid proglottids, preparing the adult tapeworm to shed or release its eggs into the external environment. The average number of eggs the tapeworm sheds per day is said to be about 300,000 eggs (Chung, 2011).

2.4.5 Digestion in *T. solium*

T. solium like other cestodes have no known digestive system. They get their nutrients through the absorptive functions of the tegument. The tegument possesses microtubules which function similarly to microvilli found in certain organisms.

2.4.6 *T. solium* habitat

The habitat of the pork tapeworm is actually dependent on the life cycle stage (Chung, 2011). There are different life cycle stages with propensity for specific habitats. The adult tape worm is found in the small intestine of the human host. The gravid proglottids and eggs are found in human faeces which could be released into the environment. Not much is known about the survival of the eggs in the environment (Gweba *et al*., 2010), however cold temperatures are known to be detrimental to the eggs (Garcia et al., 2003; Roberts and Janovy, 2009; Chung, 2011). The oncospheres are found in the gut and tissue of the pig and the next stage which is the cysticercus stage is found in the muscle and brain of both the intermediate and primary hosts (Scuitto *et al*., 2000; Pawlowski, 2002; Prasad *et al*., 2007; Chung, 2011).

2.5 Taeniasis/Cysticercosis Complex

2.5.1 *T. solium* infections

T. solium infections are referred to as neglected parasitic zoonoses which mostly affect developing countries (Mwape *et al*., 2012) and have been strongly linked to poverty, poor hygiene and sanitation, free pig management and lack of meat inspection (Garcia *et al*., 1998; Mafojane *et al*., 2003; Phiri *et al*., 2003). *Taenia solium* infects both its intermediate and definitive hosts, causing cysticercosis and taeniasis, resulting in negative consequences. Unlike the tape worm's close relative, *T. saginata*, its cysticercal stages have the ability to infect both pigs and humans, resulting in porcine and human cysticercosis. Both porcine and human cysticercosis have negative economic, agricultural, and health impacts. On the other hand, the human host is still capable of being infected with the adult tape worm resulting in taeniasis.

Taeniasis may mostly remain undetected because it is asymptomatic, but it is very dangerous because it could result to the life threatening disease of the nervous system; neurocysticercosis in humans. Cysticercosis and taeniasis are directly linked, although the link between the two diseases was not quite understood until Kuchenmaister in 1995, demonstrated the recovery of immature tape worms from condemned prisoners who were fed cysticercosis infected pork (Garcia, 2003).

2.5.1.1 Porcine cysticercosis

Cysticercosis is known as the disease resulting from infection with the cysticerci in its host (O'neal *et al*., 2012). Pigs being the major intermediate hosts could become infected with the cysticerci when they ingest infective *T. solium* eggs from the environment. From the life cycle, it is clear that the infective eggs are shed alongside faeces of an individual having taeniasis. Contact between infective faeces and pigs therefore becomes the start point of transmission in the intermediate host. Conditions that favour access of pigs to faeces therefore play a major role in the transmission cycle. Such conditions include, poor sanitation, indiscriminate disposal of faeces in the external environment, free range pig keeping or back yard pig keeping and direct feeding of pigs with human faeces. Due to the coprohagic nature of pigs, they ingest the infective eggs from contaminated external environment and then eventually develop cysticercosis as the parasite eggs travel through the digestive tract, and develop into larvae within the musculature.

The cysticerci cause veterinary problems in the pig host. More often than not, porcine cysticercosis has been found to be asymptomatic (Chung, 2011); the reason being that most pigs are slaughtered for consumption and do not live long enough for the cysticerci to degenerate thereby presenting with symptoms (Garcia *et al*., 2003). Nevertheless, some

symptoms may be observed if the pig is heavily infected and the infection is well disseminated in its system.

Porcine cysticercosis is prevalent in resource poor communities where pig keeping and production is emerging as an additional income source. There is a vast array of studies on the prevalence of porcine cysticercosis reported worldwide, particularly in parts of Africa, Asia, India, and Latin America. Extensive studies on porcine cysticercosis reported in Africa include; studies in South Africa (Krecek *et al*., 2012), Nigeria (Onah and Chiejina, 1995; Gweba *et al*., 2010; Karshima *et al*., 2013), Tanzania (Ngowi *et al*., 2004) and Cameroon (Assana *et al*., 2001; Pouedet *et al*., 2002; Shey- Njila *et al*., 2003), just to mention a few. High porcine cysticercosis is indicative of frequent occurrence of *T. solium*- taeniasis (Murrell and Pawlowski, 2005).

Porcine cysticercosis has a devastating effect on agriculture and on the overall economy because infected meat is destroyed and cannot be consumed. Zoli *et al*. (2003) reported an estimate of animal losses resulting from porcine cysticercosis to be about 25 million Euro in some parts of West and Central Africa.

2.5.1.2 Human cysticercosis

Human cysticercosis is a parasitic zoonotic disease caused by metacestodes (cysticerci or larvae) of *T. solium*, also known as *Cysticercus cellulosae* (Garcia *et al*., 2003; Kaliaperumal *et al*., 2005; Weka *et al*., 2013). Cysticercosis is acquired through faecal-oral route by ingestion of food, vegetables or water contaminated by the infective ova of *T. solium* (Garcia *et al*., 2003; Kaliaperumal *et al*., 2005). Humans are the definitive host of the parasite and so they do harbour the adult tapeworm which makes them carriers, posing a risk to other individuals around them, their environment and even to themselves. Humans are also

reported to be accidental intermediate hosts (Pouedet *et al*., 2002). The possibility of internal autoinfection in an individual with taeniasis has been reported, where by regurgitation of proglottids into the stomach or reverse peristalsis occurs (Garcia *et al*., 2003; Naik *et al*., 2011). Once the infective eggs are ingested, they undergo series of transformations within their human host and develop into cysticerci which are eventually lodged in different internal organs of the host. Cysts can be lodged in the muscle, subcutaneous tissue, eye and heart, but have a high affinity for the CNS. On this basis, clinical presentations therefore are dependent on the affected organ (Kraft, 2007), giving rise to cysticercosis. Human cysticercosis can therefore be broadly categorized into extraneural cysticercosis and neurocysticercosis (Garcia *et al*., 2003).

Extraneural cysticercosis:

When cysticercosis occurs outside the CNS, it is considered extraneural. More often than not, extraneural cysticercosis causes no major symptoms. Cardiac, subcutaneous, muscular and ophthalmic cysticercosis are all classically extraneural:

1. Subcutaneous cysticercosis

   This presents with painless nodules usually found in the anus and chest. The nodules are most times movable and small in size and may remain for a few months or even years before gradually disappearing. This condition is commonly observed in Asia and Africa, but is rare in Latin America (Garcia *et al*., 2003).

2. Cardiac cysticercosis

   Occasionally, cysts may be lodged in the heart, therefore resulting in cardiac cysticercosis, which accounts for five percent of infected patients (Rabiella, 1982). Cardiac cysticercosis is said to be asymptomatic (Garcia *et al*., 2003) and extremely

rare. Occasionally, demonstration of cysts in the heart is accidental or incidental during an autopsy or a heart surgery (Eberly *et al*., 2008). A recent case report of a 17 year old boy from Cameroon indicated finding of a sessile cystic cardiac mass during an ongoing hypertension investigation. This finding was linked with cardiac cysticercosis after series of radiological and histological examinations (Shogan *et al*., 2008).

3. Ophthalmic cysticercosis

   Ophthalmic cysticercosis results from lodgment of *T. solium* cysticerci in the eye. It is reported that ocular or adnexal involvement occurs in about 13-16% of patients (Kaliaperumal *et al*., 2005). Even though ophthalmic cysticercosis is not as common as NCC, the tapeworm responsible is reported as the "the most common intraorbital parasite" (Rahalkar *et al*., 2000). The degree of visual disturbance depends to a large extent on the part of the eye affected (Cardenas *et al*., 1992; Garcia *et al*., 2003). Kruger-Leite *et al*. (1985) reported 35% of the cysts in the sub retinal space, 22% in the sub conjuctival space, 22% in the vitreous humour, 5% in the anterior segment and 1% in the orbit.

4. Muscular cysticercosis

   Muscular cysticercosis is sometimes detected in NCC patients. It is asymptomatic and also incidental in finding when radiographic investigations are done for other reasons (Garcia *et al*., 2003). The thigh and muscles are major predilection sites in muscular cysticercosis, in which case, the cysts appear as ellipsoidal dots under radiography (Garcia *et al*., 2003). In cases where parasite burden is very high, muscular pseudohypertrophy may result.

Neurocysticercosis (NCC):

Ndimubanzi *et al*. (2010) described NCC as the most frequently reported helminthic infection of the CNS. NCC is a neurologic infection which occurs when *T. solium* cysticerci are present in the brain or CNS. The cysts are viable and are responsible for subsequent inflammatory reactions and responses in the body. NCC can be conveniently divided into parenchymal and extraparenchymal forms. The extraparenchymal forms can cause intraventricular, subarachnoid, intraocular and spine disease. Once the cysts develop in the brain, they are able to evade destruction by the host immune system through a number of postulated means. Some studies have suggested that the blood- brain barrier protects the cysticerci (Flisser, 1994; Carpio, 2002), and this alongside its immune evasion mechanism helps it to remain a very long time, even years within its host. At such initial viable stage, the cysts do not cause much inflammation within the surrounding tissues since it is evasive, as such, the disease remains asymptomatic at this point.

The immune evasion by the parasite is due to a variety of substances possessed by the metacestode. One of such substances called taeniastatin is a parasite serine proteinase inhibitor. Others are paramyosin, sulfated polysaccharides, and secretory proteases all of which have a major function of either inhibiting or diverting host inflammatory responses (White *et al*., 1997). Taeniastatin is able to inhibit normal cellular immune defenses by interfering with macrophage function and adequate proliferation of lymphocytes. Secretory proteases are also said to degrade cytokines and host molecules. It is also believed that humoral antibodies are unable to destroy mature cysticerci.

Symptoms of NCC:

Symptoms depend on the number and location of the cysticerci and the extent of inflammation. Symptoms also vary from asymptomatic to life threatening (Coyle and Tanowitz, 2009). As a result of the intelligent evasive mechanism of the parasite, symptoms do not appear on time and so individuals could carry the cysts undetected and without any symptoms for a very long time. Symptoms only begin to appear once the parasite is no longer able to evade the host's immune system and consequently the host's immune and inflammatory cells launch an attack on the cysticerci, thereby leading to a series of symptoms. Some studies also postulate that by blocking the circulation of cerebrospinal fluid, cysticerci could also stimulate symptoms (Garcia *et al*., 2003).

The most common presentation or manifestation of NCC is epileptic seizures. For some patients, epilepsy is the sole manifestation of the disease with about 70% of them having recurring seizures (Garcia *et al.*, 2003). As the immune system attacks the cysts, an immune mediated response occurs and host inflammatory molecules such as substance P attack the cysts which then degenerate and result to seizures (Coyle and Tanowitz, 2009). NCC is reported to be responsible for about 30% of acquired epileptic cases and or late onset of epilepsy in endemic regions (Murrell, 2005). In fact, reports of Garcia *et al*. (1991) suggest that the sudden onset of seizures among apparently healthy persons is strongly suggestive of NCC. Calcified cysts in NCC patients are strongly linked to development and maintenance of seizures. Calcified granulomas have also been implicated in increasing the risks of ongoing seizures (Coyle and Tanowitz, 2009). A case series by Carpio and Hauser (2002) involving individuals with an initially mild infection showed that about 50% of such patients who presented with seizures, had further seizures (epilepsy).

Another clinical presentation of NCC which varies in about 20-30% of cases is intracranial hypertension and hydrocephalus (Garcia *et al*., 2003). Giant cysts could occasionally result from growth of smaller cysts; they could act as tumours and compress cerebral structures therefore resulting in intracranial hypertension.

Other symptoms of NCC include; oedema related motor deficits, stroke which is more frequent in subarachnoid NCC, acute encephalitis and non-encephalitic forms of NCC (commonly found in children and teenagers), mild cognitive dysfunction and compromise of the spine.

Psychotic manifestations and depression have also been identified as symptoms. Forlenza *et al*. (1997) carried out a study on psychiatric manifestations in a certain group of in-patients and found that there was an increased risk of cysticercosis among the in-patients having mental disorders compared to those with other psychiatric disorders. There was a high prevalence of cysticercosis in this group of in-patients. NCC is associated with considerable morbidity and could in extreme cases, lead to death.

2.5.1.2 T. solium- taeniasis

Taeniasis simply refers to infection with the adult tapeworm and it only occurs in humans; it could be caused by *T. solium, T. saginata* or *T. asiatica*. However, in this context, *T. solium* taeniasis is being discussed. In contrast to cysticercosis, taeniasis is relatively innocuous, with the adult stages of these cestodes infecting the small intestines of humans and causing a few specific symptoms such as abdominal pain and nausea (Pawlowski and Schultz, 1972), loss of appetite, weight loss and general uneasiness (OMS/OPS, 1993). Taeniasis is usually asymptomatic and it has been reported that the presence of gravid proglottids passed in faeces daily or about twice or thrice a week is the most noticeable sign of taeniasis (Hidalgo, 2007).

Sometimes, the gravid proglottids actively migrate out of the host, though not as spontaneously as seen in *T. saginata* proglottid migration. Taeniasis on its own causes no life threatening condition, however because it is linked with cysticercosis, it puts individuals at risk of cysticercosis. The most important risk factor for the acquisition of cysticercosis is the proximity of a tapeworm carrier. Gravid proglottids filled with the eggs expelled from tapeworm carriers serve as a new source of infection for intermediate hosts, particularly in developing countries where sanitary conditions are poor. Therefore early detection and adequate treatment of taeniasis is important for the prevention of cysticercosis.

2.6 Epidemiology of *T. solium*

2.6.1 Worldwide distribution

T. solium is cosmopolitan and has a worldwide distribution. *Taenia solium* infection is exacerbated by poor sanitation and infection rates are high in areas with large faecal human contamination, and free grazing of pigs. Human cysticercosis is prevalent in areas where people eat raw or undercooked pork, practice back yard pig keeping, use untreated wastewater for irrigation purposes and where there is inadequate sanitation and poor personal hygiene. It has been estimated that more than 50 million pigs and about 6 million humans have cysticercosis, while the annual incidence of human taeniasis is put between 10 to 50 million (Craig, 1995; Crompton, 1999). Within these estimates, Bern *et al*. (1999) estimates that approximately 400,000 display symptomatic disease, while OMS/OPS (1997) reports that 50,000 deaths occur annually as a result of NCC.

Cases of *T. solium* infections are now emerging and even re-emerging in developed countries and this is being attributed to immigration and increased influx of people across borders (Ortega- Herrera *et al*., 2004; Anderson, 2010, Chung, 2011). Prevalence of *T. solium*

infections is on the increase in America due to influx of migrant farmer carriers from neighbouring Central and Latin American countries (Anderson, 2010). In the Western hemisphere, *T. solium* is mostly found in South and Central America, and is also widely distributed throughout countries in Africa, Asia and parts of India (Chung, 2011). Canada, America, Argentina, Guiana, Uruguay and Paraguay which previously appeared to be free, seem to in recent times have issues of re-emergence of *T. solium* (Chung, 2011).

2.6.1.1 Latin America

A lot of research efforts have been put into the Latin American region, and this has generated extensive data on the cysticercosis-taeniasis complex in the region. Prevalence rates vary from country to country, however, data show that there is a very high risk of *T. solium* infection for many Latin American residents (Sarti *et al*., 1994; Rodriguez *et al*., 1999; Garcia *et al*., 1999; Bragazza *et al*., 2002; Costamagna *et al*., 2002; De Arruda Pinto *et al*., 2002; Agudelo-Florez and Palacio, 2003; Taico *et al*., 2003; Flisser *et al*., 2003, 2005; Coral-Almeida *et al*., 2014).

2.6.1.2 Europe

The most part of Europe is free from *T. solium* infections. However, due to immigration, travel and tourism, some regions in Europe have reported the acquisition of *T. solium* infection locally; Overbosch *et al*. (2002) in a survey demonstrated that eleven out of a total of 45 diagnosed NCC cases were actually autochthonous.

2.6.1.3 Asia

Cysticercosis and taeniasis have only recently received much attention in Asia, though it is reported to have been in existence for a very long time. *Taenia solium* infections are prevalent

in Western Irian Jaya, Republic of Indonesia (Simanjuntak and Widarso, 2004) and Bali. *Taenia solium* infections have also been reported in Taiwan province of China, Kingdom of Thailand and Republic of Korea. Anantaphruti *et al*. (2010) reported sympatric occurrences of *T. solium*, *T. saginata* and *T. asiatica* in Kanchanaburi, Thailand with a 0.6% prevalence by the Kato Katz technique. Nakao *et al*. (2002) also did a careful and detailed molecular and phylogenetic analysis of isolates of *T. solium* cysticerci from China, Thailand, Irian Jaya, Taiwan and a host of other locations. A recent survey by Xu *et al*. (2010) reported that cysticercosis and taeniasis were both prevalent in a helminth endemic community in Leyete, Philippines.

Rajshekhar (2004) reports that cysticercosis is prevalent in nearly all of the Republic of India. The report reveals that NCC accounts for a significant percentage (8.7-50%) of patients with onset of seizures. Interestingly, the solitary form of cysticercosis is peculiar in India with about 70% of NCC patients having a solitary cysticercosis granuloma (Prabhakaran *et al*., 2004; Prasad *et al*., 2008). Taeniasis prevalence in India is between 0.5 to 2%, though a survey in a pig rearing community, Uttar Pradesh reported a taeniasis prevalence of 18.6% (Prasad *et al*., 2007). Another study reported a rather high prevalence of taeniasis among pig farmers somewhere in Nepal (Rajshekhar, *et al*., 2003). Recently, Okello *et al*. (2014) also reported 26.7% taeniasis prevalence in Lao People's Democratic Republic, a Southeast Asian country.

2.6.1.4 America

Most cases of *T. solium* cysticercosis reported in America have been attributed to immigration and influx of people from *T. solium* endemic regions. It has been reported that a proportion out of the rising incidence in the western states in America are autochthonous

(Murell and Pawlowski, 2005). In retrospect, a look at hospital records in Oregon from 1995-2000 revealed that five people out of the 89 patients diagnosed with cysticercosis had not travelled or lived outside the United States (Engels *et al*., 2003). Dorny *et al*. (2004) reported a death rate of 3.9 per million population over a twelve year period (1989-2000) in California, USA, majority of whom were "foreign born, predominantly in the United Mexican states".

2.6.1.5 Africa

Taeniasis and cysticercosis are both emerging and expanding in Africa (Zoli *et al*., 2003). Extensive reviews done in Western and Central Africa by Zoli *et al*. (2003) report regions of hyperendemicity and a gross under estimation of human cysticercosis prevalence by simply relying on the prevalence of porcine cysticercosis alone. Of recent, interest in *T. solium* infections has gained grounds in Africa as a result of the public health significance posed by human cysticercosis/taeniasis and the consequential economic and agricultural burdens felt in endemic regions. This has become the driving force behind recent increase in researches tailored to capture the burden of cysticercosis and taeniasis in many affected African communities. Information on porcine cysticercosis in Africa is easily accessible than human *T. solium* infections due to the growing interests among veterinarians and agriculturists. Limitations of extensive studies on human *T. solium* infections in Africa are attributed to inaccessibility and costs of modern diagnostic techniques, lack of adequate surveillance, monitoring and reporting systems (Murrell and Pawlowski, 2005). However, available researches report varying prevalence among countries using different diagnostic techniques. Extensive data are available on *T. solium* infections in Benin, Togo, Cameroon, South Africa

and Tanzania. Few data are available in regions of Nigeria, Kenya, Burkina Faso, Ghana and Egypt (Onah and Chiejina, 1995; Gweba *et al*., 2010; Abdo *et al*., 2010).

Porcine cysticercosis prevalence between 20-40% has been reported in Eastern and Southern Africa by Mafojane *et al*. (2003). There are also increasing concerns in Eastern and Southern Africa about exacerbation of taeniasis-cysticercosis complex in many regions as a result of increased pork consumption and rapid expansion of small holder pig farming (Murrell and Pawlowski, 2005). Phiri *et al*. (2003) documented a tripling of pig populations in Uganda, Tanzania, Kenya, Zambia, Zimbabwe and Mozambique.

The prevalence of *T. solium* infections in Africa is summarized in Table 2.1; the information was obtained from an extensive review done by Zoli *et al*. (2003) on cysticercosis in Africa, using different diagnostic approaches. This present review, has however included more recent updates on both cysticercosis and taeniasis since the last review done by Zoli *et al*. (2003). Thomas (2004) conducted a study on human cysticercosis in Mayo- Dannay, Cameroon, using antigen-detecting ELISA as a diagnostic technique. Krecek *et al*. (2012) also conducted a prevalence study on porcine cysticercosis by use of antibody-detecting ELISA in some parts of Eastern Cape, South Africa. A number of recent studies on the prevalence of *T. solium* infections have also been conducted in Tanzania; Mwang'onde *et al*. (2012) and Mwanjali *et al*. (2013) carried out community-based prevalence studies on human cysticercosis in Mbulu district and Mbeya regions in Tanzania respectively, using a variety of diagnostic techniques. There have also been recent studies conducted in parts of Eastern Zambia on prevalence and incidence of *T. solium* infections also using a vast array of diagnostic techniques (Mwape *et al*., 2012; Mwape *et al*., 2013).

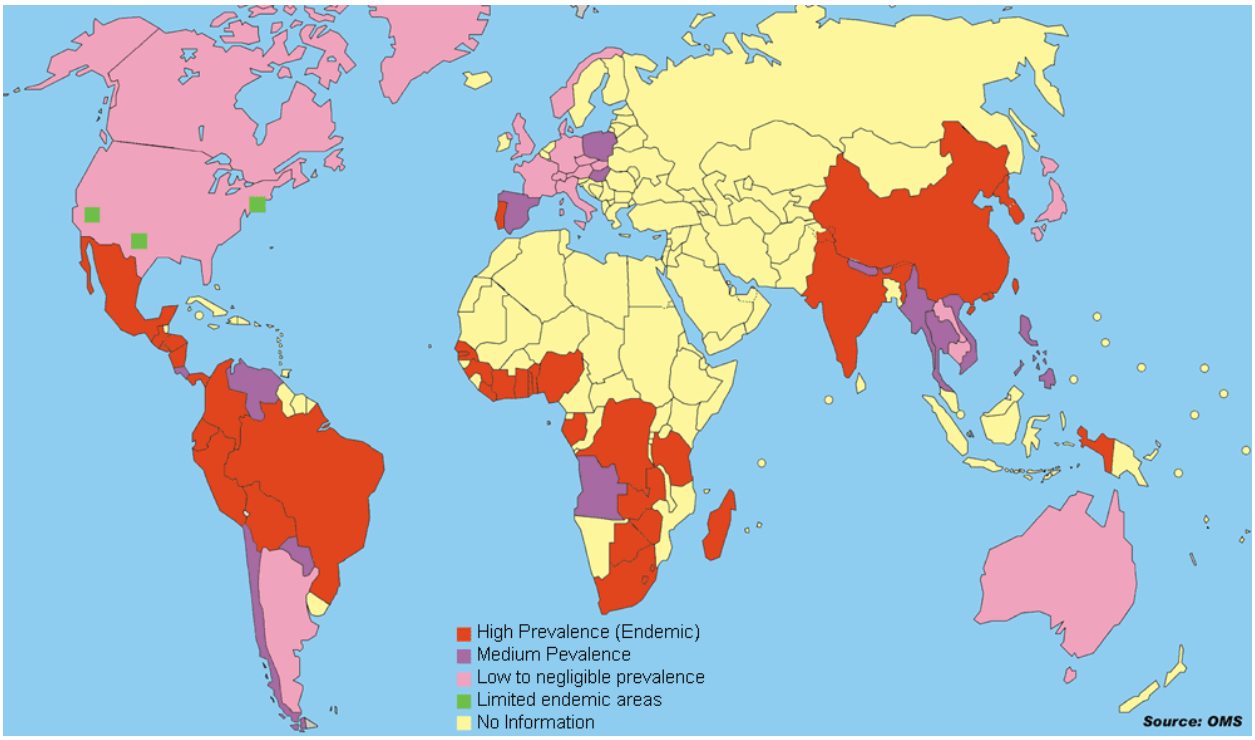

Fig 2.3: Worldwide distribution of human cysticercosis

Source: WHO, 2002

Table 2.1 Prevalence of *T. solium* Infections in Different Regions of Africa

Country	*T. solium* infection	Diagnostic Assay	Prevalence (%)	Reference
Cameroon				
Western Cameroon	Porcine cysticercosis	Ag ELISA	11.0	Pouedet *et al*., 2002
		Ab ELISATI	21.8	
			6.1	
North	Human cysticercosis	Ag ELISA	2.05	Zoli *et al*., 2002
North West	Porcine cysticercosis	TI	4.44	Shey Njila *et al*., 2003
		Ag ELISA	27.7	
Bafou	Human cysticercosis	M-AbELISA	0.4	Nguekam *et al*., 2003
Bamendou	"	"	1.0	
Fonakeku	"	"	3.0	
Mayo-Dannay	Human cysticercosis	Ag-ELISA	0.49-4.12	Thomas, 2004
Nigeria				
Nsukka	Porcine cysticercosis	MI	20.5	Onah & Chiejina, 1995
	Taeniasis	HR	8.7	
South Africa				
Eastern Cape	Porcine cysticercosis	Ab ELISA	57	Krecek *et al*., 2012
Tanzania				
Mbulu district	Human cysticercosis	IgG WB	16.3	Mwang'onde *et al*., 2012
Mbozi, Mbeya	Human cysticercosis	Ag ELISA	16.7	Mwanjali *et al*., 2013
		Ab ELISA	45.3	
	NCC	CT Scan	54.6	
	Taeniasis	Coprology	1.1	
		CoAg ELISA	5.2	
		EITB	4.1	
Zambia				
Eastern Zambia	Taeniasis	Co Ag ELISA	6.3	Mwape *et a*l., 2012
	Human cysticercosis	Ag ELISA	5.8	
Eastern Zambia	Human cysticercosis	Sero Ag	12.2-14.2	Mwape *et al*., 2013
		Sero Ab	33.5-38.5	
	Taeniasis	CoAg ELISA	11.9	

Key: Ag ELISA- Antigen ELISA; Ab ELISA- Antibody ELISA; TI: Tongue inspection; M-Ab ELISA- monoclonal Ab ELISA; MI-meat inspection; HR-hospital records; IgG WB- Immuno globulin G western blotting; CT- computed tomography; CoAg ELISA- coproAg ELISA

Adopted from Zoli *et al*. (2003) and modified

Table 2.1 continued: Prevalence of *T. solium* Infections in Different Regions of Africa

Country	*T. solium* infection	Diagnostic Assay	Prevalence (%)	Reference
Benin	Human cysticercosis	Ab ELISA	1.3	Houinato *et al*., 1998
Burkina Faso	Human cysticercosis	Ab ELISA	CR	Preux *et al*., 1996
	Porcine cysticercosis	Meat Inspection	0.6	Coulibaly and Yameogo, 2000
	Human cysticrcosis	Ag ELISA	10.3	Carabin *et al*., 2009
Ghana	Porcine cysticercosis	Meat Inspection	11.7	Permin *et al*., 1999
Ivory Coast	Porcine cysticercosis	Meat Inspection	2.5	Mishra & N'depo, 1978
	Human cysticercosis	Ab ELISA	CR	Heroin *et al*., 1972
Senegal	Porcine cysticercosis	Meat Inspection	1.2	Zoli *et al*., 2003
	Human cysticercosis	Ab ELISA	CR	Collomb *et al*., 1964
Togo	Human cysticercosis	Ab ELISA	2.4	Dumas *et al*., 1989
	Porcine cysticercosis	Meat Inspection	17	Dumas *et al*., 1990
Angola	Porcine cysticercosis	Meat Inspection	0.6-8	Kama, 1998
Chad	Porcine cysticercosis	Meat Inspection	6.8	Graber &Chailloux, 1970
Congo	Porcine cysticercosis	Meat Inspection	8.1	Chartier *et al*., 1990
	Human cysticercosis	*Detection of cysts	3	Fain, 1997
Rwanda	Porcine cysticercosis	Meat Inspection	10-30	Thienpont *et al*., 1959

Key: Ab ELISA- Antibody ELISA; *At autopsy; CR- case report

Adopted from Zoli *et al*. (2003), and modified.

2.7 Prevalence of *T. solium* Infections in Nigeria

Despite the fact that consumption of pigs by certain tribes, culture and religion in Nigeria is a taboo, pig production and consumption is gradually being embraced by many people in pig-raising areas. This could be attributed to the fact that pig rearing serves as either a major or an additional source of income, and pork is also a cheaper source of animal protein for many. Animal protein consumption in Nigeria is said to be less than 8%, and this is way below the Food and Agricultural Organization (FAO) recommended intake (Niang and Jubrin, 2001).

Pigs are the natural intermediate hosts of *T. solium* and they play an important role in the life cycle of the parasite, as such pork consumption is considered a risk factor of *T. solium* infections. Despite the risks associated with pork consumption in many pig raising areas in Nigeria, not much has been documented about taeniasis and cysticercosis. However, between the two hosts, reports on porcine cysticercosis appear to be more readily available than that on *T. solium* infection in the human host.

Onah and Chiejina (1995) reported a porcine cysticercosis prevalence of 20.5% in Nsukka area of Enugu state Nigeria. The same study by Onah and Chiejina (1995) also reported a taeniasis prevalence of 8.6% and a high epilepsy prevalence of 37 per 1000 among subjects in Nsukka, using hospital records.

Another study by Biu and Ijudai (2012) reported a porcine cysticercosis prevalence of 9.5% in Northern Nigeria. In consonance, Gweba *et al*. (2010) also reported a porcine cysticercosis prevalence of 14.4% in Zuru, Nigeria.

A recent study by Biu and Hena (2008) reported on prevalence of human taeniasis in Maiduguri, Nigeria, but only recorded a prevalence of 4.2% for *T. saginata*-taeniasis, there

was no occurrence of *T. solium* in the samples they examined. A more recent survey conducted in Ibi Local Government Area of Taraba state, Nigeria by Karshima *et al*. (2013) also reported a porcine cysticercosis prevalence of 6.25% in slaughtered pigs.

One of the few studies documented on human cysticercosis was conducted in Jos, Plateau state Nigeria by Weka *et al*. (2013). The survey was conducted among farmers in Jos and reported a human cysticercosis prevalence of 9.6% using IgG antibody ELISA technique.

2.8 Diagnosis of *T. solium* Infections

The need for prompt, reliable and feasible diagnostic methods for cysticercosis and taeniasis has led to advancement in this area of research. Microscopic diagnostic methods (coprology), immunological and serological methods (for detection of both antibodies and antigens) and lately molecular diagnostic techniques have all been reported for cysticercosis and taeniasis.

These different diagnostic techniques have also been validated under different research conditions with defined specificities and sensitivities. Diagnostic methods all have their peculiar advantages and disadvantages; however, based on the need, methods could be combined in order to arrive at a proper diagnosis of the disease.

The purpose for diagnosis also influences the suitability and choice of the test method; for instance, rapid, reliable screening methods are employed for cross sectional surveys involving massive populations, however diagnosis of NCC particularly for the sake of drug administration and treatment requires very precise imaging methods such as computerized tomography or magnetic resonance imaging for detection of cysts.

2.8.1 Diagnosis of taeniasis

Taeniasis is defined as infection with the adult stages of any of the human tapeworms. The three major human tapeworms include *T. solium*, *T. saginata* and *T. asiatica*. Morphologically, the tapeworms look alike and have to be critically scrutinized in order to differentiate them. This is particularly so between *T. saginata* and *T. solium* whose eggs cannot be distinguished because they look very similar. Therefore, very specific methods are required to arrive at the actual causative human tapeworm, particularly where the three tapeworms occur sympatrically.

2.8.1.1 Microscopic diagnosis of taeniasis

Traditionally, diagnosis of human taeniasis depends on microscopic or coprological examination of the faecal sample for taeniid ova. Such an approach is not very sensitive because the ova of the beef tapeworm (*T. saginata*) and that of the pork tapeworm (*T. solium*) are actually indistinguishable under the light microscope (Schantz and Sarti, 1989; Guezala *et al*., 2009).

Despite this disadvantage, coprological stool examination has for a long time remained the routine diagnostic method for identifying ova of *Taenia spp* till date. Although coprology shows a high specificity (Praet *et al*., 2013), it has got a rather low sensitivity (Allan *et al*., 1996; Flisser, 2006; Somers *et al*., 2006). Due to the disadvantage of this technique, it cannot be used for clinical follow-up of taeniasis patients living in areas where there is sympatric occurrence of the parasites (Praet *et al*., 2013).

Allan *et al.* (1993) reported that even with large multiple faecal samples, sensitivity of stool microscopy does not exceed 60 to 70%; other complementary methods therefore have to be used.

2.8.1.2 Immuno diagnostic test for taeniasis

The coproantigen Enzyme linked immunosorbent Assay (copro Ag ELISA) was developed by Allan *et al*. (1990) and serves as a reliable alternative or complementary test to microscopy. The development of the coproantigen ELISA test has immensely improved diagnostic potential and is presently described as the most reliable approach for epidemiological studies/surveillance of taeniasis (Allan and Craig, 2006). Coproantigens have been defined as parasite-specific products that are present in the host faeces and are associated with the metabolism of the adult parasite (Praet *et al*., 2013). These coproantigens are present independent of eggs or other parasitic parts such as proglottids. This makes it more advantageous over conventional microscopy which relies solely on the presence of the parasite eggs. Copro Ag ELISA can therefore detect taeniasis cases even when eggs/ova are absent from truly positive faeces that may have turned out negative by microscopy as a result of intermittent shedding of the parasite eggs. Coproantigens can also be used for early detection because they are produced by both mature and immature worms (Allan *et al*., 1990; Machnicka *et al*., 1996); this is such a significant advancement in taeniasis diagnosis (Allan *et al*., 2003; Praet *et al*., 2013). The copro Ag ELISA can also be used to indicate and monitor treatment success, because the products soon become undetectable shortly after the adult worms have been removed (Praet *et al*., 2013). Different assay formats have been described which affect the sensitivity of the assay. The assay is said to detect as little as 35ng protein/ml of the antigenic extracts of the adult parasite (Praet *et al*., 2013).

The copro Ag ELISA developed by Allan *et al*. (1990) used hyperimmune rabbit IgG (polyclonal antibodies) directed against the parasite. This test is however believed to be genus-specific and not necessarily species-specific (Allan *et al*., 2003; Ito and Craig, 2003). Guezala *et al*. (2009) developed their own coproantigen ELISA by modifying the procedures of Allan *et al*. (2003).

In the modified ELISA test, the researchers made use of a combination of polyclonal hyper immune rabbit IgG directed against *T. solium* crude somatic extract and rabbit IgG raised against *T. solium* adult ES antigens. The adult tapeworm-developed crude somatic extract, also known as whole worm extract was used as the capture anti-body while the rabbit IgG raised against *T. solium* adult ES antigens was enzyme conjugated.

The sensitivity of the copro Ag ELISA is dependent on the assay format used and the quality of the hyper immune rabbit IgG; a higher sensitivity is attributed to a high titre of rabbit serum (Praet *et al*., 2013). At present, rabbit anti-*Taenia*, anti-serum is not commercially available, so antibody titres and avidity vary from laboratory to laboratory (Praet *et al*., 2013).

2.8.1.3 Molecular characterization of Taenia spp.

The discovery of molecular techniques in different fields of science has advanced greatly and this has helped in developing new, reliable and up – to date approaches even in the field of parasitology. Molecular approaches are now used routinely for the accurate identification of many parasites including taeniid tapeworms (McManus, 2006; Campbell *et al*., 2006).

DNA approaches have proved very useful in molecular epidemiological surveys of many parasitic organisms. Identification and systematics of parasites have been immensely impacted by molecular techniques. Molecular approaches have also been employed in

accurate and reliable diagnosis of infections, epidemiology and transmission dynamics of different pathogens, population genetics and also drug resistance and vaccine development studies (Campbell *et al.*, 2006).

Many years ago, Flisser *et al*. (1988) described and demonstrated the use of DNA hybridization in specific detection of *Taenia saginata*. This was a milestone in the use of molecular techniques for detection of taeniid tapeworms.

Mulvey *et al*. (1991) described a study on genetic vaccination in *Fascioloides magna* and *Ascaris* and this was one of the first notable studies on the application of genetic markers in parasitology. Since then a lot of new molecular tools have been developed and much progress has been made in the field of molecular parasitology.

Many copro DNA tests have been developed and even modified in order to overcome limitations of morphological identification of taeniid cestodes. Some of such copro DNA tests include use of DNA probes, which were one of the first molecular approaches used (Rishi and McManus, 1988; Flisser *et al*., 1988; Chapman *et al*., 1995; Gonzalez *et al*., 2000). Polymerase chain reactions (PCR) or PCR coupled to restricted fragment length polymorphism (RFLP) have also been described (Mayta *et al*., 2000; Gonzalez *et al*., 2002; Rodriguez-Hidalgo *et al*., 2002; Yamasaki *et al*., 2002). Other molecular approaches besides PCR and restriction fragment length polymorphism (PCR-RFLP) include, single strand confirmation polymorphism (SSCP) and use of whole genomes as applied in analysis of random amplified polymorphic DNA (RAPD) and microsatellites.

Polymerase Chain Reaction (PCR):

Different formats and types of the PCR assays have been employed in differential diagnosis of taeniasis involving the different human tapeworms. Yamasaki *et al*. (2004) described the use of multiplex PCR for differential diagnosis of taeniasis and cysticercosis. Multiplex PCR with cytochrome C oxidase subunit 1 gene was employed and this yielded evident differential products unique for *T. saginata, T. asiatica* and American/African and Asian genotypes of *T. solium*.

Similarly, Anantaphruti *et al*. (2010) also analyzed stool samples by multiplex PCR; cytochrome C oxidase subunit 1 (cox 1) gene in mitochondrial DNA of both worms and ova in stools were amplified by multiplex PCR and subsequently analyzed.

Praet *et al*. (2013) developed a novel real-time PCR for diagnosis of taeniasis by amplifying the internal transcribed spacer 1 region/gene of the parasites. Eom *et al*. (2011) also used conventional copro PCR format for differential diagnosis of taeniasis caused by *T. solium* and *T. saginata*. Holger *et al*. (2008) described a nested PCR for specific diagnosis of *T. solium* taeniasis.

Restriction Fragment Length Polymorphism (RFLP):

Restriction fragment length polymorphism actually detects genetic polymorphisms in amplified PCR products which have been digested by restriction enzymes with specific recognition sites. Bowles and McManus (1993) and Gasser and Chilton (1995) have successfully used PCR – RFLP of the ITS rDNA to define *Echinococcus spp* and distinguish *Taenia spp* respectively.

Single Strand Conformation Polymorphism (SSCP):

Orita *et al*. (1989) have described the dependence of electrophoretic mobility of single stranded DNA on its size and structure, and this is believed to be the principle behind the SSCP technique. As a result of self-annealing between nucleotides of single stranded molecules, such molecules take up confirmations that vary on the basis of length, location and numbers, therefore serving as a measure of differentiation (Campbell *et al*., 2006). PCR-based SSCP could be used to indicate intra-specific variation among organisms and this has been demonstrated by Gasser *et al*. (1999) to show intra-specific variation of some *Taenia spp* using mitochondrial nad 1 and cox 1 genes.

Random Amplified Polymorphic DNA (RAPD):

The principle of random amplified polymorphic DNA is bordered around the use of short primers, typically 10 bases long, to amplify random anonymous genomic sequences (Williams *et al*., 1990). These primers of arbitrary sequences are available in commercial kits. RAPD analysis more or less detects variation in fragment size but is insensitive to sequence variation, therefore variation is defined by presence or absence of a fragment (Campbell *et al*., 2006). Maravilla *et al*. (2003) and Vega *et al*. (2003) have successfully demonstrated some genetic diversity in *T. solium* using RAPD technique.

Microsatellites:

Uses of microsatellites have been described as a very valuable tool in investigation of population structure and genetics (Campbell *et al*., 2006). Microsatellites are actually defined as "Short randomly repeated DNA motifs" which are widely distributed throughout most of

the eukaryotic genome (Tautz and Renz, 1984). Variation in number could occur at microsatellite loci, which is often hyper variable as a result of high mutation rates.

Hyper variability at microsatellite loci has been described by Nakao *et al*. (2003) in *E. multiculolaris* which is believed to have little genetic diversity as detected by other less polymorphic genetic markers.

2.8.2 Diagnosis of human cysticercosis

Diagnosis of human cysticercosis is quite challenging because the disease is usually asymptomatic (with the exception of seizures manifesting in cases of neurocysticercosis or cysticeri found in the eyes of superficial tissues) (Flisser and Gyorkos, 2007). However, immunodiagnostic techniques and the use of computerized tomography/magnetic resonance imaging has been described (Flisser and Gyorkos, 2007; Coyle and Tanowitz, 2009).

2.8.2.1 Immunodiagnostic assays for human cysticercosis

Over the years, various immunodiagnostic techniques have been employed for seroepidemiological surveys of human cysticercosis. These techniques are either antibody-based or antigen-based. The antibody-based immunodiagnostic techniques include:

Immunoelectrophoresis (IEP):

Immunoelectrophoresis improves the resolution of an immunodiffusion test. It is a type of precipitation reaction used to separate and identify antigens in a mixture by electrophoresis (Bauman, 2006).

This immunodiagnostic technique was first applied in diagnosis of NCC in 1975 (Flisser and Gyorkos, 2007). Cystic fluid, scolex and cystic wall were used as antigenic fractions derived

from cysticerci in the assay. The obtained cysticerci were punctured and the fluid was drained out, centrifuged and used at a concentration of 5mg/ml (Flisser *et al*., 1975). The scolex and cystic wall were homogenized in 3Mkcl, centrifuged and dialyzed in a 15mg/ml-adjusted saline and protein concentration. All processed antigenic fractions were then tested against sera of NCC suspected cases. The results revealed that the proportion of positive sera, particularly with antigenic fractions; scolex and wall increased with likelihood of NCC (Flisser and Gyorkos, 2007). The specificity and sensitivity of the scolex antigenic fraction was reported to be 98 and 62% respectively. Results therefore concluded that positive sera practically established NCC diagnosis, though in only about half of the patients having NCC (Flisser *et al*., 1975).

Western Blot (WB):

Western blot is a very valuable technique used for detecting antibodies against multiple antigens in a complex mixture. WB can detect more antibody types and are subject to less misinterpretation. The procedure basically involves three steps which are; electrophoresis, blotting and ELISA. Antigens in solution are separated by gel electrophoresis, where by each protein present in the antigens is resolved into a single band thereby producing a pattern of different protein bands. By absorption of the solution, the protein band patterns are transferred into an overlying nitrocellulose membrane, which is subsequently cut into strips. The strips are washed and an enzyme-labeled antibody solution is added for some time. Colour changes develop amidst series of washes.

Use of WB for NCC diagnosis was first published in 1986. High species-specific peptides binding to serum antibodies were detected with isoelectric points between pH 4.6 and 3.9 (Gottstein *et al*., 1986). A lentil – lecithin (LL column) was used to prepare a partially

purified crude extract of cysticerci. Serum glycoprotein bands (GP) having different molecular masses were discovered to be specific for human cysticercosis (Tsang *et al*., 1998).

The WB – LLGP was said to have 100% specificity, while sensitivity was influenced by the number of cysticerci located in the brain. A sensitivity of 98% was found with three or more cysticerci, while 65% sensitivity was reported for one or two cysticerci. A recent study by Sato *et al*. (2006) successfully evaluated purified *Taenia solium* glycoproteins and recombinant antigens in serodiagnosis of human and porcine cysticercosis.

Enzyme Linked Immunosorbent Assay (ELISA):

Soon after ELISA was developed as a diagnostic tool in medicine (Voller *et al*., 1976), it was standardized and employed in the detection of NCC-specific antibodies. Antigens obtained from crude extracts (CE) of cysticerci and a partially purified antigen B (AgB) fraction previously obtained from IEP were subjected to ELISA test, and assayed with sera of surgically confirmed NCC cases. Sensitivity of IgG antibody detection was 85 and 80% for both CE and AgB respectively, while specificity was 100% for CE and 96% with AgB (Espinoza *et al.*, 1986). Antigenic – based immunodiagnostic techniques have also been developed for both NCC and taeniasis; the most commonly used technique is the ELISA technique. The copro antigen ELISA techniques for taeniasis has been described previously (2.8.1.2).

ELISA (for NCC):

Antibody-detecting ELISA (Ab- ELISA) tests have proved very useful in mass screening of populations for different diseases. However, just like any other diagnostic test, Ab-ELISA has a short coming of not necessarily indicating a current or active disease. This has led to

the development of antigen-detecting ELISA test, however only a few researches have been published in this area.

The available and most recent studies are monoclonal – based antigen detection ELISA where monoclonal antibodies are used in a capture ELISA. This assay has been used with both sera and cerebrospinal fluid (CSF) of NCC patients (Garcia *et al*., 2002; Fleury *et al*., 2003). Seropositivity of 4% was detected using this assay among 27 NCC patients having hydrocephalus, while there was 0% seropositivity among 29 patients with calcifications only. This indicated the presence of the live parasite in patients with hydrocephalus, secondary to NCC (Garcia *et al*., 2002).

2.8.3 Diagnosis of porcine cysticercosis

Cysticercosis in pigs can be detected by direct visual observation of the cysts or metacestodes in muscles and tissues and also by ELISA methods using blood samples. As previously explained, blood samples of infected pigs can be examined for the presence of antibodies or parasite antigens using ELISA techniques.

2.8.3.1 Diagnosis of T. solium metacestodes

Metacestodes can be detected in both live and dead pigs by tongue inspection and palpation and by post mortem examination or meat inspection.

Tongue palpation is said to be of much diagnostic value only in cases of heavy infection in the pigs. Where infection is mild or low, diagnosis by tongue inspection is difficult and differentiation from large sacrocysts becomes complicated (OIE terrestrial manual, 2008).

Post mortem examination or meat inspection is the main diagnostic method for metacestodes. This is because the parts of the meat can be inspected for the presence of cysts and incisions could also be made to enhance diagnosis.

Initially, metacestodes appear to be very small, typically 1mm, therefore necessitating slicing of the tissues in the laboratory. Theoretically, cysts can be visualized and palpated in the tongues of pigs with heavy infections as early as two weeks after infection. At six weeks, cysts are mature and so readily visible; they appear oval, about 10 x 5mm or larger. They have a translucent and delicate membrane and a host capsule enclosing the pale fluid. The cystic fluid contains a scolex which is visible as a white dot. A large number (85 – 100%) of cysts detected during meat inspection are said to be dead (OIE terrestrial manual, 2008). *Taenia solium* cysts are said to survive for many years in the human brain and humans exhibit symptoms only when there is degeneration of such cysts.

The cysts are preferentially distributed in its host; as such they have predilection sites and are known to die more rapidly in the muscular predilection sites. The appearance of degenerating cysts differs; the fibrous tissue capsule could thicken and change from translucent to opaque. The white fluid gradually becomes colloid, while greenish or yellowish caseous material fills the cyst cavity making it appear very gross. The cyst also increases in size and becomes more visible in the meat than its original viable form (OIE terrestrial manual, 2008).

General meat inspection procedures consist of:

1. Visual inspection of the carcass, its cut surfaces and internal organs.
2. Inspection of predilection sites such as internal and external masseters.

3. Visual examination and palpation of the freed tongue.
4. Examination of the heart cavity. One length-wise incision of the heart through the left ventricle and interventicular septum. Muscles of the diaphragm should be visually examined and incised.
5. The brachii and gracillis muscles of the pigs are incised deeply and examined in African countries because it is believed that more of the parasites lodge in the legs of free ranging pigs.

The initial incision into any tissue is considered the most important; this could determine the need for additional incisions. Once cysts are found in common predilection sites, then further incisions could be made in other suspect tissue (OIE terrestrial manual, 2008).

2.9 Prevention and Control of *T. solium* Infections

The International Task Force for Disease Eradication (ITFDE, 1993) has named *T. solium* cysticercosis as one of only six potentially eradicable diseases. Certain characteristics of the parasite *T. solium* make its eradication a possibility and these include the fact that:

1. The life cycle requires humans as its primary host.
2. The only source of infection in pigs (being the natural intermediate host) is taeniasis in humans.
3. There is no known significant wildlife reservoir.
4. Domestic pigs which are the natural intermediate hosts can be managed.
5. Safe and effective drugs are available for treatment and practical intervention for both human taeniasis and porcine cysticercosis (ITFDE, 1993; Schantz *et al*., 1993).

The major targets or prevention and control of *T. solium* infections are tapeworm carriers and infected pigs. This is because of the important role they play in transmission of the parasite; therefore, effective, sustainable and practical approaches for control should aim at preventing both human infection and environmental contamination with the parasite eggs and the supply of measly/infected meat to consumers. Some of such measures include:

1. Detection and treatment of human tapeworm carriers and mass treatment of populations with praziquantel or niclosamide. This could reduce environmental contamination of the parasites if there is continuous maintenance and effective intensive surveillance (Cruz *et al*., 1989; Garcia *et al*., 2002).
2. Prevention and control of indiscriminate disposal of stool. There should be safe disposal of stool.
3. Education and intervention measures targeted at behavioural change. Education is an integral part of control programmes.
4. Regular assessment of sanitary conditions of pig slaughter premises and meat inspection to prevent circulation of infected pork. Transmission of *T. solium* infections could be blocked by confiscation of infected pig carcasses (Joshi *et al*., 2003) by the right governing bodies in charge of meat inspection. This is also important in instances of home pig slaughter for commercial purposes; therefore meat at both public and private slaughter slabs going out into the populace should be inspected.
5. Upscale of general hygiene is important for control of *T. solium* infections.

2.10 Treatment of *T. solium* Infections

Human tapeworm infections could be treated with niclosamide or praziquantel. Except for NCC, cysticercosis occurring as a result of cysts lodging in other human organs is considered benign and does not necessarily require any specific treatment (Garcia *et al*., 2003). Antiparasitic drugs are believed to be effective against cysticerci lodged in the human brain, but at the cost of complications such as acute inflammation (Garcia *et al*., 2003). However, patients with giant cysts are said to benefit from such antiparasitic treatment or they stand a chance of dying as a result of progression of the disease (Del Brutto *et al.*, 1992; Proano *et al*., 2001). Albendazole or praziquantel can be used to treat human cysticercosis cases. Albendazole treatment (15mg/kg daily) with simultaneous administration of steroids or 15 days of praziquantel (50mg/kg daily) have proven effective (Garcia *et al*., 2003). Pretell *et al*. (2001) have postulated shorter regimens of praziquantel for 1 day or 3 days of albendazole for patients with only one lesion.

For treatment of porcine cysticercosis, benzimidazole, oxfendazole is said to be very effective (more than 95%) in killing the cysts with a single dose of 30mg/kg (Garcia *et al*., 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

A community-based descriptive cross-sectional survey was conducted in Kaduna metropolis, Nigeria, to determine the prevalence of *Taenia solium* infections. Kaduna metropolis is characterized by a high population density of 1,570,331 with household population of 314,066 (NPC, 1998; FRN, 2009). Kaduna metropolis is divided into Kaduna North, Kaduna South, Igabi and Chikun Local Government Areas. This study however was carried out in Kaduna South and Chikun Local Government Areas of Kaduna Metropolis known to be involved in small holder pig farming and pork consumption. A pilot survey in the area revealed that residents were very much involved in small holder/ back yard pig farming and there was lack of regular use of veterinary services by free-range pig farmers. Some of the residents were also involved in home slaughter of pigs for sale within the communities, since due to religious beliefs, there were no pig abattoirs. A lot of commercial outlets for pork consumption were found in these communities. In addition to pigs, other animals such as cattle, poultry and goats were also reared and the people practise subsistence farming. The study area was divided into four clusters for proper sampling: Sabon-Tasha and Television village, Ungwan-Romi and Gonin-Gora, Baranawa and Narayi, Nassarawa and Kakuri.

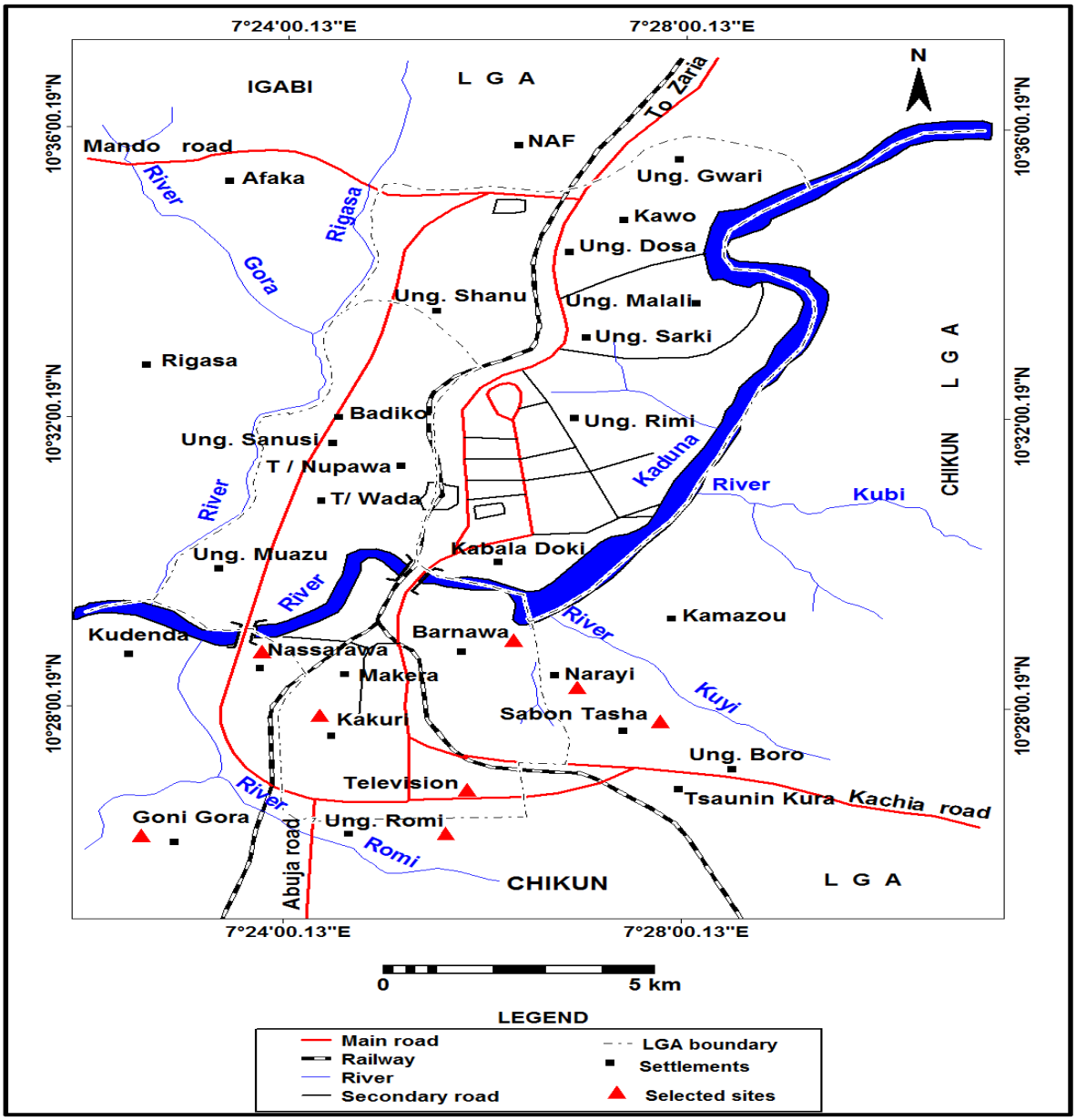

Fig. 3 1: Kaduna metropolis showing selected sampling sites

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3.2 Study Design

Simple random sampling approach was employed in the selection of communities within the study population and based on inclusion criteria and willingness; participants were recruited for the study.

3.2.1 Inclusion criteria

Residents of parts of Kaduna South and Chikun Local government Areas in the metropolis who ate pork and/or were in close contact with people who atr or reared pigs.

3.2.2 Exclusion criteria

Residents of Kaduna metropolis who did not eat pork and were not in close contact with people who ate or reared pigs.

3.3 Ethical Approval

The study was approved by the Kaduna State Ministry of Health, Nigeria (MOH/ADM/744/VOL.1) and consent was sought from the local district authorities in the area of survey. Prior to sample collection, a forum was organized within the selected communities in the study area to explain the purpose of the study and get the attention of willing participants. Participants were recruited for the survey based on their free will to participate in the study. Written consent was then sought from the willing participants and minors involved in the study had written informed consent of either their parents or guardians.

3.4 Sample Size

The sample size for the aspect of the prevalence study was calculated using the following equation (Araoye, 2004):

$$N = \frac{z^2 pq}{L^2}$$

$$N = \frac{(1.96)^2 \text{ x } 0.5 \text{ x } 0.5}{(0.05)^2} = 384$$

Where N = sample size

z = score for a given confidence interval usually set at 1.96 for 95% C.I.

p = prevalence value of 50% (0.5) was used (Araoye, 2004)

q = (1-p) = 1- 0.5 = 0.5

L = permissible error of estimation which is taken as 0.05 (5%)

A total of 750 samples made up of 300 blood & 450 faecal samples were obtained from human subjects.

3.4.1 Determination of sample size for pig slaughter slab study

A convenient, purposive sampling technique was used for this aspect of the survey at the selected slaughter slabs, thereby arriving at the following samples collected:

1. Ninety (90) environmental samples comprising; 25 Water samples, 50 soil samples and 15 drainage samples collected from slaughter slab premises.

2. Pig carcasses for meat inspection: 240 meat parts were obtained comprising of tongues, livers, lungs, hearts, masseters, thigh and shoulder muscles from a total of 43 pig carcasses slaughtered at premises.

3.5 Questionnaire Design and Administration

Structured, self-administered questionnaires were designed and used to acquire relevant information from the subjects on clinical history, socio demography, environmental and risk factors associated with human cysticercosis/taeniasis in the study area (Appendix I). Information obtained included the following: pig husbandry practises, sanitation practises, household facilities, associated cysticercosis symptoms, recent antiparasitic treatment, slaughter house facilities and sanitary conditions, slaughtering and handling processes, available veterinary services, and pig sources, among others. A total of 750 questionnaires were administered to the respondents and collected after appropriate answers were provided.

3.6 Collection of Samples

Three hundred human blood (300) and four hundred and fifty (450) human faecal samples were obtained from human subjects who had been administered questionnaires. The samples were labelled to tally with that recorded on the questionnaire.

3.6.1 Blood samples

Ten (10) ml of venous blood was obtained intravenously from each individual into sterile vacutainer tubes and transported to the Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna, Nigeria, in cool box containing ice packs. The blood samples were allowed to clot standing at 4ºC in a refrigerator. The sera were obtained by centrifuging at

3200 × g for 5mins and then aliquoted into 1.8ml cryovials and stored at -20ºC until analysed for cysticercal antibodies using IgG ELISA technique.

3.6.2 Faecal samples

Each respondent was given a clean, wide screw capped container and instructed on how to pass the stool sample into the container. All specimen containers were appropriately labeled before dispatching. Upon collection, faecal specimens were then transported on ice packs to the laboratory in the Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna, Nigeria, where the preliminary analysis was carried out. The faecal samples were aliquoted into two parts using stool tubes. The first part was preserved with 10% formalin and kept at room temperature (25ºC) for coproscopic examination and coproantigen enzyme linked immunosorbent assay (Copro Ag-ELISA), while the second part was preserved in 95% ethanol and stored at 4ºC in a refrigerator for further molecular studies.

3.7 Laboratory Analysis of Blood and Faecal Samples

3.7.1 Detection of IgG antibodies in sera using indirect ELISA technique

The IgG antibodies to cysticercosis were detected in sera using cysticercosis microwell ELISA kit (Diagnostic Automation INC, California). Frozen sera were allowed to thaw and 1:64 dilution of the sera was made using the dilution buffer provided in the ELISA kit. The wells used were broken off and placed in a strip holder and then appropriately labeled. One hundred (100µl) of the pre-diluted negative and positive control each was added to wells labeled as 1 and 2 respectively. This was followed by addition of one hundred microliter (100µl) of each of the diluted test sera into the remaining appropriately labeled wells. The wells were incubated at room temperature of 20°C for 10 minutes. The contents of the wells

were shaken and washed out three times with the diluted wash buffer after incubation and then blotted dry by slapping against paper towels to remove excess moisture. Two drops of the enzyme conjugate made up of Protein A conjugated to peroxidase were then added into each well and incubated for the second time at room temperature (25ºC) for 5 minutes. The contents of the well were again shaken and washed with wash buffer. Excess moisture was removed by slapping against paper towels. Two drops of the chromogen tetramethylbenzidine (TMB) substrate solution was added to every well and incubated at room temperature (25º) for 5 minutes. Two drops of the stop solution, 0.7 M phosphoric acid was added to the wells and mixed by tapping the strip holder. Results were read using an ELISA reader. All wells were read at 450/650 - 620nm. Absorbance reading greater than 0.3 OD (optical density) units was considered as a positive result, while absorbance reading less than 0.3 OD units was considered a negative result based on the cut off provided by the kit manufacturers (Diagnostic Automation INC, California).

3.7.2 Coprology of faecal specimens

The formol ether concentration technique was used following methods of Cheesebrough (2001). Two (2g) of faeces was thoroughly emulsified with 7mls of 10% formol saline and strained through gauze in a small plastic funnel into a test tube. Three (3mls) of ether was added and the mixture shaken vigorously for one minute. The mixture was then centrifuged at a speed of 4000 × g for 3 minutes. The debris on the surface and at the interface between the ether and formalin was loosened from the wall of the tube with an applicator stick, with the last drop allowed to run back. The upper part of the tube was wiped clean of fatty debris. The small deposit was shaken up and poured unto a slide using a pasteur pipette. This was

viewed under the microscope for taeniid ova and other parasite cysts and ova present as well (Cheesebrough, 2001).

3.7.3 Copro-antigen detection in faecal specimens by ELISA technique

An in-house coproantigen detection ELISA (copro Ag-ELISA) was performed on the stool samples as described by Mwape *et al*. (2012). The stool samples stored in 10% formalin were processed by mixing together equal amounts of Phosphate Buffered Saline (PBS) and stool sample. This was allowed to soak for one hour amidst intermittent shaking and centrifuged at 2000 × g for 30 minutes. The supernatant was then used in the ELISA.

Polystyrene ELISA plates (NuncH Maxisorp) were coated with the capturing hyper-immune rabbit anti-*Taenia* IgG polyclonal antibody diluted at 2.5 mg/ml in carbonate-bicarbonate buffer (0.06 M, pH 9.6). After coating, the plates were incubated for 1h at 37ºC, washed once with PBS in 0.05% Tween 20 (PBS-T20) and all wells were blocked by adding blocking buffer (PBS-T20+ 2% New Born Calf Serum). After incubating at 37ºC for 1h and without washing, 100 ml of the stool supernatant was added and plates were incubated for 1h at 37ºC followed by washing five times with PBS-T20. A biotinylated hyper-immune rabbit IgG polyclonal antibody diluted at 2.5 mg/ml in blocking buffer was used as the detector antibody. One hundred microliters (100µl) were added and the plates were incubated for 1h at 37ºC and then washed 5 times. One hundred micolitres (100µl) of streptavidin-horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10,000 in blocking buffer were added as conjugate. It was incubated at 37ºC for 1h and washed 5 times, then 100 µl of ortho phenylenediamine (OPD) substrate (prepared by dissolving one tablet in 6 ml of distilled water and adding 2.5 ml of hydrogen peroxide) was added. The plates were incubated in the dark for 15 minutes at room temperature and the reaction was stopped by adding 50 µl of

sulphuric acid (4 N) to each well. The plates were read using an automated spectrophotometer at 490 nm with a reference of 655 nm. To determine the test result, the optical density (OD) of each stool sample was compared with the mean of a series of 8 reference *Taenia* negative stool samples plus 3 standard deviations (cutoff).

3.8 Assessment of Sanitary Conditions of Pig Slaughter Slabs

Two privately owned, slaughter slabs located within the communities at homesteads in Nassarawa and Gonin-Gora were visited twice a week for a period of six months between January and July, 2013 at slaughtering times, in order to carry out post-mortem inspection on pig carcasses and assess the environmental and sanitary conditions of the slaughter slab premises.

3.8.1. Environmental inspection

The environmental inspection was carried out as described in previous studies by Diaz *et al.* (1992) and Gweba *et al.* (2010); the slaughter premises were inspected for the presence of necessary facilities such as structure/ type of pig abattoir, location of the slaughter slab, type of slaughter slab, exposure of the slaughter premises, presence of cracks on walls and floors of premises, average number of pigs slaughtered per day, method of slaughtering, pig sources and availability of veterinary services, water source, toilet/drainage facilities available and availability of routine meat inspection. Water, soil and open drainage samples were collected within the slaughter slab environment for laboratory analysis. Twenty five water samples were collected from main sources supplying the slaughter site and from water stored in containers for domestic purposes since the slabs were at the backyard within the homesteads. One litre (1L) of water was collected into sterile bottles and appropriately labeled. Fifty soil

samples were collected strategically, at least two meters apart (Gweba *et al*., 2010), within the slaughter slab environments and from nearby refuse dumpsites where free range pigs were seen scavenging and where humans sometimes defaecated. Fifteen samples from open drainages and spent slurry within the slaughter premises were also collected for laboratory analysis. All environmental samples collected were placed in an ice box and transported to the laboratory where they were examined microscopically for the presence of taeniid ova. The soil, water and drainage samples were analysed using centrifugation and sedimentation techniques and taeniid ova were identified based on microscopic characteristics (Gweba *et al*., 2010). Butchers and individuals handling pork at the slaughter sites were observed and then interviewed verbally and with a questionnaire form in order to ascertain their knowledge, attitude and behavioural practices in relation to their awareness of mechanisms of transmission, prevention and public health significance of cysticercosis and taeniasis. Faecal samples of butchers were collected and assayed for *T. solium* coproantigens as reported in section 3.7.3.

3.8.2. Meat inspection

Meat inspection was conducted following standard procedures (Faleke and Ogundipe, 2003; Kyvsgaard and Murrell, 2005). Meat inspection basically involved post-mortem inspection of the tongues by palpation and dissection of predilection sites of the metacestodes of *T. solium*. A total of 43 pig carcasses were obtained during the sampling period from the two slaughter slabs, and cysticerci predilection sites were collected for critical examination in the laboratory. A total of two hundred and forty samples obtained from predilection sites in pork comprising 43 tongues, 33 masseter muscles, 25 thigh muscles, 10 neck/shoulder muscles, 43 livers, 43 lungs and 43 hearts were obtained after evisceration of the pigs. Tongues were

palpated and observed for the presence of cysticerci on their entire ventral surface; the entire surfaces of meat samples collected were observed for the presence of cysticerci and a dissecting set was further used to make incisions on apparently safe meat surfaces and muscles to further check for cysticerci embedded in the muscles. Live cysts were identified as fluid-filled, translucent with invaginated visible scolices (Onah and Chiejina 1995, Kyvsgaard and Murrell, 2005). Meat inspection procedures (Kyvsgaard and Murrell, 2005; OIE terrestrial manual, 2008) consisted of:

i. Visual inspection of the carcass, its cut surfaces and the organs within it.

ii. The external and internal masseters muscles were examined and two incisions made into each, the cuts being parallel to the bone and right through the muscle.

iii. The freed tongue was examined visually and palpated for *T. solium* cysts

iv. The pericardium and heart were examined visually; externally and then internally after cutting through the inter ventricular septum. The heart was incised once; lengthwise through the left ventricle and inter ventricular septum, exposing the interior and cut surfaces for examination. Incisions were made from the base to the apex and deeper incisions were made into the left ventricle. Meat samples with no observable cysticerci were recorded as negative samples, while positive meat samples which had the cysts were counted and carefully removed into a sterile container and kept frozen at -20˚C until required.

3.9 Molecular Studies of *Taenia* DNA in Faecal Samples

3.9.1 Extraction of *Taenia* DNA directly from faeces

Faecal specimens which tested positive to both coprology and coproantigen ELISA were further subjected to molecular studies. *Taenia* DNA was extracted directly from ethanol-stored faecal specimens with the use of QIAamp stool mini kit (Qiagen, Hilden, Germany), with slight modification. Two hundred milli grammes (200mg) of each solid stool sample was weighed into separate 1.5ml microcentrifuge tubes and placed on ice for 10mins. Two hundred microliters (200µl) of watery stool samples were used where samples were in liquid state instead of solid. One thousand microliters (1000µl) stool lysis buffer (ASL) was added to each stool sample on ice, and thoroughly vortexed. This was allowed to stand at room temperature for fifteen to thirty minutes. Bashing beads (S6003-50, Zymo Research) (not provided with the extraction kit) were added to the mixture and vortexed until thoroughly and properly homogenized. The suspension was then heated in a heating block for 10mins at 100˚C. The heated suspension was vortexed for a minute and then centrifuged at 14,000 rpm for 5mins. One inhibitex tablet was added to each sample suspension and immediately vortexed continuously until the tablet was completely suspended or dissolved. The suspension was incubated for 5mins at room temperature to allow inhibitors adsorb to the inhibitex. The samples were centrifuged at a speed of 14,000rpm for 10mins to pellet inhibitors bound to inhibit matrix. The entire resultant supernatant was pipetted into a new 1.5ml micro centrifuge tube and the pellet discarded. The samples were then centrifuged at a speed of 14,000rpm for 3mins. Thirty microliters of (30µl) of proteinase K was pipetted into a new appropriately labeled 1.5ml micro centrifuge tube. Four hundred microlitres (400µl) of each of the previously centrifuged supernatant was dispensed into the tubes containing

proteinase K. Four hundred microliters (400µl) of buffer AL (lysis buffer provided in the extraction kit) was then added to each of the tubes containing proteinase K and supernatant. The resultant mixture was thoroughly vortexed to mix and then briefly spinned in a centrifuge to collect drops from the sides of the tubes. The mixture was incubated at 100˚C for 1h. Four hundred microliters (400µl) of absolute ethanol was then added and mixed properly by vortexing. The suspension was again briefly centrifuged to remove drops from the insides of the tubes. The samples were then carefully transferred to appropriately labeled spin columns provided in the extraction kit. The samples were centrifuged in the spin columns at 14,000rpm for 2mins. The columns were removed and placed in new 2ml collection tubes while flow through was discarded alongside the old collection tubes. The spin columns were carefully opened and 700µl of wash buffer (AW1) was added and the cap closed and centrifuged at 14,000rpm for 2mins. The QIAamp spin columns were placed in new 2ml collection tubes and the flow through discarded again. The QIAamp spin columns were carefully opened and 700µl of a second wash buffer (AW2) was added. The caps were closed and contents centrifuged at 14,000rpm for 3mins. The collection tubes containing filtrate were discarded as QIAamp spin columns were placed in new 2ml collection tubes and centrifuged with empty tubes at 14,000rpm for 2mins. The QIAamp spin columns were transferred into newly labeled 1.5ml microcentrifuge tubes and 50µl elution buffer (AE) was added directly to the QIAamp membrane. The caps were closed and the QIAamp spin columns were incubated at room temperature (23ºC) for 2mins. The columns were subsequently centrifuged at 14,000rpm for 2mins to elute DNA. Thirty microliters (30µl) of AE was added again directly unto the surface of the same QIAamp membrane in the same microcentrifuge tube and incubated again at room temperature (23ºC) for 2mins. The columns were then centrifuged at 14,000rpm for 2mins to elute more DNA. The spin columns

were discarded and the flow through in the 1.5ml microcentrifuge tubes were appropriately labeled as extracted DNA. All extracted DNA were frozen at -21˚C until needed for downstream polymerase chain reaction assays.

3.9.2 Polymerase chain reaction (PCR)

3.9.2.1 *Copro PCR for amplification of T. solium DNA isolated from faeces*

Copro PCR for specific detection of *T. solium* involved the use of *T. solium* specific primers targeting both the mitochondrial DNA (mtDNA) and nuclear DNA, which were cytochrome oxidase c (*Cox1*) sub unit 1 and internal transcribed spacer I (*ITSI*) region respectively. The *T. solium*-specific primers TsolCox1F1 and TsolCoxR1 were synthesized (Inqaba, South Africa, GenBank accession number, AB086256) and used such that a fragment inside the *Cox 1* sequence was amplified as described by Eom *et al.* (2011). The *T. solium*-specific primers, TsolITS_145F and TsolITS_230R (Inqaba, South Africa, GenBank accession number, EU747662) were chosen such that a 100bp fragment inside the *ITS1* sequence was amplified as described by Praet *et al.* (2013). A cocktail was then prepared (while working on ice in an ice box) for a 25µl reaction volume and composed of the following:

1. PCR master mix (Top Taq master mix, Qiagen, Helden Germany for *Cox 1* primers, and PCR master mix, Thermo research, for *ITS1* primers).
2. 0.2µg/µl of bovine serum albumin.
3. 1µM of each forward and reverse primer set.
4. Nuclease free water (Qiagen, Helden Germany for *Cox1* primers and Thermoresearch for *ITS1* primers).

5. 5.0µl of extracted DNA.

Twenty five microlitres (25µl) of the cocktail was dispensed into different PCR tubes, briefly centrifuged to let down all drops and then placed into a thermocycler (Applied Biosystems 9902,USA) where conditions were adjusted to bring about amplification of DNA with each specific primer set used. Following series of optimization and gradient PCR by using varying annealing temperatures at same time, the PCR working conditions with each primer set was arrived at and used as follows:

1. PCR conditions for DNA amplification with *Cox 1* primers

   The PCR working conditions consisted of initial denaturation at 95˚C for 5mins, followed by 35 amplification cycles consisting of denaturation at 94˚C for 30s, annealing at 55˚C for 30s, extension at 72˚C for 90s and final extension at 72˚C for 5min.

2. PCR conditions for DNA amplification with *ITS1* primers

   The PCR working conditions consisted of initial denaturation at 95˚C for 15mins, followed by 50 amplification cycles consisting of denaturation at 95˚C for 15s, annealing at 60˚C for 30s, extension at 72˚C for 1min and final extension at 72˚C for 7mins.

3.9.2.2 *Tsol 31 Nested PCR for specific diagnosis of T. solium-taeniasis*

Two sets of *T. solium*-specific primers designed based on the gene sequence that encodes the *T. solium* oncosphere- specific protein Tso31 (Gen Bank accession no. DQ861410) were used in a nested format to amplify the target DNA (Holger *et al*., 2008). The first pair of primers Tsol31 F1/R1 were predicted to amplify a 691bp segment of the oncosphere gene, while the

second primer pair Tsol31 F589/R294 were predicted to amplify a 234bp fragment. A cocktail was prepared for the outer and nested rounds as follows:

1. Outer PCR cocktail preparation and amplification

Outer PCR cocktail was prepared (while working on ice in an ice box) in a 25µl reaction volume and consisted of a mixture of the following:

1. PCR master mix (Top Taq master mix, Qiagen, Helden Germany).

2. 0.2µg/µl of bovine serum albumin.

3. 1µM of each forward and reverse primer set.

4. Nuclease free water (Qiagen, Helden Germany).

5. 2.0µl of extracted DNA.

Twenty five microliters (25µl) of the outer PCR cocktail was dispensed into different appropriately labeled PCR tubes, briefly centrifuged to let down all drops and then placed into a thermocycler (Applied Biosystems, 9902, USA) for amplification. Amplification consisted of initial denaturation step at 95˚C for 3min and 25 cycles each consisting of denaturation at 95˚C for 30s, annealing at 55˚C for 30s and extension at 72˚C for 1min and final extension at 72˚C for 5mins. Amplified products were characterized by agarose gel electrophoresis.

2. Nested PCR cocktail preparation

Nested PCR cocktail was prepared (while working on ice in an ice box) in a 25µl reaction volume and consisted of a mixture of the following:

1. PCR master mix (Top Taq master mix, Qiagen, Helden Germany).

2. 0.2µg/µl of bovine serum albumin.

3. 1µM of each forward and reverse primer set.

4. Nuclease free water (Qiagen, Helden Germany).

5. 1.0µl of outer PCR product as DNA template.

Twenty five microliters (25µl) of the outer PCR cocktail was dispensed into different appropriately labeled PCR tubes, briefly centrifuged to let down all drops and then placed into a thermocycler (Applied Biosystems, 9902, USA) for amplification. Amplification consisted of initial denaturation step at 95˚C for 3min and 40 amplification cycles each consisting of denaturation at 95˚C for 30s, annealing at 60˚C for 30s and extension at 72˚C for 1min and final extension at 72˚C for 5mins. Amplified products were held at 4˚C until characterized by agarose gel electrophoresis.

3.10 Characterization of Amplified DNA by Agarose Gel Electrophoresis

Amplified PCR products were characterized by electrophoresis using 1% agarose gel. One gram (1g) of agarose gel (Lonza, White scientific) was dissolved in 100ml of 10% TBE and stained with ethidium bromide (10ug/ml) for 10mins, then emptied into a gel casting apparatus with a comb affixed. The gel was allowed to set on cooling for about 20mins. After setting of the gel, the comb was carefully removed from the set gel which was then submerged in running buffer (1% TBE) contained in a buffer tank. Ten (10µl) of each PCR product was mixed with loading buffer, loaded into the wells on the gel and run at 100V for 60mins against 50bp (Thermo research) and 100bp markers(Lonza, White scientific). Stained

gels were then visualized and photographed under UV light of a transilluminator (Bio Rad Image lab) and then documented.

3.11 DNA Sequencing

Deoxyribonucleic acid sequencing basically involved recovery or extraction of amplified DNA from the PCR product using a Zymoclean^{TM} gel DNA recovery kit according to the manufacturer's instructions (Zymo research). A second PCR which is also referred to as big dye PCR was run, using a Big Dye Terminator sequencing kit and an automated sequencer (Applied Biosysytems Co., Grand Island, New York, USA). Resultant clean sequences indicated by the clarity of the nucleotide peaks in the electrophoregram were subsequently used for phylogenetic analysis.

3.11.1 DNA recovery from gels using a Zymoclean^{TM} gel DNA recovery kit

The amplified DNA fragment required was excised directly from the agarose gel using a clean scalpel and transferred into a 1.5ml micro centrifuge tube. Thirty (30µl) of agarose dissolving buffer (ADB) was added to 100mg of the agarose gel slice. The mixture was incubated at 55˚C for 15mins until the gel slice was completely dissolved. The melted agarose solution was then transferred to a zymo- spin column in a collection tube. The mixture was centrifuged for 60s at 14,000× g and the flow through discarded. Two hundred (200µl) of DNA wash buffer was added to the column and centrifuged at a speed of 14,000× g for 30s. The flow through was discarded and the wash step repeated. Six (6µl) of DNA elution buffer was added directly to the column matrix. The column was placed in a 1.5ml tube and centrifuged for 60s at 14,000× g to elute DNA.

3.11.2 Big dye terminator sequencing

The primer walking method was employed to obtain direct sequences for each of the amplified fragments. Cyclic sequencing from both ends was performed using a big dye terminator sequencing kit (Applied Biosystems Co., New York, USA). The reaction products were electrophoresed on an automated DNA sequencer (Model 377, Applied Biosystems).

3.12 Phylogenetic Analysis of Sequenced DNA

The resultant reaction products (Clean DNA sequences) were edited, aligned and trimmed using the Bioedit programme (version 5.0.6, Biosoft, Missouri, USA) and Clustal W computer programme (Thompson *et al*., 1994) respectively. Using the BLAST searches, the sequences obtained were identified by comparing them with those of *Taenia solium* (Cameroon West) deposited in the GenBank database, with accession number FN995666.1.

Using the DNAsp programme (Deoxyribonucleic acid sequence polymorphism, version 5.10) and PAUP (phylogenetic analysis using parsimony and other methods, version 5.0) phylogenetic analyses was done using Neighbour joining, Maximum likelihood and Bayesian methods. Neighbour joining, Maximum likelihood and Bayesian trees were all constructed to show the clades of *T. solium* isolated from Kaduna metropolis, Nigeria and similarities or differences in the topology of the different trees. The evolutionary distances were computed by Kimura's two parameter method. The trees were evaluated using the bootstrap test based on 1000 resamplings. Phylogenetic trees were out group-rooted using nucleotide sequences from *Echinococcus granulosus*.

3.13 Statistical Analysis

All collected data were entered into Microsoft Excel 2011 and analysed by Statistical Package for Social Scientists (SPSS), 2013 version. Bivariate analysis was then performed by Chi-Square test to check for the marginal association between *T. solium* infections and factors/variables in the study population.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of *T. solium* Infections and Associated Socio demographic/Risk Factors in Kaduna Metropolis

The overall prevalence of human cysticercosis and *T. solium*-taeniasis in Kaduna metropolis, Nigeria is presented in Figure 4.1. Out of 300 human sera examined for IgG antibodies to cysticercosis, 43 samples tested positive giving a cysticercosis prevalence of 14.3%, while 40 of 450 faecal samples tested positive by copro Ag- ELISA giving a *T. solium*-taeniasis prevalence of 8.8%.

The prevalence of human cysticercosis and *T. solium*-taeniasis across the study locations within Kaduna metropolis is presented in Figure 4.2. The Nassarawa/Kakuri cluster recorded the highest prevalence of 23.3% and 13.6% for both human cysticercosis and *T. solium*-taeniasis respectively. The Barnawa/Narayi cluster recorded the least prevalence of 8% and 4% for human cysticercosis and *T. solium*-taeniasis respectively.

The prevalence of taeniasis detected by coproscopic examination and copro Ag-ELISA tests using human stool samples is presented in Table 4.1. Six (1.3%) out of 450 faecal samples presented with *Taenia* ova by coproscopic examination, while 40 (8.8%) tested positive by copro Ag-ELISA test. Plate I shows the microscopic view of an embryonated *Taenia* ovum in a stool specimen.

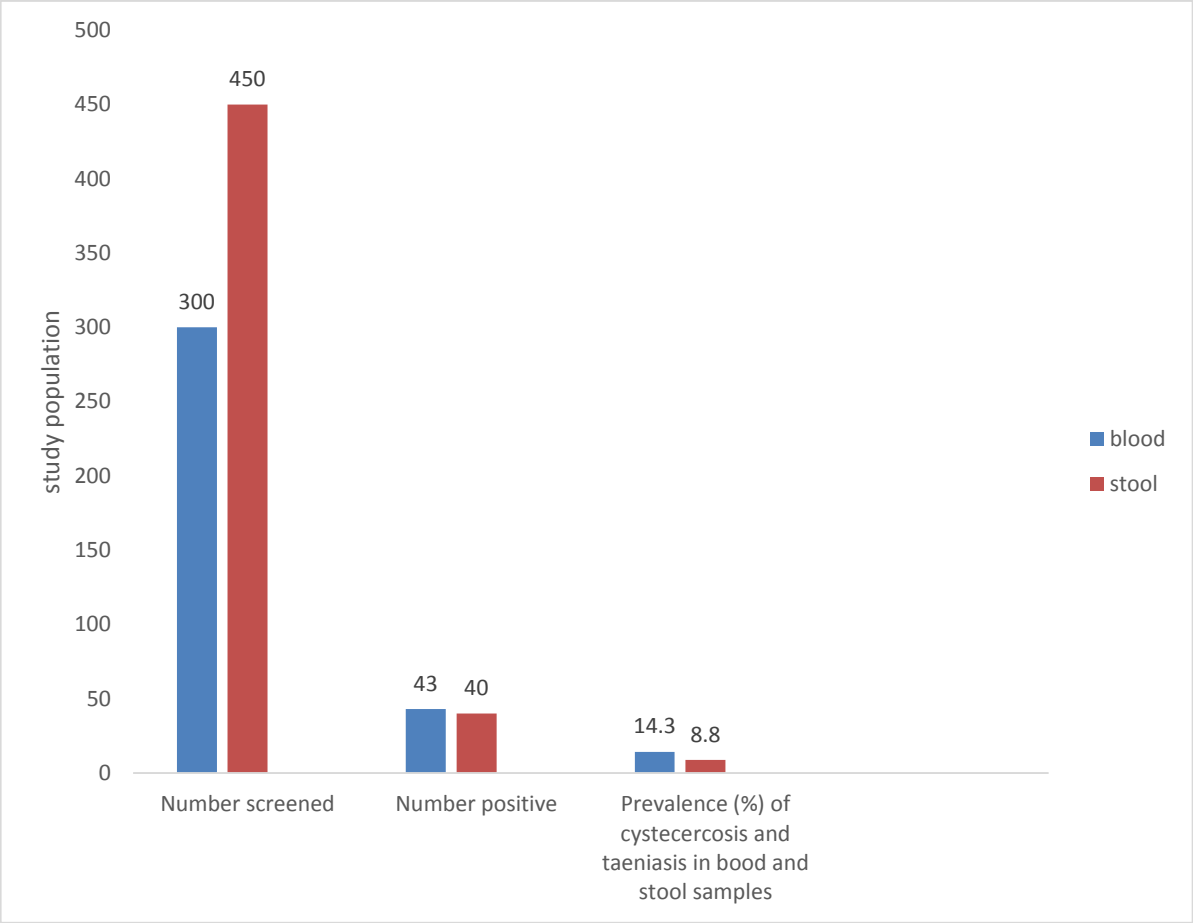


Fig. 4.1 Prevalence of human cysticercosis and *Taenia solium*-taeniasis in Kaduna metropolis, Nigeria.

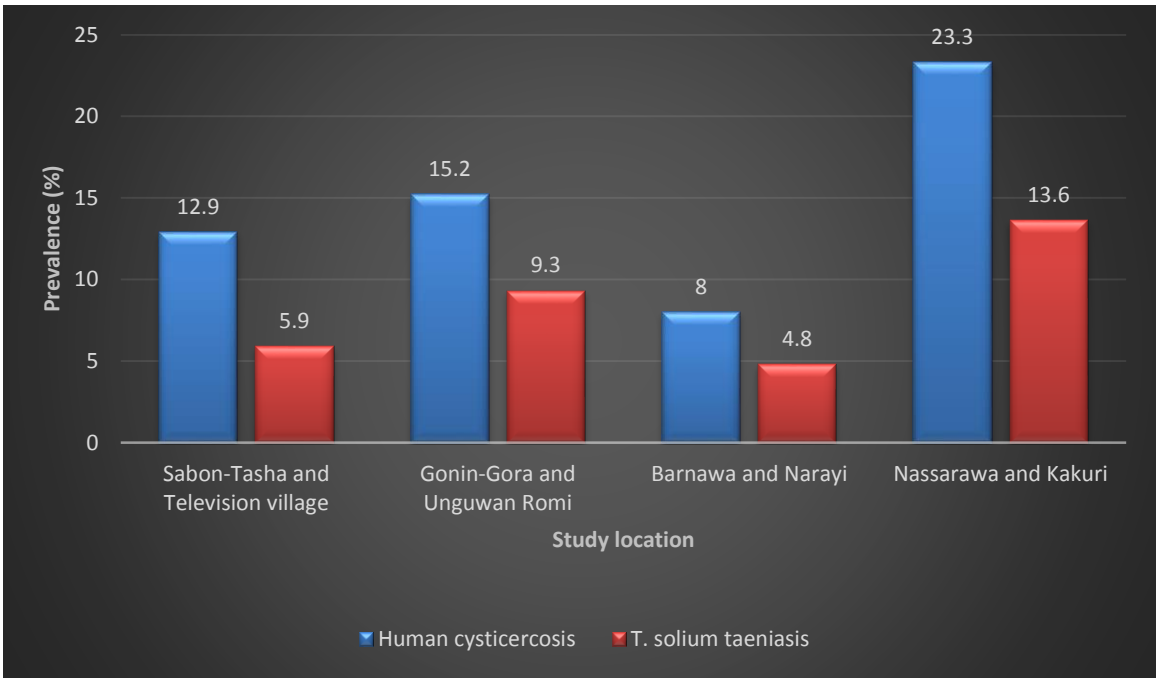


Figure 4.2. Prevalence of human cysticercosis and *T. solium*- taeniasis in relation to study locations in Kaduna metropolis.

Table 4.1. Prevalence of *T. solium* Detected by Coproscopic Examination and Copro Ag- ELISA

Diagnostic test	No. positive	Prevalence (%)
Coproscopic examination	6	1.3
Copro Ag-ELISA	40*	8.8

* Inclusive of all six samples which tested positive by coproscopic examination

Plate I: Microscopic view of *Taenia* ovum in human stool sample
Magnification: ×40

The seroprevalence of human cysticercosis in relation to some socio demographic factors, knowledge of cysticercosis and pork cooking methods among respondents in the study population is presented in Table 4.2. The seroprevalence of cysticercosis was slightly higher in female than male. The highest seroprevalence with respect to occupation was recorded among butchers and farmers. The least seroprevalence of cysticercosis (4.65%) was observed amongst those who boiled and then fried their pork before consumption, while the highest seroprevalence was observed among respondents who preferred their pork either roasted or barbecued.

Table 4.3 presents the bivariate analysis, indicating significant association between some variables in the study population. There was significant association ($p < 0.05$) between method of pork preparation and cysticercosis, method of pork preparation and knowledge of cysticercosis among the respondents, and also between family history of epileptic seizures and cysticercosis. The odds ratio for history of epilepsy and cysticercosis seropositivity was 2.2.

Table 4.4 shows the prevalence of *T. solium*-taeniasis in relation to some socio demographic factors among respondents and their knowledge about taeniasis. Prevalence was higher among male than female. The prevalence also varied among the age groups, with the highest prevalence (11.1%) observed among adults 41 years and above, while the least prevalence (8.2%) was observed among age group 20 years and below. With regards to occupation, the highest taeniasis prevalence (12.4%) was observed among civil servants and business men, this was followed by 8.4% among farmers and butchers and 6.7% among students.

Table 4.2. Seroprevalence of Human Cysticercosis in Relation to Sociodemographic Factors, Cysticercosis Knowledge and Pork Cooking Methods of Respondents in Kaduna Metropolis, Nigeria

Variables	No. Examined	No. Positive	Prevalence (%)
Sex			
Male	164	22	13.40
Female	136	21	15.40
Age			
11-30	130	17	13.07
31-50	141	18	12.77
51 & above	29	13	44.82
Occupation			
Business men	73	11	15.07
Civil servants	83	9	10.83
Farmers & butchers	73	14	19.18
Students	60	9	15.00
Knowledge of cysticercosis			
Good	54	4	7.41
Fair	24	5	20.83
Poor	222	34	15.31
Preferred Method of pork preparation			
Boiled only	194	26	13.40
Boiled & fried	43	2	4.65
Roasted/barbecued	63	15	23.80

Table 4.3.Bivariate Analysis Showing Associations between Variables in the Study Population

Variables	Seropositivity of cysticercosis	Knowledge of cysticercosis
Age	0.235	ND
Sex	0.618	0.943
Method of pork preparation	0.044*	0.007*
Pork preparation at home or purchase from vendors	0.245	0.460
Regular hand washing habit after toilet use	0.191	0.746
Regular self-deworming	0.718	0.443
Family history of epilepsy	0.048* [OR: 2.2; CI: 1.02-3.58]	0.529

Key: Values within the table represents probability values using Chi square
CI: Confidence Interval
*Significant association between the specified variable, 95%CI, $p<0.05$
OR: Odds Ratio, ND-not determined

Table 4.4. Prevalence of *T. solium*-Taeniasis in Relation to Sociodemographic Factors and Knowledge of Taeniasis among Respondents

Parameter	No. Examined (n= 450)	No. positive	Prevalence (%)
Sex:			
Male	270	28	10.4
Female	180	12	6.7
Age (years):			
20 & below	171	14	8.2
21-40	216	19	8.8
41 & above	63	7	11.1
Occupation:			
Farmers & butchers	119	10	8.4
Civil servants & Business men	137	17	12.4
Students	194	13	6.7
Knowledge of taeniasis:			
Good	82	14	17.1
Poor	368	26	7.1

Taeniasis prevalence in relation to eating habits and hygienic practices of respondents is presented in Table 4.5. In relation to preferred method of cooking or preparing pork, the highest taeniasis prevalence was observed among respondents who barbecued their pork (15.8%) before consumption, this was followed by a prevalence of 8.6% representing those who preferred their pork boiled. The least prevalence (1.3%) was observed among respondents who applied two heat treatment methods of boiling and then frying their pork before consumption.

A higher prevalence (14.0%) was also observed among respondents who patronized shebeens and street vendors for their ready-to-eat pork, than among those who consumed home-prepared pork (4.3%). There was a higher taeniasis prevalence (16.2%) among respondents who did not practice regular washing of their hands, than among those who reported regular washing of their hands (7.6%) particularly after toilet use.

The risk factors associated with *T. solium*-taeniasis in Kaduna metropolis, using the Chi square analysis are as presented in Table 4.6. Backyard pig keeping was significantly associated with the occurrence of *T. solium*-taeniasis ($p < 0.05$). The knowledge of taeniasis among the respondents was also found to be significantly associated with the occurrence of taeniasis. There was very significant association ($p=0.001$) between *T. solium*-taeniasis and each of the following parameter: regular pork consumption, hand washing habit after toilet use, epilepsy and the main source of ready-to-eat pork (either home prepared or street vended pork). The measure of the strength of association (odds ratio) and confidence intervals are also presented in the table.

Table 4.5. Prevalence of *T. solium*-Taeniasis in Relation to Eating/Dietary Habits and Hygiene Practices of Respondents

Parameter	No. examined (n= 450)	No. positive	Prevalence (%)
Preferred method of pork preparation:			
Roasted/barbecued	95	15	15.8
Boiled only	278	24	8.6
Boiled & Fried	77	1	1.3
Source of ready to eat pork:			
Home	235	10	4.3
Shebeens/street vendor	215	30	14.0
Type of toilet facility used:			
Pit latrine	115	14	12.2
Water cistern	292	24	8.2
Defaecate in open air	43	2	4.7
Regular practice of hand washing with soap and water [a]:			
Yes	382	29	7.6
No	68	11	16.2
Main drinking water source:			
Pipe-borne	240	21	8.8
Well/borehole	159	16	10.1
Rivers/streams	51	3	5.9

Key: [a] After toilet use

Table 4.6. Risk Factors Associated With *T. solium*-taeniasis in Kaduna Metropolis, Nigeria

Parameter	p	X^2	O.R.	95% CI
Backyard pig keeping	0.013	6.617	2.30	1.17-4.37
Regular pork consumption	0.001*	52.07	2.43	1.53-4.92
Hand washing habit with soap after toilet use	0.001*	10.22	2.32	1.25-4.96
Epilepsy	0.001*	17.02	4.89	2.16-11.10
Knowledge about taeniasis	0.006	7.42	2.56	1.28-5.14
Source of ready to-eat pork	0.001*	52.59	1.89	1.75-4.72

Key: O.R.: Odds ratio

CI: Confidence Interval
X^2: Chi square values
p: probability values
*: p=0.001 (very significant association)

4.2 Assessment of Sanitary conditions of Pig Slaughter Slabs in Gonin-Gora and Nassarawa

A brief description of the structure of the slaughter slabs, available sanitary facilities and on-site activities within the slaughter slab premises is presented in Table 4.7. The two slaughter slabs were make-shift slabs, consisting of rough concrete/cemented floors where slaughtering, dressing and meat handling were done under unsanitary conditions. Both slaughter premises were located at the backyard within the homes. The slaughter slab premises in Gonin-Gora was located within the enclosed yard, while the slaughter slab premises at Nassarawa were exposed. There was an open refuse dump a few meters away from the exposed slaughter premises in Nassarawa. The pigs slaughtered at Gonin-Gora were sourced majorly from semi intensive pig farms where the pigs had routine veterinary care and sometimes from free ranging pigs. Pigs slaughtered at Nassarawa were mainly free ranging pigs. Slaughter facilities and procedures were substandard and no mechanized equipment was used for the slaughtering process. Routine meat inspection was absent at both slaughter slabs. Intestinal contents of the pigs were not cleaned out with the use of an anal plug as is ideal, and all meat parts were handled on same spot with the intestinal contents. Flies were found hovering around raw meat, constituting a nuisance at both slaughter slab premises. Meat displayed for sale at slaughter slab premises was kept at ambient temperature (30ºC) in the open, until all the meat was sold by the end of the day. No chilling or freezing practices were adopted immediately after slaughtering at the premises. Sanitizers were not used as part of the routine clean-up procedures at the end of the day, after the slaughtering procedure was completed.

Table 4.7. Structure, Sanitary Facilities and On-site Activities at Pig Slaughter Slabs in Gonin-Gora and Nassarawa in Kaduna Metropolis, Nigeria

Structure, Facilities & on site activities	Slaughter slab 1	Slaughter slab 2
Location	Backyard at a home-stead in Gonin-Gora.	Backyard at a home-stead in Nassarawa.
Structure of slaughter premises	Bare cemented floor inside an enclosed residential yard served as slaughter slab. Floors at premises had visible cracks.	Bare, rough concrete floor used as slaughter slab. Premises were exposed and without a fence. Concrete floors and walls were visibly cracked.
Toilet system available at premises	Pit latrine	Pit latrine
Water supply at premises	Well	Well
Drainage facility/waste disposal	Open drainage for liquid waste.	Open drainage used for liquid waste. Solid waste was disposed in refuse dump opposite slaughter site.
Frequency of pig slaughtering	Once a week	Once a week
Pig source/management system at point source	Semi-intensive farms and free ranging pigs	Free ranging pigs
Availability of Veterinary services at source	Routinely at intensive farms, seldom for free range pigs	No veterinary services
Routine meat inspection at slaughter slabs by official authorities	Absent	Absent
Rodent and pest control	Absent	Absent
Use of standard equipment	Machetes were used for slaughter, scalding was done with hot water and shearing with a razor blade. Meat was held up at ambient temperature without chilling. Intestinal contents were cleaned out manually without anal plugs.	Same as slaughter slab 1.
Cleaning procedures	Water & detergents were used to wash off soiled slabs. No sanitizers used.	Plain water was used for washing up soiled slab. No detergents or sanitizers were used for clean-up.

Key: Slaughter slab 1: located at Gonin-Gora
Slaughter slab 2: Located at Nassarawa

The sociodemographic factors and medical history of the butchers working at both slaughter slab premises are highlighted in Table 4.8. The butchers working at both slaughter slab premises were all male, with eight (80%) of them having no formal education, while 20% had formal education to at least secondary school level. None of the butchers at both slaughter slab premises practiced regular deworming of them selves with anti parasitic drugs. None of the butchers reported a history of epileptic seizures, however, three (3) out of the ten (10) butchers tested positive to taeniasis at the time of the study by the copro Ag ELISA test.

Table 4.9 presents the knowledge, behaviour and hygienic practices of butchers in relation to *T. solium* infections. Thirty percent (30%) of the butchers reported washing their hands regularly after using the toilet, the remaining 70% did not. The butchers at both slaughter slabs did not wear protective clothing such as aprons and hand gloves while slaughtering the animals. All the butchers at both slaughter slab premises consumed pork on a regular basis. Four of the butchers were observed roasting and eating pork in the course of duty. Forty percent (40%) of the butchers preferred their pork boiled, 25% preferred theirs both boiled and fried, while 40% preferred their pork barbecued. The butchers' knowledge of *T. solium* and its public health significance was generally poor; only 20% of the butchers had a fair knowledge of *T. solium*. Eight (80%) of the butchers had no idea about *T. solium* and the role of inadequately cooked pork in transmission of the parasite metacestodes to humans.

Table 4.8. Sociodemographic Factors and Medical History of Butchers Working at Slaughter Slab Premises

Sociodemographic factors and medical history		Outcome n=10
Sex:	Male	10 (100%)
	Female	0
Marital status:	Married	5(50%)
	Single	5(50%)
Educational Level:	Informal	8(80%)
	Formal	2(20%)
Regular deworming of self:	Yes	0
	No	10 (100%)
Tested positive to taeniasis by Copro Ag-ELISA at time of study:	Yes	3 (30%)
	No	7 (70%)
History of epilepsy:	Yes	0
	No	10(100%)

Key:Copro Ag-ELISA:Copro antigen enzyme linked immunosorbent assay

Table 4.9. Knowledge, Behaviour and Hygiene Practices of Butchers in Relation to *T. solium* Infections

Factors		**Outcome (%) (n= 10)**
Boiling/Treatment of drinking water:	Yes	0
	No	10 (100)
Regular hand washing after toilet use:	Yes	3(30)
	No	7 (70)
Regular hand washing after handling pork with:	Soap	9(90)
	Sanitizer	0
	Water only	1(10)
Wearing of hand gloves and aprons at work:	Yes	0
	No	10(100)
Regular consumption of pork:	Yes	10(100)
	No	0
Roasting and eating pork while handling raw pork:	Yes	4 (40)
	No	6 (60)
Method of pork processing for consumption:	Boiled	4(40)
	Boiled& fried	2 (20)
	Roasted	4 (40)
Knowledge on public health significance of *Taenia solium*:	Yes	2 (20)
	No	8 (80)

Some parasites were detected in environmental samples collected from within and around the slaughter premises (Table 4.10). Ova of *Taenia spp, Hookworm, A. lumbricoides* and larvae of *S. stercoralis* were detected in soil samples collected from a refuse dump site some few meters away from the exposed slaughter slab premises at Nassarawa. No parasites were detected in water samples used for domestic purposes and slaughter processes at both Nassarawa and Gonin-Gora slabs. Ova of hookworm and *A. lumbricoides* were both detected in soil samples collected at sites within the Nassarawa slaughter slab premises and in open drainage samples (half a metre away from the slaughter slab) collected at Gonin-Gora slaughter slab premises.

The prevalence of porcine cysticercosis and the distribution of cysticerci in meat parts examined at home-based slaughter slabs in Gonin-Gora and Nassarawa are presented in Table 4.11. Out of a total of 43 pigs examined by post mortem inspection at the two home slaughter slabs, four (4) were positive with viable *Cysticercus cellulosae,* giving an overall prevalence of 9.3%.The pig carcasses examined at Nassarawa and Gonin-Gora slaughter slabs had cysticercosis prevalence of 9.4% (n=32) and 9.1% (n=11) respectively. Out of a total of 240 meat parts, a total of 34 cysticerci were counted from common predilection sites which included, tongue, masseter, thigh muscles and the heart and also from behind the neck and shoulder muscles which are uncommon predilection sites. Cysts were detected on the surface of the tongue of only one of the four positive pigs inspected during the survey. No cysts were seen in the lungs and liver of all the pigs slaughtered during the survey. The highest number of cysticerci were recovered from the neck and shoulder muscle 11(32.4 %,), followed by the heart 9(26.5%), thigh muscles 7(20.6%), tongue 4(11.8%), and masseter muscles 3(8.8%) respectively.

Table 4.10. Parasites Detected in Environmental Samples Collected at Pig Slaughter Slab Premises

Environmental samples screened	Parasites detected	Sampling site
Soil samples (n=50)	*Taenia* ova	Refuse dump*
	Fasciola ova	Refuse dump
	Hook worm ova	Refuse dump, slaughter slab 2
	A. lumbricoides ova	Refuse dump, slaughter slab 2 premises
	S. stercoralis larvae	Refuse dump
Water samples (n= 25)	NP	Slaughter 1 & 2 premises
Open drainage (n= 17)	*A. lumbricoides* ova	Slaughter slab 1 premises
	Hookworm ova	

Key: NP: No parasite seen
* Refuse dump site was few meters away from exposed slaughter slab premises at Nassarawa.
Slaughter slab 1: Gonin-Gora, slaughter slab 2: Nassarawa.

Table 4.11. Prevalence of Porcine Cysticercosis and Distribution of *Cysticercus cellulosae* in Pig Carcasses at Nassarawa and Gonin-Gora

Location of slaughter slab	Total no. of pigs examined	Total no. of pigs positive/prevalence (%)	Predilection site (PS) examined for *Cysticercus Cellulosae* (n=240)	No. of carcasses with *C. cellulosae* at corresponding PS	Total no. of viable cysts distributed in corresponding PS (n=34)
NS	32	3(9.4)	Tongue	1	4
			Masseter muscle	2	3
			Thigh muscles	3	7
			Heart	2	6
			*Neck& shoulder muscle	-	0
			Lungs & Liver	-	0
GG	11	1 (9.1)	Tongue	-	0
			*Neck& shoulder muscle	1	11
			Heart	1	3
			Masseter & thigh	-	0
			Lung & liver	-	0
Total	43	4 (9.3)			

Key: NS- Nassarawa slab
GG- Gonin-Gora slab
*Non routine predilection sites
PS – Predilection sites

4.3 Molecular Diagnosis of *T. solium* by Copro PCR

A 100 bp diagnostic band was detected for nuclear gene; internal transcribed spacer region 1 (*ITS1*), while a 500 bp diagnostic band was detected for mitochondrial cytochrome oxidase c subunit 1 (*cox 1*) gene, as depicted in Figures 4.3 and 4.4. No DNA amplification was achieved in the nested PCR conducted using two sets of *T. solium*-specific primers designed based on the gene sequence that encodes the *T. solium* oncosphere- specific protein Tsol 31 (Gen Bank accession no. DQ861410). All the *Taenia* egg positive cases clearly demonstrated the typical diagnostic bands for both *cox 1* and *ITS 1* genes.

The confirmation of *T. solium cox 1* query sequences by comparison with a similar known gene sequence from Cameroon West (Accession number: FN995666.1), using the basic logical alignment search tool (BLAST) is presented in Table 4.12. The e-value, which is the "expect" value is presented in the table as 0.0. The e-value accounts for the significance of the alignment between the known gene sequence and the query sequences; the low value reported here indicates a low probability of the alignment occurring as a result of mere random chance. The table also presents the accession numbers accorded to the annotated *T. solium* sequences (out of the fifteen clean sequences obtained from this study) after successful deposition at the NCBI GenBank. The nucleotide compositions of the new *T. solium* sequences were biased toward T and A, with T being the most favoured nucleotide and C being the least favoured.

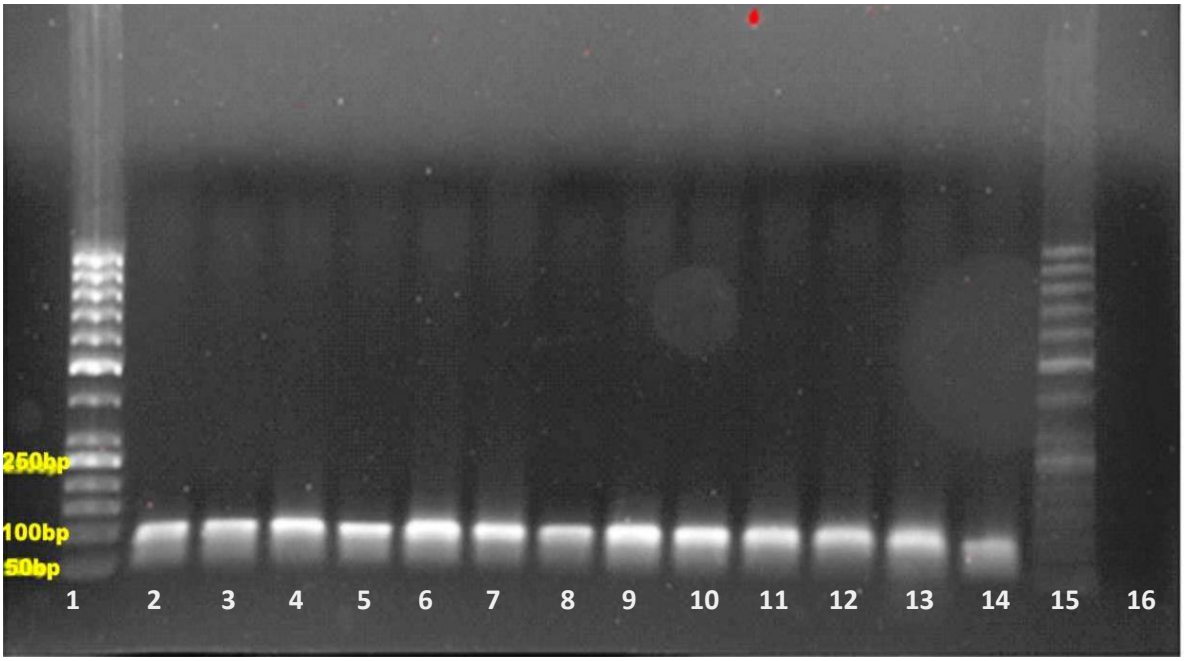


Fig. 4.3. PCR Amplification of *ITS 1* region of *T. solium*

Lanes 1 & 15: 50bp DNA markers.

Lane 2: Positive *T. solium* control.

Lanes 3-14: Amplicons of *T. solium* DNA.

Lane 16: Negative control

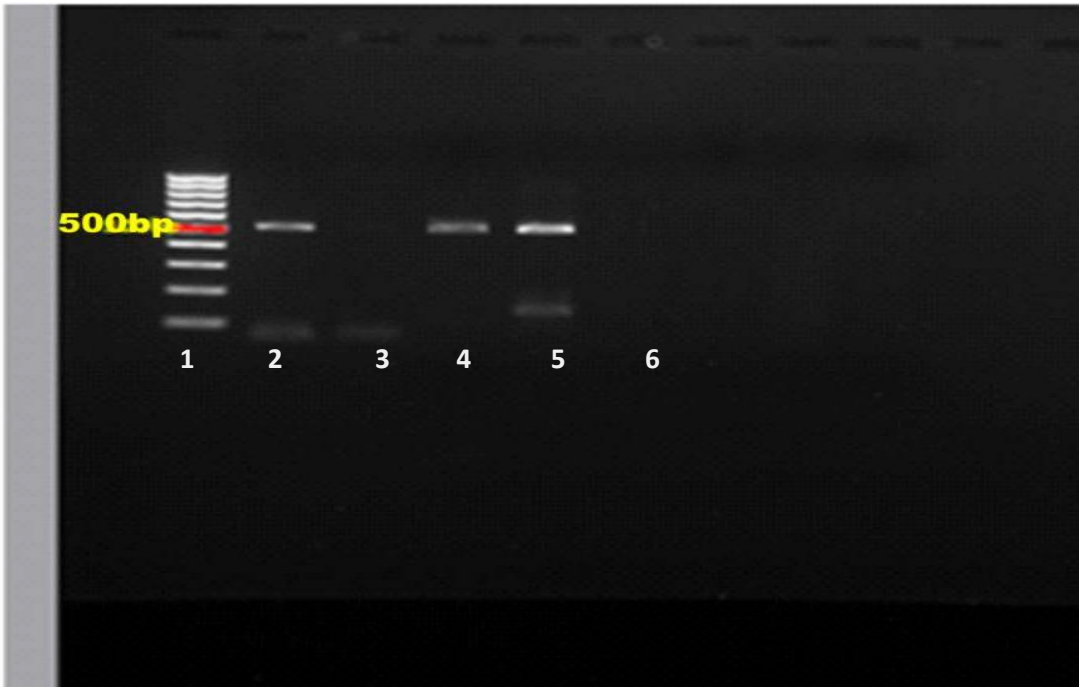


Fig. 4.4. PCR Amplification of *mt cox 1* region of *T. solium*

Lane 1: 100bp DNA marker,

Lane 2: Positive *T. solium* control

Lanes 3-5: Amplicons at expected 500bp band.

Lane 6: Negative control

Table 4.12. Confirmation of *T. solium mtcox1* Gene Sequences Using the Basic Logical Alignment Search Tool (BLAST)

Sequence name/ order	Query cover (%)	%Similarity (e =0.0)	Accession number
T. solium mtcox1 Cameroon West*	100	100	FN995666.1
Tsol GG2 TTAATTTTTTAAAACCTTATTATAATGTGA TTTCTTTGGATTTCATTGGATTGTTATAAA TTTTTGATTACTAATGATGGTATAATAATG ATTTTCTTTTTTTT……..A	100	100	KM030022.1
Tsol NAS TTAATTTTTTAGAACCTTATTATAATGTGA TTTCTTTGGATTTCTTTGGATTGTTATAAA TTTTTGATTACTAATGATGGTATAATAATG ATTTTCTTTTTTTT……..A	100	100	KM030023.1
Tsol BAR TTAATTTTTTAGAACCTTATTATAATGTGA TTTCTTTGGATTTCTTTGGATTGTTATAAA TTTTTGATTACTAATGATGGTATAATAATG ATTTTCTTTTTTTT……..T	100	100	KM030024.1
Tsol ST1 TTAATTTTTTAAAACCTTATTATAATGTGA TTTCTTTGGATTTCATTGGATTGTTATAAA TTTTTGATTACTAATGATGGTATAATAATG ATTTTCTTTTTTTT……..A	100	100	KM030025.1

*GenBank reference sequence which query sequences were compared to.

KM030022.1-KM030025.1: Accession numbers assigned to annotated *T. solium cox1* sequences obtained within Kaduna metropolis, Nigeria. *Tsol GG2*: Gonin-Gora, *Tsol NAS*: Nassarawa, *Tsol BAR*: Barnawa, *Tsol ST1*: Sabon-Tasha.

e=0.00 refers to Expect value.

No deletions or insertions of nucleotides were observed in the *T. solium cox 1* gene sequences obtained, however, very minimal intra-specific variation occurred in terms of nucleotide differences. Table 4.13 shows the intra-specific variations in the *cox 1* genes, indicating the variable sites in the sequences. Only three variant nucleotide positions were detected among the fifteen *cox 1* sequences. Out of the fifteen *T. solium* sequences, only five sequences exhibited this reported limited variation. The variable sites were detected at positions 20 and 53 of *Tsol GG2, Tsol GG2a* and *Tsol ST1* and positions 415 of *Tsol Nas* and *Tsol Nas c*.

Findings of the study present rooted phylogenetic trees showing evolutionary relationships of *T. solium* from the study locations and other geographical regions, inferred from the *mt cox1* sequences. A common out group, *Echinococcus granulosus* was used for all the phylogenetic trees (Fig. 4.5, Appendix XIX, Appendix XX). The phylogenetic trees constructed exhibited similar topologies for *T. solium* from Kaduna metropolis and from other parts of the world pooled together from the GenBank, hence they were collapsed into one Neighbour joining tree. The neighbour joining tree is presented in Fig. 4.5, which depicts the similar topologies observed in the Bayesian (Appendix XIX) and Maximum likelihood trees (Appendix XX), indicating the B/NJ/ML nodal boot strap values for all the trees as presented in the NJ tree. Two distinct clades and genotypes of *T. solium* from Kaduna metropolis and other geographical regions in the world were recognized on the phylogenetic trees, with 100% bootstrap values of 1000 replicates, indicating maximal nodal support. The phylogenetic tree separated the isolates from India, China and Philippines into a single cluster, while isolates from Barnawa, Sabon-Tasha, Gonin-Gora and Nassarawa combined with those from Tanzania, Mexico, Mozambique, Cameroon and Peru to form another single cluster. Further still, the African and American clusters were further sub grouped into three

other clades; *T. solium* isolates BAR (Barnawa), ST2 (Sabon-Tasha 2), GG1 (Gonin-Gora 1) and GG2 (Gonin-Gora 2) combined with isolates from Tanzania, Mexico, Mozambique, Cameroon and Peru to form a single cluster within the African and American clade, while isolate ST1 branched off to form another sub group, sharing a common ancestral line with the cluster mentioned above. Isolate NAS (Nassarawa) formed a single sub clade on its own, although it still shares a common recent ancestor with the other African/Latin American clade.

The pairwise comparison (by maximum parsimony) of the nucleotide distances between the *T. solium cox 1* sequences from the different study locations in Kaduna metropolis is presented in Table 4.14. There was little or no difference observed in the nucleotide distances between closely related or highly similar sequences.

The pairwise nucleotide distance comparison between *T. solium* from Kaduna metropolis and other parts of the world is shown in Table 4.15. There was also little or no difference in nucleotide distances between closely related *T. solium* sequences, such as sequences of the African and Latin American origin and also between sequences obtained from different Asian regions. Nucleotide distances were however farther apart when compared between *T. solium* from Africa/Latin America and Asia.

Table 4.13: Multiple alignment of partial *cox 1* sequences of *T. solium* from Kaduna Metropolis Indicating Variant Nucleotide Positions

Sequences				20 ↓		53 ↓	415 ↓
Tsol GG1	TTA	ATT	TTT	TAG	AAC	TTA	TAT
Tsol GG2c	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Tsol NAS	. . .	. . .	. . .	. . .	. . .	. . .	. **T** .
Tsol BAR	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Tsol GG2	. . .	. . .	. . .	. . **A**	. . .	. **A** .	. . .
Tsol BAR a	. . .	. . .	. . .	. . .	. . .	. . .	. . .
TsolGG2 b	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Tsol NAS b	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Tsol NAS a	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Tsol NAS c	. . .	. . .	. . .	. . .	. . .	. . .	. **T** .
Tsol GG2 a	. . .	. . .	. . .	. . **A**	. . .	. **A** .	. . .
Tsol ST2	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Tsol ST1	. . .	. . .	. . .	. . **A**	. . .	. **A** .	. . .
Tsol ST1 a	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Tsol ST2 a	. . .	. . .	. . .	. . .	. . .	. . .	. . .

Similar sequences are represented by same colour. A-adenine, T- thymine, C- cytosine, G-guanine. All nucleotides identical to the *TsolGG1* nucleotides are represented by dots. Variant nucleotides are shown in bold, coloured alphabets; **A**, **T**. Space in between triplet codons does not indicate gaps but sequences which are not displayed. *Tsol GG2*: Gonin-Gora, *Tsol NAS*: Nassarawa, *Tsol BAR*: Barnawa, *Tsol ST1*: Sabon-Tasha.

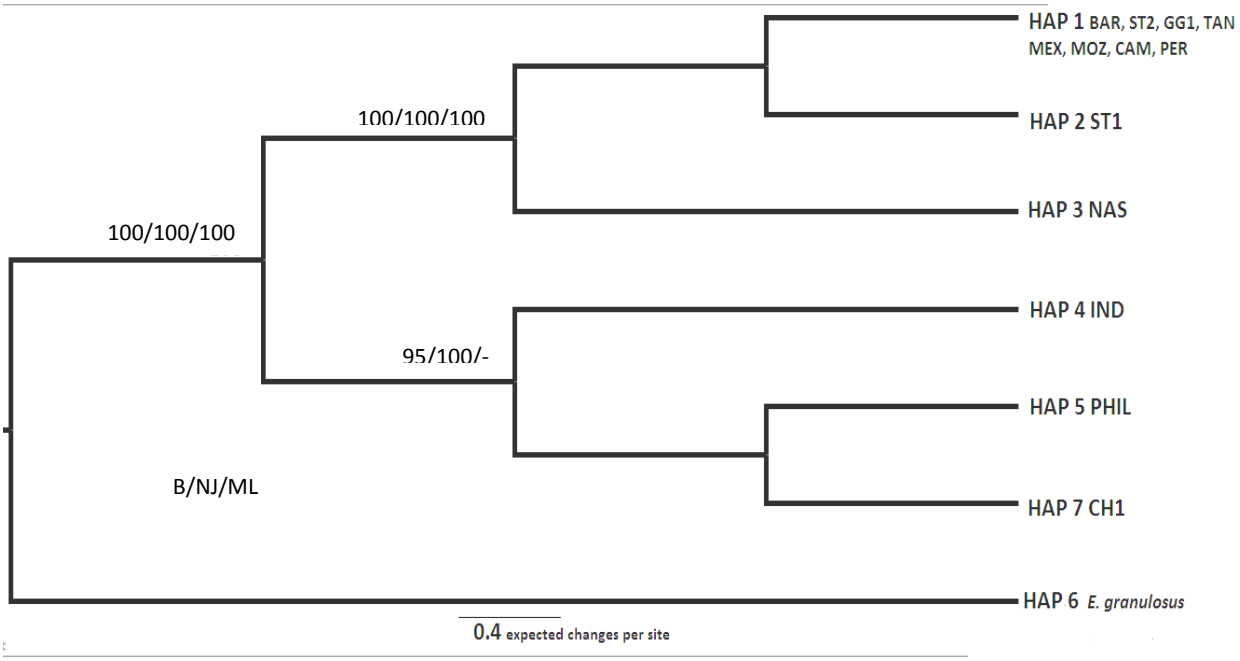


Fig 4.5 The Neighbour joining tree inferred from *cox 1 T. solium* genes from Kaduna metropolis and other parts of the world.

Key: Scale bar represents the rate of nucleotide substitutions per nucleotide site. Node values represent bootstrap confidence level of 100 based on 1000 replicates. B- Bayesian, NJ- neighbor joining, ML- maximum likelihood. HAP- Haplotypes, Bar-Barnawa, ST2- Sabon-Tasha 2, ST1- Sabon-Tasha 1, GG1- Gonin-Gora 1, GG2- Gonin-Gora 2, Nas- Nassarawa, TAN-Tanzania, MEX-Mexico, MOZ-Mozambique, CAM- Cameroon, PER- Peru, IND-India, PHIL- Philippines, CH1: China

Table 4.14. Pairwise Comparison of Nucleotide Distances of *T. solium* Sequences from Kaduna Metropolis, Nigeria

	Tsol-Bar	Tsol- ST 1	Tsol-ST 2	Tsol-GG1	Tsol-GG2
Tsol-ST1	0.002				
Tsol-ST 2	0.000	0.002			
Tsol-GG 1	0.000	0.002	0.000		
Tsol-GG 2	0.000	0.000	0.000	0.000	
Tsol-Nas	0.002	0.005	0.002	0.002	0.002

Key: Bar-Barnawa,
ST1- Sabon-Tasha,
ST2- Sabon-Tasha,
GG1- Gonin-Gora,
GG2- Gonin-Gora,
Nas- Nassarawa,

Table 4.15. Pairwise Comparison of Nucleotide Distances of *T. solium* Sequences from Kaduna Metropolis, Nigeria and Other Parts of the World

	Bar	ST1	ST2	GG1	GG2	Nas	Tan	Chi	Ind	Mex	Per	Phil
ST1	0.003											
ST2	0.000	0.003										
GG1	0.000	0.003	0.000									
GG2	0.000	0.000	0.000	0.000								
Nas	0.003	0.005	0.003	0.003	0.003							
Tan	0.000	0.003	0.000	0.000	0.000	0.003						
Chi	0.713	0.720	0.713	0.713	0.713	0.706	0.713					
Ind	0.713	0.720	0.713	0.713	0.713	0.706	0.713	0.016				
Mex	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.713	0.713			
Per	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.713	0.713	0.780		
Phil	0.713	0.720	0.713	0.713	0.713	0.706	0.713	0.014	0.03	0.713	0.125	
E	0.792	0.799	0.792	0.792	0.792	0.792	0.784	0.792	0.200	0.792	0.245	0.204

Key: Bar-Barnawa, ST1- Sabon-Tasha, ST2-Sabon-Tasha, GG1- Gonin-Gora, GG2- Gonin-Gora, Nas-Nassarawa, Tan- Tanzania, Chi-China, Ind-India, Mex-Mexico, Per- Peru, Phil-Philippines, E-*Echinococcus granulosus*

CHAPTER FIVE

5.0 DISCUSSION

5.1 Seroprevalence of Human Cysticercosis and Associated Risk Factors

This study has established and reported a human cysticercosis prevalence of 14.3% which is relatively higher than 1.3%, 2.4% and 9.6% reported in Benin Republic, Togo (Zoli *et al*., 2003) and Jos, Nigeria respectively (Weka *et al*., 2013). Garcia-Noval *et al*. (2001) reported that in most cysticercosis endemic communities, more than 10% of the general population are seropositive and seropositivity can even be as high as 25%. The prevalence reported in this survey is not as high as anticipated by Garcia-Noval *et al*. (2001), it is however closer to 16.3% reported in Mbulu community in Tanzania (Mwang'onde *et al*., 2012) but far less than 40.8% reported in Burundi (Newell *et al*., 1997), and 45.3% prevalence (n=830) reported in Mbozi district of Tanzania (Mwanjali *et al*., 2013), both of which also used the antibody ELISA techniques.

Different diagnostic methods have been employed for detecting human cysticercosis, a few of which include, detection of antibodies or antigens in sera and use of computerized tomography for detection of cysts lodged in organs such as the brain. The choice of method depends largely on suitability; antibody ELISA has been very useful in large population screening. This study made use of IgG antibody ELISA technique which has a high specificity and sensitivity (Cortez Diagnostic Kit, INC, California).

In addition to diagnostic method used, a barrage of factors interacting together may likely account for the disparity in prevalence reported across the different study locations in this survey. Nassarawa and Kakuri had the highest prevalence of both cysticercosis and taeniasis,

while Barnawa and Narayi had the least prevalence. Some of such factors include, knowledge of the disease among residents in the different areas, their behaviour and the different levels of exposure to risk factors present in these different areas. The levels and degrees of these observed risk factors as pertaining to the study locations such as; free range pig rearing, regular consumption of pork, barbecuing and road side sale of ready-to-eat pork by vendors could possibly be responsible for the varying prevalence observed among the different locations. From observation, Nassarawa had a larger pig population compared to the other locations studied, thus, this in conjunction with other factors such as unsanitary conditions, pig husbandry practices and eating habits might explain the higher prevalence observed in the given location. Furthermore, the Nassarawa and Unguwan-Romi clusters seemed to have more of slum areas compared to the other sites included in the study, indicating poor hygiene, thereby exacerbating the the cysticercosis-taeniasis complex.

There was a slightly higher cysticercosis prevalence in female than male and this could be attributed to the habit of women tasting food on fire before it is thoroughly cooked. Despite the slightly higher prevalence reported in female than male, there was no statistically significant difference in the cysticercosis seroprevalence between sexes, indicating that both male and female were equally predisposed to infection and that sex is not a risk factor. This corroborates findings reported in Chaco region, Bolivia (Carrique-Mas *et al*., 2001), which showed no significant difference in cysticercosis seroprevalence between the two sexes. In contrast however, some studies in Guatemala (Garcia-Noval *et al*., 2001), Tanzania (Mwanjali *et al*., 2013) and Plateau, Nigeria (Weka *et al*.,2013) have reported statistically significant prevalence in female than male, with the study conducted in Plateau, Nigeria reporting that female were 1.7 times more prone to infection than male (Weka *et al*., 2013).

Another study in Vietnam reported higher infection rates in male than in female (Willingham *et al*., 2003), this could be attributed to male social habits of eating pork prepared in shebeens along with their peers. Pork prepared for such commercial purposes have the tendency to be undercooked therefore increasing the chances of acquiring taeniasis and eventually cysticercosis.

Farmers and butchers recorded the highest cysticercosis seroprevalence than all other occupation reported in this survey, this may be attributed to the fact that farmers and butchers often come in direct contact with infected pigs in the course of duty. Seroprevalence was very high among respondents who preferred their pork roasted, while the least seroprevalence was observed among respondents who both boiled and fried their pork before consumption. This could be attributed to the fact that roasted pork is more likely to be undercooked than pork both boiled and fried. This study reported a significant association between cysticercosis seropositivity and method of pork preparation before consumption. Consumption of pork infected with *T. solium* cysticerci is crucial in the transmission cycle of taeniasis and eventually cysticercosis. Adequate heat source is therefore required to sufficiently cook pork before consumption to take care of the cysts present in the meat, reducing the risk of taeniasis. The method of pork preparation was found to be associated with knowledge of cysticercosis. This implies that knowledge on transmission and control of *T. solium* could influence pork preparation methods. Therefore, acquisition and application of the right knowledge about transmission, prevention and control of cysticercosis could prevent or reduce exposure to risks or habits which may enhance transmission of the parasite.

The findings of this present study also revealed that cysticercosis seropositivity is associated with epileptic seizures. Epileptic seizures had been linked to NCC particularly where the

dying cyst incites a host inflammatory reaction which results in the seizures (Coyle and Tanowitz, 2009). The odds ratio of 2.2 in this study indicates that individuals with cysticercosis are 2.2 times more likely to have seizures than those without cysticercosis therefore indicating that seizures are a clinical manifestation of NCC. Studies in Cameroon (Zoli *et al*., 2003) and Western India (Vora *et al*., 2008) revealed that 44.6% and 35.7% respectively of people having epileptic seizures were cysticercosis seropositive by Ab-ELISA. This study is in agreement with other studies that had indicated a strong association between cysticercosis and epileptic seizures, indicating that NCC is a major cause of epilepsy (Nash *et al*., 2004; Montano *et al*., 2005; Foyacca-Sibat *et al*., 2009; Ndimubanzi *et al*., 2010; Blocher *et al*., 2011; Mwanjali *et al*., 2013).

5.2 Prevalence of *T. solium* Taeniasis and Associated Risk Factors.

Contrary to studies which have reported that *T. solium* taeniasis tends towards a low prevalence, typically less than 1% even in endemic regions (Allan *et al*., 1996), this study has established and shown a high taeniasis prevalence of 8.8% in Kaduna metropolis, Nigeria. The taeniasis prevalence of 8.8% reported in this survey is higher than those reported elsewhere in Africa. In Togo, prevalence of 0.09-0.26% had been reported by Dumas *et al*. (1990). Newell *et al*. (1997) also reported a prevalence of 0.22% among school children in Burundi. Vondou *et al*. (2002) reported a prevalence of 0.13%, where four (4) out of 3,109 stool samples were *Taenia* spp, with 3 (0.1%) further identified as *T. solium*. A prevalence of 3.8% had been reported among school children in Conakry by Gyorkos *et al*. (1996). In Nigeria, Onah and Chiejina (1995) reported a prevalence of 8.7% using hospital records of patients. In Kenya, Eastern Zambia and Tanzania, prevalence of 8.6%, 4-10%, 6.3% and 5.2% had been reported respectively (Wholgemut *et al*., 2010; Mwape *et al*., 2012; Mwanjali

et al., 2013). In India, higher prevalence of 30.3% (among NCC patients) and 18.6% had been reported by Kumar *et al.* (2006) and Prasad *et al.* (2007) respectively.

The disparity in prevalence figures as reported above by different researchers may be due to the sensitivities and specificities of the different diagnostic techniques used. The prevalence of taeniasis detected by microscopy (1.3%) in this present study was less than that detected by copro Ag-ELISA (8.8%). Before the advent of copro Ag-ELISA, microscopic detection of *Taenia* ova and identification of proglottids were the only means of diagnosis of taeniasis. Coprology as a diagnostic technique is not sensitive enough, though specific, it does not differentiate between taeniid species. It had been reported that even with multiple samples and concentration of large volumes of stool sample, sensitivity of stool microscopy does not exceed 60 - 70% (Allan *et al*., 1993), in addition to this, the intermittent shedding of the taeniid ova, hampers the accuracy of this technique (Guezala *et al*., 2009). Epidemiologic studies have reported that copro-Ag ELISA is 2.6 times more sensitive than microscopy (Allan *et al*., 1996; Allan *et al*., 2003; Flisser *et al*., 2006). The in- house copro-Ag assay used in this study had reliable sensitivity and specificity estimated at 96-98% and 98-100% respectively (Allan *et al*., 2003; Allan *et al*., 2006).

Despite the lack of significant difference between taeniasis and some study parameter in population, the record of different prevalence rates within the different categories of these studied parameter only reflects the fact that there was exposure to conditions that favour transmission and sustenance of *T. solium* among certain categories in the population. For example, although there was no statistically significant association between taeniasis prevalence and gender/age, prevalence appeared slightly higher in male than female and among age group of 41years and above. Such sex and age prevalence may be attributed to

social habits of these two groups who tend to socialize with their peers at commercial outlets or shebeens where they eat pork and drink locally brewed alcohol known as *burukutu*.

A large proportion of the study population (368/450) had a poor knowledge of taeniasis, with a high number of positive cases (24). This is actually expected due to the poor knowledge of taeniasis in the population. The higher prevalence among the group of respondents with good knowledge of the disease is not actually due to knowledge without practice, but probably as a result of fewer return of respondents (82) in this category.

The reported prevalence of taeniasis in this study is an indication of existence of risk factors in the study area. This is also evidenced in the strong association ($p=0.001$) observed between taeniasis and several parameters such as medical history, eating habits, and hygienic practices observed in the study population. The transmission cycle of *T. solium* could be considered a vicious circle because the occurrence of taeniasis could lead to porcine and human cysticercosis, thereby worsening the condition of the first and sustaining the cycle.

The findings of this study have shown a significant association ($p< 0.05$) between *T. solium* taeniasis and the following factors: backyard pig rearing, regular pork consumption, hand washing habit, epilepsy, knowledge of taeniasis and source of ready-to-eat pork. Backyard pig keeping which involves free ranging of pig could increase the risk of porcine cysticercosis due to coprohagic habits of pigs and eventually result to taeniasis in humans, particularly where pork is undercooked. In line with this, studies have shown that contact with swine and *T. solium* carriers could promote and maintain transmission of *T. solium* (Murrell, 2005; Prestes – Carneiro *et al*., 2006; Weka *et al*., 2013). Regular consumption of pork was found to be associated ($p=0.001$) with taeniasis in this survey and this concurs with another study conducted in North India where regular pork consumption particularly when undercooked

was also found to be associated with taeniasis (Prasad *et al*., 2007). There is therefore an increased risk of taeniasis among those who consume pork regularly than those who do not. Although this present study did not find preferred method of pork preparation before consumption to be associated with taeniasis, there was a strong association (p=0.001) between source of ready-to-eat pork and taeniasis. The source of the ready-to-eat pork is suggestive of its method of preparation; pork prepared for large gatherings and at shebeens could have the tendency to be inadequately cooked because it is prepared in large quantities. Likewise, street vended pork in the study area was basically roasted and therefore the tendency to have it undercooked or inadequately heat processed was also anticipated. These could be likely reasons for the higher prevalence of taeniasis observed among those who patronized shebeens and street vendors as compared to those who prepared their pork at home (Table 4.5).

The findings of this study corroborate studies that had found hand washing habit to be associated with occurrence of *T. solium* infections (Mwanjali, *et al*., 2013; Weka *et al*., 2013), with a higher prevalence among those who did not practise regular washing of hands with soap. An aberration in the lifecycle of *T. solium* has been linked to humans becoming accidental intermediate hosts of the parasite; this occurs through faecal oral route involving contaminated food, water and dirty hands thereby resulting in cysticercosis in humans (Garcia *et al*., 2003). Since NCC occurs as a result of ingestion of taeniid ova by faecal oral route, proper washing of hands with soap might break the transmission cycle and prevent further infection. Knowledge of taeniasis was also found associated with occurrence of taeniais in this survey and this corroborates findings of Flisser *et al*. (2006) which also implicated knowledge of *T. solium* lifecycle with occurrence of taeniasis.

5.3 Assessment of Sanitary Conditions of Pig Slaughter Slabs in Kaduna Metropolis, Nigeria

Despite the fact that Nigeria has existing abattoir law meant to ensure proper management and livestock slaughtering to protect people's health, production, handling, sales and consumption of poor quality animal food products have been identified as serious public health problems. This has been attributed to the influence of several factors affecting different segments of livestock industry (Okoli *et al*., 2005).The law forbids the slaughter and sale of livestock for human consumption in locations other than abattoirs (Nwanta *et al*., 2008), however, home slaughter, distribution and sale of pork is common practice in parts of Kaduna metropolis, Nigeria.

It is reported that under normal circumstances, many abattoirs and slaughter slabs in developing countries are poorly constructed, and have poor slaughter and meat inspection facilities (Mkupasi *et al*., 2011). As in other studies in Nepal (Ghimire *et al*., 2013), Tanzania (Mdegela *et al*., 2010) and Imo State, Nigeria (Okoli *et al*., 2006) this study reported poor hygiene and substandard conditions within the two home slaughter premises/slabs. The home slaughter conditions were substandard because they were makeshift and not constructed to actually meet the requirements of ideal slaughter premises. Such substandard conditions could lead to ease of microbial contamination because proper slaughter facilities are lacking, since hygiene is poor. For instance, the bare concrete floor was used as slaughter slabs at both slaughter premises, which was unhygienic. Visible cracks were also observed in the walls and floors and these could serve as hideouts for insects, rodents and other vectors. The slaughter premises at Nassarawa was not fenced and so it was exposed to rodents which could easily find their way through to the premises. A few metres away from this same slaughter

site, was an open refuse dump site where people were seen defaecating and where pigs were also found scavenging; this seemed to be evidence of a well sustained transmission cycle of the parasite. The presence of poor sewage and open drainages observed in the study area also led to breeding and hovering of flies which could contaminate food and water within the premises.

The lack of pipe borne water in this study supports similar findings in Zuru, Nigeria and Nepal respectively where wells served as major sources of water at slaughter slabs (Gweba *et al*., 2010; Ghimire *et al*., 2013); water from such alternative sources could be contaminated on account of direct exposure to environmental factors.

The free ranging of pigs and lack of routine veterinary services observed in this study could result to inadequate care of the animals, reducing their productivity and exposing them to disease agents, which may become hazardous to the animals, to humans and the environment (Christensen, 1996; Okoli *et al*., 2006). The health and proper husbandry of livestock is therefore essential for optimization of their productivity as well as promoting good health in their human consumers (Okoli *et al*., 2006).

Lack of meat inspection is critical in the transmission of *T. solium* to humans (Gweba *et al*., 2010) and exacerbates the cysticercosis-taeniasis complex. In home slaughtering, absence of meat inspection is an important predisposing factor because of the risk of creating urban foci or point source of infection as the case may be.

In Nigeria, it is believed that male, illiterates and school drop-outs dominate butchery (Okoli *et al*., 2006), and this fact has been corroborated by this study. This may be attributed to the fact that butchery is tasking and requires a lot of physical strength. This study also agrees

with findings in Ghana and Nepal, where 64% and 80% of butchers respectively had no formal education (Adzitey *et al*., 2011; Ghimire *et al*., 2013). This may be attributed to the fact that no "knowledge" is required as qualification requirement for butchery.

A health education intervention study in Mexico and Southern Tanzania revealed that knowledge of *T. solium* played a significant role in preventing transmission in the human – pig cycle (Sarti *et al*., 1997; Ngowi *et al*., 2011). The poor knowledge of *T. solium* among the butchers in this survey was evident in their behaviour, such as poor hand washing practices, drinking untreated water and handling of raw pork with bare hands without the use of protective covering such as hand gloves and aprons. A similar finding in Nepal also reported that butchers neither wore gloves nor masks while handling raw meat and only 30% of them wore an apron regularly at work (Ghimire *et al*., 2013). Soaps, sanitizers and detergents are known to clean surfaces thoroughly and also reduce microbial population on contaminated hands and surfaces; however, their roles were undermined as they were not used by the butchers.

Butchers having taeniasis in this study pose a serious risk of cysticercosis both to themselves and to others in close contact with them. Despite the prevalence of taeniasis reported among butchers in this study, there was no reported history of epilepsy and this is related to the fact that having taeniasis is not necessarily indicative of the presence of cysticercosis-related epilepsy. However, it had been reported that the most important risk factor for acquisition of cysticercosis is the proximity of a tapeworm carrier (Coyle and Tanowitz, 2009).

Taeniid ova detected in soil from refuse dump site indicates practice of defaecating in the open by *Taenia* carriers around the study area and this could maintain transmission in the intermediate host.

The prevalence of porcine cysticercosis in Nassarawa home slaughter premises was higher than that at Gonin-Gora. This could be attributed to the fact that pigs slaughtered at Nassarawa were sourced from free ranging pigs which had no routine veterinary care, unlike pigs slaughtered at Gonin-Gora site which were mainly sourced from semi intensive farms and reportedly had routine veterinary care. Porcine cysticercosis is caused by the metacestodes of *T. solium* which is also referred to as *Cysticercus cellulosae*. The prevalence of porcine cysticercosis suggests the existence of an active transmission cycle of the parasite between pigs and humans (Saravanan *et al*., 2014). The reported 9.3% prevalence of porcine cysticercosis at the slaughter premises in this study was higher than 2.5% reported in Nsukka (Onah and Chiejina, 1995), closer to 9.5% reported in Northern Nigeria (Biu and Ijudai, 2012) but much lower than 14.40% reported in Zuru, Nigeria (Gweba *et al*., 2010). Higher prevalence rates had been reported in Cameroon and South Africa (Zoli *et al*., 2003; Krecek *et al*., 2012). These varying prevalence rates have been attributed to a host of factors of which diagnostic techniques used is inclusive.

The tongue examination technique in live pigs is said to have a lower sensitivity than the post mortem meat inspection (Gweba *et al*., 2010), although it is said to be 100% specific (Da Silva *et al*., 2012). This is because several predilection sites are examined at post mortem and incisions made where necessary, in contrast to only the tongue in lingual palpation (Gweba *et al*., 2010). Detailed necroscopy had been recommended as a more reliable diagnostic method in pigs with low infection (Da Silva *et al*., 2012), but is impractical for routine inspection because it is time-consuming, expensive and could lead to commercial losses (Gonzalez *et al*., 1990; Pinto *et al*., 2000). The absence of cysticerci in the liver and lungs of pigs in this study corroborated with the findings of a similar study in Nsukka, Nigeria

(Onah & Chiejina, 1995), but contradicted the findings of the survey in Zuru, Nigeria where cysts were found in the liver and lungs during routine meat inspection (Gweba *et al*., 2010). The findings of this study revealed a high cystic affinity for neck and shoulder muscles which are non-routine predilection sites and supports other studies which reported muscles of the heart, tongue and masseter as common predilection sites (Garcia *et al*., 2001; Da Silva *et al*., 2012).

5.4 Molecular Diagnosis and Genotyping of *T. solium* in Kaduna Metropolis

In recent times, molecular tools have impacted greatly in the field of parasitology where it has proved useful for diagnosis of parasitic infections, epidemiology, transmission dynamics, population genetics, drug resistance and vaccine development. As is widely recognized for many parasites, knowledge of life cycles, mechanisms and dynamics of transmission and infection, all form the basis for effective control strategies. A review by Donald (2006) indicated a number of molecular approaches used in differential diagnosis of closely related taeniids. Some of these molecular approaches include; direct comparison of PCR- amplified DNA sequences, random amplified polymorphic DNA-PCR (RAPD-PCR), and restriction fragment length polymorphism (RFLP).

This study has successfully used highly specific *T. solium* primers targeting both nuclear and mitochondrial genes to confirm *T. solium* taeniasis in carriers. Copro PCR is said to be useful in the confirmation of copro-Ag positive samples (Mathis and Deplazes, 2006) as proven in this survey. Due to the fact that PCR is expensive and technical (Mathis and Deplazes 2006; Guezala *et al*., 2009) and is reliant upon the presence of parasite material, it is only sensible that the technique be used on faecal samples that have been screened for the presence of copro-Ag or directly on taeniid eggs recovered from faecal and environmental samples as

done in this study. Copro PCR has been useful in speciation and accurate diagnosis of closely related *Taenia* worms such as *T. solium* and *T. saginata*. The successful combination of PCR targeting different genetic markers and further analysis of the amplicons as applied in this study identified *T. solium* DNA directly from faeces. The success of PCR depends largely on a number of issues because of the technicality of the process. One of such issues is the proper isolation of DNA from faecal matter, which necessitated the modification of the DNA isolation protocol used in this survey, in order to ensure that the final elute had enough of the *T. solium* DNA for further use in downstream applications. The introduction of bashing beads was to help in breaking open ova present in the faeces thereby improving lysis. The diagnostic 100 and 500bp bands obtained in the PCR assay targeting *ITS1* and *cox1* genes respectively, verifies the success of the modified DNA isolation process and the use of PCR as a diagnostic tool. This study is therefore in consonance with findings of Hancock *et al.* (2001) and Campbell *et al.* (2006) which reported that both mtDNA and nuclear DNA are advantageous as multicopy genes and appropriate for use in PCR assays and other molecular approaches.

Studies on genetic diversity or variation in *T. solium* have not been examined closely and there is no record of such studies in Nigeria. This study is therefore one of the first notable applications of the use of genetic markers in genotyping *T. solium* in Nigeria. The study applied the use of two gene markers from mitochondrial and nuclear genes; cytochrome oxidase c sub unit 1 (*cox 1*) and internal transcribed spacer region 1 (*ITS1*) for identification of *T. solium* obtained from humans in Kaduna metropolis. Various genetic markers have been useful in phylogenetic analysis among closely related groups (Campbell *et al*., 2006). However, mtDNA is considered the most suitable and the best neutral marker for such

analysis. Many reasons have been attributed to this; it is maternally inherited, evolves fairly rapidly, has the most of the substitution of the nucleotides occurring at neutral sites and is said not to be responsible for morphological changes (Osawa *et al*., 1999). Mitochondrial genes are amongst the most popular markers and so are frequently used for phylogenetic studies, population differentiation/ genetics and evolutionary biology (Campbell *et al*., 2006; Jia *et al*., 2010). Mitochondrial genomes are small, typically less than 20,000bp and are maternally inherited (Kandil *et al*., 2010). Mitochondrial DNA sequences are used to infer phylogeny of *T. solium* because they are known to evolve faster than nuclear genes and so considered to be the best neutral markers with high levels of variability and without high host- related selection pressure (Campbell *et al*., 2006).

In line with this, studies by Wolsteinholme (1992) and Campbell *et al.* (2006) also reported that because of these desirable characteristics of mtDNA, they are more commonly and frequently used for phylogenetic studies and are better for discrimination of species that are closely related. *ITS* is considered the most commonly used nuclear DNA fragment and comprises of *ITS1* and *ITS 2*, however, in comparison with mtDNA, there is no sufficient variability in direct sequence analysis, as such, other complementary tools such as PCR, RFLP, single- stranded confirmation polymorphism (SSCP), random amplified polymorphism DNA (RAPD) and use of microsatellites are recommended with the use of nuclear genes (Campbell *et al*., 2006).

The uploaded *T. solium* DNA from this survey were no longer considered query sequences, but due to the successful deposition at the NCBI Gen Bank, have become a library of known sequences from Nigeria, information which before now was not available on the genbank database.The T and A bias in the nucleotide composition of *T. solium* from Kaduna

metropolis is not surprising as this is in consonance with other flatworm mtDNAs (Johnston, 2006). It has been reported that T-A bias is not an unusual occurrence amongst cestodes and is in fact higher in *Taenia* species than is found in the sister genus *Echinococcus* (Nakao *et al*., 2003). Nucleotide composition plays an important role because it may affect the amino acid composition in the mitochondrial protein (Johnston, 2006).

Within the genus *Taenia*, DNA sequence variation has been used to distinguish one species from another and to infer phylogeny (Bowles and McManus, 1994; Gasser and Chilton, 1995; Okamoto *et al*., 1995; De Queiroz and Alkire, 1998). However, little is known about intra-specific variation of *Taenia solium* (DNA sequence variation within the species). The findings of this study demonstrated much similarity and minimal variability in *mt cox 1* sequences of *T. solium* across the different study locations in Kaduna metropolis. This observed minimal variation is in agreement with findings of Campbell *et al*. (2006) which revealed that mtDNA markers such as *cox 1*, *cob* and *nad 1* sequences of *T. solium* have little or no genetic variability. The three variant nucleotides detected at positions 20 and 53 of *Tsol GG2, TsolGG2a* and *Tsol ST1* where G and T were substituted for A respectively, occurred as a result of transitional and transversional substitutions. Transitions are said to appear more often in genomes as a result of molecular mechanisms, oxidative deamination and tautomerization (Eberserger *et al*., 2002). A case of single nucleotide polymorphism (SNP) is however attributed to the variant nucleotide found in position 415 of *Tsol Nas* and *T sol Nas c* sequences from Nassarawa. "A single nucleotide polymorphism is a DNA sequence variation occurring within a population (e.g. 1%) in which a single nucleotide- A, T, C or G in the genome or other shared sequence differs between members of a biological species"(www.wikipedia.org/wiki/single-nucleotide-polymorphism). For example the last

triplet codon of *Tsol Nas* and *Tsol Nas c* differ in the single nucleotide T when compared with all the other *T. solium* sequences obtained, hence resulting in the triplet codon TTT instead of TAT; clearly a transversional substitution has occurred where A has been substituted for T in the new triplet codon. Approximately two out of three SNPs are said to be transitions (Collins e*t al*., 1994), transversions could also be associated with SNPs as observed in this study.

Although this study has reported very minimal genetic variation in *mt cox1* sequences of *T. solium* in circulation in Kaduna metropolis, such intra-specific variation still proves very useful and could be exploited in understanding evolutionary changes, genetic differentiation and speciation of *T. solium* within the study area. Such minimal intra-specific variation shows that the *mt cox 1* gene studied is highly conserved, therefore concurring with studies by Jia *et al*. (2010) and Kandil *et al*. (2010). Among the three genes coding for cytochrome oxidase, Kandil *et al*. (2010) reported *cox 1* to be the most highly conserved, thus conferring on it its importance in several phylogenetic studies/analysis (Traversa *et al*., 2007).

A study by Rishi and McManus (1988) demonstrated intraspecific sequence variation between *T. solium* cysts obtained from Zimbabwe, Mexico and India, without any variation at all in the Indian strains. Other studies have also demonstrated inter- specific variation among *Taenia* species using short *cox 1* and *nad 1* sequences with a significantly reduced variability among the *T. solium* sequences (Okamoto *et al*., 1995; Gasser *et al*., 1999). Due to the same/similar sequences shared between the *T. solium* isolates in this study, it can be said that *T. solium* isolates in Kaduna metropolis share a common, recent ancestor and seem to be associated with geographical location. There is therefore a high possibility of the isolates being introduced into the population from a common source.

The limited genetic variability amongst *T. solium* demonstrated in the *mt cox 1* in this study, could be attributed to the fact that tapeworms are monoecious and capable of self-fertilization which may supress genetic variability within populations, hence there could be a tendency of having genetically identical progeny (Campbell *et al*., 2006), which could greatly impact on tapeworm populations.

Partial and complete genome sequencing of mtDNA of *T. solium* worldwide have revealed two genotypes/clades of *T. solium* and these are: the Asian and the American/African types (Okamoto *et al*., 2001; Ito *et al*., 2002; Nakao *et al*., 2002; Eom *et al*., 2011). The phylogenetic analyses using the Bayesian, Neighbour-Joining and Maximum Likelihood trees in this study infer that *T. solium* in circulation in Kaduna metropolis is the African/ American type. These findings support the fact that *T. solium* diverged into two genetic groups and that the Africa/ American types share related origin and thus have a common ancestor. Although the genetic variations within the *T. solium* sequences from Kaduna metropolis were very small, a close look at the *T. solium* clusters inferred from the phylogenetic tree shows that, clusters BAR, ST2, GG1 and GG2 could have diverged from the common ancestral line after ST1, which also diverged soon after NAS. This implies that in the African/Latin American group, isolates NAS from Nassarawa would have first diverged from the common ancestral line, however, the genetic variation between the three sub- groups within this clade is minimal. Isolate ST1 from Sabon-Tasha are more closely related to the clusters from Barnawa, Sabon-Tasha 2, Gonin-Gora 1 and 2 than to isolate NAS from Nassarawa, because they share a more recent common ancestor. Similarly, in the Asian group, the Indian isolate first diverged from the common ancestral line before isolates from Philippines and China as indicated in the phylogenetic tree.

Taenia solium from Kaduna metropolis and South America, Tanzania, Mexico, Mozambique, Cameroon and Peru appear to be very closely related despite the fact that they are highly separated geographically. Similarly, Nigeria and Latin America are not geographically close, yet this present result shows that *T. solium* circulating in Kaduna metropolis and Latin America share similar sequences, suggesting that the isolates may have been introduced into these regions from Europe during the colonial age (Nakao *et al*., 2010). It seems that *T. solium* prevalent in Africa and Latin America have the same origin and this has been attributed to domestication of pigs and the role of migration among humans from infected countries (Okamoto *et al*., 2001; Nakao *et al*., 2003).

A pairwise comparison/distance matrix of *cox 1* DNA sequences of *T. solium* from different locations in Kaduna metropolis also reiterates the close relationship between the sequences earlier inferred by the phylogenetic trees. Therefore, the phylogenetic analysis have consistently revealed similarities between closely related strains of *T. solium*. There was no distance observed between very closely related strains having no variability at all. The negligible nucleotide distance observed between some of the *T. solium* sequences in Kaduna metropolis could be attributed to the earlier observed minimal variation existing between the isolates. Similarly, the pairwise comparison of the nucleotide sequence distances between *T. solium* in Kaduna metropolis, Latin America and other parts of Africa revealed little and almost negligible distances. However, the nucleotides distances observed between *T. solium* from Kaduna metropolis, India, Philippines and China are very far apart and this is attributed to the difference in geographical location; genotypes differ according to geographical regions. This study agrees with Okamoto *et al.* (2001) that *T. solium* in Africa and South/Latin America seem to share similar sequences and as such also have the same origin,

which is believed to originate from the domestication of pigs and the human-to-pig cycle. It is believed that European activities played a key role in this (Okamoto *et al*., 2001), as European isolates of *T. solium* were extensively introduced into Latin America and Africa alongside pigs and humans (Epstein and Bichard, 1984). Genetic divergences of these African and South American samples in both *cox1* and *cyt b* suggest that populations introduced were already variable and originated from Europe (Nakao *et al*., 2002). Studies by Epstein and Bichard (1984), also asserted that pigs were introduced into Latin America by Spanish and Portuguese colonists and that Southern African pigs were actually from imported European stock.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This community based-epidemiologic survey was aimed at obtaining baseline information on the prevalence, risk factors, and genetic variation of *T. solium* present in parts of Kaduna metropolis, Nigeria, where pork is consumed. A total of 300 human blood samples were tested for cysticercosis by IgG antibody ELISA. Four hundred and fifty human faecal samples were tested for the presence of *T. solium* by coproscopic examination, coproantigen ELISA and PCR assays using highly species-specific primers. The study also assessed the sanitary conditions of some home-pig slaughter slabs which were involved in killing and sale of pork to interested individuals. Within the pig slaughter slab premises, pig carcasses were examined for the presence of *T. solium* metacestodes which are responsible for porcine cysticercosis.

This study has established human cysticercosis and taeniasis prevalence of 14.3% and 8.8% respectively in Kaduna metropolis, Nigeria. The Nassarawa/Kakuri cluster recorded the highest prevalence of 23.3% and 13.6%, while Barnawa/ Narayi recorded the least prevalence of 8 and 4% for both human cysticercosis and *T. solium*-taeniasis respectively. Pork preparation method and history of epileptic seizures were found to be associated with human cysticercosis, while factors associated with taeniasis include; hand washing practice, regular consumption of pork, main source of ready-to-eat pork, backyard pig rearing and knowledge of taeniasis/cysticercosis. Sanitary conditions of the home slaughter premises in this study were unsatisfactory and home slaughter facilities were substandard. Porcine cysticercosis

prevalence of 9.3% was detected among home slaughtered pigs by post- mortem meat examination. Among butchers operating the home slaughter, 30% taeniasis prevalence was also detected. Poor slaughter slab conditions, existence of poor knowledge of *T. solium* infections/poor hygiene and lack of meat inspection were also reported in this survey.

The *mt cox 1* sequences in this study demonstrated a very high level of homogeneity, with minimal variation among *T. solium* populations from the different study locations in Kaduna metropolis. The results also further revealed that *T. solium* in circulation in Kaduna metropolis is basically the African/ Latin American type.

6.2 Conclusion

Based on the findings of this study, human cysticercosis and taeniasis are prevalent in areas within Kaduna metropolis, Nigeria where pork is consumed and humans are in close contact with pigs. Porcine cysticercosis was also present among home slaughtered pigs examined within the study area. The high prevalence of *T. solium* infections in the study area could therefore be attributed to incriminating factors likely to exacerbate the already existing cysticercosis-taeniasis complex. Such factors include; poor hygiene, poor pig husbandry practices, lack of meat inspection particularly in home-slaughter settings and poor knowledge of *T. solium* among risk groups. Home slaughter of pigs may enhance the sale and consumption of measly pork by unsuspecting individuals, thereby contributing to the prevalence of *T. solium* infections, and subsequently creating urban foci in such households, however, this needs to be further investigated on a larger scale.

The *mt cox I* sequences of *T. solium* populations from the different study locations in Kaduna metropolis, Nigeria were highly similar and therefore it can be inferred that they share a

common ancestor/origin. Out of a total of 15 *T. solium cox 1* sequences, only 3 variant nucleotide positions were detected among 5 of the sequences indicating very minimal intra-specific variation and a highly conserved *cox 1* gene. The five sequences exhibiting the limited variation were probably introduced from a common source, because the isolates originated from within a common area; three isolates from Gonin-Gora and Sabon-Tasha exhibited same type of sequence variation, while two isolates from Nassarawa region exhibited same type of sequence variation.The findings of this study agree with the fact that *T. solium* isolates are divided into two genotypes based on geographical distributions; Asian and African/Latin American types. The inference of these genotypes from the *cox 1* genes analyzed here, indicates that *T. solium* population in Kaduna metropolis, Nigeria is the African/ Latin American type; this result was highly supported by the high bootstrap values and similar topologies of the Bayesian, NJ and ML phylogenetic trees.

6.3 Recommendations

1. Appropriate and effective community based intervention needs to be conducted in the affected areas by Kaduna State Ministry of Health e.g. Mass deworming of the populace.

2. Kaduna State Ministry of Health should intensify awareness on proper cooking of pork meat before consumption and discourage consumption of roasted meat.

3. Local Government Health authorities should educate the populace on the link between consumption of measly pork and development of taeniasis, cysticercosis and eventually epilepsy.

4. Home slaughter of pigs should be considered a potential component of intervention and control programs in order to equip individuals involved with a better understanding of the risks associated with animal husbandry and human behavioral practices.

5. There is the need to further investigate the role of home slaughter in creating urban foci for cysticercosis and taeniasis on a larger scale.

6. Further analysis of *T. solium* DNA from other pig raising areas in Kaduna State is important to obtain genetic maps of the parasite in Kaduna State.

7. Further studies on the intra-specific variation of *T. solium* in Kaduna State and other pig raising regions in Nigeria are recommended using other DNA markers, including whole genome markers such as microsatellites.

8. There is the need to establish a link between existing intra-specific variation of *T. solium* in the study area, with infectivity/pathogenicity of the parasite and clinical manifestation of *T. solium* diseases as is peculiar to the study area in both humans and pigs.

6.4 Contribution to Knowledge

Findings from this survey contribute to data on human cysticercosis prevalence and risk factors in Nigeria where the disease is under recognized and adequate information is lacking. To the best of our knowledge, this is the first study conducted so far, on taeniasis-cysticercosis complex within Kaduna metropolis, with a focus on both primary and secondary hosts, therefore, the findings of the study proves valuable for planning effective prevention

and control strategies in Nigeria as a whole. Studies on genetic diversity or variation in *T. solium* from Kaduna State have not been previously examined and there is no record of such studies in Nigeria. This study is therefore one of the first notable applications of the use of gene markers in genotyping *T. solium* in Nigeria, therefore providing basic information about the *T. solium* genotype in Kaduna, Nigeria, which is useful for diagnostic and vaccine studies. The *mt cox 1 T. solium* sequences (Kaduna Metropolis, Nigeria) obtained from this study have been successfully deposited in the NCBI GenBank as a library of sequences from Nigeria for reference purposes for other researchers; the GenBank accession numbers accorded range from KM030022.1- KM020025.1. This study has therefore provided easily assessable genetic information on *T. solium* from Nigeria (providing useful information for use in primer synthesis), which prior to now, has not been available on the NCBI GenBank.

Findings from the study could serve as an important tool in evaluation of effective drug treatments, diagnostics, vaccines and interpretation of epidemiology of taeniasis and cysticercosis in Kaduna metropolis, Nigeria.

REFERENCES

Abdo, R.N.B., Sayed, S.M.A, Hussein, A.A.A. and Arafa M.I. (2010). Occurrence of *Taenia solium* and cysticercosis in man in Egypt. *Veterinary World*, 3(2) 57-60.

Adzitey, F., Teye, G.A. and Dinko, M.M. (2011). Pre and post-slaughter animal handling by butchers in the Bawku Municipality of the Upper East Region of Ghana. *Livestock Research for Rural Development*, 23:39.

Agudelo – Florez, P. and Palacio, L.G. (2003). Prevalence of *Taenia solium* antibodies in humans and in pigs in an endemic area of Columbia. *Revista de Neurologia*, 36: 706 – 709.

Allan, J.C. and Craig, P.S. (2006). Coproantigens in taeniasis and echinococcosis. *Parasitology International*, 55: S75 – S80.

Allan, J.C., Avila, G., Garcia – Noval, J., Flisser, A. and Craig, P.S. (1990). Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology*, 101 (pt 3): 473 – 477.

Allan, J.C., Mencos F., Garcia-Noval, J., Sarti, E. and Flisser, A. (1993). Dipstick dot ELISA for the detection of *Taenia* coproantigens in humans. *Parasitology*, 107 (1): 79-85.

Allan, J.C., Velasquez-Tohom, M., Garcia-Noval, J., Torres-Alvarez, R. and Yurrita, P. (1996). Epidemiology of intestinal taeniasis in four, rural, Guatemalan communities. *Annals of Tropical Medicine & Parasitology*, 90: 157–165.

Allan, J.C., Wilkins, P.P., Tsang, V.C. and Craig, P.S. (2003). Immunodiagnostic tools for taeniasis. *Acta Tropica*, 87: 87–93.

Anantaphruti, M.T., Okamoto, M., Yoonuan, T., Sagauankiat, S., Kusolsuk, T., Sato, M., Sako, Y., Waikagul, J. and Ito, A. (2010). Molecular and serological survey on taeniasis and cysticercosis in Kanchananburi province, Thailand. *Parasitology International*, 59:326-330.

Anantaphruti, M.T., Waikagul, J., Yamasaki, H. and Ito, A. (2007). Cysticercosis and taeniasis in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 38:151-158.

Anderson, J. (2010). Neurocysticercosis diagnosis and vaccine development against the tapeworm, *Taenia solium* (Master's Thesis), Uppsala University Handledare, Sweden.

Araoye, M.O. (2004). *Research Methodology with Statistics for Health and Social Sciences*. Nathadex publishers, Ilorin, Nigeria, p. 118.

Arora, D.R., and Arora, B.B (2012). *Medical Parasitology*. 3rd Edition, CBS Publishers & Distributors, Pvt. Ltd. India, p 129.

Assana, E., Zoli, P.A., Sadou, H.A., Nguekam, V.L., Pouedet, M.S.R., Dorny, P., Brandt, J. and Geerts, S. (2001). Prevalence de la cysticercose porcine dans le Mayo – Danay (Nord Cameroon) et le Mayo – Kebbi (Sud-ouest du Tchad). *Revue d' Elevage Et de Medicine Veterinaire des pays Tropicaux*, 54: 123 – 127.

Baer, J.G (1940). The origin of human tapeworms. *Journal of Parasitology*, 26: 127 – 134.

Bauman, R.W. (2006): *Microbiology*. Pearson Benjamin Cummings Publishers, San Francisco, United States of America, pp. 502-505.

Berkvens, D., Speybroeck, N., Praet, N., Adel, A., and Lesaffre, E. (2006). Estimating disease prevalence in a bayesian framework using probabilistic constraints. *Epidemiology*, 17:145-153.

Bern, C., Garcia, H.H., Evans, C., Gonzalez, A.E., Verastegui, M., Tsang, V.C.W. and Gilman, R.H. (1999). Magnitude of the disease burden from neurocysticercosis in a developing country. *Clinical Infectious Diseases,* 29:1203 – 1209.

Biu, A.A. and Hena, S.A. (2008). Prevalence of human taeniasis in Maiduguri, Nigeria. *International Journal of Biomedical and Health Sciences*, 4(1): 25 – 27.

Biu, A.A. and Ijudai, J. (2012). Prevalence and morphometric studies on porcine cysticercosis in Adamawa state, Nigeria. *Sokoto Journal of Veterinary Sciences,* 10(1): 28-31.

Blocher, J., Schmutzhard, E., Wilkins, P.P., Gupton, P.N. and Schaffert, M. (2011). A cross-sectional study of people with epilepsy and neurocysticercosis in Tanzania: clinical characteristics and diagnostic approaches. *PLos Neglected Tropical Diseases*, 5: e1185 doi: 10.1371/journal.pntd.0001185.

Bowles, J. and McManus, D.P. (1993). Rapid discrimination of *Echinococcus* species and strains using a polymerase chain reaction- based RFLP method. *Molecular and Biochemical Parasitology*, 57 (2): 231-239.

Bowles, J. and McManus, D.P. (1994). Genetic characterization of the Asian *Taenia*, a newly described taeniid cestode of humans. *American Journal of Tropical Medicine& Hygiene,* 50: 33-44.

Bragazza, L.M., Vaz, A. J., Passos, A.D. Takayanagui, O.M., Nakamura, P.M., Espindola, N.M., Pardini A. and Bueno. E.C. (2002). Frequency of serum anti-cysticercus antibodies in the population of a rural Brazilian Community (Cassia dos Coqueiros Sp) determined by ELISA and immunoblotting using *Taenia Crassiceps* antigens. *Revista do Instituto de Medicina Tropical de Sao Paulo,* 44: 7 – 12.

Cameron T.W.M. (1956). *Parasites and parasitism*. John Wiley and Sons, New York, pp 1-46.

Campbell, G., Garcia, H.H., Nakao, M., Ito, A., and Craig, P.S. (2006). Genetic variation in *Taenia solium*. *Parasitology International*, 55: 121-126.

Carabin H., Krecek, R.C. and Cowan, L.D. (2006). Estimation of the cost of *Taenia solium* cysticercosis in Eastern Cape Province, South Africa. *Tropical Medicine and International Health*, 11:906-910.

Carabin, H., Milogo, A., Praet, N., Hounton, S., Tarnagda, Z., Ganaba, R., Dorny, P., Niteina, P., Cowan, L.D. (2009). Seroprevalence to the antigens of *T.solium* cysticercosis among residents of three villages in Burkina Faso: a cross-sectional study. *Plos Neglected Tropical Diseases*, 3(11): 555.

Cardenas, F., Quiroz, H., Plancarte, A., Meza, A., Dalma, A. and Flisser, A. (1992). *Taenia solium* ocular cysticercosis: findings in 30 cases. Annals Ophthalmology, 24: 25-28.

Carpio, A. and Hauser, W.A. (2002). *Neurocysticercosis and Epilepsy*. In: Singh, G., Prabhakar, S. (Eds.), *Taenia solium* cysticercosis: from basic to clinical science. CAB International, pp 211 – 220.

Carrique-Mas, J., Iihoshi, N., Widdowson, M.A., Roca, Y. and Morales, G.(2001). An epidemiological study of *Taenia solium* cysticercosis in a rural population in the Bolivian Chaco. *Acta Tropica*, 80: 229–235. doi: 10.1016/S0001-706X(01)00161-9.

Chapman, A.V., Vallejo, K., Mossie, G., Ortiz, D., Agabian, N. and Flisser, A. (1995). Isolation and characterization of species- specific DNA probes from *Taenia solium* and *Taenia saginata* and their use in an egg detection assay. *Journal of Clinical Microbiology*, 33: 1283-1288.

Chartier, C.M. and Ndakala, N.O. (1990). Les helminthes du porc domestique en Ituri, Hait – Zaire. *Annales De La Societe Belge De Medicine Tropicale*, 70: 213 – 225.

Cheesbrough, M. (2001). Laboratory Practice in Tropical Countries. Part 1. Publ. Tropical Health Technology, printed by Cambridge University Press, UK. pp. 228-230.

Christensen, S.G. (1996). An Overview of food safety situation for the human population in African developing countries: A veterinary public approach. In: '*Veterinary Medicine impact on human health and nutrition in Africa',* R. Luidberg (Ed.), SIPATH Uppsala, pp 111-120.

Chung, A. (2011). *Taenia solium* online. Animal Diversity Web Accessed May 06, 2014 at http://animaldiversity.ummz.umich.edu/accounts//.

Collins, D.W. and Jukes, T.H. (1994). Rates of transition and transversion in coding sequences since the human-rodent divergence. *Genomics,*20 (3): 386-396.

Collomb, H., Lariviere, M. and Phillips, Y. (1964). Foyer de la cysticercose de la Basse Casamance (Senegal). *Bulletin Et Memoires De La Faculty du Medecine Pharmacologica de Dakar,*12: 148 – 155.

Coral – Almeida, M., Rodriguez – Hidalgo, R., Celi-Erazo, M., Garcia, H.H., Rodriguez, S., Devleesschauwer, B., Bernitez – Ortiz, W., Dorny, P. and Praet, N. (2014). Incidence of human *Taenia solium* larval infections in an Ecuadorian endemic area: implications for disease burden assessment and control. *Plos Neglected Tropical Diseases,* 8(5): 2887.

Costamagna, S., Vis ciarelli, E. and Casas, N. (2002). Epidemiolagia de las parasitosis en Bahia Blanca (Provincia de Buenos Aires) Argentina 1994/1999 *Parasitologia Latinoamericana*, 57: 103 – 110.

Coulibaly, N.D and Yameogo, K.R. (2000). Prevalence and control of zoonotic diseases: collaboration between public health workers and veterinarians in Burkina Faso. *Acta Tropica,* 76:53 – 57.

Coyle, C.M. and Tanowitz, H.B. (2009). Diagnosis and treatment of neurocysticercosis. *Interdisciplinary Perspectives on Infectious Diseases*, 2009:180742.

Craig, P. S., Rogan, M.T. and Allan, J.C. (1996). Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. *Advances in Parasitology*, 38: 170-250.

Crompton, D.W.T. (1999). How Much human helminthiasis is there in the world? *Journal of Parasitology,* 85: 397 – 403.

Cruz, M., Davis, A., Dixon, H., Pawlowski, Z.S. and Proano, J. (1989). Operational studies on the control of *Taenia solium*-taeniasis/cysticercosis in Ecuador. *Bulletin of the World Health Organization,* 67: 401 – 407.

Da Silva, M.R.M., Uyhara, C.N.S., Silva, F.H., Espindola, M.N., Politi, MD, Vaz, A.J., Meirelles, F.V. and Mala, A.A.M. (2012). Cysticercosis in experimentally and naturally infected pigs: parasitological and immunological diagnosis. *Pesquisa Veterinaria Brasileira, 32*(4):297-302.

De Arruda Pinto, P., Pereira, L., Manuel, L.G., Vaz, A. and Mutuko, K.P. (2002). Cysticercosis occurrence and sanitary risks in groups of inspected and non-inspected swine in Brazil. *Parasitolagia Latino Americana,* 57: 129 – 133.

De Queiroz, A. and Alkire, N.L. (1998). The phylogenetic placement of *Taenia* cestodes that parasitize humans. *Journal of Parasitology,* 84:379-383.

Deckers, N. and Dorny, P. (2010). Immunodiagnosis of *Taenia solium* taeniasis/cysticercosis: review. *Trend in Parasitology,* 26: 137-144.

Del Brutto, O.H., Sotelo, J., Aguirre, R., Diaz-Calderon, E., Alarcon, T.A. (1992). Albendazole therapy for giant subarachnoid cysticerci. *Archives of Neurology*, 49:535- 538.

Diaz, F., Garcia, H.H., Gilman, R.H., Gonzales, A.E., Castro, M., Tsang, V.C., Pilcger, J.B., Vasquez, L.E., Lescano, M. and Carcamo, C. (1992). Epidemiology of taeniasis and cysticercosis in a Peruvian village. *American Journal of Epidemiology*, 135: 875-882.

Donald, P.M. (2006). Molecular discrimination of taeniid cestodes. *Parasitology International*, 55:S31-S37.

Dorny, P., Brandt, J. and Geerts, S. (2004). Immunodiagnostic approaches for detecting *Taenia solium.Trends in Parasitology*, 20: 259-260.

Dorny, P., Brandt, J. and Geerts, S. (2005). Chapter 4. Detection and Diagnosis. In: K.D. Murrell (Ed.) WHO/FAO/OIE Guidelines for Surveillance, Prevention and control of taeniasis and cysticercosis. Paris, OIE, pp 45-55.

Dumas, M., Grunitzky, K., Belo, M., Dabis, F., Denian, M., Bouteille, B., Kassan kogno, Y., Catanzano, G. and Prestre – Alexandre, M. (1990). Cysticercosis et neurocysticercose: enquête épidémiologique dans le nord du Togo. *Bulletin De La Societe De Pathologic Exotique*, 83: 263 – 274.

Dumas, M., Grunitzky, K., Dabis, F., Deniau, M., Bouteille, B., Belo, M., Prestre – Alexandre, M., Catanzaro, G., Darda, M. L. and D' Almeida, M. (1989). Epidemiological study of neuro – cysticercosis in West Africa. *Acta Leid*, 57: 191 – 196.

Durga, D.J., Mahendra, M., Vang Johansen, M., Willingham, A.L. and Sharma, M. (2003). Improving meat inspection and control in resource-poor communities: the Nepal example. *Acta Tropica*, 87(1): 119.

Eberly, M.D., Soli, E.K., Bannister, S.P., Tavaf – Motamen, H. and Scott, J.S. (2008). Isolated cardiac cysticercosis in an adolescent. *Paediatric Infectious Diseases Journal*, 27: 369 – 371.

Ebersberger, I., Metzler, D., Schwarz, C. and Pabbo, S. (2002). Genome wide comparison of DNA sequences between humans and chimpanzees. *American Journal of Human Genetics*, 70 (6): 1490-1497.

Engels, D., Urbani C., Belotto, A., Meslin, F. and Savioli, L. (2003). The control of human neurocysticercosis: which way forward? *Acta Tropica*, 87: 177 – 182.

Eom, K.S and Rim, H.J. (1993). Morphological Description of *Taenia asiatica sp*. *Korean Journal of Parasitology*, 31: 1-6.

Eom, K.S., Chai, J., Yong, T., Min, D., Rim, H., Kihamia, C., and Jeon, H. (2011). Morphologic and genetic identification of *Taenia* tapeworms in Tanzania and DNA Genotyping of *Taenia solium*. *Korean Journal of Parasitology, 49* (4): 399-403.

Epstein, H. and Bichard, M. (1984). Pig. In: *Evolution of domesticated animals* (Ed. Mason, I.L.), Longman, London, pp.145-161.

Espinoza, B., Ruiz-Palacios, G., Tovar, A., Sandoval, M.A., Plancarte, A. and Flisser, A. (1986). Characterization by enzyme-linked immunosorbent assay of the humoral immune response in patients with neurocysticercosis and its application in immunodiagnosis. *Journal of Clinical Microbiology*, 24:536-541.

Fain, A. (1997). Human helminthic infections, In: Janssens, P.G., Kivits, M. and Vuylsteke J. (Eds). Health in Central Africa since 1885; past, present and future. *King Baudouin Foundation*, 1: 485 – 500.

Faleke, O.O. and Ogundipe, G.A.T. (2003). *Taenia solium* cysticercosis and human taeniosis in Oyo State, Nigeria. *Nigerian Veterinary J*ournal *24,* (3): 60-64.

Fleury, A., Gomez, T., Alvarez, I., Meza, D., Huerta, M.I., Chavarria, A., Carrillo – Mezo, R.A., Loyd, C., Dessein, A., Preux, P.M., Dumas, M., Larralde, C., Scuitto, E. and Fragoso, G. (2003). High prevalence of calcified silent neurocysticercosis in a rural village in Mexico. *Neuroepidemiology*, 22: 139 – 145.

Flisser, A. (1994). Taeniasis and cysticercosis due to *Taenia solium*. In: Sun, T. (Ed.) *Progress in Clinical Parasitology*. CRC Press. Boca Paton, Florida, pp. 77 – 116.

Flisser, A. (2006). Where are tapeworms? *Parasitology International,* 55(Suppl):S117-S120.

Flisser, A. and Gyorkos, T.W. (2007). Contribution and immunodiagnostic tests to epidemiological/intervention studies of cysticercosis/taeniosis in Mexico. *Parasite Immunology*, 29: 637-649.

Flisser, A., Reid, A., Garcia – Zepeda, I. and McManus, D.P. (1988). Specific detection of *Taenia saginata* eggs by DNA hybridisation. *Lancet*, 332: 1429 – 1430.

Flisser, A., Rodriguez, C.R. and Willingham, A.L. (2006). Control of taeniasis/cysticercosis complex future developments. *Veterinary Parasitology,* 139: 283-292.

Flisser, A., Sarti, E., Lightowlers, M. and Schantz, P. (2002). Neurocysticercosis: regional status, epidemiology, impact and control measures in the Americas. *Acta Tropica*, 87: 43 – 51.

Flisser, A., Tarrab, R., Willms, K. and Larralde, C. (1975). Immunoelectroforesis y doble inmunodifusion en el diagnostic de la cysticercosis cerebral humana. *Archives of Investigative Medicine (Mexico)*, 6: 1-12.

Flisser, A., Vazquez – Mendoza, A., Martinez – Ocana, J., Gomez – Colin, E., Leyva, R.S. and Medina – Santillan, R. (2005). Short Report: evaluation of a self-detection tool for tapeworm carriers for use in public health. *American Journal of Tropical Medicine and Hygiene*, 72: 510 – 512.

Forlenza, O.V., Filho, A.H.G., Norbrega, J.P.S., Machado, L.R., Barros, N.G., Camargo, C.H.P. and Silva, M.F.G. (1997). Psychiatric manifestations of neurocysticercosis: a study of 38 patients from a neurology clinic in Brazil. *Journal of Neurology, Neurosurgery and Psychiatry*, 62(6): 612 – 616.

Foyaca-Sibat, H., Cowan, L.D., Carabin, H., Targonska, I. and Anwary, M.A. (2009). Accuracy of serological testing for the diagnosis of prevalent neurocysticercosis in outpatients with epilepsy, Eastern Cape Province, South Africa. *PLoS Neglected Tropical Diseases,* 3: e562. doi: 10.1371/journal.pntd.0000562.

Federal Republic of Nigeria (FRN) (2009). Federal Republic of Nigeria Official Gazette. Legal notice on Publication of 2006 census Final Results. The Federal Government printers, Abuja-Nigeria. 2nd February.

Garcia, H.H., Martinez, M., Gilman, R.H., Herrera, G., Tsang, V.C.W., Pilcher, J.B., Diaz, F., Verastegui, M., Gallo, C., Porras, M., Alvarado, M., Narango, J., Mirawa, E and Cysticercosis working group, Peru. (1991). Diagnosis of cysticercosis in endemic regions. *Lancet*, 338: 549 – 551.

Garcia, H.H., Araoz, R., Gilman, R.H., Valdez, J., Gonzalez, AE, Gavidia, C., Bravo, ML, Tsang, VCW, the Cysticercosis Working Group in Peru. (1998). Increased prevalence of cysticercosis and taeniasis among professional fried pork vendors and the general population of a village in the Peruvian highlands. *American Journal of Tropical Medicine and Hygiene*, 59:902-905.

Garcia, H.H., Gilman, R., Gonzalez, A.E., Pacheco, R., Verastegui, M. andTsang, V.C.W. (1999). Human and porcine *Taenia solium* infection in a village in the highlands of Cusco, Peru. *Acta Tropica,* 73: 31 – 36.

Garcia, H.H., Gonzalez, A.E., Evans, C.A.W. and Gilman, R.H. (2003): *Taenia solium* cysticercosis. *Lancet*, 362: 547-556.

Garcia, H.H., Gonzalez, A.E., Gilman, R.H., Bernal, T., Rodriguez, S., Pretell, E.J., Azuerra, O., Parkhouse, R.M.E., Tsang, V.C.W., Harrison, I.J.S. and the Cysticercosis working Group in Peru. (2002). Circulating parasite antigen in patients with hydrocephalus secondary to neurocysticercosis. *American Journal of Tropical Medicine and Hygiene*, 66: 427-430.

Garcia, H.H., Gonzalez, A.E., Gilman, R.H., Palacios, L.G., Jimenez, I., Rodrigues, S., Verastegui, M., Wilkins, P. and Tsang, V.C. (2001). Short report: transient antibody response in *Taenia solium* infection in field conditions: a major contributor to high seroprevalence. *American Journal of Tropical Medicine & Hygiene*, 6:31-32.

Garcia-Noval, J., Moreno, E., De Mata, F., Soto de Alfaro, H., Fletes, C., Craig, P.S. and Allan, J.C. (2001). An epidemiological study of epilepsy and epileptic seizures in two rural Guatemalan communities. *Annals of Tropical Medicine& Parasitology*, 95:167-75.

Gasser, R. B., Zhu, X. and McManus, D.P. (1999). NADH dehydrogenase subunit 1 and cytochrome C oxidase subunit I sequences compared for members of the genus *Taenia* (cestoda). *International Journal of Parasitology*, 29: 1965 – 1970.

Gasser, R.B. & Chilton, N.B. (1995). Characterization of taeniid cestode species by PCR-RFLP of ITS2 ribosomal DNA. *Acta Tropica*, 59: 31-40.

Gasser, R.B., Zhu, X. and Woods, W. (1999): Genotyping *Taenia* tapeworms by single-strand conformation polymorphism of mitochondrial DNA. *Electrophoresis*, 20:2834-2837.

Ghimire, L., Dhakal, S., Pandeya, Y.R., Chau Lagain, S., Mahato, B.R. and Singh, D.K. (2013). Assessment of pork handlers' knowledge and hygienic status of pig shops of Chitwan district: focusing campylobacteriosis risk factors. *International Journal of Infectious Microbiology*, 2(1); 17-21.

Gonzalez, A.E., Lama, V., Gilmar, R.H., Tsang, V.C., Pilcher, J.B., Chavera, A., Castro, M., Montenegro, T., Verastegui, M. and Miranda, E. (1990): Prevalence and comparism of serological microscopy and tongue examination for the diagnosis of porcine cysticercosis in Peru. *American Journalof Tropical Medicine and Hygiene*, 43:194-199.

Gonzalez, L.M., Montero, E., Harrison, L.J.S., Parkhouse, R.M.E. and Gárate, T. (2000). Differential diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR. *Journal of Clinical Microbiology*, 38:737-744.

Gonzalez, L.M., Montero, E., Puente, S., Lopez – Velez, R., Hernandez, M., Scuitto, E., Harrison, L., Parkhouse, R.M. and Garate, T. (2002). PCR tools for the differential diagnosis of *Taenia saginata* and *Taenia solium* taeniasis/ cysticercosis from different geographical locations. *Diagnostic Microbiology and Infectious Diseases*, 42: 243 – 249.

Gottstein, B., Tsang, V.C. and Schantz, P.M. (1986). Demonstration of species – specific and cross reactive components of *Taenia solium* metacestodes antigens. *American Journal of Tropical Medicine and Hygiene*, 35: 308 – 313.

Graber, M. and Chailloux, A. (1970). Existence au Tchad de la ladrerie porcine á*Cysticercus cellulosae*. *Revue Elevage Et De Medecine Veterinaire Des Pays Tropicaux*, 23: 263 – 274.

Guezala, M.C., Roderiguez, S., Zamora, H., Garcia, H.H., Gonzalez, A.E., Tembo, A., Allan, J.C., and Craig, P.S. (2009). Development of a species-specific coproantigen ELISA

for human *Taenia solium*-taeniasis. *American Journal of Tropical Medicine & Hygiene,* 81 (3): 433-437.

Gweba, M., Faleke, O. and Junaidu, A. (2010). Some risk factors for *Taenia solium* cysticercosis in semi intensively raised pigs in Zuru, Nigeria. *Veterinaria Italiana,* 46(1): 57-67.

Gyorkos, T.W., Camara, B., Kokoskin, E., Carabin, H. and Prouty, R. (1996). Enquête de la prévalence parasitaire chez les enfants d'age scolaire en Guinée (1995). Santé 6, 377-381.

Hancock, K., Broughel, D.E., Moura, I.N.S., Khan, A., Peniaze, N.J., Gonzalez, A.E., Garcia, H.H., Gilman, R.H. and Tsang, V.C.W. (2001). Sequence variation in the cytochrome oxidase 1, internal transcribed spacer 1 and TS14 diagnostic antigen sequences of *Taenia solium* isolates from south and central America, India and Asia. *International Journal of Parasitology*, 31: 1601-1607.

Heroin, P., Loubiére, R. and Doucet, J. (1972). Un case de cysticercose chez les maladies neurologiques en milieu hospitalier á lomé, Togo. *Annals of Medicine International*, 146: 419 – 422.

Hidalgo, R.I.R. (2007). The epidemology of *Taenia spp*. and cysticercosis in Ecuador,(Ph.D Thesis), Universiteit Gent, Belgium.

Hoberg, E., Alkire, N.I., De Queiroz, A. and Jones, A. (2001). Out of Africa, origin of the *Taenia* tapeworms in humans. *Proceedings Royal Society of London*. B 268: 781 – 787.

Hoberg, E.P. (2002). *Taenia* Tapeworms: Their biology, evolution and socio economic significance. *Microbes and Infection,* 4:859 – 866.

Holger, M., Robert, H.G., Prendergast 3,E., Castillo, J. B., Tinoco, Y.O., Garcia, H.H., Gonzalez, A.E. and Sterling 3, C.R. (2008). Nested PCR for specific diagnosis of *Taenia solium* taeniasis. *Journal of Clinical Microbiology,* 46(1): 286-289.

Houinato, D., Ramanan Kandrasana, B., Adjide, L., Melaku, Z., Josse, R., Avode, G., Dumas, M. and Bouteille, B. (1998). Seroprevalence of cysticercosis in Benin. *Transaction of the Royal Society of Tropical Medicine and Hygiene,* 92: 621 – 624.

International Task Force for Disease Eradication (1993). Recommendations of the International Task Force for Disease Eradication. *Morbidity, Mortality Weekly Report,* 42 (RR – 16): 1 – 46.

Ito, A. and Craig, P.S. (2003). Immunodiagnostic and molecular approaches for detection of taeniid cestode infections. *Trends in Parasitology*, 19: 377 – 381.

Ito, A., Nakao, M. and Wandra, T. (2003). Human cysticercosis in Asia. *Lancet*, 362: 1918 – 1920.

Ito, A., Nakao, M., Okamoto, M., Sako,Y. and Yamasaki, H. (2002): Chapter 5. Mitochondrial DNA of *Taenia solium*: from basic to applied science. In: Singh, G., Prabhakar, S. (Eds.), *Taenia solium* cysticercosis. CABI Press, Oxson, pp.47-55.

Ito, A., Yamasaki, H., Nakao, M., Sako, Y., Okamoto, M., Sato, M.O., Nakaya, K., Margono, S.S., Ikejima, T., Kassuku, A.A., Afonso, S. M.S., Ortiz, W.B., Plancarte, A., Zoli, A., Geerts, S. and Craig, P.S. (2003). Multiple genotypes of *Taenia solium*-ramifications for diagnosis, treatment and control. *Acta Tropica,* 87: 93-101.

Jia, W., Yan, H., Guo, A., Zhu, X., Wang, Y., Shi, W., Chen, H., Zhan, F., Zhang, S., Fu, B., Lttlewood, T.J. andCai, X. (2010). Complete mitochondrial genomes of *Taenia multiceps*, *T. hydatigena* and *T. pisiformis*: additional molecular markers for a tapeworm genus of human and animal health significance. *BioMed Central Genomics,* 11: 447.

Johnston, D.A. (2006). Genomes and genomics of parasitic flatworms. In: Maule, A.G., Marks, N.J. (Eds.). *Parasitic flatworms: molecular biology, biochemistry, Immunology and physiology*. CAB International, Wallingford, pp 37-68.

Joshi, D.D., Maharjan, M., Johansen, M.V., Willingham, A.L. III and Sharma, M.C. (2003). Improving meat inspection and control in resource poor communities: the Nepal example. *Acta Tropica*, 87: 119 – 12 7.

Kaliaperumal, S., Rao, V.A. and Parija, S.C. (2005). Cysticercosis of the eye in south India-A case series. *Indian Journal of Medical Microbiology,* 23 (4): 227 – 230.

Kama, K.L. (1998). Les cysticercoses porcines et bovines dans le Sud de L'Angola. In: *Abstract Book of the 9th International Conference Association of Institutions of Tropical Verterinary Medicine,* (p. 62). Harare, 14 – 18 September.

Kandil, O.M., Mahmoud, M.S., Allam, N.A.T. and El namaky, A. H. (2010). Mitochondrial Cytochrome C oxidase subunit 1 (cox 1) gene sequence of the *Hymenolepsis* species. *Journal of American Science,* 6 (12): 640-647.

Karshima, N.S., Bobbo, A.A., Udokainyang, A.D. and Salihu, A.A. (2013). *Taeniasolium* cysticercosis in pigs slaughtered in IBI Local Government Area of Taraba State, Nigeria. *Journal of Animal Sciences Advances,* 3(3): 109 – 113.

Kraft, R. (2007). Cysticercosis: An emerging parasitic disease. *American Family Physician,* 75: 91 – 98.

Krecek, R.C., Mohammed, H., Michael, L.M., Schantz, P.M., Ntanjana, L., Morey, L., Were, S.R. and Willingham, A.L. III. (2012). Risk factors of porcine cysticercosis in the Eastern Cape province, South Africa. *PLoS ONE,*7 (5):e37718. 1-5.

Kruger – Leite, E., Jalkh, A.E., Quroz, H. and Schepen, C.L. (1995). Intraocular cysticercosis. *American Journal of Ophthalmology*, 99: 252 – 257.

Kumar, A., Khan, S.A., Khan, S., Das, S.A., and Negi, K.S. (2006). A study of neurocysticercosis in the foothills of the Himalayas. *International Journal of Infectious Diseases*, 10: 79—82.

Kyvsgaard, N.C. and Murrell, K.D. (2005). Prevention of taeniosis and cysticercosis. In: WHO/FAO/OIE guidelines for the surveillance, prevention and control of taeniosis/cysticercosis, Murrell, K.D. ed. OIE, Paris, 57-72.

Machnicka, B., Dziemian, E. and Zwierz, C. (1996). Factors conditioning detection of *Taenia saginata* antigens in faeces. *Applied Parasitology,* 37:99-105.

Mafojane, N.A., Appleton, C.C., Krecek, R.C., Michael, L.M. and Willingham, A.L. (2003). The current status of neurocysticercosis in Eastern and southern Africa. *Acta Tropica,* 87: 25-33.

Maravilla, P., Soaza, V., Valera, A., Romero – Valdovirios, M., Lopez – Vidal, Y., Dominquez – Alpizar, J., Ambrosio, J., Kana, S. and Flisser A. (2003). Detection of genetic variation in *Taenia solium*. *Journal of Parasitology*, 89 (6): 1250 – 1254.

Mathis, A., and Deplazes, P. (2006). Copro- DNA tests for diagnosis of animal taeniid cestodes. *Parasitology International*, 55:S87-S90. doi: 10.1016/j. parint.200.5.11.012.

Mayta, H., Talley, A., Gilman, R.H., Jimenez, J., Verastegui, M., Ruiz, M., Garcia, H.H. and Gonzalez, A.E. (2000). Differentiating *T. solium* and *T. saginata*infections by simple hematoxylin – eosin staining and PCR – restriction enzyme analysis. *Journal of Clinical Microbiology*, 38: 133 – 137.

McManus, D. P. (2006). Molecular discrimination of taeniid cestodes. *Parasitology International*, 55:S31-S37.

Mdegela, R.H., Laurence, K. and Nonga, H.E. (2010). Occurrence of thermophilic *Campylobacter* in pigs slaughtered at Morogoro slaughter slab, Tanzania. *Tropical Animal Health Production,* 43: 83-87.

Mishra, G.S. and N' Depo, A.E. (1978). Les cystiticerques des animaux abbatus ál'abatoire de Port-Bouet (Abidjan). *Revue Elevage Et De Medecine Veterinaire Des Pays Tropicaux*. 31: 63 – 71.

Mkupasi, E.M., Ngowi, H.A. and Nonga, H. (2011). Prevalence of extra-intestinal porcine helminth infections and assessment of sanitary conditions of pig slaughter slabs in Dar es Salaam city, Tanzania. *Tropical Animal Health Production*, 43: 417-423.

Montano, S.M., Villaran, M.V., Ylquimiche, L., Figueroa, J.J. and Rodriguez, S. (2005). Neurocysticercosis: association between seizures, serology, and brain CT in rural Peru. *JAMA Neurology,* 65: 229–233. doi: 10.1212/01.wnl.0000168828.83461.09.

Mulvey, M., Aho, J.M., Lydeard, C., Leberg, P.L., Smith, M.H. (1991). Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. *Evolution*, 45: 1628-1640.

Murrel, K. (2005). Epidemology of taenisis and cysticercosis In: Murrel K (Ed.) Guidelines for the surveillance, prevention and control of taeniasis/cysticercosis. WHO/FAO/OCE, pp 27 – 43.

Murrell, K.D. (2005). Epidemiology. In: Murrell, K.D., (Ed). WHO/FAO/OIE Guidelines for the surveillance, prevention and control of taeniasis and cysticercosis. Paris, OIE (Available at http://www.oie.int/eng/publicat/ouvrages/A_taeniosis.htm). Accessed April, 2014. Pp 27-43.

Murrell, K.D. and Pawlowski, Z. (2005). Capacity building for surveillance and control of zoonotic diseases. *In*: *FAO Corporate Document Repository. FAO Animal Production and Health Proceedings, FAO/WHO/OIE Expert and Technical Consultation* (pp 37 – 46), Rome, 14 – 16 June 2005.

Mwang'onde, B.J., Nkwengulila, G. and Chacha, M. (2012). The serological survey for human cysticercosis prevalence in Mbulu District, Tanzania. *Advances in Infectious Diseases*, 2: 62-66.

Mwanjali, G., Kihamia, C., Kakoko, D.V.C., Lekule, F., Ngowi, H., Johansen, M.V., Thamsburg, S.M., Willingham, A.L. III. (2013). Prevalence and risk factors associated with human *Taenia solium* infections in Mbozi district, Mbeya region, Tanzania. *PLoS Neglected Tropical Diseases, 7*(3):e2102.

Mwape, K.E., Phiri, I.K., Praet, N., Muma, J.B., Zulu, G. Bossche, P.V., Deken, R., Speybroeck, N., Dorny, P., and Gabriel, S. (2012). *Taenia solium* infections in a rural area of Eastern Zambia- a community based study. *PLoS Neglected Tropical Diseases,* 6:1-9.

Mwape, K.E., Phiri, I.K., Praet, N., Speybroeck, N., Muma, J.B., Dorny, P.and Gabriel, S. (2013). The incidence of human cysticercosis in a rural community of Eastern Zambia. *PLoS Neglected Tropical Diseases, 7* (3):1-7.

Naik, D., Srinath, M.G. and Kumar, A. (2011). Soft tissue cysticercosis – ultrasonographic spectrum of the disease. *Indian Radiology Imaging*, 21: 60 – 62.

Nakao, M., Okamato, M., Sako, Y., Yamasaki, H., Nakaya, K. and Ito, A (2002). A phylogenetic hypothesis for the distribution of two genotypes of the pig tapeworm *Taenia solium* worldwide. *Parasitology*, 124:657-662.

Nakao, M., Sako, Y. and Ito, A. (2003). The mitochondrial genome of the tapeworm *Taenia solium*: a finding of the abbreviated stop codon U. *Journal of Parasitology*, 89: 633-635.

Nakao, M., Yanagida, T., Okamota, M., Knapp, J., Nkouawa, A., Sako, Y. and Ito, A. (2010). State of the art *Echinococcus* and *Taenia* phylogenetic taxonomy of human pathogenic tapeworms and its application to molecular diagnosis. *Infection, Genetics and Evolution*, 10: 444 – 452.

Nash, T.E., Del Brutto, O.H., Butman, J.A., Corona, T. and Delgado-Escueta, A. (2004). Calcific neurocysticercosis and epileptogenesis. *JAMA Neurology*, 62: 1934–1938. doi: 10.1212/01.WNL.0000129481.12067.06.

Ndimubanzi, P.C., Carabin, H., Budke, C.M., Nguyen, H. and Qjan, Y.J. (2010). A systematic review of the frequency of neurocysticerosis with a focus on people with epilepsy. *PLoS Neglected Tropical Diseases*, 4: e870.

Newell, E., Vyungimana, F., Geerts, S., Van Kerckhoven, I. and Tsang, V.C.W. (1997). Prevalence of cysticercosis in epileptics and members of their families in Burundi. *Trans Royal Society of Tropical Medicine,* 91: 389–391. doi: 10.1016/S0035-9203(97)90251-0.

Ngowi, H. A., Kassuku, A.A., Maeda, G.E., Boa, M.E., Carabin, H. and Willingham, A.L. III (2004). Risk factors for the prevalence of porcine cysticercosis in Mbulu District, Tanzania. *Veterinary Parasitology*, 120: 275 – 283.

Ngowi, H.A., Mkupasi, E.M., Lekule, F.P., Willingham, A.L. III. and Thamsborg, S.M. (2011). Impact of farmer education on their knowledge, attitudes, and practices in southern Tanzania: a case for *Taenia solium* control. *Livestock Research for Rural Development*, 23: 2.

Nguekam, J.P., Zoli, A.P., Vondou, L., Poudet, S.M.R., Assana, E., Dorny, P., Brandt J., Losson, B. and Geerts, S. (2003). Kinetics of circulating antigens in pigs experimentally infected with *Taenia solium* eggs. *Veterinary Parasitology*, 111: 323 – 332.

Niang, T. and Jubrin, S. (2001). Quarterly News Letter of the Nigeria Agricultural Question and Answer Service 1 (3): 1 – 6.

National Population Commission (NPC) (1998): Analytical Report of the 1991 Population Census of the Federal Republic of Nigeria, NPC Abuja-Nigeria. 3rd April.

Nwanta, J.A., Onunkwo, J.I., Ezenduka, V.E., Phil-Eze, P.O. and Egege, S.E. (2008). Abattoir operations and waste management in Nigeria. *Sokoto Journal of Veterinary Sciences, 7*(2): 61-67.

O'meara, S. (2010). Veterinary Advice Online- *Taenia* tapeworm life cycle. Retrieved (02/19/2014) from www.pet-informed-veterinary-advice-online.com/*Taenia*.html.

O'neal, S.E., Townes, J.M., Wilkins, P.P., Noh, J.C., Lee, D., Rodriguez, S., Garcia, H.C. and Stauffer, W.M. (2012).Seroprevalence of antibodies against *Taenia solium*

cysticerci among refugees resettled in United States. *Emerging Infectious Diseases,* 18(3): 431-438.

Odermatt, P. (2014). Introduction to medical parasitology. Health Care and Management in Tropical Countries, Swiss Tropical and Public Health Institute, Ecosystem Health Sciences Unit, Basel, Switzerland. pp 75-76.

Office International Des Epizooties Terrestrial Manual (2008). Cysticercosis. World Organization for Animal Health, Paris, France, pp 1216 – 1226. www.oie.int.

Okamoto, M., Ito, A., Kurosawa, T., Oku, Y., Kamya, M. and Agatsuma, T. (1995). Intraspecific variation of isoenzymes in *Taenia taeniaformis*. *International Journal of Parasitology,* 25:221-228.

Okamoto, M., Nakao, M., Sako, Y., and Ito, A. (2001). Molecular variation of *Taenia solium* in the world. *Southeast Asian Journal of Tropical Medicine & Public Health,* 32(2):90-93.

Okello, A., Ash, A., Keokhamphet, C., Hobbs, E., Khamlome, B., Dorny, P., Thomas, L. and Allen, J. (2014). Investigating a hyper-endemic focus of *Taenia solium* in northern Lao PDR. *Parasites and Vectors*, 7:134.

Okoli, C.G., Okoli, I.C., Okorondu, U.V. and Opara, M.N. (2006). Environmental and public health issues of animal food products delivery system in Imo state, Nigeria. *Online Journal of Health Allied Sciences*, 2:1-11.

Okoli, I.C., Aladi, N.O., Etuk, E.B., Opara, M.N., Anyanwu, G.C. and Okeudo, N.J. (2005). Current facts about the animal food products safety situation in Nigeria. *Ecology of Food and Nutrition*, 44: 359-373.

OMS/OPS (1993). Epidemiologia y control de la taeniasis y cysticercosis en America Latina. WHO Version 2.0, PNSP/9128.

Onah, D.N. and Chiejina, S.N. (1995). *Taenia solium* cysticercosis and human taeniasis in the Nsukka area of Enugu state, Nigeria. *Annals of Tropical Medicine Parasitology*, 89: 399-407.

Organizacio Mundial de la Salud/Organizacion Panamericana de la Salud. (1997). Informe de una reunion de consulta de la OPS/OMS Sobre el complejo taeniasis/cysticerosis. WHO HCT 1/AIE, p1 – 5.

Orita, M., Suzuki, Y., Sekiya, T. and Hayashi, K. (1989). Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics*, 5: 874-879.

Ortega – Herrera, R., Fernandez – Segura, M.E. and Gomez, I. (2004). Immigrante ecuatoriana con cefalea. *Enfermedades Infeciosas y Microbiologia Clinica,* 22: 248 – 249.

Osawa, S., Su-Z. H, Kim, C. G., Okamoto, M., Tominaga, O. and Imura, Y. (1999): Evolution of the carabid ground beetles. *Advances in Biophysics*, 36:65-106.

Overbosch, D., Oosterhuis, J.W., Kortbeck, L.M. and Garcia – Albca, E. (2002). Neurocysticercosis in Europe. In: P. Craig and Z. Pawlowski (Eds.), Cestode zoonoses: echinococcoses and cysticercosis, Amsterdam IOS Press, pp 33 – 40.

Pal, D.K., Carpio, A. and Sander, J.W.A.S. (2000). Neurocysticercosis and epilepsy in developing countries. *Journal of Neurology, Neurosurgery and Psychiatry*, 68:137-143.

Pawlowski, Z. and Schultz, M.G. (1972). Taeniasis and cysticercosis (*TaeniaSaginata*). *Advances in Parasitology*, 10:269 – 343.

Pawlowski, Z.S. (2002). *Taenia solium:* Basic biology and transmission. In: *Taenia solium* cysticercosis; from basic to clinical science. Singh G and Prabhakar S. (Eds.) CABI Publishing. Willingford, pp. 201 – 213.

Permin, A., Yelifari, L., Bloch, P., Steenhard, N.P. and Nansen, P. (1999). Parasites in cross – bred pigs in the upper East region of Ghana. *Veterinary Parasitology*, 87: 63 – 71.

Phiri, I., Ngowi, H., Afonso, S., Matenga, E., Boa, M., Mukaratirwa, S., Githigia, S., Saimo, M., Sikasunge, C., Maingi, N., Lubega, G.W., Kassuku, A., Michael, L., Siziya, S., Krecek, R.C., Noormahomed, E., Vilhena, M., Dorny, P. and Willingham III, A.L. (2003). The emergence of *Taenia solium* cysticercosis in Eastern and Southern Africa as a serious agricultural problem and public health risk. *Acta Tropica* 87:13-23.

Pinto, P.S.A., Vaz, A.J., Germano, P.M.L. and Nakamura, P.M. (2000). ELISA test for diagnosis of cysticercosis in pigs using antigens of *Taenia solium* and *Taenia crassiceps* cysticerci. *Revista do Instituto de Medicina Tropical* de*Sao Paulo*, 42:71-79.

Pouedet, M.S.R., Zoli, A.P., Nguekam, L., Vondou, L., Assana, E., Speybroeck, N., Berkvens, D., Dorny, P., Brandt, J. and Geerts, S. (2002). Epidemiological survey of swine cysticercosis in two rural communities of West-Cameroon. *Veterinary Parasitology*, 106: 45 – 54.

Prabhakaran, V., Rajshekar, V., Murrel, K.D. and Dommen, A. (2004). Confirmation sensitive immunoassay improve the serodiagnosis of solitary *Cysticercus* granuloma in Indian patients. *Transaction of Royal Society of Tropical Medicine and Hygiene*, 101: 570 – 577.

Praet, N., Verweij, J.J., Mwape, K.E., Phiri, I. Muma, J.B., Zulu, G., Lieshout, L., Rodriguez – Hidalgo, Benitez – Ortiz, W., Dorny, P. and Gabriel, S. (2013). Bayesian modelling to estimate the test characteristics of coprology, coproantigen ELISA and a novel real-time PCR for the diagnosis of taeniasis. *Tropical Medicine and International Health*, 00: 1 – 7.

Prasad, A., Gupta, R.K., Nath, K., Prodhan, S., Tripathi, M., Pandey, C.M. and Prasad, K.N. (2008). What triggers seizures in neurocysticercosis? An MRI based study in pig farming community from a District of North India. *Parasitology International*, 55: 166 – 171.

Prasad, K.N., Prasad, A., Gupta, R.K., Pandey, K.M., and Singh, U. (2007). Prevalence and associated risk factors of *Taenia solium* taeniasis in a rural pig farming community of north India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 101: 1241-1247.

Prestes-Carneiro, L.E., Freitas, S.B.Z., Zago, S.C.S.Z., Miguel, N.A., Primo, O.B., Iha, A.H., Espindola, N., and Vaz, A.J. (2006). Taeniosis- cysticercosis complex in individuals of a peasants' settlement (Teodoro Sampaio, Pontal of Paranapanema, SP, Brazil). *Memorias Instituto Oswaldo Cruz,* 101: 15-20.

Pretell, E.J., Garcia, H.H., Gilman, R.H., Saavedra, H., Martinez, M. (2001). Cysticercosis Working Group in Peru. Failure of one day praziquantel treatment in patients with multiple neurocysticercosis lesions. *Clinical Neurology and Neurosurgery*, 103: 175-177.

Preux, P.M., Melaku, Z., Druet – Cabanal, M., Avode, G., Grunitzki, E.K., Bouteille, B., Cruz, M. and Dumas, M. (1996). Cysticercosis and neurocysticercosis in Africa: current status. *Neurological Infections and Epidemiology,* 1: 63 – 68.

Proano, J.V., Madrazo, I., Avelar, F., Lopez-Felix, B., Diaz, G. and Grijalva, I. (2001). Medical treatment or neurocysticercosis characterized by giant subarachnoid cysts. *New English Journal of Medicine*, 345: 879-885.

Rabiela, M.T., Rivas, A., Rodriguez, J., Castillo, S. and Cancino, F.M. (1982). Anatomopathological aspects of human brain cysticercosis. In: Flisser A., Willms, K., Laclette, J.P., Larralde, C., Ridaura, C., and Beltran, F. eds. Cysticercosis: present state of knowledge and perspectives. New York: Academic Press, pp 179-200.

Rahalkar, M.D., Shetty, D.D., Kelkar, A.A., Kinare, A.S. and Ambardekar, S.T. (2000). The many faces of cysticercosis. *Clinical Radiology*, 55: 668-674.

Rajshekhar, V., Joshi, D.D., Doanh, N.Q., De, N.V. and Zhou, X.N. (2003). *Taenia solium* taeniasis/ cysticercosis in Asia: epidemiology, impact and issues. *Acta Tropica*, 87: 53 – 60.

Rajshekhar, V. (2004).Epidemiology of *Taenia solium* taeniasis/cysticercosis in India and Nepal. SouthEast Asian Journal of Tropical Medicine and Public Health, 35 (Suppl 1): 247-251.

Rahalkar, M.D., Shetty, D.D., Kelkar, A.B., Kelkar, A.A., Kinare, A.S. and Ambardekar, S.T. (2000). The many faces of cysticercosis. *Clinical Radiology*, 55: 668-674.

Richards, F. Jr., and Schantz, P.M. (1985). Cysticercosis and taeniasis. *New England Journal of Medicine*, 312:787-788.

Rishi, A.K., and McManus, D.P. (1988). Molecular cloning of *Taenia* genomic DNA and characterization of taeniid cestodes by DNA analysis. *Parasitology*, 97:161-176.

Roberts, L and Janovy, J. (2009). Gerald D. Schmidt and Larry S. Roberts *Foundation of Parasitology*. New York N.Y. McGraw Hill. pp. 89-154.

Rodriguez – Hidalgo., Geysen, D., Benitez – Ortiz, W., Geerts, S. and Brandt, J. (2002). Comparison of conventional techniques to differentiate between *Taenia solium* and *Taenia saginata* and an improved polymerase chain reaction – restriction fragment length polymorphism assay using a mitochondrial 12SrDNA fragment. *Journal of Parasitology*, 88: 1007 – 1011.

Rodriguez Canul, R., Fraser, A., Allan, J.C., Dominigue-Zalpizar, J.L., Argaez, RF. and Craig, P.S. (1999). Epidemiological study of *Taenia solium* – taeniasis/cysticercosis in a rural village in Yucatan State, Mexico. *Annals of Tropical Medicine and Parasitology*, 93: 57 – 67.

Saravanan, B.C., Manjunathachar, H.V., Tewan, A.K., Karthik, K., Tamilmahan, P. and Sudhakar, N.R. (2014). Prevalence of porcine cysticercosis in Bareilly, northern India. *Veterinary World*, 7:281-283.

Sarti, E., Flisser, A., Schantz, P.M., Gleizer, M., Loya, M., Plancarte, A., Avilla, G., Allan, J., Craig, P., Bronfman, M. and Wijeyaratni, P. (1997). Development and evaluation of health education intervention against *Taenia solium* in a rural community in Mexico. *American Journal of Tropical Medicine & Hygiene*, 56: 127-132.

Sarti, E., Schantz, P.M., Plancarte, A., Wilson, M., Gutierrez, I., Aguilera, J., Roberts, J. and Flisser, A. (1994). Epidemiological investigation of *Taenia solium* taeniasis and cysticercosis in a rural village of Michoacán State, Mexico. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88: 49 – 52.

Sato, M.O., Sako, Y., Nakao, M., Yamasaki, M., Nakaya, K. and Ito, A. (2006). Evaluation of purified Taenia solium glycoproteins and recombinant antigens in the serologic detection of human and swine cysticercosis. *Journal of infectious Diseases*, 194: 1783-1790.

Schantz, D.M. and Sarti, E. (1989). Diagnostic methods and epidemiological surveillance of *Taenia solium* infection. *Acta Leidensia*, 57: 153 – 163.

Schantz, P.M., Cruz, M. Sarti, E. and Pawlowski, Z. (1993). Potential eradicability of taeniasis and cysticercosis. *Bulletin Pan American Health Organization*, 27:397-400.

Schantz, P.M., Wilkins, P.P. and Tsang, V.C.W. (1998). Immigrants imaging and immunoblots: "The emergence of neurocysticercosis as a significant health

problem" *In*: W.M., Scheld, W.A. Craig and J.M. Hughes, (Eds.) Emerging Infections 2, ASM Press, Washington DC, pp. 213-242.

Scuitto, E., Fragoso, G., Fleury, A., Laceliette, J.P., Sotelo, J., Aluja, A., Vargas, L. and Larralde, C. (2000). *Taenia solium* disease in humans and pigs: an ancient parasitosis disease rooted in developing countries and emerging as a major health problem of global dimensions. *Microbes and Infections,* 2: 1875 – 1890.

Shey – Njila O., Zoli, P.A., Awah – Ndukum J., Nguekam, J.P., Assana, E., Byambas, P., Dorny, P., Brandt, J. and Geerts, S. (2003). Porcine cysticercosis in village pigs of northwest Cameroon. *Journal of Helmithology*, 77: 351 – 354.

Shogan, P.J., Yasmer, J.F. and Monson, M. (2008). Cardiac cysticercosis. *American Journal of Roentgenology*, 192: w212 – w213.

Simanjuntak, S.G. and Widarso, H.S. (2004). The current situation of *Taenia solium* taeniasis/ cysticercosis in Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 36: 240 – 246.

Somers, R., Dorny, P., Nguyen, V.K., Dang, T.C., Goddeeris, B. and Craig, P.S. (2006). *Taenia solium* taeniasis and cysticercosis in three communities in North Vietnam. *Tropical Medicine and International Health*, 11:65 – 72.

Sreedevi, C. (2013). Diagnosis of *Taenia solium* metacestode infection in pigs: A review 1:302. *International Journal of Veterinary Health Sciences and Research.* 1(3): 1 – 7.

Taico, F., Lopez, T., Gonzalez, A.E., Garcia, H.H. and Gilman, R. (2003). Epidemiologia de la cysticercosis porcina en tres caserios de la provincia de Zarumilla, Tumbes. *Revista de Investigacion Veterinaria de Peru,* 14: 166 – 173.

Tautz, D. and Renz, M. (1984). Simple DNA-sequences of *Drosophila virilis* isolated by screening with RNA. *Journal of Molecular Biology*, 172: 229-235.

Thienpont, D., De Keyser, J., Vandervelden, M. and Kageruka, P. (1959). La cysticercose cérébrale du porc. *Annales De La Societe Belge De Medecine Tropicale,*39: 507-514.

Thomas, I.R. (2004). Prevalence of human *Taenia Solium* cystercisosis in Mayo – Danay division Cameroon (Masters of Science in Tropical Animal Health). Prince Leopold Institute of Tropical Medicine, Department of Animal Health, Antwerp, Belgium, pp 1-10.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids research*, 22:4673-4680.

Traversa, D., Constanzo, F., Iorio, A., Aroch, I. and Lavy, E. (2007). Mitochondrial cytochrome c oxidase subunit 1 (cox 1) gene sequence of *Spirocerca lupi* (Nenatoda, Spirurida): Avenues for implications. *Veterinary Parasitology*, 146: 263-270.

Tsang, V.C.W., Brand, A.J. and Boyer, A.E. (1998). An enzyme –linked immunoelectrotransference blot assay by glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *Journal of Infectious Diseases*, 159: 50 – 59.

Vega, R., Pinero, D., Ramanankandrasana, B., Dumas, M., Bouteille, B., Fleury, A., Scuitto, E., Lavralde, C. and Fragoso, G. (2003). Population genetic structure of *Taenia solium* from Madagascar and Mexico: implications for clinical profile diversity and immunological technology. *International Journal of Parasitology*, 33: 1479 – 1485.

Vondou, L., Zoli, A., Nguekam, P., Pouedet, S., Assana, E., Kamga Tokam, A.C., Dorny, P., Brandt, J., and Geerts, S. (2002). Taeniasis/cysticercosis due to *Taenia solium* in the Menoua (west Cameroun). *Parasite*, 9: 271-274.

Vora, S.H., Motghare, D.D., Ferreira, A.M., Kulkarni, M.S. and Vaz, F.S. (2008.) High prevalence of cysticercosis in a rural village in western India. *Tropical Medicine and Health*, 36: 137–138. doi: 10.2149/tmh.2007-22.

Weka, R.P., Ikeh, E., Kamani, J. (2013). Seroprevalence of antibodies (IgG) to *Taenia solium* among pig rearers and associated risk factors in Jos metropolis, Nigeria. *Journal of Infections of Developing Countries*, 7(2):067-072.

White, A.C Jr., Robinson, P. and Kuhn, R.C. (1997). *Taenia solium* cysticercosis: host – parasite interactions and immune response. *Chemical Immunology*, 66: 209 – 230.

Whitehouse, D. (2014). Tapeworm evolution revealed (BBC News World Edition, Science/Nature). Retrieved from: news.bbc.co.uk/2/hi/science/nature/2040756.stm.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18: 6531-6535.

Willingham, A.L. III., De, N.V., Doanh, N.Q., Cong, L.D. and Dung, T.V. (2003). Current status of cysticercosis in Vietnam. *Southeast Asian Journal of Tropical Medicine and Public Health, 34: 35–50.*

Wohlgemut, J., Dewey, C., Levy, M., and Mutua, F. (2010). Evaluating the efficacy of teaching methods regarding prevention of human epilepsy caused by *Taenia solium* neurocysticercosis in Western Kenya. *American Journal of Tropical Medicine & Hygiene*, 82: 634–642.

Wolsteinholme, D.R. (1992). Animal mitochondrial DNA structure and evolution. *International Review of Cytology*, 141:173-216.

World Health Organization. (2002). Control of neurocysticercosis. In Fifty Fifth World Health Assembly, provisional agenda, Item 14. 2. A. 56/10.

Xu, J.M., Acosta, L.P., Hou, M., Manalo, D.L., Jiz, M., Jarilla, B., Pablo, A.O., Ovleda, R.M., Langdon, G., McGarvey, S.T., Kurtis, J.D., Friedman, J.F. and Wu, H. (2010). Seroprevalence of cysticercosis in children and young adults living in a helminth endemic community in Leyete, the Philippines. *Journal of Tropical Medicine*, 2010: 1-6.

Yamasaki, H., Nakao, M., Sako, Y., Nakaya, K., Sato, M.O., Mamuti, W., Okamoto, M and Ito, A. (2002). DNA differential diagnosis of human taeniid cestodes by base excision sequence scanning; thymine base reader analysis with mitochondrial genes. *Journal of Clinical Microbiology*, 40: 3818 – 3821.

Yamasaki, H., Allan, J.C., Sato, M.O., Nakao, M., Sako, Y., Nakaya, K., Qiu, D., Mamuti, W., Craig, P.S. and Ito, A. (2004). DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. *Journal of Clinical Microbiology*, 42: 548-553.

Zoli, A., Shey-Njila, O., Assana, E., Nguekam, J.P., Dorny, P., Brandt, J. and Geerts, S. (2003). Regional status, epidemiology and impact of *Taenia solium* cysticercosis in western and central Africa. *Acta Tropica*, 87: 35-42.

APPENDICES

Appendix I: Questionnaire form used for data retrieval from respondents

1. Sex (a) M (b) F
2. Age _____
3. Marital Status (a) Married (b) Single (c) Other
4. Occupation_____
5. Do you have a toilet system in your household _____(a) Yes (b) No
6. If yes to the question above, then what type?

   (a). Pit Latrine (b) Water system (c) Other
7. Do you and your household members practice thorough hand washing after toilet use? (a) Yes (b) No.
8. Drinking water source (a) Tap/pipe (b) Well/borehole (c) Rivers/Stream (d) Others_____
9. Do you boil your drinking water? (a) Yes (b)No
10. Do you or any member of your household have epilepsy? (a) Yes (b) No
11. Do you or your household enjoy/eat pork? (a) Yes (b) No.
12. How do you enjoy it? (a) Cooked (b) Cooked & Fried (c) Roasted (d) Other _____
13. Do you prepare it yourself or visit a commercial outlet? _____
14. Do you rear pigs within your home? (a) Yes (b) No
15. Do your pigs roam freely or are they penned up? _____
16. Do you have a vegetable farm in close proximity to your pigsty?

(a) Yes (b) No

17. Have you ever heard of cysticercosis? (a) Yes (b) No

18. Do you know how one acquires it? (a) Yes (b) No

19. Do you have an idea on how to prevent it? (a) Yes (b) No

20. Have you been recently de-wormed or on any antiparasitic treatment?

(a) Yes (b) No

Appendix II: Consent form for research participation

RESEARCH TITLE: PREVALENCE AND MOLECULAR STUDIES OF *TAENIA SOLIUM* AND ASSESSMENT OF SANITARY CONDITIONS OF SOME PIG SLAUGHTER SLABS IN KADUNA METROPOLIS, NIGERIA

Dear respondent,

I am conducting a research on the epidemiology of *Taenia solium* (pork tapeworm which causes cysticercosis and taeniasis) in Kaduna metropolis, Nigeria. Taeniasis is an infection with the adult tapeworm, while cysticercosis is caused by the larval stage of the pork tapeworm. *T. solium* infections are found to be associated with people who eat prok or are in close contact with pigs. Transmission of cysticercosis is by accidental ingestion of eggs in contaminated food or water.

I would like to seek your consent to participate in this study, if you eat pork or are in close contact with pigs. You would be briefly educated on the life cycle of the tapeworm, mode of transmission and your role in the study, to enable you decide if you want to participate in the study or not. The specimens collected and any other relevant information provided would be treated confidentially, and would not be used for any other purpose outside the intended study. Results would also be conveyed to you as appropriate at the end of the research. This research proves to be useful for future preventive, control and intervention programmes (on *T. solium* infections) which will be to the benefit of the community.

Please fill in the consent form if you wish to be a part of the study. You may also decide to drop out of the study at any point in time, as you wish.

Thank You for your anticipated cooperation.

Sincerely,

Edia-Asuke, U.A. (Mrs).

+2348023567998/ agnesasuke@gmail.com

I _____ do give my consent to participate in the above study, given the terms stated above.

_____

Sign/Date

Appendix III: Ethical Permit from Kaduna State Ministry of Health, Nigeria

MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

Independence Way,
P.M/;B 2014
Kaduna.
Kaduna State, Nigeria.

MOH/ADM/744/VOL.I/

31st May, 2013

Department of Microbiology
Ahmadu Bello University
Samaru Zaria
Kaduna state

PERMISSION TO CONDUCT RESEARCH

I have been directed to convey the Ministry's approval to EDIA-ASUKE, UREGWU AGNES a student from Department of Microbiology, Faculty of Science, Ahmadu Bello University Zaria, Kaduna State Nigeria, to conduct a research on the Topic: **"EPIDEMIOLOGY OF HUMAN CYSTICERCOSIS AND SANITARY ASSESSMENT OF PIG SLAUGHTER SLABS IN KADUNA METROPOLIS NIGERIA"** for the award of Ph.D. It is expected that necessary assistance be accorded him/her in the process of the research, please.

However, it is mandatory for the researcher to submit a copy of his/her research finding(s) to the office of the Honourable Commissioner, Ministry of Health, as soon as it is concluded. Accept the assurances of the Honourable Commissioner's highest regards.

F. A. KURAH (MRS)
SECRETARY, ETHICAL COMMITTEE

Appendix IV

Bare concrete floor used as slaughter slab at one of the slaughter slab premises in Kaduna metropolis

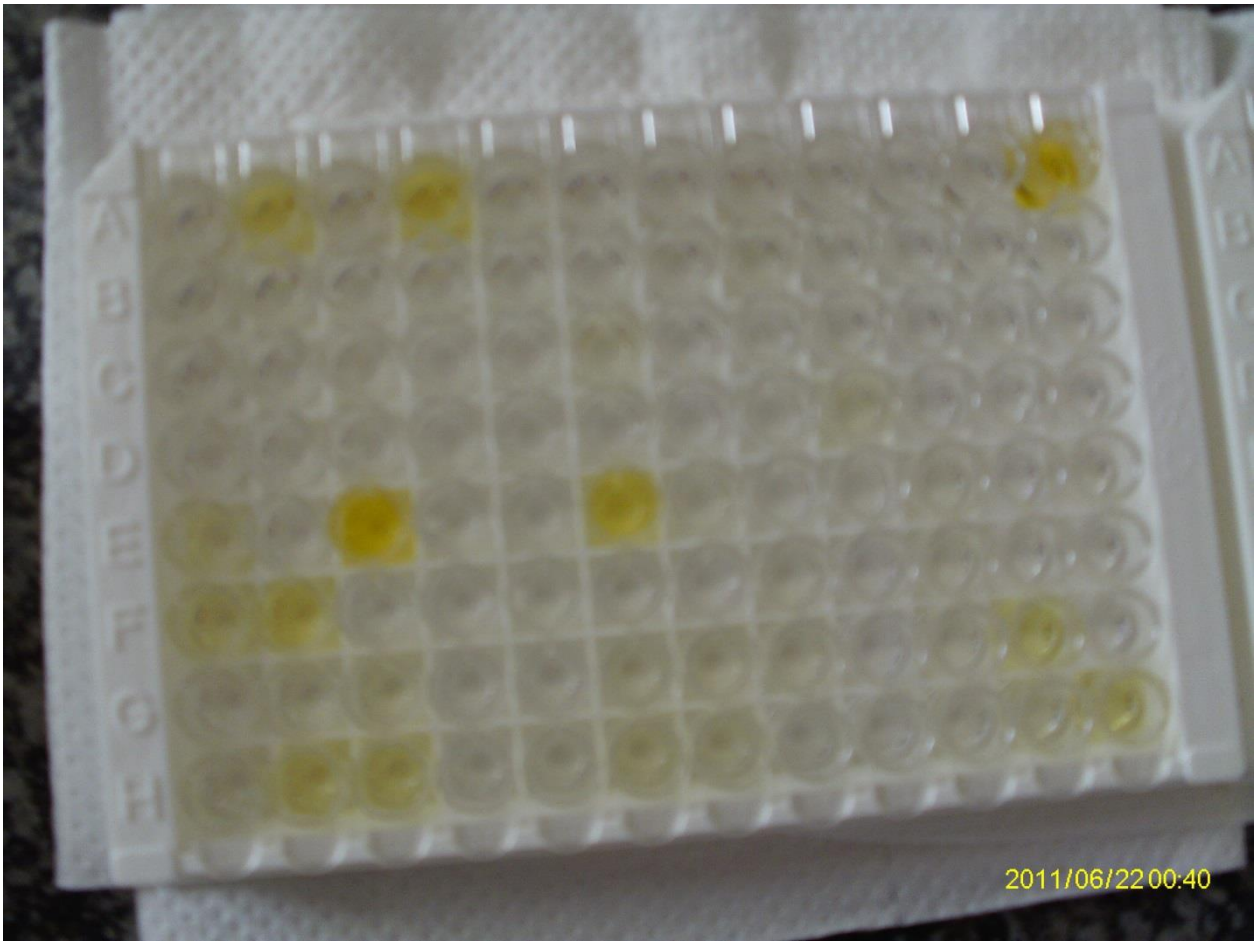
Appendix V

Absence of protective covering such as apron and hand gloves by butcher at pig slaughter premises.

Appendix VI

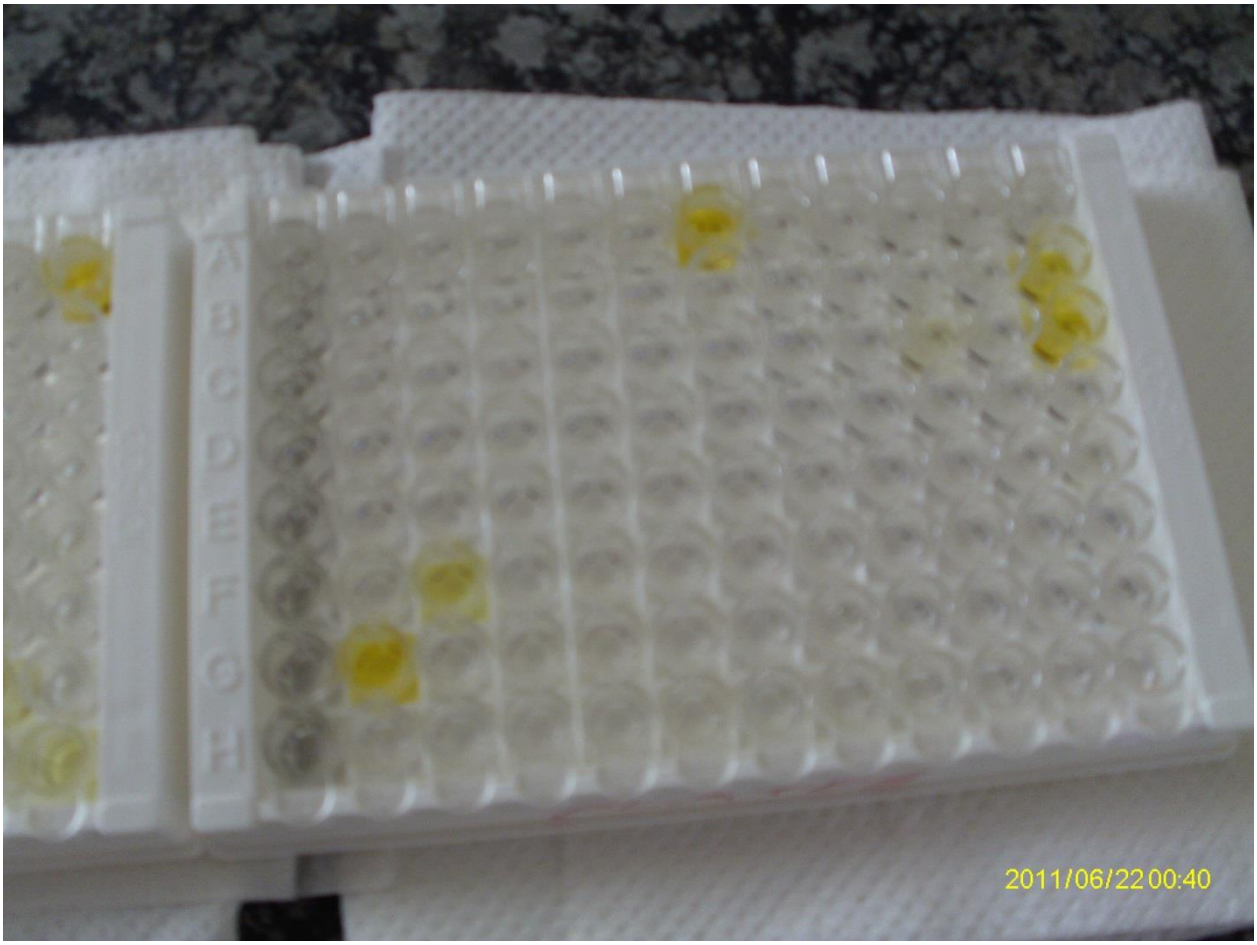
T. Solium cysticerci in infected pork

APPENDIX VII



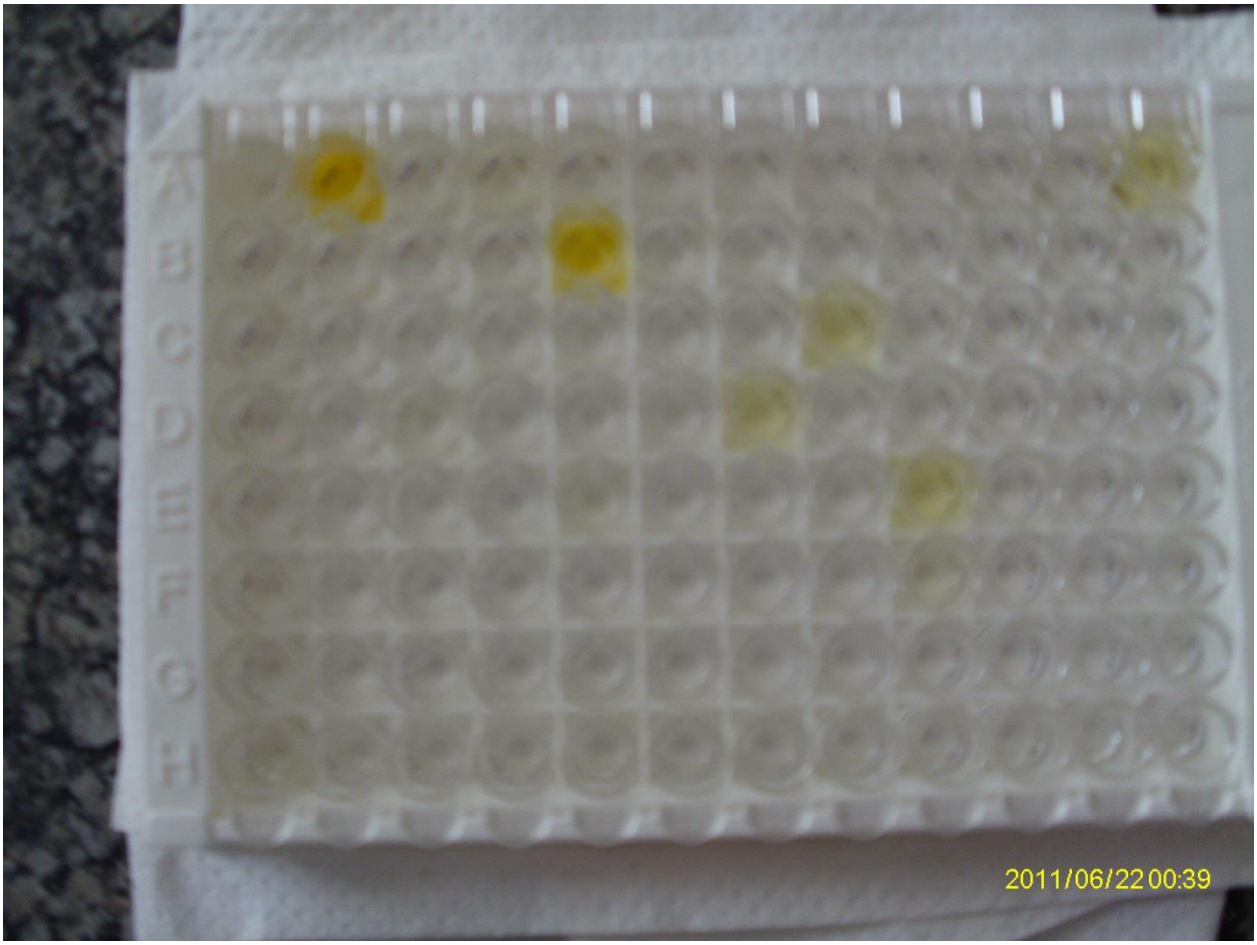
First Microwell plate with coloured reactions indicating positive results in ELISA test.

Appendix VIII



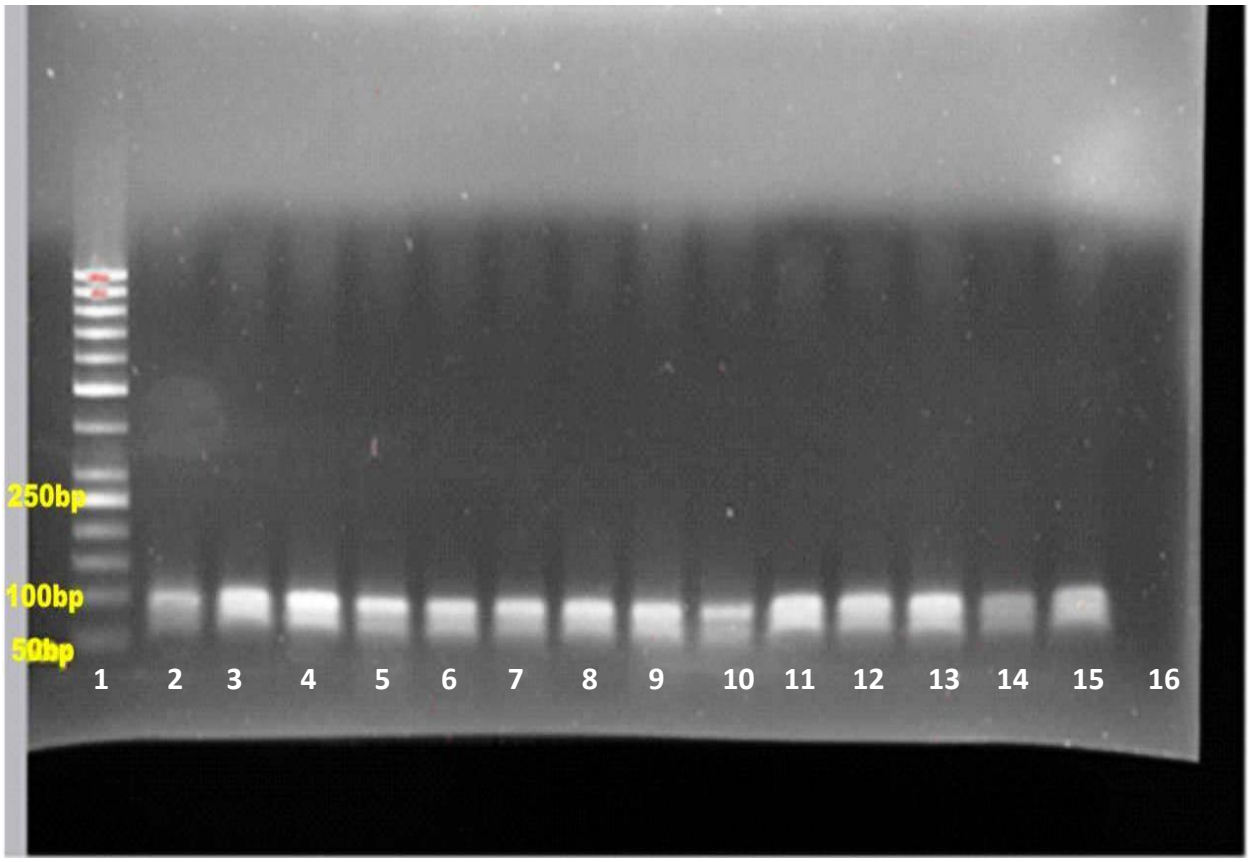
Second Microwell plate with coloured reactions indicating positive results in ELISA test.

Appendix IX



Third Microwell plate with coloured reactions indicating positive results in ELISA assay.

APPENDIX X



PCR Amplification of *ITS 1* region of *T. solium* from 14 stool samples

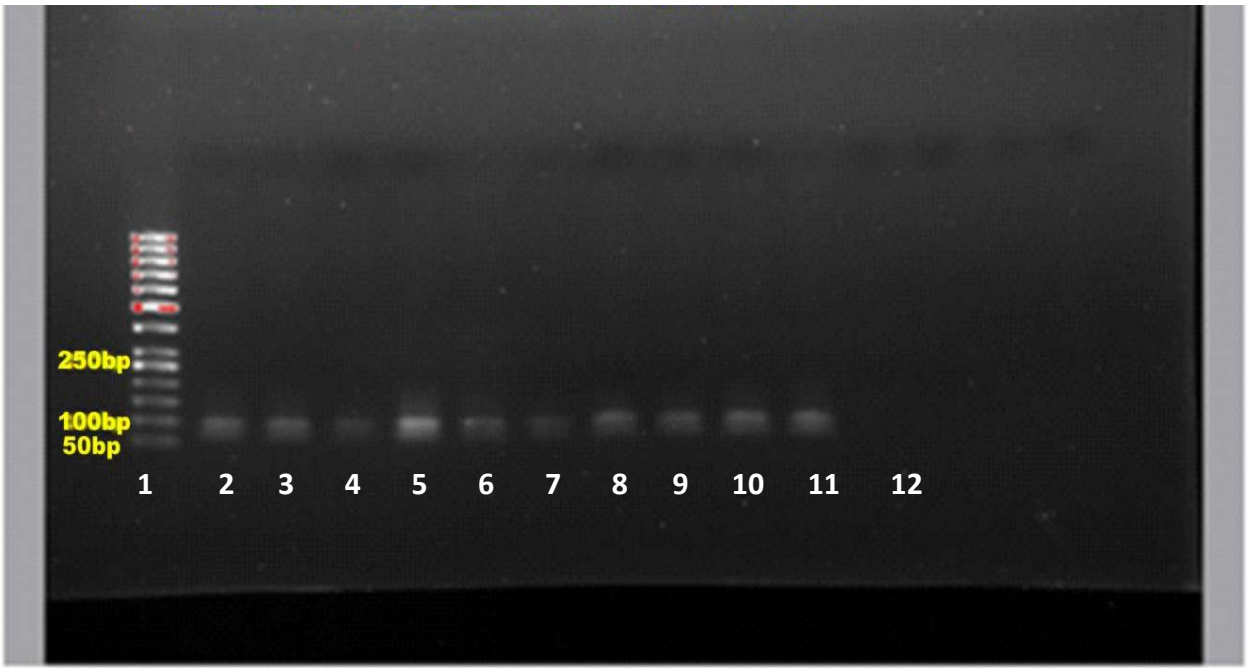
Lane 1: 50bp DNA marker.

Lane 2: Positive *T. solium* control.

Lanes 3-15: Amplicons of *T. solium* DNA.

Lane 16: Negative control

Appendix XI



PCR Amplification of *ITS 1* region of *T. solium* from 9 stool samples

Lane 1: 50bp DNA marker.

Lane 2: Positive *T. solium* control.

Lanes 3-11: Amplicons of *T. solium* DNA.

Lane 12: Negative control

APPENDIX XII

PCR Amplification of *cox 1* region of *T. solium* DNA from stool samples

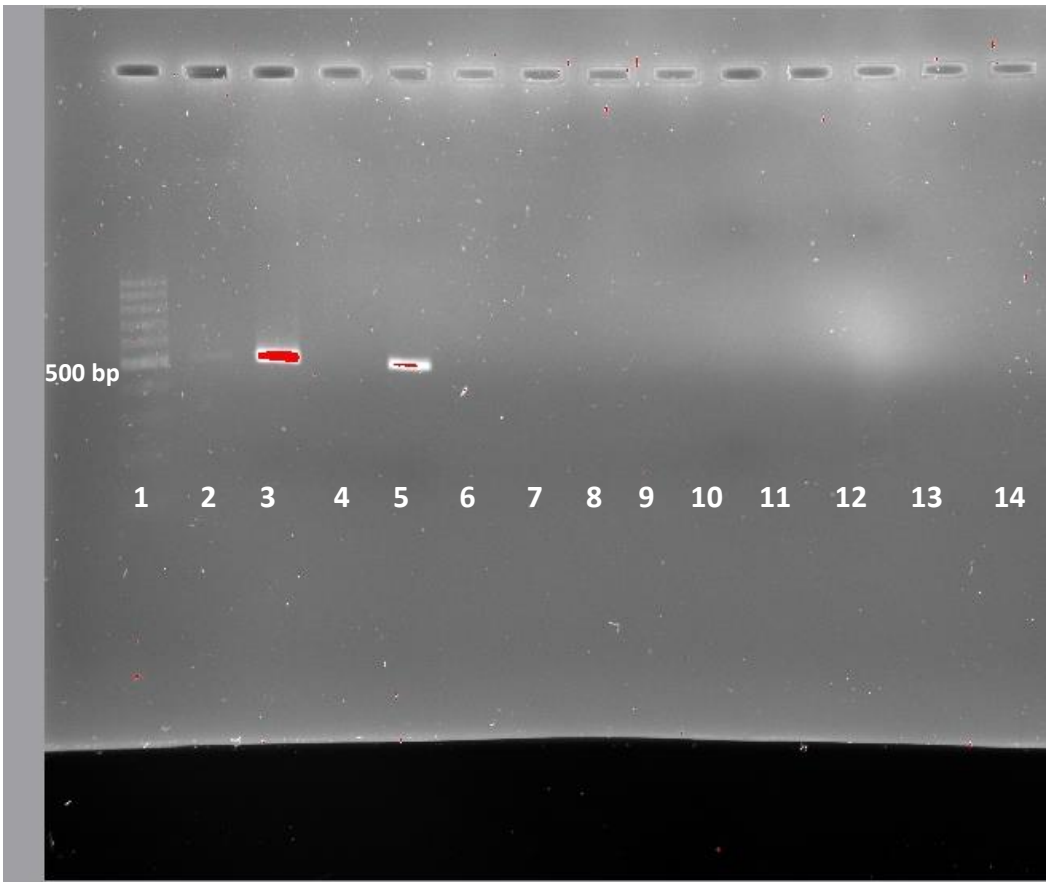
Lane 1: 100bp DNA marker.

Lanes 2-3: Amplicons of *T. solium* DNA.

Lane 4 and 5: Negative results

Lane 6: Negative control

Appendix XIII



PCR Amplification of *cox 1* region of *T. solium* DNA from some stool samples

Lane 1: 100bp DNA marker.

Lanes 2, 3 and 5: Amplicons of *T. solium* DNA.

Lanes 4, 6, 7 - 13: Negative results

Lane 14: Negative control

Appendix XIV

PCR Amplification of *T. solium cox 1* genes from eight stool samples

Lane 1: 100bp DNA marker.

Lane 2: Amplicons of *T. solium* DNA.

Lanes 3 and 8: Negative results

Lane 9: Negative control

Appendix XV

PCR Amplification of *T. solium cox 1* genes from stool samples

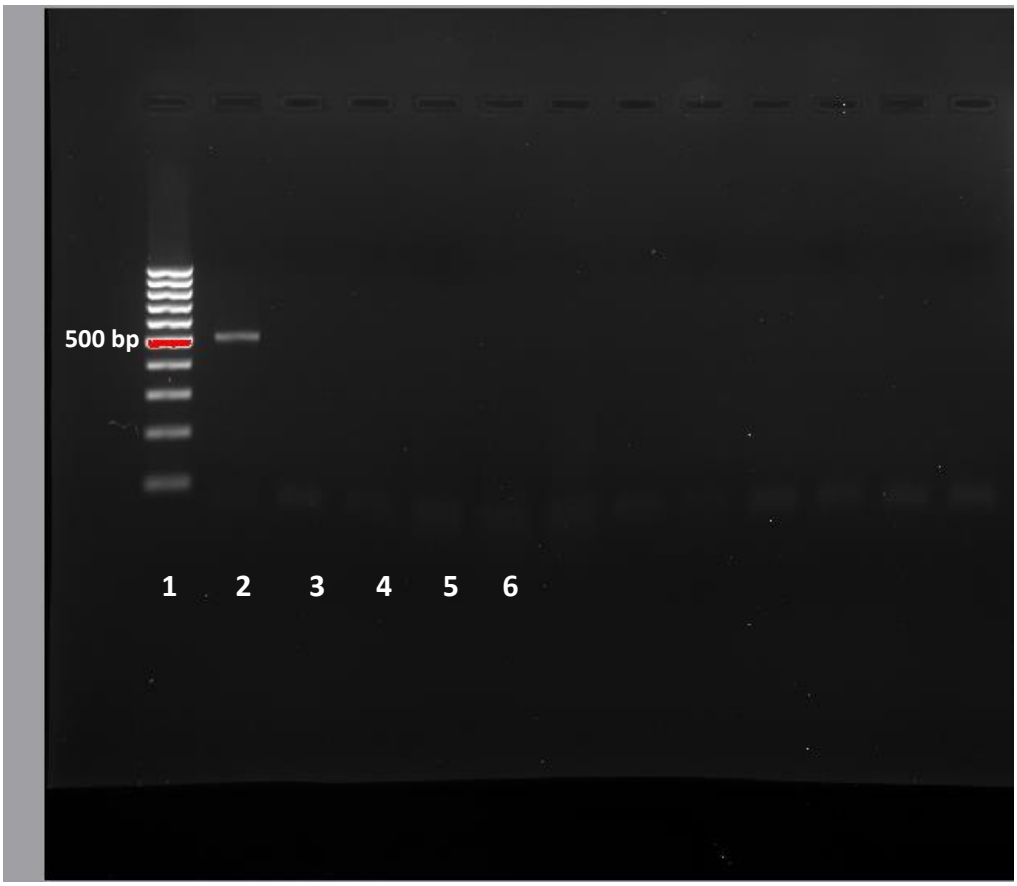
Lane 1: 100bp DNA marker.

Lanes 2-3: Amplicons of *T. solium* DNA.

Lanes 4-7: Negative results

Lane 8: Negative control

Appendix XVI



PCR Amplification of *T. solium cox 1* gene showing diagnostic 500bp band

Lane 1: 100bp DNA marker.

Lane 2: Amplicon of *T. solium* DNA.

Lane 3 - 5: Negative results

Lane 6: Negative control

Appendix XVII

PCR Amplification of *T. solium cox 1* genes from stool smples

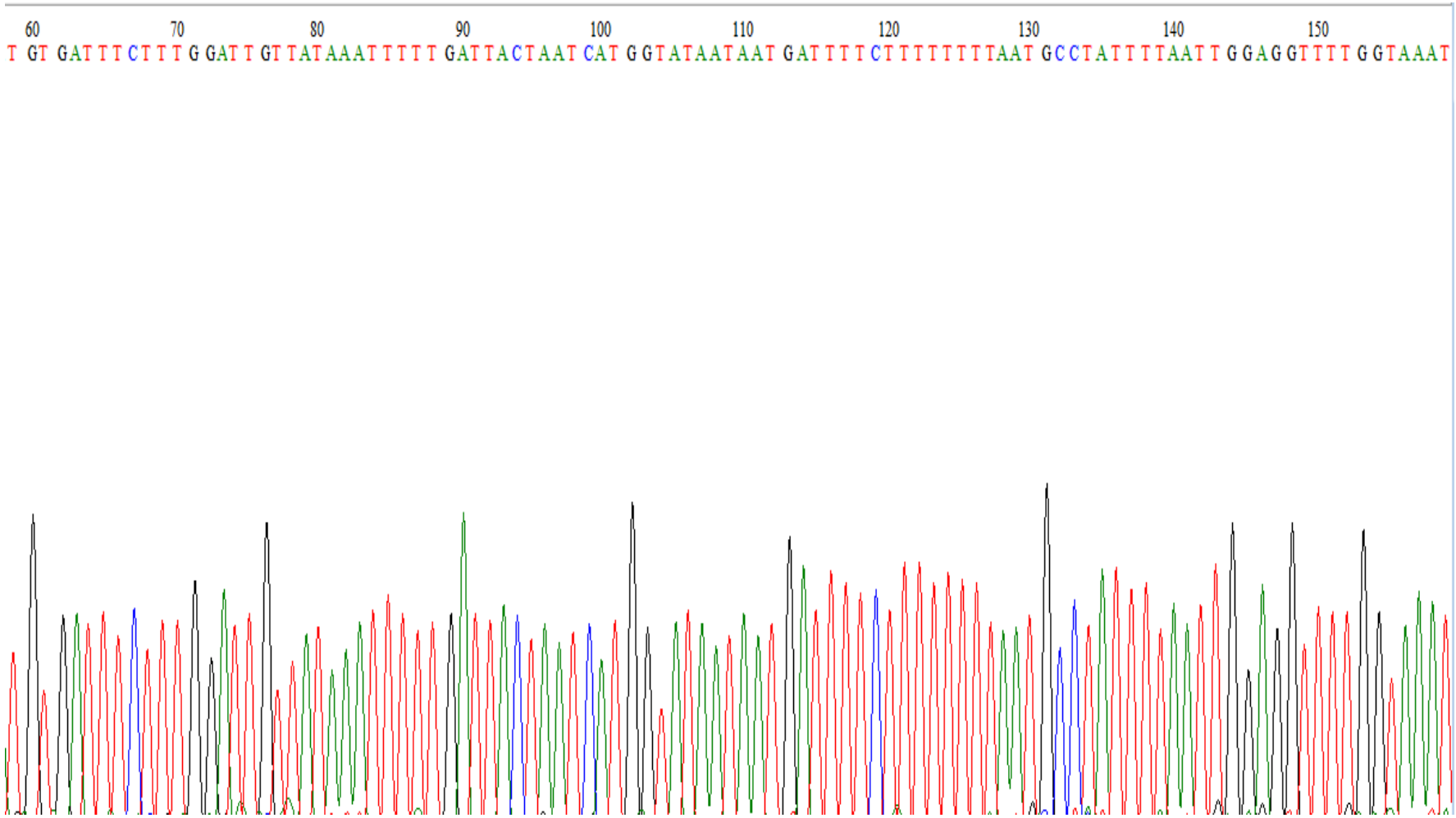
Lane 1: 100bp DNA marker.

Lanes 2,3,5 and 7: Amplicons of *T. solium* DNA.

Lane 4 and 6: Negative results

Lane 8: Negative control

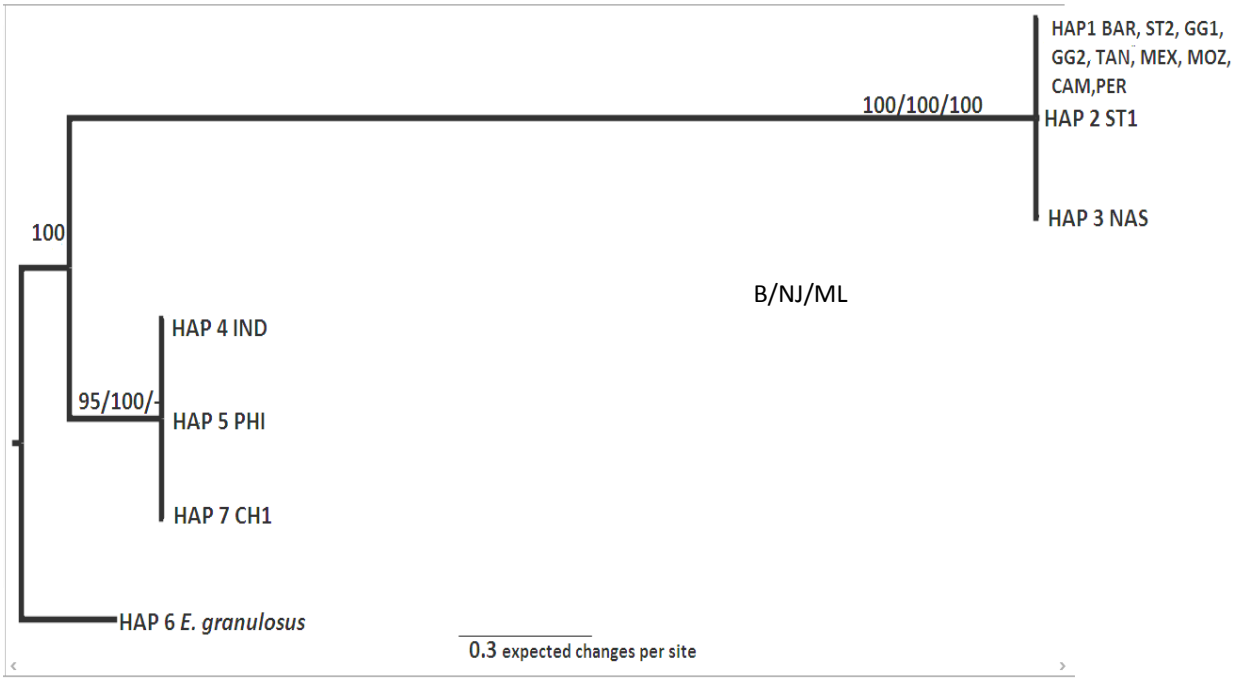
APPENDIX XVIII: Chromatogram of *cox 1* sequence of *T. solium* from Kaduna metropolis, Nigeria



Chromatogram with nucleotide peaks showing patterns of *cox 1* clean sequence of *T. solium* from Goningora, Kaduna metropolis.

Red, black, green and blue peaks represent nucleotide bases, Thymine (T), Guanine (G), Adenine (A), and Cytosine (C) respectively.

APPENDIX XIX

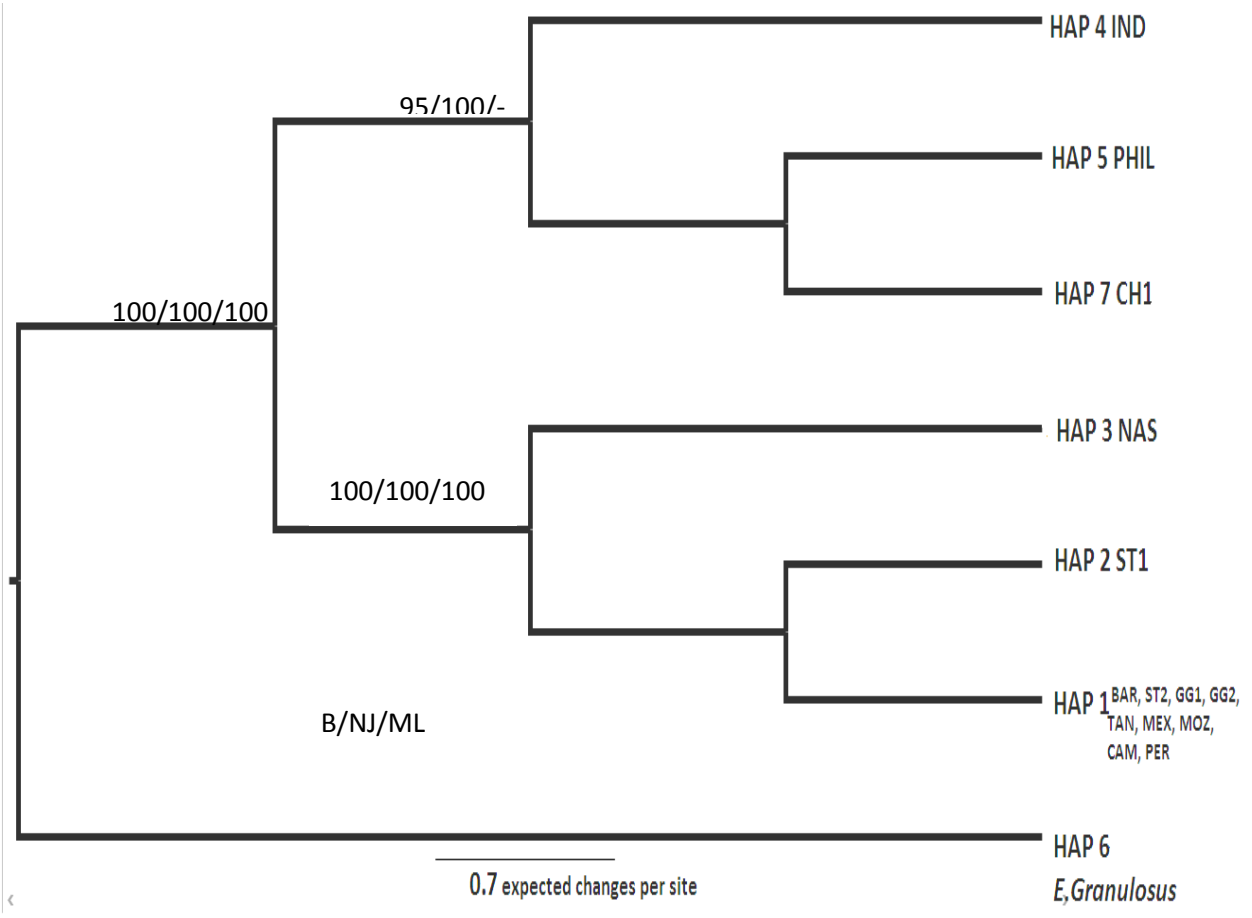


The Bayesian phylogenetic tree inferred from the mitochondrial *cox 1* gene of *T. solium* isolates from Kaduna metropolis and other parts of the world.

Key: Scale bar represents the rate of nucleotide substitutions per nucleotide site. Node values represent bootstrap confidence level of 100 based on 1000 resampling. B:Mr Bayes, ML: Maximum Likelihood, NJ: Neighbour joining boot strap values. HAP- Haplotypes

Bar-Barnawa, ST2- Sabon-Tasha 2, ST1- Sabon-Tasha 1, GG1- Gonin-Gora 1, GG2- Gonin-Gora 2, Nas-Nassarawa, TAN-Tanzania, MEX-Mexico, MOZ-Mozambique, CAM- Cameroon, PER- Peru, IND-India, PHIL-Philippines, CH1: China

APPENDIX XX



Maximum Likelihood phylogenetic tree inferred from *cox 1 T. solium* genes from Kaduna metropolis and other parts of the world.

Key: Scale bar represents the rate of nucleotide substitutions per nucleotide site. Node values represent bootstrap confidence level of 100 based on 1000 replicates. B_ Bayesian, NJ- neighbor joining, ML- maximum likelihood, HAP- haplotypes, Bar-Barnawa, ST2- Sabon-Tasha 2, ST1- Sabon-Tasha 1, GG1- Gonin-Gora 1, GG2- Gonin-Gora 2, Nas- Nassarawa, TAN-Tanzania, MEX-Mexico, MOZ-Mozambique, CAM- Cameroon, PER- Peru, IND-India, PHIL- Philippines, CH1: China