# DETECTION OF TRICHINELLA ANTIBODIES IN HUMANS AND SLAUGHTERED PIGS IN KADUNA METROPOLIS, NIGERIA

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## ABSTRACT

Trichinellosis is a parasitic zoonotic disease caused by a nematode of the genus *Trichinella*. It is cosmopolitan and affects most mammals, birds and reptiles. It is spread by eating raw, undercooked meat or meat products containing the trichinae worm. In this study, trichinellosis in patients attending three hospitals in Kaduna metropolis, Nigeria and slaughtered pigs at pig abattoir in Angwan Television, Kaduna South Local Government Area, Kaduna was detected. Risk factors associated with *Trichinella* infection were also determined using close-ended structured questionnaire administered to the patients. A total of 472 serum samples were processed and tested serologically for antibodies to *Trichinella* by ELISA using excretory-secretory (E/S) antigen. There was an overall human prevalence of 16.13% while Prevalence of 13.4% was observed in the pigs studied. Out of the 186 pig serum samples collected, 56 were from juvenile pigs while 130 were obtained from adult pigs. Adult pigs recorded a higher prevalence of 14.6% than the juvenile pigs, 10.7%. Male pigs had a prevalence rate of 12.6% which was slightly lower than that of the female (14.1%). The survey also revealed that out of 100 pig farmers and owners 90% had scavenging pigs, 98% had seen rodents around and within their piggeries, 85% had no rodent control programme, 70% feed their pigs with food leftovers, 4% had seen their pigs interact with stray pigs, 16% feed their pigs with dead animals and 10% had seen snakes around their piggeries. Only 2% of the respondents were aware of trichinellosis while 30% knew of pork-borne diseases. Significant association existed between all the risk factors in the backyard pig farms visited at $p \leq 0.001$ with trichinellosis except for seeing of rodents around and within pig pens. There was no significant association in the demography of the human population studied. However, male participants had 16.1% prevalence while female participants recorded 16.5%. Respondents within the age range 40-49 years had the highest

prevalence of 21.2%. Association of risk factors with trichinellosis was assessed and the type of meat eaten was significantly associated ($\chi^2$ = 19.192 p=0.002) with trichinellosis with beef eaters, having the highest prevalence of 29.5%, pork eaters 11.4%, poultry eaters 8.0%. Eating of hamburger/sausages revealed a significant association ($\chi^2$=4.091, p=0.043). Respondents knowledge of how *Trichinella* infects pigs was significantly associated with trichinellosis ($\chi^2$ = 6.348, p=0.042). The survey for farm management practices associated with trichinellosis was carried out using close-ended structured questionnaire administered to 100 pig farmers. The result showed the distribution of pig owners based on farm management practices as extensive system (55%), semi-intensive (40%) and 5% intensive system; Prevalence rates of 96.4%, 70.0% and 0.0% respectively were recorded and showed a significant association at $p \leq 0.001$ ($\chi^2$=32.89, p<0.001).The study has established a serological evidence of trichinellosis in humans and slaughtered pigs in Kaduna metropolis, Nigeria. ELISA test was recommended to be included in the routine tests in hospitals and in slaughterhouses for quick detection of trichinellosis.

# CHAPTER ONE

## 1.0 INTRODUCTION

Throughout much of the world, *Trichinella* spp has been found to be the causative agent of human trichinellosis, a disease that is not only a public health hazard by affecting human patients but also represents an economic problem in porcine animal production and food safety (Gottstein *et al*., 2009)

Trichinellosis, trichinosis or trichiniasis is a parasitic zoonotic disease caused by tissue –dwelling nematode of the genus *Trichinella.* It is cosmopolitan in distribution and affects a number of mammals. The organism is acquired by eating *Trichinella*-infected meat and meat products; especially eating raw or undercooked pork or wild game infected with the larvae of a species of the roundworm *Trichinella spiralis;* commonly called the trichina worm (Pozio and Murrell, 2006).

Raw meat, undercooked meat, ham, pork sausage and other meat products containing encysted Trichinella larvae cause human trichinellosis when ingested (Zimmermann,1970;Gajadhar *et al*.,2006). The cyst acts as a "nurse cell" and become digested. The released larvae invade intestinal mucosa and are then carried to tissues via intestinal lymphatic or mesenteric venules (Makendonka and Douglas, 2006).

Human Trichinellosis has variable clinical manifestations ranging from asymptomatic to fatal. In the United States, it has caused hundreds of preventable cases of illness and occasional deaths (Roy *et al*., 2001).It begins with a sensation of general body discomfort, headache, fever, chills, diarrhoea, abdominal pain, pyrexia, oedema of the eyelids and face. These present as the principal syndrome of the acute stage, which has the attendant complications of myocarditis, thromboembolic disease, encephalitis and ultimately death (Leslie *et al*., 1998; Dupouy-Camet, 2000).

Due to the predominantly zoonotic importance of the infection, the main efforts in many countries have focused on the control of *Trichinella* from the food chain. The most important source of human infection worldwide is the domestic pig (Gottstein, *et al*.,2009) and *Trichinella spiralis* is the species most adapted to domestic and wild swine (Pozio and Murrell, 2006).

*Trichinella spiralis* is a parasitic nematode (roundworm) which is found in many warm – blooded carnivores and omnivores including pigs (swine) (Gamble, 2001). The parasite was first identified under the microscope in 1835 by James Paget, who first observed the larval form of the parasite. The parasite was named and published in a report by his professor, Richard Owen, who is now credited for the discovery of the larval form of *T. spiralis* (Owen, 1835)

*Trichinella spiralis* has a long standing association with pork products around the world. The concept which many people have about the need to cook pork thoroughly is based on the risk of becoming infected with this parasite. This concern is well founded in history. A National Institute of Health report, published in 1943 found in 16.2% of the U.S population to be infected (1 out of every 6 people).This type of information led to considerable publicity on the danger of eating pork (Gamble, 2001).

*Trichinella spiralis* is most commonly associated with domestic pigs and it is most important to humans due to its worldwide (cosmopolitan) distribution and high pathogenicity (Pozio *et al.*, 2002). The other species and types have low relative infectivity for pigs and are primarily of importance because they occur in game animals (Gamble, 2001).

Exposure of domestic pigs to *Trichinella* is said to be limited to just a few risk factors which include feeding of animals with products contaminated with the parasites;

exposure to living or dead rodents or other wild life infected with trichinae; or cannibalism among pigs within an infected herd (Gamble, 2001).

While at present the incident and prevalence of infection due to *Trichinella* are reportedly low in Western Europe and the United States, common source outbreak continue to occur (Landry *et al.*, 1992; McAuley, 1992: Pozio *et al.*, 1993; Robert *et al.*, 1994), sometimes resulting in death (Schantz and Mcauley, 1991) but more often causing clinical conditions that present as baffling signs and symptoms and thus usually go undiagnosed (Pawlowski, 1983).

The re-emergence of a parasite like *Trichinella* probably results from a change in either the ecology of the host, the parasite or both, because the zoonotic parasite thrives within a continuum involving wild animals, domestic animals (pigs, for example) and man (Daszak *et al.*2000*).*Underlying causal factors which lead to these changes include encroachment from human activity in the form of agricultural intensification, environmental degradation and translocations of animals, human travel and export of food. The role played by the sylvatic *Trichinella* species is also important. As a result, the resurgence has increased, and has led to activated public health concerns and research intensification (Murrell and Pozio, 2000).

Estimation of the likely impact of trichinellosis in non-industrialized countries with reference to health, social and economic cost is very difficult. There is considerable uncertainty regarding the prevalence and the significant underestimation of incidence because of the lack of access to standard approaches to diagnosis and reporting of trichinosis in animals and humans. In the same vein, the effect of globalization in exacerbating the risk of spreading trichinellosis are moderated not only

through the movement and travel of people but also the increase in movement of livestock, wildlife and potentially infectious food products (Gottstein *et al.,* 2009).

Only sporadic information is available on the prevalence of trichinellosis in South America, Africa and Asia, but these limited reports suggest high infection rates in pigs and humans (ICT, 2012). Less than 100 human cases have been documented in Africa (Ethiopia, Kenya, Senegal and Tanzania). In all cases, infections were derived from wildlife (Willingham, 2010).

However, the increasing popularity of raising pigs and pork consumption (especially undercooked pork) in Africa has raised concern that trichinellosis could emerge as a problem. A survey of domestic pigs in Nigeria indicated 5.21% of the pigs were positive for *T. spiralis* in pigs slaughtered at abattoirs in Oyo state. All positive pigs were said to be those allowed to scavenge (Akinboade *et al.*, 1984). There is now a high demand for pork in Lagos State and its environs in Nigeria for either direct consumption or for industrial uses (Adesehinwa, *et al*., 2003).

## 1.1 Statement of research problem

Trichinellosis has been a public health threat for more than 150 years but it was not included among re-emerging zoonosis. However, its dramatic re-emergence in many areas around the world over the past 10-20 years, in spite of veterinary/public health efforts to eradicate it, necessitate its being dubbed a re–emerging zoonosis today (Murell and Pozio, 2000).

Until recently, outbreaks predominantly occurred due to consumption of *Trichinella spiralis*-infected pork in local, single-source outbreaks. However and increasingly, the mass-marketing of meat and meat products can distribute the parasite throughout a large population. In addition, the increase in cases caused by sylvatic

*Trichinella* species, either directly through game meat or through contacts with domestic animals, is increasingly important (Murell and Pozio, 2000).

*Trichinella spiralis* is ubiquitous and it is adjudged the most important etiological agent to cause disease in humans (Pozio and Murrell,2006) and in fact only one other parasite, *Toxoplasma gondii* is found more ubiquitously (Li,1991;Schantz and Mcauley,1991;Singh *et al*.,1991).

About 11 million people are infected with Trichinellosis worldwide (Pozio *et al*., 2002). The European Union (EU) spends about US$750 million yearly for meat inspection for *Trichinella* (Pozio, 1998). The economic impact of swine and human trichinellosis in the United States is also substantial (an estimated 1 billion US$ annually). These efforts are primarily for health regulatory activities to prevent infection which includes the utility costs to treat pork (freezing, heating, curing, etc) (Harr and Brown, 1992, Roberts *et aI.*1994; Bruschi *et al.,* 1999). These direct and indirect costs have made trichinellosis one of the most costly of all parasitic zoonosis (Murrell and Pozio, 2000).

Pig production and marketing in Nigeria is entirely in the hands of traditional middlemen. The government intervention is limited to areas of disease surveillance and some information gathering in a few major towns, with no direct participation or regulation. Hence the Nigerian pig production and management system is essentially indigenous with strong cultural and religious influences (Ajala and Adesehinwa, 2007).

## 1.2 Justification for the study

More pork is consumed than any other meat in the world (FAO, 2014). In 1998, it represented 39% of the world's total meat consumption compared to 26.5% for beef and 28% for poultry. Currently pork accounts for over 36% of the world meat intake. It is closely followed by poultry and beef with about 33% and 24% respectively (FAO,

2014). This positive development is mostly noticed in developed countries. Pork consumption varies widely among countries and regions with per capita intake ranging from 2kg/year in many African countries to 60kg/year in Germany and Spain (Adebisi, 2008).

Pork from domestic pigs and related products remain the most important source of *Trichinella* infection in humans, especially when pigs are raised under free–range or backyard production conditions (Gottstein *et al.,* 2009). A practice that is common and predominant in our communities.

*Trichinella* infection in humans is strongly associated with the consumption of raw or undercooked meat; thus cultural factors such as traditional dishes based on raw or undercooked meat or meat-derived products play an important role in the epidemiology of the disease (Gottstein *et al*., 2009).

Outbreak of infection with the parasitic nematode *Trichinella spiralis* occurred with great frequency around the world prior to the 1960s (Bailey and Schantz, 1990; Robert *et al.,* 1994). *Trichinella spiralis* outbreaks occur most frequently within a community or among family members (Pawlowski, 1983; Roberts *et al*., 1994).

Epidemiological studies are highly needed to determine the presence and extent of the parasite in Africa not only in pigs and humans but also in other livestock. The active surveillance for trichinellosis at slaughterhouses is not usually done in Africa (Willingham, 2010).

The prevalence of *T. spiralis* has declined considerably in reared pigs in USA. The lowest prevalence rates in domestic pigs are found in countries where meat inspection programme has been in place for many years (including countries of the

European Union notably Denmark and the Netherlands); these countries consider themselves essentially free of trichinae (Gamble, 2001). This study seeks to find out if the same scenario can be said about a developing country such as Nigeria with particular reference to Kaduna Metropolis.

In 2007, Pozio reported that reliable epidemiological information on animal and human infections is not uniformly collected around the world. Hence only a limited number of countries have implemented an official recording system for human and/or animal infections in the last 50 years. In most countries, only fragmentary information exists.

The state of knowledge on livestock production, management and marketing in Nigeria comes from studies on cattle, sheep, goats and poultry. There is dearth of literature on pig marketing, production and management in Kaduna State, Nigeria (Ajala and Adesehinwa, 2007).

## 1.3 Aim of the Study

The aim of this research was to detect *Trichinella* antibodies in humans and slaughtered pigs in Kaduna Metropolis, Nigeria.

## 1.4 Objectives of the study

The specific objectives of this study are:

1. To determine the prevalence of *Trichinella* antibodies in humans and slaughtered pigs.
2. To establish the risk factors associated with the transmission of *Trichinella* infection in pigs and in humans.

3. To identify farm management practices associated with the transmission of trichinellosis.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Trichinellosis (*Trichinella* spp)

Trichinellosis or trichinosis is a zoonotic disease caused by the parasitic nematodes of the genus *Trichinella*. It is worldwide in distribution and affects most mammals, some birds and reptiles (Soulsby, 1982; Andrews *et al*., 1995).

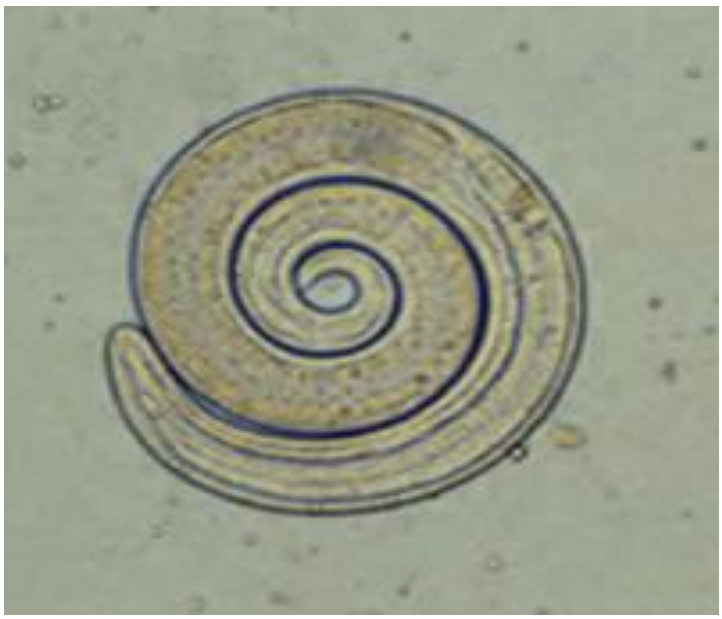


Fig 2.1: Diagram of *Trichinella spiralis* (www.google.com/search?q=trichinellaworm)

It is a food borne parasitic disease that is spread primarily by the consumption of raw or undercooked meat containing trichinae, the infective immature (larval) stage of the worm (Anon, 2009).

This nematode worm of the genus *Trichinella* and family *Trichinadae* are one of the most widespread zoonotic pathogens in the world. Infection by *Trichinella* spp. has been detected in domestic and /or wild animals of all continents with the exception of Antarctica, where there is no record of the parasite (Pozio and Murell, 2006).

*Trichinella* has been reported recently in birds and Crocodiles in Africa (Jean *et al.,* 2002). The death of a captive European brown bear was associated with the consumption of trichinous rat (Lynn and Susanne, 2004)

According to current nomenclature, there are eight distinct types of species, each of which has been assigned a number (Pozio and Rosa, 1991; Pozio *et al.*, 1992). *Trichhinella spiralis* is designated $T_1$; *T. britovi*; T3; *T.nativa* $T_2$ ; and *T.nelsoni* $T_7$ others have number designations only; $T_{5;}$ $T_6$ ; and$T_8$ although their precise species group has not been classified (Pozio *et al*., 1992) *T. pseudospiralis* ($T_4$) infects birds of prey (Wheeldon *et al.*, 1982) and other mammals such as the Tasmanian devil. One human infection with *T. pseudospiralis* has been documented (Ainsworth, 1993).

However Murrell and Pozio (2000), aver that new taxonomic scheme lists ten *trichinella* genotypes where seven are at the species level. (*T. spiralis, T nativa, T. britovi, T. pseudospiralis, T. murrelli, T. nelsoni and T. Papuae*). Five species or types of *Trichinella* are encapsulating. These include *Trichinella spiralis* T1; *T.nativa,* T2; *T.britovi,* T3; *T.nelsoni,* T7 and *T.murrelli.* The non encapsulating species include *T.papue,* and *T.zimbabwensi. T.pseudospiralis,* T4; primarily infects raptorial birds.

The cosmopolitan distribution of *T. spiralis* over five continents is unique because the parasite has been introduced passively by humans from Eurasia to North and South America, Africa and New Zealand. Murrell and Pozio (2000).

Gamble (2001), observed that *T. spiralis* has a long standing association with pork products, not only in the U.S but around the world. The concept which many people have about the need to cook pork thoroughly is based on the risk of becoming infected with this parasite.

Before now outbreaks of trichinellosis mostly resulted from consumption of *T. spiralis* – infected pork, however in recent years, species of *trichinella* infecting wildlife have caused outbreaks, either directly through game meat or indirectly through spill-over to domestic animals. Also infected herbivores (horses, cattle, sheep and goats) have in recent years been found to be the source of outbreaks (Willingham, 2010).

## 2.2 Historical Perspectives

### **2.2.1** History of Trichinellosis.

Outbreaks of infection with the parasitic nematode *T. spiralis*, occurred with great frequency in the U.S and elsewhere in the world prior to the 1960s (Roberts *et al.*, 1994; Bailey and Schantz, 1990), changing the course of history in some cases. For example, one small epidemic in 1897 resulted in the deaths of three Swedish explorers delaying by 12 years the discovery of the geographic North Pole (Capo and Despommier, 1996).

Owen (1835) stated that the parasite was first identified under the microscope in 1835 by James Paget, who first observed the larval form of the parasite. The parasite was named and published in a report by his professor, Richard Owen, who now has the credit for the discovery of the larval form of *T. spiralis.*

Murrell *et al.* (1986) opined that Trichinellosis, though dating back to antiquity was clearly evident in 1860 when Fredrick Zenker showed the transmission from animal to man.

At the beginning of the 20th century conservative estimates revealed a 2.5% infection rate in U.S pigs. Even more alarming were post mortem surveys conducted in the 1930s. A National Institute of Health report published in 1943 found 16.2% of the U.S population to be infected (1 out of every 6 people) this type of results lead to considerable publicity on the dangers of eating pork (Gamble, 2001).

Pozio (2007) observed that the condition has been seen in some parts of Africa. In Oyo state, Nigeria Akinboade *et al*., (1984) carried out a study of trichinellosis in pigs using the pepsin digestion technique and recorded a prevalence of 5.21% while a survey of trichinellosis in one hundred wild African giant rats conducted in Maiduguri in the arid region of Northern Eastern Nigeria revealed a prevalence rate of 16% (Mbaya *et al.,* 2010); and a survey carried out to determine the seroprevalence of trichinellosis in slaughtered pigs in Bodija abbatoir Oyo State Nigeria using ELISA recorded a prevalence of 15.04% (Adediran and Uwalaka, 2012). Momoh (2012), recorded a prevalence of 26.6% from a survey in Markurdi, Benue State, Nigeria.

## 2.3 Life Cycle

The life cycle of all species of the genus *Trichinella* principally comprises of two generations in the same host and includes a very broad range of host species (mammals, birds and reptiles) although only humans become clinically affected (Gottstein *et al.,* 2009).

Capo and Despommier (1996) earlier stated that there are three phases of infection and that parasites in each of the three phases of the infection cause the disease condition (La Rosa *et al*., 1993): First – through fourth stage larvae and adults in the

enteral phase; First – stage Newborn Larvae (NBL) that travel through the bloodstream and enter the tissue in the migratory phase; and first – stage larvae after they enter the tissue in the parenteral phase.

Following delivery by the gravid female worm, which lives within the intestinal mucosa of the host, NBL migrate directly into predominantly lymphatic blood vessels of the host. This allows them to be transported to the predilection sites (highly oxygenated muscles), where they penetrate them (Gottstein *et al.,* 2009).

The ingested First stage larva is freed from the surrounding tissue by the action of pepsin and hydrochloric acid in the stomach. It is then carried to the small intestine where it invades the columnar epithelium (Despommier, *et al*., 1987) shortly thereafter, about 10-28 hours after ingestion, molts four times to become the adult worm (Capo *et al*., 1984; Bailey and Schantz, 1990; La Rosa, *et al*., 1993). About 30 -34 hours after ingestion mating ensues and 5 days later NBL are shed (La Rosa *et al.,* 1993).

Despommier (1983); Wakelin and Deham, (1983) revealed that the number of newborn larvae (NBL) produced is dependent on the immune status of the infected host while Pozio *et al.* (1992); Pozio *et al. (*1993) noted that the number of NBL also depends on the infecting species of the parasite. It is estimated that 500 to 1,500 NBL are shed over the life span of an adult female worm before combination of immune responses force their expulsion from the small intestine (Wakelin and Denham, 1983). Immunity appears to be lifelong and is thought to involve the synergistic action of humoral and cell – mediated mechanisms.

In muscle nurse cells, parasites larvae can survive for years (up to 40 years in humans and over 20years in animals e.g polar bears) (Froscher *et al.,* 1988).

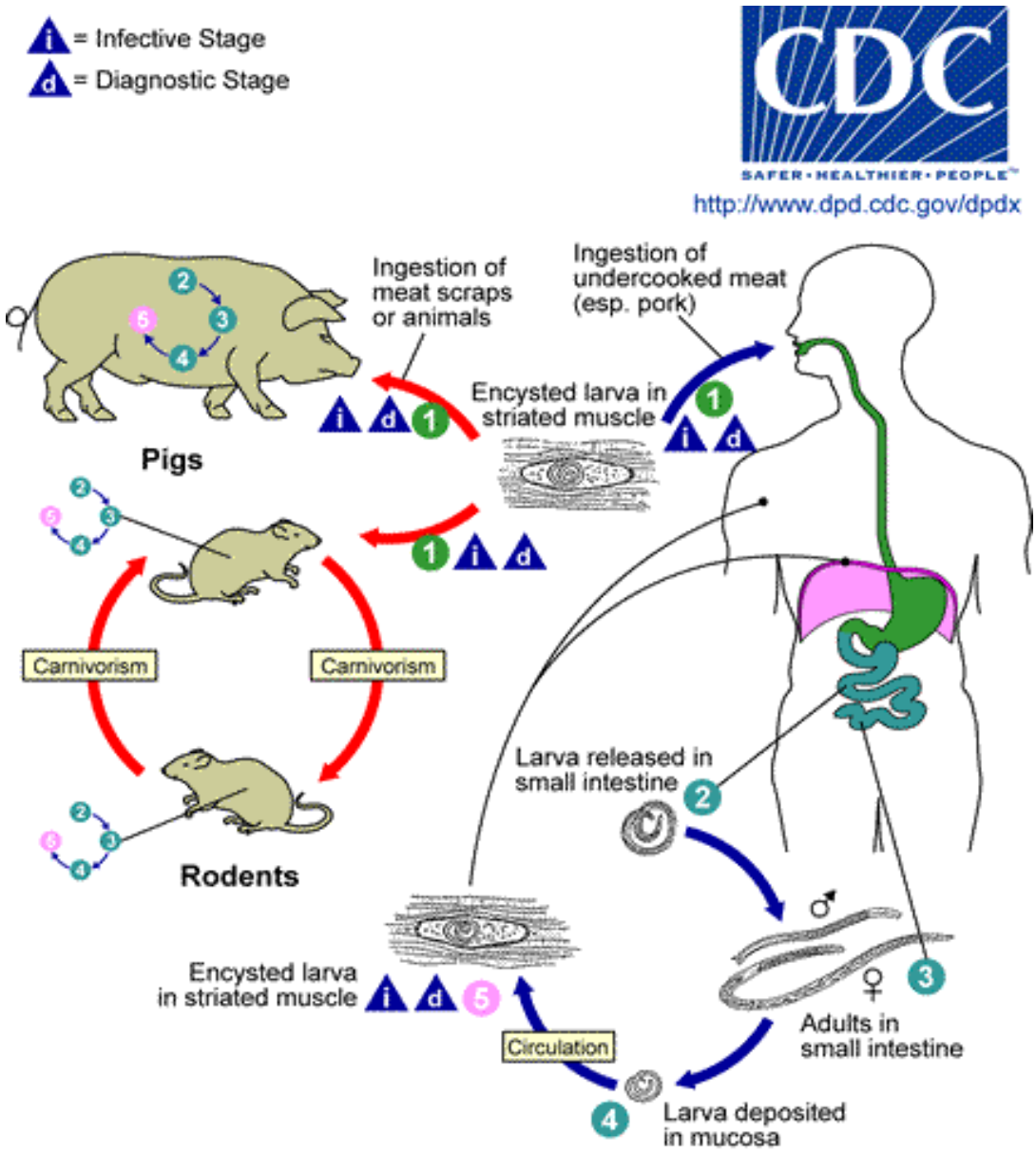



FIG.2.2 LIFE CYCLE OF *TRICHINELLA SPIRALIS*

Culled from http://www.dpd.cdc.gov/dpdx

## 2.4 Transmission of trichinellosis

*Trichinella* is transmitted from one animal to another through the ingestion of the flesh containing *Trichinella* larvae (Soulsby, 1982). In the same vein, humans get infected by eating raw or uncooked meat containing encysted *Trichinella* larvae. The cyst also called the 'nurse cell' becomes digested. The larvae released by this enzymatic action in digestion, invade the intestinal mucosa which are then carried to tissues via intestinal lymphatic or mesenteric venules (Makendonka and Douglas; 2006).

According to Zoonoses report UK (2009). The main sources of human infection are undercooked or raw meat products from horses, pigs (including wild bears) and other mammals from countries where the disease is present. The trichinae which are present in the striated muscles can be killed by thorough cooking. The disease cannot be transmitted from person -to- person.

An outbreak of trichinellosis occurred in Izmir, Turkey between January and March 2004. The outbreak was cursed by the consumption of raw meat balls made of beef deceptively minced with pork infected with *Trichinella britovi* (Akkoc *et al*., 2009)

## 2.5 Epidemiology

Carnivorism is key to understanding the epidemiology of *Trichinella* because the only way the parasite can gain entrance into a host is by being ingested along with raw or partially cooked, infected muscle tissue (unless for laboratory accidents) Capo and Despommier, 1996).

In the wild, the vast majority of all carcasses are consumed by scavengers and this infection is widespread within a given biome and throughout the world. (Dissamarn and Indrakamhang, 1985; Cobo, *et al.*, 1988).

### 2.5.1 Epidemiology in Animals

Several species of *Trichinella* are found in warm blooded carnivores and raptorial birds. In North America, there are five known species of *Trichinella.* These include *Trichinella spiralis*, *T.nativa*, *T. pseudospiralis*, *Trichinella* T-5 and *Trichinella* T-6.*T. spiralis* is most commonly associated with domestic pigs. The other species and types mentioned have low relative infectivity for pigs and are primarily of importance because they occur in game animals (Gamble, 2001).

These parasites of genus *trichinella* are present on all continents except Antarctica where no investigation of these parasites have been released or carried out so far. Most of the species with the exception of the *T.spiralis*, parasites predominantly affect wild animals. A switch from wild animals to domestic animals occurs when there is an improper management in segregating husbandry and wildlife. Domestic Cycles and the Sylvatic Cycle can function either independently from each other or interactively (Pozio, 2007).

The most common sources of meat are domesticated animal species such as cattle, pigs and poultry. Others are buffaloes, sheep and goats. In some parts of the world, camels, yaks, horses, ostriches and game animals are consumed as meat. Exotic animals such as snakes, crocodiles and lizards are also eaten in some regions of the world. But for many centuries, poultry, cattle, sheep, goats and pigs supplied man with meat and are the main sources of animal protein for humans. Pork is the most widely eaten meat in the world and it represents more than 36% of the world's meat intake whereas poultry and beef account for about 33% and 24% respectively (FAO, 2014).

*Trichinella* spp. prevalence in pigs varies from country to country, and regionally within countries. The lowest prevalence ratios in domestic swine are found in countries where meat inspection programs have been in place for many years. In some instances, countries with long–standing inspection programs consider themselves

essentially free from *Trichinella* in domestic swine. In countries of Eastern Europe, higher prevalence rates of *Trichinella* have been reported in pigs and this is supported by higher numbers of cases of human trichinellosis. Increased prevalence of *Trichinella* infection in pigs in some Balkan countries is the result of changes from large government run farms to small holdings where pigs are raised outdoors. (ICT, 2012)

Exposure of domestic pigs to *Trichinella* is limited to just a few risk factors which include: feeding of animal waste products contaminated with the parasite; exposure to living or dead rodents or other wildlife infected with trichinae; or cannibalism among pigs within an infected herd. Other means of transmission such as tail biting or coprophagy are not important (Gamble, 2001).

The domestic cycle of the transmission and the distribution of the disease in animals refer to the pattern where the focus is on the swine herd fed, e.g. uncooked pork scraps, carrion, garbage or the pigs can feed on carcasses that are not promptly removed from the farm; transmission can also become domestic via synathropic animals living near the swine herd like rats and mustelides. (Gottstein, *et al.*, 2009)

Batelli *et al*., (1993) found that accidental infection of some domesticated herbivores also occurs. In fact, in one outbreak five people died and several others are infected when an infected horse from Texas was shipped to Paris, France and consumed as "steak tartare" (Ancelle *et al*., 1985). The parasites used that herbivore as vehicle or Trojan horse to enter their host.

### 2.5.2 Epidemiology of trichinellosis in humans

*Trichinella* infection in humans is strongly associated with consumption of raw or undercooked meat. Thus cultural factors such as traditional dishes based on raw or undercooked meat or meat–derived products play an important role in the epidemiology of the disease. Hence it can then be inferred that when a population uniquely consumes

well-cooked meat, trichinellosis cases are lacking or very scarce despite persistent wildlife transmission (Pozio, 2007).

Overall, domestic pork and related products remain the most important source of *Trichinella* infection in humans especially when pigs are raised under extensive or backyard productions conditions (Gottstein *et al.*, 2009).

Another important source of infection related to local meat consumption habit is found in France and Italy where most trichinellosis cases have been due to the consumption of raw horse meat a peculiarity related to French culture and later introduced to certain areas in Italy (Boireau *et al.,* 2000; Pozio, 2001).

China and the Slovak republic have dog meat as source of infection for several foci (Dubinsky *et al.,* 2001, Liu and Boireau, 2002). In Romania, the highest prevalence of trichinellosis in humans occurred in the Transylvanian region where the local ethnic group maintains the food habit of eating raw meat (Blaga, *et al*., 2007).

In Israel, Lebanon and Syria, where the Judaic and Muslim religions forbid the consumption of pork, human cases of trichinellosis have been observed following the consumption of pork from wild boars among the Christian Arab community and some immigrants from Thailand (Eisenman and Einat, 1992; Olaison and Ljungstrom, 1992).

Pozio (2007), reported that in Algeria and Senegal, trichinellosis has been documented only in Europeans because the majority of the population is Muslim. However, the muslim population is not exempt from acquiring the disease as shown by the occurrence of large outbreak of trichinellosis from the consumption of minced beef illegally mixed with pork of unknown origin in Turkey (Akkoc *et al.*, 2009). There is no documentary information on trichinellosis in the muslim population of Nigeria but with increase in consumption of meat from herbivores which are also hosts of the

parasite and consumption of hamburgers and sausages, the likely infection with the parasite is possible.

Hunters, poachers, their relatives and their friends are at risk of trichinellosis infection when raw or undercooked meat from game animals is not tested for *Trichinella* before consumption. (CDC, 2004; Ancelle *et al.*, 2005)

The mass movement of humans with their own peculiar food practices which includes the eating of raw meat, the importation of not–controlled meat from high risk and non–endemic countries coupled with new food practices including raw meat eating resulted in outbreaks in Denmark, Germany, Italy, Spain and the United Kingdom.

Also the increasing population of international travelers has resulted in many reports of tourist who became infected with *Trichinella* while traveling or hunting in endemic areas and on reaching their home countries developed the disease. In most of these instances, diagnosis was difficult because infections appeared as isolated cases (McAuley; *et al.*, 1991; Nakamura *et al*., 2003; Pozio and Marucci, 2003; Gallardo, *et al*., 2007).

## 2.6 Clinical trichinellosis

There are so many factors that modify the clinical picture of trichinellosis, cases in a single individuals challenge clinicians who are unaware of the life cycle and epidemiology of *T. spiralis* because the symptoms mimic those of other diseases (Compton *et al*., 1993, Murrell and Bruschi, 1994).

The length of the incubation period is strongly related to the number of larvae ingested which in turn, usually determines the severity of the disease. Patients have also been found to vary in their symptoms according to time elapsed from the ingestion of infected meat and the infecting species of *Trichinella* (Kefeni and Bero, 1992, Murrell and Bruschi, 1994).

Gould, (1970), Pawlowski (1983) and Kim, (1991) reported that host immunity, age, sex and general health of the infected individual are important factors in the outcome of the disease. Principally, a *Trichinella* infection in the human host can be divided into two phases: an intestinal (or enteral) phase and a muscular (or parenteral or systemic) phase. Infections with low intensities can be asymptomatic, but burdens higher than a few hundred larvae can initially cause gastroenteritis associated with diarrhoea and abdominal pain.

The pathology is caused by the larvae released into the intestinal mucosa, which subsequently migrate to the blood vessels, by means of which they spread throughout the body until reaching the striated skeletal muscles (Gosttstein, *et al*., 2009).

### 2.6.1 Enteral Phase

The first week of the enteral phase in patients with moderate to severe infection is associated with upper abdominal pain, diarrhoea or constipation, vomiting malaise and low-grade fever all of which can vary in severity and last only a few days (Gould, 1970, Pawlowski, 1983; Murrell and Bruschi, 1994).

This clinical presentation is not restricted to trichinellosis but to other enteral disorders. (e.g food poisoning or uncomplicated indigestion), and hence easily misdiagnosed (XU,1993). Patients usually do not go for medical advice at this stage of the infection but request medical help only when the nature of the symptoms becomes parenteral (systemic) (Kociecka, 1993).

### 2.6.2 Parenteral Phase

Weeks 2-6 after onset of infection, the enteral phase still persists but symptoms abate. However in mild infection arising from the injection of low numbers of larvae in muscles symptoms of the migratory and parenteral phases are usually the first to be

detected clinically since patients experience no symptoms during the enteral phase (Gould, 1970; Pawlowski 1983; Capo and Dickson, 1996).

Mild to moderate infection can produce the following signs and symptoms: diffuse myalgia in 30 – 100% of patients, a paralysis-like state (10-35%), periorbital and or facial oedema (15–90%), conjunctivitis (55%), fever (30 to 90%), headache (75%), skin rash (15 – 65%), difficulties in swallowing (35%) or in opening the mouth, insomnia, weight loss, peripheral nerve sensations, hot flashes, coryza, hoarseness (5–20%), bronchitis (5-40%). Splinter hemorrhages of the nail beds and or the retinae, visual disturbances, and paralysis of the ocular muscles.(Capo and Despommier, 1996).

After week 2 of the parenteral phase of infection, most patients have developed specific serum antibodies against the secreted antigens of the larvae in muscle (Ljungstrom, 1983).

Patients with severe infection are often the first cases of an epidemic because they are often the first to be diagnosed based on the presentations with the classical signs and symptoms of the disease (Capo and Dickson, 1996).

Gottstein *et al*. (2009) describe two stages of clinical trichinellosis which are: Acute and Chronic – Stage Trichinellosis.

### 2.6.3 Acute – stage trichinellosis

This stage cuts across the enteral and parenteral phases of the parasites. Clinical manifestations include nonspecific signs such as uneasiness, headache, fever, fever–associated chills and occasionally gastrointestinal disorders. The fever usually last for 1-3 weeks, depending on the infection dose and severity of disease. Other clinical features includes: pyrexia, eyelid or facial oedema and myalgia.

There is the correlation between eosinophil levels and muscles enzymes such as lactate dehydrogenase and creatine phosphokinase which has been observed in patients

of this disease showing that muscle damage may have been mediated indirectly by these activated granulocytes (Ferracciolli *et al*., 1988). Later on there is the abating of eosinophilia with a resulting long–term low though there is still elevated level for a period of weeks to month.

### 2.6.4 Chronic – Stage Trichinellosis

Complication at this stage includes encephalitis and secondary infections (bronchopneumonia and sepsis). These usually result later on during infection most likely within the transitional phase up to the chronic stage of the diseases (weeks 3-4 after infection) or later. Neurological complications which are rather rare in trichinellosis patients, show multiple small sub acute cortical infarcts upon MRI with gadolinium diethlenetriamine penta-acetic acid (Gd-DTPA) enhancement (Feydy *et al*., 1996; Gottstein *et al*., 2009)

The electroencephalogram shows a total deceleration of the cortical electric activity without critical aspects. Patients with such complications show consciousness disorders or excessive excitement and commonly somnolence and apathy. Anisocorial, facial nerve paresis, and Babinsky's reflexes have also been observed in such severe cases. Most brain abnormalities visualized by computed tomography or MRI as well as the clinical signs and symptoms disappear in 4–8 weeks post infection. Pneumonia and pleuritis of bacterial etiology may appear at the last stage of the disease (Pawlowski, 1983). After glucorticosteroid treatment, the respiratory disturbances regress with days. Chronic trichinellosis may yield persistent formication, numbness and excessive sweating months or even years after the acute stage as well as impaired muscle strength and conjunctivitis, which may persist up to 10 years after infection (Harms, 1993, Pielok, 2001), in persons who had not been treated early in the acute phase of infection.

Even though clinical differences among persons infected with different species of *Trichinella* have been observed, it has not been possible to attribute these differences to the species of the pathogen because the number of infecting larvae ingested by each person was generally unknown. However, *T. spiralis* infections could be more severe than those caused by other *Trichinella* species. Nevertheless these differences should be considered with caution due to the lack of knowledge on the infecting dose for each patient (Gottstein *et al*., 2009, Bruschi and Murrell, 2002).

## 2.7 Diagnosis of trichinellosis

A number of techniques and assays have been developed to detect *Trichinella* parasites and or *Trichinella* antibodies. These techniques as useful as they may be come with a number of limitations which has led to the advancement of more newer techniques that lead to the effective detection of trichinellosis in animals and humans.

The oldest method and one still being used in some laboratories, is called the compression method or a muscle biopsy in which the piece(s) of tissue (> 1 gram) is pressed between two thick glass slides (the compressorium) and viewed under the microscope for the presence of worms. This method will reveal larvae in heavy infections. The pieces of meat need to be obtained from predilection sites of the parasites in the animals. In pork it can be collected from the pillars (crus muscle or hanging tender loins of the diaphragm) of the pig.

Some limitations of this technique are that it can show larvae usually in heavy infections, if the diagnoses is attempted before larvae begin to coil. (i.e up to 2 weeks after larvae enter the muscle cell then there is the risk of confusing the worm with fragments of muscle tissue.( Capo and Dickson, 1996,Gamble, 2001). Also this technique may not be suitable for detecting *Trichinellosis* in a living person couple with the fact that it is laborious and time consuming.

An improvement over the compression method is the pooled pepsin digestion method. Samples of tissue collected from sites where parasites concentrate such as the diaphragm, masseters or tongue are finely minced and then subjected to digestion in acidified pepsin (1% HCL – 1% pepsin for 1 hour at 37$^{o}$C). This process will release the larvae from their nurse cells and makes them more easily observed under the microscope (Dordevic, 1991, Gamble, 2001). This method though good for detecting older larvae that are not susceptible to the digestion procedure is not useful in detecting young larvae that can be destroyed by this process.(Capo and Dickson, 1996). The use of pepsin digestion is laborious, time consuming and poor at detecting very low level infections (Van Knapen, 2000). Also pepsin powder can be carcinogenic and allergenic to humans (CRLFP, 2006).

Routine histopathological examination of the biopsy sample is also used in demonstrating the presence of muscle larvae. Absence of larvae in sections or changes in muscle tissue, however, do not rule out infection since infection may be light or larvae may simply be missed because of their uneven distribution in muscle tissue. The absence of a capsule and the presence of a straight (i.e non-spiraled) worms in the complex indicate that the infection is ongoing. A mature capsule and a coiled parasite indicate an older infection that may have been acquired some time previous to admission to the clinical setting (Capo and Dickson, 1996).

Immunoassay techniques are used in detecting circulating antigens. This technique is not available in most laboratories. It is useful for diagnosis during the parenteral phase, when standard serological tests designed to detect specific antibodies have yet to become positive (Froscher *et al*., 1988). Detection of circulating antigen however, might be a useful confirmatory test (Dzbenski, 1994).

DNA – based tests have also been carried out (Pozio *et al*., 1992, Larosa, *et al.*, 1993). DNA sequences amplified by PCR have been identified and are specific for *T.spiralis* and other *Trichinella* species as well. This diagnostic test called new generation of diagnostic test is not yet available commercially. (Capo and Dickson, 1996).

Tests that are carried out to detect antibodies to *Trichinella* are now very useful to the diagnosis of trichinellosis. By 14 days after infection many patients suffering from clinical symptoms seek medical assistance.

Immunofluorescence–based assays and Enzyme-linked immunosorbent Assays (ELISA) for IgG antibodies may be positive. (Ljungstrom, 1983; Dordevic, 1991) and remain positive for years (Froscher *et al*., 1988).

The sensitivity of the IgG-ELISA reaches 100% on day 50. The test remains positive for more than two years in 88% of infected people (Morakote *et al*., 1992). Other immunoglobulins (IgA and IgE) behave in a similar manner, but test to detect them have lower sensitivity.

Gamble, (2001) opined that ELISA is an alternative method of testing pigs for trichinae infection. It is an indirect method which looks for antibodies to the parasites in pig blood. According to him, ELISA has been used extensively for testing in both pre- and post- slaughter applications and is an extremely useful tool for determining or monitoring infection in herds.

Among all conventional serodiagnostic methods, ELISA is the most commonly used approach for the detection of *Trichinella* infection in humans. Indirect Fluorescent antibody test is more time–consuming and expensive than ELISA. Also in indirect immunoflurescent test there are cross-reactions with antibodies directed against filariae

(*Onchocerca* spp) and *Schistosoma mansoni* which have been observed in blood samples of *Trichinella*-negative patients (Robert *et al*., 1996; Gottstein *et al*., 2009).

## 2.8 Treatment and Prevention

### 2.8.1 Treatment of trichinellosis in humans

After appropriate diagnosis has been carried out, immediate therapy has to be started as early as possible to curtail the threat of the infection on the patient. Intensive care treatment with all available supportive therapies is necessary.

Drugs administered to trichinellosis patients include: anthelmintics, glucorticosteroids and preparations that compensate for protein and electrolyte loss. Anthelmintics are the principal drugs for the treatment of trichinellosis (Gottstein *et al*., 2009). Specific treatment for the parasite with various benzimidazoles (Mebendazole or albendazole) is also important (Capo and Despommier, 1996)

Anthelmintics include primarily albendazole (Zentel, Smith-Kline Beecham) and Mebendazole (Vermox, Tanssien Pharmaceutica, Beerse Belgium) (Gottstein, *et al*., 2009), secondarily pyrantel (Combatrin, Pfizer) can also be administered (Dupouy-Camet, 2007).

According to Gottstein *et al*. (2009), the recommended dose for albendazole is 400mg twice daily for 8-14 days; for mebendazole, it is 200 to 400mg three times a day for 10 days. Both treatment scheme are suitable for adults and pediatric dosages; although contraindications exist in pregnant women and not recommended for infants (aged < 2years) (Horton, 1993).

Steroids e.g prednisolone administered at a dose of 30mg/day to 60mg/day for 10 to 15 days for severe symptoms are the standard choice of chemotherapy. Prednisone was shown to be safe and to alleviate symptoms due to active tissue larvae (Shimoni *et al*., 2007). Pyrantel Pamoate (Combatrin) is administered in a single dosage

of 10 to 20mg/kg of body weight, repeated for 2 to 3 days and may be used during pregnancy and in infants, it is however active only against worms in the gut and it has no effect against newborn and muscle larvae (Dupoy-Camet, 2007).

As a general rule, the later a treatment is prescribed, the higher the probability that the infected person would have already harboured viable larvae in their muscles which can then survive for years despite treatment with possible persistent myalgia (Pozio, *et al*., 2001). Prolonged treatment with steroids is not recommended, although symptoms may recur when treatment is suspended (Pawlowski, 1983). Long-term sequelae must be treated symptomatically as they arise (Capo and Despommier, 1996).

### 2.8.2 Prevention of trichinellosis in humans

The prevention of trichinellosis in humans is based on three main approaches: (i) educate the consumer on the dangers of consuming raw or semi-raw meat and meat products from both domestic and sylvatic animals that can be hosts for *Trichinella* parasites if not properly tested for *Trichinella* larvae upon meat inspection. (ii) rearing of pigs in modern, industrialized, indoor pigsties under strict veterinary control and use of certified feedstuff and (iii) control of all susceptible animals (domestic or sylvatic) by any standardized method of slaughtering or after hunting (Gottstein *et al*., 2009).

The consumption of raw or rare infected meat from game animals or from pigs raised in situations that favour the existence of rodent populations is the most frequent source of infection by any species of *Trichinella.* (Pozio, 1993). All meat from animals which might contain *Trichinella* larvae but cannot be tested by an appropriate laboratory method should be treated by a procedure that has been found to inactivate *Trichinella* prior to distribution for human consumption.(Gamble *et al.,* 2000, Gamble *et al.,* 2007).

Three methods have been proven to reliably inactivate *Trichinella* larvae in meat: (i) cooking to reach a core temperature of not less than 71$^{o}$C for at least 1 minute (i.e the meat must change the colour from pink to gray, and muscle fibers are easily separated from each other.) (ii) freezing and (iii) irradiation. Methods for the preparation of meat and meat products that are not considered safe include: (i) cooking by using microwave ovens and (ii) curing, drying or smoking. Hunters should be educated on the proper preparation of game meat which should follow the same guidelines issued to consumers. Particular caution should be given to the presumed presence of freeze-resistant *Trichinella* species or genotypes in game meats (Gottstein *et al*., 2009). Irradiation is recommended for sealed packaged food only (Gamble *et al*., 2000).

### 2.8.3 Prevention of *Trichinella* infections in domestic animals

Infection of pig herds by *T.spiralis* is usually perpetrated by the animals scavenging on infected rodents population or, less commonly by cannibalism of sick animals (Cuperlovic, 1991; Murrell and Bruschi, 1994). Pigs that have developed immunity following the first infection and experiencing a second infection, expel some of their worm burden soon afterwards as first-stage infective larvae and it is therefore suspected that coprophagy within the barnyard community of pigs may be yet another means by which naïve animals are infected (Capo and Despommier, 1996).

Feeding of raw meat scraps from local abattoirs to farm animals is illegal in the United States but no doubt occurs whenever the economic situation dictates, since steam cooking scrapes brings additional cost on most farmers which they cannot easily afford.

In other countries where these controls on domestic farm practices are less rigid, feeding raw pork scraps to livestock may be widespread and these meats “scraps” pose

an invaluable source of meat for the human population. Also the disposal of carcasses of furbearing animals by feeding their remains to farm animals has inadvertently spread *T.spiralis* to a large population of consumers with no malicious intentions on the farmers' part who were unaware of the broad host range of this parasitic nematode (Facelli, 1993).

Prevention at the grassroots level depends on animal husbandry and on withholding of uncooked meat in the feed of all farm animals, especially pigs. Microscopic inspection of portion of pig muscle tissue (directly or by the pooled digestion test) can control infection at the level of the slaughter house (Dordevic, 1991).

The use of ELISA for swine trichinellosis is now approved for the certification of pork in the U.S.A by the U.S Department of Agriculture. However, because there are several options available to meat packers for the certification of pork, it is difficult to convince industry that slaughterhouse testing is cost effective because trichinellosis is a low-prevalence disease in the U.S.A (<0.01%) (Bailey and Schantz, 1990). These inspection programs, however, are in place in most European countries (Dordevic, 1991; Hinz, 1991).

The category of pigs at highest risk for *Trichinella* infection are those raised on small holdings with minimal confinement. These so called "backyard pigs" are often fed food scraps or other forms of meat-containing waste and have access to rodents and wildlife. To worsen the problems, pigs raised here are generally not sold through retail marketing channels and therefore are not subjected to reliable methods of veterinary inspection. Free-ranging pigs are also at risk for this zoonosis. This degree of risk is of utmost important to "organic" or "green" pig producers, who provide products to consumers seeking meat from animals raised under natural conditions (Gamble *et al*.,2000, Gamble *et al*., 2007).

# CHAPTER THREE

## 3.0 MATERIALS AND METHOD

### 3.1 Study area

Kaduna State occupies part of the central position of the Northern part of Nigeria (with Kaduna as its capital) it is located in the North West Geo-Political Zone of Nigeria and shares common borders with Zamfara, Katsina, Niger, Kano, Bauchi and Plateau States. To the Southwest it shares a border with the Federal Capital Territory. The global location of the state is between longitude of 30″ East of the Greenwich meridian also 0900 and 11 30″ North of the equator. The state occupies an area of 48,473.2 square Km with a population of 6,006,562 people {2006 census} (KSGIS, 2008).

The samples for this research were obtained from the main pig abattoir and three hospitals in Kaduna Metropolis (fig 3.1). They are:

a. Pig slaughterhouse in Angwan Television **(PSH)** Kaduna.

b. General Hospital, Sabon-Tasha **(GHS)** Kaduna.

c. Dr Gwamna Awan General Hospital, Kakuri **(GAH)** Kaduna.

d. Barau Dikko Specialist Hospital **(BDH)** Kaduna.




Fig 3.1. Map showing blood sample collection sites in Kaduna metropolis.

Adapted from:http:// www.googlemaps.com. Accessed: 25th January, 2014.

KEY:

**BDH:** Barau Dikko Specialist Hospital.

**GAH:** Dr Gwamna Awan General Hospital, Kakuri.

**GHS:** General Hospital,Sabon-Tasha.

**PSH:** Pig slaughterhouse in Angwan Televis

### 3.2 Sample size determination

The sample size for this study was determined using the equation by Thrusfield (1995) and a reported 5.21% prevalence from a study of domestic pigs in Oyo State, Nigeria (Akinboade *et al.*, 1984)

$$n=\frac{z^2pq}{d^2}$$

Where, n=sample size for blood

z=statistics for a level of 95% confidence interval=1.96

p=prevalence (5.21%)

d=precision (allowable error) =5%=0.05

q=1-p

thus, $n=\frac{(1.96)^2X0.0521X(1-0.0521)}{(0.05)^2}$ =75.9,

However 186 blood samples were obtained from pigs at slaughter in pig abattoir, in addition, 100 blood samples were obtained from slaughtered pigs at pig farms.

For human: prevalence of 12% from a study in Mexico (Arriga *et al.*, 1991;www.krenet.it/ict)

Thus, $n=\frac{(1.96)^2X0.12X(1-0.12)}{(0.05)^2}$ =162.3, However 186 human blood samples were obtained.

On the whole a total of 472 blood samples were obtained.

**3.3 Inclusion Criteria (for humans)**: Meat eaters attending some hospitals in Kaduna metropolis.

**3.4 Exclusion Criteria (for humans)**: Non meat-eaters attending some hospitals in Kaduna metropolis.

### 3.5 Ethical Approval

The ethical approval for this work was sought and obtained from the Ministry of Health, Kaduna State, Nigeria (Appendix III-VI)

### 3.6 Sampling Plan

The pig slaughterhouse was visited daily for twenty four days and blood samples were collected from eight of the pigs slaughtered on each day of visit. The pigs were randomly selected by skipping one consecutive slaughtered pig and collecting blood samples from the next on queue. Close-ended structured questionnaire were administered to 100 pig owners/farmers/butchers who slaughter their pigs in their backyard farms.

For human: With the aid of doctors and Laboratory Scientists blood samples were collected from patients sent to the laboratory for tests. Patients were administered the questionnaire alongside after their informed consents were sought.

### 3.7 Sample Collection and Processing

Blood samples were obtained into labeled sample bottles (Vacutainer) free of anticoagulant. The sex, age (juvenile or adult), and source (extensive, intensive or semi-intensive) of the sampled pigs were recorded. The samples were transported to the laboratory in an ice-packed box. Centrifugation was done at 3000rpm for 10 minutes to obtain the sera. The sera were dispensed into clean labeled sample bottles and stored at -20$^{o}$C

### 3.8 Administration of Structured Questionnaire

The respondents included human patients who were sent to laboratory for tests. Pork eaters and non-pork eaters were surveyed with the aid of medical laboratory scientists. Pig owners and pork handlers were also surveyed, to know the source (extensive, semi-intensive or intensive), age and sex of the pigs and other ancillary questions.

### 3.9 Detection of *Trichinella* Antibodies using ELISA Technique

#### 3.9.1 (A) Procedure for Detection of *Trichinella* Antibodies in Pig Sera

**Kit Components (Reagents) included the following:**

-Three (3) 96-well Microplates coated with *Trichinella* Excretory/Secretory (E/S) Antigen (ID.vet, 2011).

-Concentrated Conjugate (10x).

-Positive Control.

-Negative Control.

-Dilution Buffer 2.

-Dilution Buffer 3.

Concentrated Wash Solution (20x).

-Substrate Solution.

Stop Solution ($H_2S0_4$ 0.5M)

Other required materials but not provided with the kit included:

-Mono and Multi-channel micropipettes.

-Disposable tips.

-96-well microplate reader.

-Distilled water.

-Manual wash system.

*3.9.1.1 Performance of Test*

Exactly 190µl of Dilution Buffer 2 and 10µl of the Negative Control were added to wells $A_1^2$ and $A_1^3$ after wells $A_1^1$, $A_2^1$ and $A_3^1$ were left as blanks. Also 190µl of Dilution Buffer 2 and 10µl of the Positive Control were added to wells $A_1^4$ and $A_1^5$. Then 190µl of Dilution Buffer 2 and 10µl of each sample were added to the remaining wells. The microplates were then incubated for 45 minutes at room temperature ($28^0C \pm 2^0C$). The contents of the wells were then emptied and each well was washed three times with about 300µl of the Wash Solution. Drying of the wells in between washings was avoided. Conjugate 1x was prepared by diluting the Concentrated Conjugate 10xto 1:10 in Dilution Buffer 3. Exactly 100µl of the Conjugate 1x was now added to each well and plates were again incubated at room temperature for 30 minutes. The wells were emptied again and each was washed 3 times about 300µl of the Wash Solution. Drying of the wells in between washings was prevented. Also 100µl of the Substrate Solution (tetramethylbenzidine:TMB) was added to each well next. Incubation of plates was carried out for 15 minutes at room temperature but this time in the dark.Exactly 100µl of the Stop Solution was added to each well to stop the reaction. Visual reading was done and an ELISA platereader was used to read and record the Optical Density (O.D) at 450 nm. Visual reading indicated that:In positive samples, a blue solution appeared in the wells which later became yellow after addition of the stop solution. Negative samples showed no coloration as specified by the kit manufacturers (ID.vet, 2011).

**3.9. 2 (B) Procedure for Detection of *Trichinella* Antibodies in Human Sera.**

**Reagents**

-Test Strips [Two (2) 96-microwell plates containing *Trichinella* antigens each in a test strip holder].

-Enzyme Conjugate.

-Positive Control.

-Negative Control.

-Chromogen (TMB).

-Wash Concentrate (20x).

-Dilution Buffer.

-Stop Solution.

Materials required but not provided with the kit included:

-Micropipettes

-Squeeze or Wash bottle for washing strips

-Reagent grade water and graduated cylinder

-Tubes for sample dilution

-Absorbent paper.

*3.9.2.1 Performance of Test*

The plates were labeled accordingly with wells $A_1^1$ and $A_2^1$ left as blanks. 100µl of the negative control was added to wells $A_1^2$ and $A_1^3$each and same quantity of the positive control to wells $A_1^4$ and $A_1^5$ each. Exactly 100µl of the diluted (1:64) test samples was added to the remaining wells. The dilution was done by diluting 5µl of sera in 315µl dilution buffer. Care was taken not to dilute the positive and negative controls since they were supplied prediluted. The plates were incubated at room temperature ($28^0C \pm 2^0C$) for 10 minutes. The contents of the plates were shaken out and washed three times with the diluted wash buffer (Wash Concentrate 20x [25ml] in 475ml of distilled water). Two drops of Enzyme Conjugate were added to each well and were again incubated for 5 minutes at room temperature. The content of each well were emptied and the wells were washed 3 times. Care was taken to remove the entire

wash buffer by slapping plates against paper towels. Two drops of the Chromogen were added to each well and incubated for 5 minutes at room temperature. Two drops of the stop solution were added and mixing was done by tapping the strip holders. Reading was carried out using an ELISA reader at 450 nm (DAI, 2013).

### 3.10 Interpretation of ELISA Tests Results

The ELISA test results were calculated based on the manufacturers' protocols and recommendations as follows:

a. Pig serum samples- for each sample, the sample positive percentage value (s/p) was calculated as $S/P = \frac{OD_{sample} - OD_{negative\ control}}{OD_{positive\ control} - OD_{negative\ control}} \times 100$

Where OD= Optical Density

The test results were interpreted as Negative, if S/P ratio $\leq$ 50%, S/P> 50% Positive.

b. Human serum samples – The results were interpreted as Positive if absorbance reading was greater than 0.3 OD (optical density) units and Negative if absorbance reading was less than 0.3 OD units.

### 3.11 Determination of Prevalence (%) of trichinellosis in Pigs and Humans

Percentage prevalence was calculated using the formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of positive serum samples for trichinellosis}}{\text{Total number of samples}} \times 100$$

$$\text{Overall prevalence for pig trichinellosis (\%)} = \frac{25}{186} \times 100 = 13.4\%$$

$$\text{Overall prevalence for human trichinellosis (\%)} = \frac{30}{186} \times 100 = 16.1\%$$

### 3.12 Statistical Analysis

The computer statistical software, Statistical Programme for Social Science version 19.0 (SPSS v 19.0) for Windows® (SPSS Inc., Chicago, IL., USA), was used for the statistical analysis of the data.

Chi-square was used to test for association between risk factors and prevalence of trichinellosis and to test the level of significance ($p<0.05$) for human trichinellosis and at $p\leq 0.001$ for farm management practices and also risk factors in pig farms. Also the relationship between prevalence of trichinellosis, the sources, sexes and ages of the pigs were established from the data using tables and chart.

# CHAPTER FOUR

## 4.0 RESULTS

In this study, overall prevalence rates of 16.1% and 13.4% being positive serum samples for *Trichinella* antibodies were obtained for the human population and pig population respectively. Prevalence of 10.7% was recorded among the juvenile pigs while 14.6% was observed among the adult pigs resulting in the overall prevalence of 13.4% in the pigs studied. Out of the 87 male pig sera examined, a prevalence of 12.6% was recorded and 14.1% for the 99 female pigs examined was observed (Table 4.1).

From the close-ended structured questionnaire administered to 100 pig owners, data were obtained which showed the distribution of pig owners based on farm management practices. Fifty-five per cent (55%) of them practiced extensive system while 40% and 5% practiced semi-intensive and intensive systems respectively. Pigs sourced from extensive farming system had prevalence of 96.4%, while those reared under semi-intensive conditions showed a prevalence of 70.0% and those under strict intensive care had no detectable case of trichinellosis. When the data were tested statistically, a significant association at $p \leq 0.001$ existed between farm management practices and trichinellosis (Table 4.2).

Table 4.3 presents the relationship between the various risk factors and trichinellosis in the study population (A total of 100 backyard slaughtered pigs from 100 pig farms). Ninety per cent (90%) had scavenging (search through waste/refuse for food) pigs, 98% had seen rodents around and within their pig pens, 85% had no rodent control programme, 70% feed their pigs with food leftovers, 4% had seen their pigs interact with stray pigs while 16% allowed their pigs to feed on dead animals. Ten per cent (10%) of the respondents had seen snakes around their piggeries. The awareness on trichinellosis was 2% of the respondents while 30% had knowledge of pork-borne

diseases. Statistically significant association ($\chi^2$ = 12.037, p = 0.001) exists between scavenging pigs and trichinellosis. Other factors that exhibited significant association ($p \leq 0.05$) with trichinellosis include absence of rodent control practice on the farms, feeding of pigs with leftovers, feeding of pigs with dead animals, interaction of stray pigs with farmers' pigs, snakes around piggeries, farmers' awareness of trichinellosis and other pork-borne diseases. The presence of rodents around and within piggeries showed no association ($\chi^2$ = 1.738, p=0.087) with *Trichinella* infection.

A total of 186 human serum samples were collected from patients from three hospitals in Kaduna metropolis.

Table 4.4 presents the demographics of the study participants in relation to the results obtained with no significant association observed ($p > 0.05$). Male participants had 16.1% prevalence while female had 16.5%. The age group 40 – 49 years had the highest prevalence of 21.2% while <10 years had no detectable infection with *Trichinella*. Individuals with no formal education had the highest prevalence of trichinellosis, 20.0%, followed by those with secondary education 19.8% and tertiary education the least, 15.2%.

Association of risk factors with trichinellosis was assessed and it was found that the type of meat eaten was significantly associated ($\chi^2$ = 19.192, p = 0.002) with trichinellosis with beef eaters having the highest prevalence of 29.0%, pork eaters had 11.4% prevalence and those who depend on poultry as meat source had prevalence of 8.0%. Bush meat and chevon eaters as well as others recorded no case of trichinellosis (Table 4.5).

Table 4.1: Prevalence of trichinellosis in pigs by age and sex.

| **Age group** | **Number of serum samples examined** | **Number of positive samples** | **Prevalence rate (%)** |
|---|---|---|---|
| Juveniles(1-2years) | 56 | 6 | 10.70 |
| Adult(>2years) | 130 | 19 | 14.72 |
| **TOTAL** | **186** | **25** | **13.44** |
| **SEX** | | | |
| Male | 87 | 11 | 12.64 |
| Female | 99 | 14 | 14.14 |
| **TOTAL** | **186** | **25** | **13.44** |

Table 4.2: Detection of trichinellosis in pigs in relation to farm management practices

| **Farm management type** | **Number of sample** | **Number positive (%)** | **$\chi 2$** | **p-value** |
|---|---|---|---|---|
| | | | 32.89 | < |
| Extensive | 55 | 53 (96.4) | 6 | 0.001** |
| Semi-intensive | 40 | 28 (70.0) | | |
| Intensive | 5 | 0 (0.0) | | |

** = significant association exists at $p \leq 0.001$

Table 4.3: Relationship between risk factors and trichinellosis

| **Risk factors from pig farms** | **Number of respondents (N=100)** | | **Number of pigs tested positive (%)** | $\chi^2$ | **p-value** |
|---|---|---|---|---|---|
| | **Factor present** | **Factor absent** | | | |
| Scavenging pigs | 90 | 10 | 52 (57.8) | 12.037 | 0.001** |
| Rodents around and within pigs | 98 | 2 | 46 (46.9) | 1.738 | 0.187 |
| No rodent control | 85 | 15 | 47 (55.3) | 15.649 | < 0.001** |
| Feed their pigs with leftovers | 70 | 30 | 27 (38.6) | 15.851 | < 0.001** |
| Pigs to feed on dead animals | 16 | 84 | 16 (100.0) | 100.00 | < 0.001** |
| Stray pigs interact with their pigs | 4 | 96 | 2 (50.0) | 48.980 | < 0.001** |
| Seen snakes around their piggeries | 10 | 90 | 3 (30.0) | 27.835 | < 0.001** |
| Awareness on trichinellosis | 2 | 98 | 1 (50.0) | 49.495 | < 0.001** |
| Aware of pork-borne diseases | 30 | 70 | 18 (60.0) | 51.220 | < 0.001** |

** = significant association exists at $p \leq 0.001$

Table 4.4: Detection of trichinellosis in humans by age, sex and educational level

| **Variable** | **No of Respondents** | **No. positive (%)** | $\chi^2$ | **p-value** |
|---|---|---|---|---|
| **Sex** | | | | |
| Male | 62 | 10 (16.1) | 0.005 | 0.945 |
| Female | 121 | 20 (16.5) | | |
| **Age**(in years) | | | | |
| <10 | 11 | 0 (0.0) | 7.227 | 0.3000 |
| 10-19 | 16 | 0 (0.0) | | |
| 20-29 | 30 | 5 (16.7) | | |
| 30-39 | 59 | 12 (20.3) | | |
| 40-49 | 33 | 7 (21.2) | | |
| 50-59 | 24 | 5 (20.8) | | |
| >60 | 13 | 1 (7.7) | | |
| **Education** | | | | |
| No formal education | 25 | 5 (20.0) | 7.450 | 0.114 |
| Primary | 36 | 3 (8.3) | | |
| Secondary | 86 | 17 (19.8) | | |
| Tertiary | 33 | 5 (15.2) | | |

Other risk factors such as pork eating, mode of cooking meat, how people like meat cooked and source of meat were tested for any relationship with trchinellosis but they were found to have no significant relationship with the disease ($p > 0.05$) (Table 4.5). The table further revealed that whether or not the respondents eat hamburger/sausages showed significant association ($\chi^2 = 4.091$, $p = 0.043$) with trichinellosis having a prevalence of 19.4% in individuals who eat hamburgers/sausages.

On awareness, respondents' knowledge of how *Trichinella* infects pigs was significantly associated with trichinellosis ($\chi^2 = 6.348$, $p = 0.042$). Other questions that sought to test respondents' awareness of *Trichinella* and its mode of transmission in pigs showed no significant association with trichinellosis ($p > 0.05$) (Table 4.6).

Table 4.5: Distribution of risk factors affecting the occurrence of trichinellosis in humans

| Risk factors | No of Respondents | No. positive (%) | $\chi^2$ | p-value |
|---|---|---|---|---|
| **Type of meat eaten** | | | | |
| Pork | 44 | 5 (11.4) | 19.192 | 0.002** |
| Beef | 78 | 23 (29.5) | | |
| Poultry | 25 | 2 (8.0) | | |
| Bush meat | 14 | 0 (0.0) | | |
| Chevon | 21 | 0 (0.0) | | |
| Others | 4 | 0 (0.0) | | |
| **Pork eating** | | | | |
| Yes | 58 | 15 (25.9) | 1.365 | 0.243 |
| No | 64 | 11 (17.2) | | |
| **Mode of cooking meat** | | | | |
| Boiled | 85 | 14 (16.5) | 5.444 | 0.066 |
| Fried | 30 | 8 (26.7) | | |
| Roasted | 61 | 5 (8.2) | | |
| **How do you like meat cooked** | | | | |
| Well cooked | 139 | 28 (20.1) | 1.745 | 0.187 |
| Undercooked | 7 | 0 (0.0) | | |
| **Source of meat** | | | | |
| Abattoir | 33 | 4 (12.1) | 5.657 | 0.130 |
| Market | 128 | 21 (16.4) | | |
| Meat hawkers | 7 | 1 (14.3) | | |
| Backyard slaughtering | 1 | 1 (100.0) | | |
| **Do you eat hamburger/sausages?** | | | | |
| Yes | 129 | 25 (19.4) | 4.091 | 0.043* |
| No | 54 | 4 (7.6) | | |

Table 4.6: Level of awareness of respondents on the causes of trichinellosis in humans and pigs

| Variable | No of Respondents | No. positive (%) | $\chi^2$ | p-value |
|---|---|---|---|---|
| **Do you know of pork-borne disease?** | | | | |
| Yes | 38 | 7 (18.4) | 0.034 | 0.854 |
| No | 101 | 20 (19.8) | | |
| **Ever heard of *Trichinella*?** | | | | |
| Yes | 20 | 3 (15.0) | 0.598 | 0.439 |
| No | 110 | 25 (22.7) | | |
| **If yes, source?** | | | | |
| Electronic media | 2 | 0 (0.0) | 0.302 | 0.582 |
| School | 15 | 2 (13.3) | | |
| **How are people affected by *Trichinella*?** | | | | |
| Eating undercooked pork | 16 | 6 (37.5) | 3.042 | 0.219 |
| Eating undercooked beef | 6 | 4 (66.7) | | |
| Don't know | 97 | 18 (18.6) | | |
| **How does *Trichinella* infect pigs** | | | | |
| Scavenging dead rodents | 13 | 6 (46.2) | 6.348 | 0.042* |
| Eating meat scraps | 16 | 0 (0.0) | | |
| Don't know | 95 | 19 (20.0) | | |
| **Have you been to slaughter house?** | | | | |
| Yes | 81 | 15 (18.5) | 0.115 | 0.735 |
| No | 73 | 12 (16.4) | | |
| **If yes, was routine inspection carried out?** | | | | |
| Yes | 29 | 8 (27.6) | 2.923 | 0.232 |
| No | 68 | 9 (13.2) | | |
| No idea | 40 | 8 (20.0) | | |

# CHAPTER FIVE

## 5.0 DISCUSSION

The prevalence rate of 13.4% positive pigs for *Trichinella* antibodies slaughtered in Kaduna metropolis as recorded in this study is slightly lower and similar to the prevalence rate of 15.0% recorded by Adediran and Uwalaka, (2012) in Oyo State, Nigeria; while Momoh (2012), recorded a prevalence of 26.6% from a survey in Markurdi, Benue State, Nigeria. This variation could be based on the cultural preferences, religious beliefs or tradition of the people inhabiting the various locations. This view point is upheld by the Food and Agricultural Organisation of the United Nations who hold that the utilization and consumption of animal species vary as cultural preferences and religious beliefs are observed (FAO, 2014).

Akinboade *et al*. (1984), reported a trichinellosis prevalence of 5.2% in Oyo State using Pepsin Digestion technique. The Pepsin Digestion Technique has the limitations of being unable to detect all larvae present in the carcasses of the animals and hence poor at detecting low level infections (Van Knapen, 2000). Powdered pepsin has also been observed to be carcinogenic and the commercial type is not readily available (CRLFP, 2006).

Mbaya *et al*. (2010) recorded a prevalence of 16.0% for trichinellosis among African giant rats in northern Nigeria. The giant rats that dwell in pig-rearing areas were those that were said to harbour the parasite. Wang and Cui (2001) in China, observed sero-prevalences varying from 0.09% to 29.6% between 1914 and 1999. Larrieu *et al.,* (2004) in Argentina, Chavez-Larrea *et al.* (2005) in Ecuador, La Rosa *et al*., (1998) in Mexico and Gamble (2000) in North America reported seroprevalences of 19.9%, 5.7%, 2.5% and 0.013% in swine respectively.

The relatively high prevalence in pigs observed in this study could be due to the predominantly extensive system of farming the animals and backyard rearing which exposed the animals to a lot of risk factors like scavenging, interactions with wild pigs and rodents like rats and reptiles like snakes. Also the awareness of pig owners and/or farmers of the disease and its transmission among pigs were very low (only 2% of respondents). Hence this deficiency in knowledge did not allow pig farmers and producers to design management system that would prevent or drastically reduce the risk of exposure to the parasite (Gamble, 2000; Gamble 2007).

In this study, there was significant statistical association between the following risk factors and trichinellosis: scavenging pigs, lack of rodent control in farms, feeding pigs with food leftovers, pigs gaining access to feed on dead animals, interactions of farmers' pigs with stray pigs, snakes around piggeries and level of awareness of the disease. This is occasioned to the extensive and semi-intensive mode of farming swine in Kaduna metropolis.

Momoh *et al.* (2013) established some risk factors associated with trichinellosis in backyard pig farms in Zaria, Kaduna State, Nigeria, to include presence of rodents, rodent control and access to dead pigs. Zaria and Kaduna metropolis are major urban cities in Kaduna state. Gamble *et al.* (1999), also established that access of pigs to live wildlife and wild carcasses on the farm were significantly associated to trichinellosis, while waste feeding was not an important risk factor in northeastern United States.

In this study, adult pigs were observed to be more infected than the juvenile pigs (14.7%) as against (10.7%) this is in tandem with the reports of Akinboade *et al.*, (1984), Larrieu*et al.,* (2004) and Adediran and Uwalaka (2012). The reason for this could be deduced from the trend that adult swine naturally tend to scavenge more than the younger ones and are most likely to be exposed to predisposing factors such as

scavenging, exposures to rodents and reptiles, interactions with wild pigs and other wild animals and carnivorism.

The prevalence in female pigs was seen to be higher (14.1%) than that of males (12.6%) a result which differed slightly from that obtained by Adediran and Uwalaka, (2012) but agreed with that of Momoh, (2012) from a study in Makurdi, Benue State Nigeria. This could be as a result of both Kaduna metropolis and Makurdi being part of the northern region of Nigeria and this could also not be unconnected with the fact that the female scavenge for themselves and their piglets and hence could cover large areas in search of food which could bring them in contact with wild pigs (Kingdon, 1989) and other predisposing factors.

The overall prevalence of 16.1% in the human population in this study indicates that trichinellosis is not only a re-emerging zoonoses but also present in Kaduna metropolis. This result is similar to that obtained in Mexico (12%) (ICT, www.krenet.it/ict), in which pigs slaughtered in the backyards were surveyed for trichinellosis.

Specificity of the ELISA and absolute sensitivity accounts for the high prevalence rate since both new and existing cases were detected. In fact ELISA can detect serum antibody titre of up to 130 weeks (Nockler *et al*., 2000) and up to 11 years after infection. The sensitivity of ELISA was reported to be higher with Excretory/Secretory antigen than with tyvelose for detecting circulating antibodies early in the infection. (Pozio, 2007)

There was no significant association ($p>0.05$) between the sex of the respondents and the infection which agrees with work of Gottstein *et al*. (2009) and that done by Capo and Despommier, (1996).

In this study, the age group of 40-49 years had the highest prevalence rate which could be that they have been exposed to the parasites earlier in their lives or may now have the economic power or income to enjoy meat delicacies more thereby increasing their chances of exposure to the parasite (Murrell and Pozio, 2000).

Individuals with no formal education had the highest prevalence of trichinellosis (20%) followed by those with secondary education (19.8%). Respondents with tertiary education had a prevalence of (15.2%) and those with primary education the least infected (8.3%). The observations are in consonance with the results obtained from China in which the rising income and changing of food preferences of the population showed an association between incidence and level of education (Murrell and Pozio, 2000).

The result also showed a significant association between trichinellosis and meat consumed a fact that has been established over time (Capo and Despommier, 1996; Gamble 2001; Pozio, 2007; Gottstein *et al*., 2009). In this study, beef eaters recorded the highest prevalence which could be due to the high number of beef eater respondents than pork eaters. However transmission of trichinellosis from cows (beef) to humans is very possible, if one considers that in Turkey with the largely muslim population, a large outbreak of trichinellosis occurred due to the consumption of minced beef illegally mixed with pork of unknown origin (Akkoc *et al*., 2008).

Also, most of the beef in Nigeria come from cow herded by the Fulani nomadic herdsmen whose livestock criss-cross a lot of forest and hinterlands and therefore, possible interactions of these cows with living and dead wildlife, even at the point of grazing, is not unlikely.

Akar (2007) revealed that *Trichinella* mainly affects pigs but has been reported in cattle. According to him, the main pathogenic species in cattle are: *Trichinella*

*spiralis and Trichinella britovi* and most affected cattle are asymptomatic and the disease occurs when beef is eaten by humans and carnivores.

A study in Canada showed that *Trichinella spiralis* infections were established in cattle by gavage and by feeding infected musculature in the ration-to obtain veal. The cattle got infected when fed with musculature camouflaged with molasses. The experimental cows in the study were fed with or maintained on grass silage or hay and grain maintenance rations (Smith, *et al.*, 1990); rations to which cattle in Nigeria are also fed with.

Pork eaters also had a significant prevalence as well as poultry eaters. It is adduced that the increase in human infections appears to be directly related to increase in swine prevalence (Murrell and Pozio 2000). It is important to note that *Trichinella* species have been recovered from a number of mammals including cows, goats, sheep, dog, giant rats, wildlife animals, reptiles and a number of raptorial birds.

Another significant association was the respondents' knowledge of how *Trichinella* infects pigs and trichinellosis in humans (p=0.042). Although this finding could be surprising not until one considers the postulation of Gottstein *et al*., (2009), that knowledge on the modes of transmission of *Trichinella* to domestic pigs allows pig farmers and producers to design management system that prevent or drastically reduce the risk of exposure to the parasite (Gamble, 2000; Gamble 2007). This supports the saying that what you don't know may be killing you.

More Hamburger and Sausages eaters were positive for *Trichinella* antibodies (19.4%) than non-eaters (7.6%) which indicated a significant association with trichinellosis (p=0.043). This could have arisen from the possibility that the meat may not be well-cooked due to the large quantity of meat involved; and economic interest of the producers or the meat may have been microwaved. Also test for trichinellosis may

not have been done on the meat. This finding agrees with Zimmerman, (1970) who recorded existence of *T. spiralis* in commercial pork sausages in the U.S.A.

Extensive farm management practices constitute a major predisposing factor in the transmission of trichinellosis among domestic pigs in Kaduna Metropolis (96.4%), this is closely followed by semi-intensive system (70.0%) and intensive (0.0%). This trend was also observed by Adediran and Uwalaka (2012) and also Momoh *et al.* (2013).

# CHAPTER SIX

# 6.0 CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion and Summary

This survey concludes that, pigs and humans in Kaduna Metropolis, Nigeria habour antibodies to *Trichinella* sp. and the prevalence in humans and in pigs is high. An overall prevalence of 13.4% and 16.1% for pigs and humans respectively were recorded. The increase in human infections appeared to be proportional to an increase in swine prevalence. A number of risk factors for swine trichinellosis and human trichinellosis were established. The type of meat eaten, hamburger/sausages and knowledge of how *Trichinella* infected pigs were important factors in the transmission of human trichinellosis. Allowing pigs to scavenge, lack of proper rodent control, feeding of pigs with food leftovers, access of pigs to dead animals, interaction of domestic pigs with stray pigs, presence of reptiles like snakes and level of awareness of pig farmers were important risk factors in the spread of pig trichinellosis. The study also revealed that extensive and semi-intensive farm management systems practiced by pig farmers and pig producers aided the transmission of the disease. The result of this study therefore, is of public health importance because meat and meat products are considered very important sources of daily protein in-take and sources for delicacies in Kaduna metropolis, Nigeria.

## 6.2 Recommendations

In view of the dangers to health and economic implications of trichinellosis in pig rearing, management and increase in consumption of various meat types by humans, the following measures are strongly recommended which will greatly reduce risk of exposure of pigs and humans to trichinae.

Pigs:

**i.** Swine should not be fed uncooked waste products, table scraps or animal carcasses especially carcasses from hunted or trapped wildlife or raw bush meat.

**ii.** Barriers should be created to eliminate or minimize exposures of swine to wildlife.

**iii.** Pig farmers should implement and maintain an effective rodent control programme.

**iv.** Piggeries should be maintained under strict hygienic conditions; dead porcine animals should be removed as soon as they are found.

**v.** Employment of intensive farm management practice for porcine animal husbandry.

**vi.** Massive public enlightenment on trchinellosis is advocated.

Humans:

i. ELISA technique should be employed in abattoirs for quick detection of trichinellosis.

ii. Freezing of all meat products from domestic and game animals especially pork before cooking to ensure death of larvae and cooking meat at $58^{o}$C for 10 minutes also annihilates the larvae. Microwaving should not be relied upon since it is not 100% effective due to “cold spots” pattern in the meat.

iii. ELISA test for trichinellosis should be included in the routine test carried out in hospitals especially in regular checkups of patients.

iv. Hunters, poachers, rangers and people who have interactions with dead or living wildlife should be tested compulsorily for antibodies to the parasites.

v. Curing of meat and meat products should be encouraged which may prove to be an effective means of inactivating trichinae larvae.

vi. Food regulatory agencies and researchers should carry out tests for the parasites in hamburgers/sausages sold to the public.

vii. More work should be carried out on trichinellosis in other domestic animals especially herbivores.

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## APPENDIX I: STRUCTURED QUESTIONNAIRE FOR HUMAN TRICHINELLOSIS.

Date ……

Sex: Male ( ) Female ( )

Age (years): <10 ( ); 10-19 ( ); 20-29 ( ); 30-39 ( ); 40-49 ( ); 50-59 ( ); >60 ( )

Educational level: Primary ( ) Secondary ( ) Tertiary ( ) others Specify……

Town …….. LGA…… Location…….

1. Which meat(s) do you eat? A. Pork ( ) B. Beef ( ) C. Poultry( ) D. Bush meat( ) E. Goat meat( ) F. Others specify…….
2. Do you know of pork- borne disease(s)? Yes ( ) No ( )
3. Have you heard of the parasite *Trichinella*? Yes ( ) No ( )
4. If yes, from which source?

Print media ( ) B. Electronic media ( ) C. School ( ) D. Others ( )

5. Do you eat pork Yes ( ) No ( )
6. How do you consume your pork?

A. Boiled ( ) B. fried ( ) C. Roasted ( ) D. Smoked ( ) E. Raw ( )

7. How do you like your meat cooked? A. Well cooked ( ). B. Under cooked
8. How do you think people are most affected by *Trichinella?*

Eating undercooked pork ( ) Eating undercooked beef ( ) Don't know ( )

9. How do you think that *Trichinella* infect pigs?

Scavenging dead rodents ( ) Eating meat scraps ( ) Eating grains ( ) Don't know ( )

10. Have you been to the slaughterhouse before?

Yes ( ) No ( )

11. If yes, is routine meat inspection carried out?

Yes ( ) No ( ) No idea ( )

12.Where do you get your pork and or other meat from? A. Abattoir ( ) B. Market C. Meat Hawkers.D Backyard slaughtering.

13.Do you eat hamburger and/or sausages? Yes ( ) No ( )

**APPENDIX II: QUESTIONNAIRE ON FARM MANAGEMENT PRACTICE AND RISK FACTORS IN PIG FARMS**

Date ……

Town …….. LGA …… Location……..

1. What type of pig farming do you practice?

A. Extensive [ ] B. Semi intensive [ ] C .Intensive [ ]

2. How do you obtain your pigs?

A .Market [ ] B .Farms [ ] C .Inheritance [ ]

3 Do you see rodents around your piggery?

A yes [ ] B. no [ ]

4. Are rats widespread or localized in your piggery?

A. Widespread [ ] B. Localized [ ]

5. Do you have any rodent control program?

A. Yes [ ] B .No [ ]

6. What type of wild animal do you see around your piggery?

A. Bush (wild) pigs [ ] B. Dogs [ ] C. Snakes [ ] D. None [ ]

7. Which of these methods do you use to dispose of dead pigs?

A. Bury in the ground [ ] B. Burning at open field [ ] C. Given to pigs to eat [ ] D. Given to dogs to eat [ ].

8. Indicates the type of feed you regularly give to your pigs.

A. Whole grain [ ] B. Commercial pigs feed [ ] C. leftovers [ ] D. Waste with meat [ ] E. Brewers spent grain [ ].

9. Do you have information about pork-borne disease[s]? Yes [ ] No [ ].

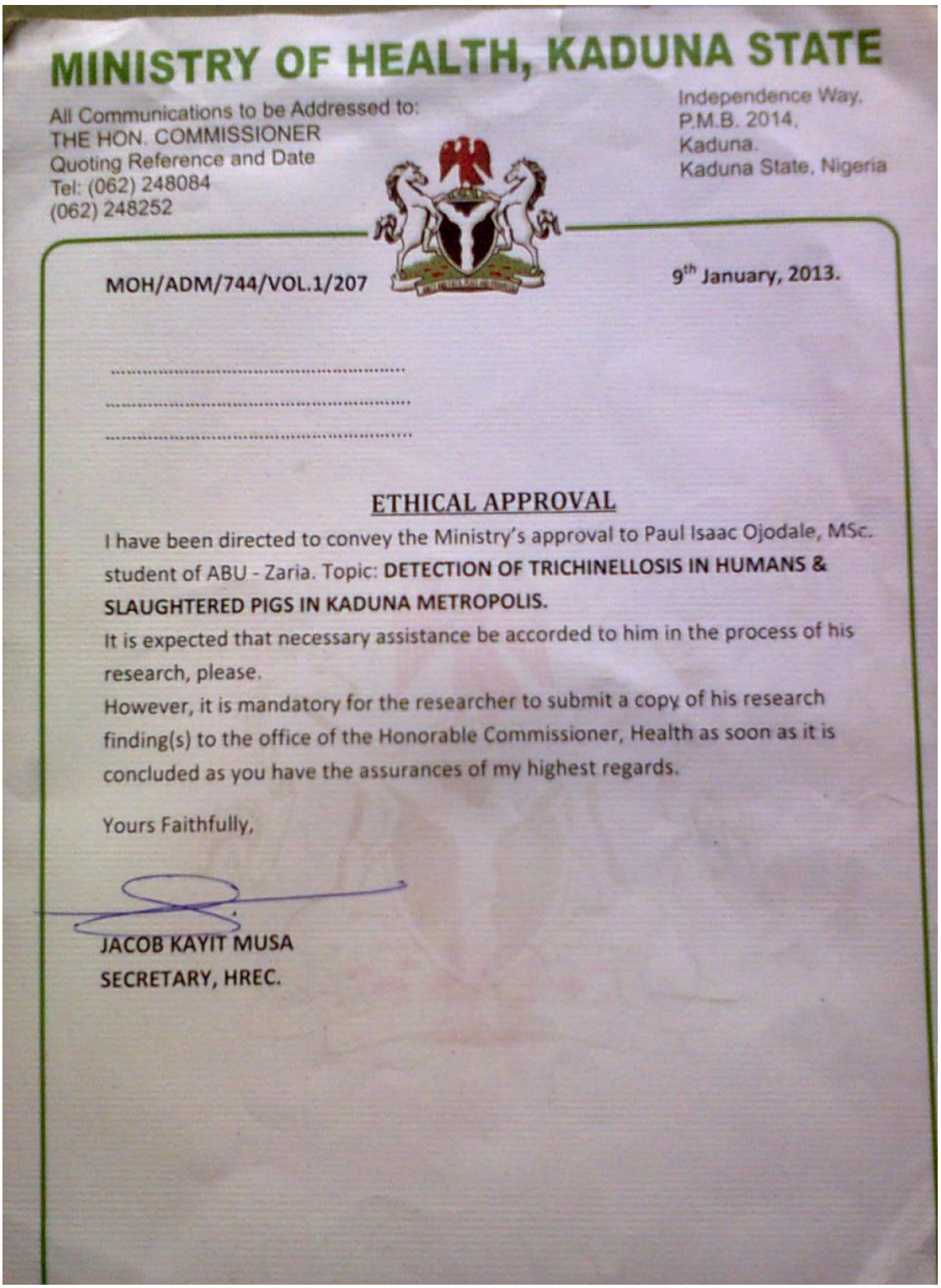
10. Have you heard of the parasite *Trichinella*? Yes [ ] No [ ]

11. If yes, from where?

A. Print media [ ] B. Electronic media [ ] C. School [ ] D. Others [ ]

12. Do you allow your pigs to scavenge? Yes [ ] No [ ]

APPENDIX III: Ministry of Health, Kaduna State: Ethical Approval



# MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 248084
(062) 248252

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

MOH/ADM/744/VOL.1/207

9th January, 2013.

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.....
.....

## ETHICAL APPROVAL

I have been directed to convey the Ministry's approval to Paul Isaac Ojodale, MSc. student of ABU - Zaria. Topic: **DETECTION OF TRICHINELLOSIS IN HUMANS & SLAUGHTERED PIGS IN KADUNA METROPOLIS.**

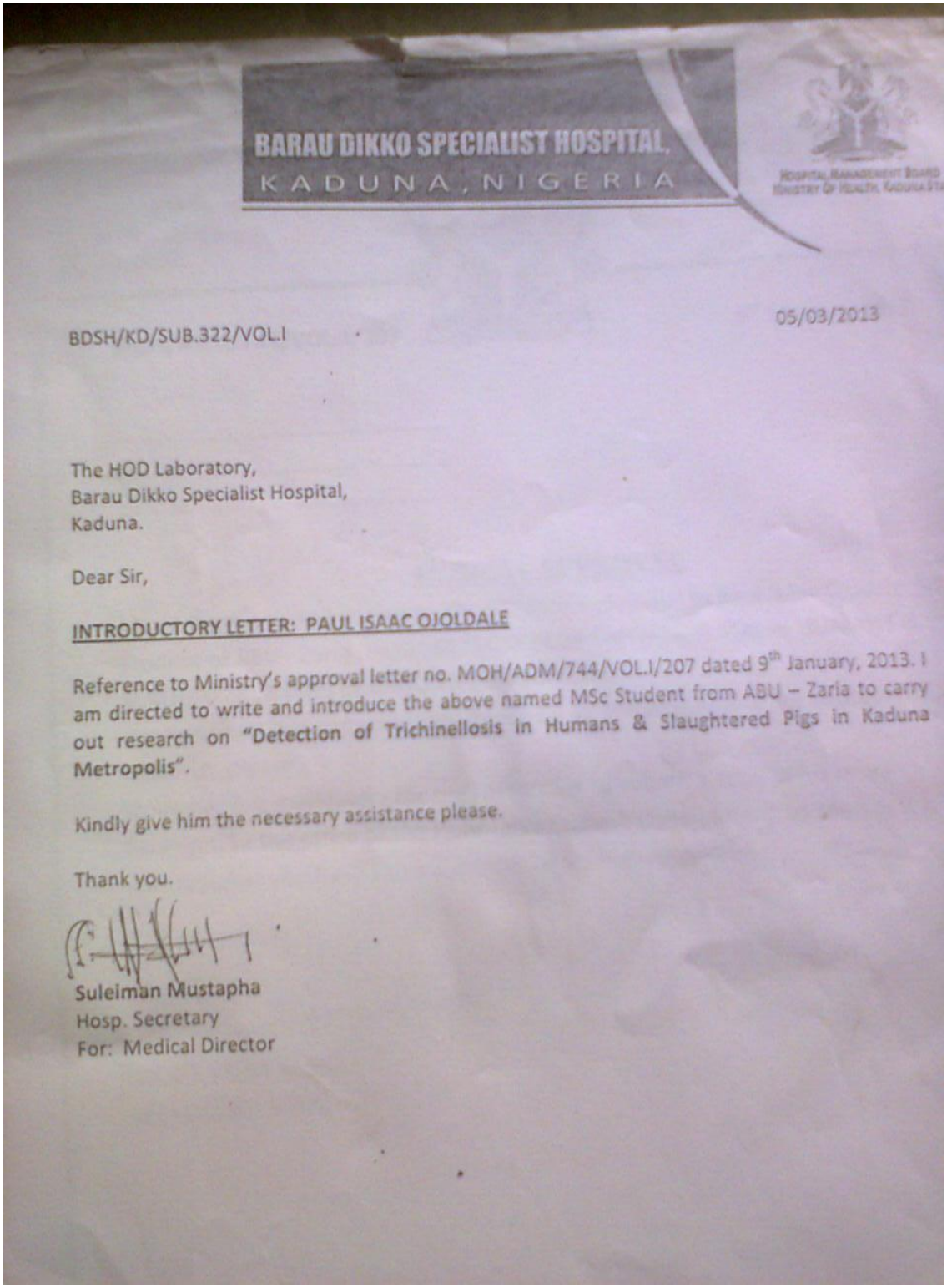
It is expected that necessary assistance be accorded to him in the process of his research, please.

However, it is mandatory for the researcher to submit a copy of his research finding(s) to the office of the Honorable Commissioner, Health as soon as it is concluded as you have the assurances of my highest regards.

Yours Faithfully,

**JACOB KAYIT MUSA**
**SECRETARY, HREC.**

APPENDIX IV: Barau Dikko Specialist Hospital Kaduna,Nigeria: Introductory Letter for Paul, Isaac Ojodale



BARAU DIKKO SPECIALIST HOSPITAL,
KADUNA, NIGERIA

BDSH/KD/SUB.322/VOL.I

05/03/2013

The HOD Laboratory,
Barau Dikko Specialist Hospital,
Kaduna.

Dear Sir,

INTRODUCTORY LETTER: PAUL ISAAC OJOLDALE

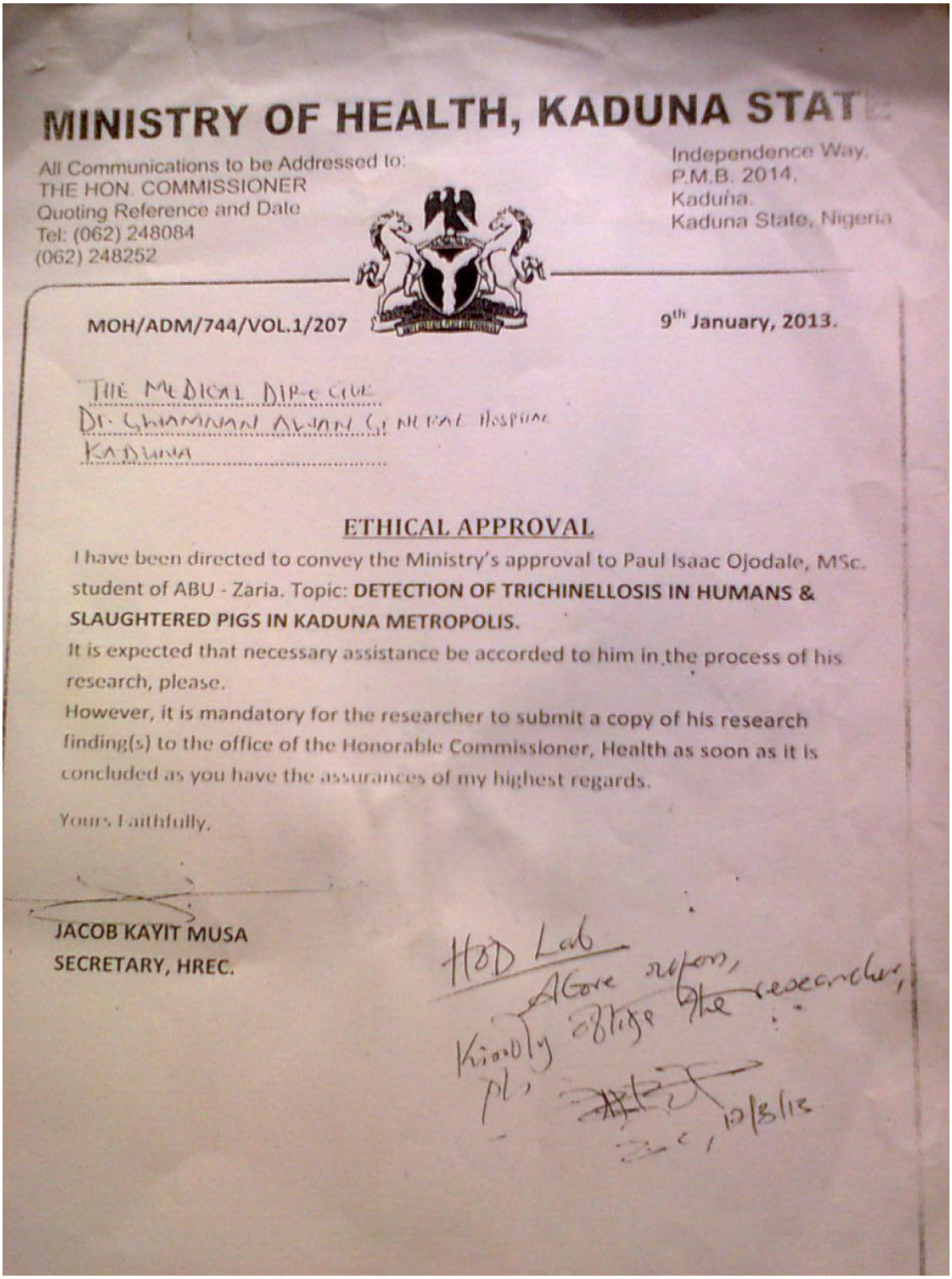
Reference to Ministry's approval letter no. MOH/ADM/744/VOL.I/207 dated 9th January, 2013. I am directed to write and introduce the above named MSc Student from ABU – Zaria to carry out research on **"Detection of Trichinellosis in Humans & Slaughtered Pigs in Kaduna Metropolis"**.

Kindly give him the necessary assistance please.

Thank you.

Suleiman Mustapha
Hosp. Secretary
For: Medical Director

APPENDIX V: Ethical Approval letter to Dr. Gwamnan Awan General, Hospital Kaduna



**MINISTRY OF HEALTH, KADUNA STATE**

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 248084
(062) 248252

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

MOH/ADM/744/VOL.1/207

9th January, 2013.

THE MEDICAL DIRECTOR
DR. GWAMNAN AWAN GENERAL HOSPITAL
KADUNA

**ETHICAL APPROVAL**

I have been directed to convey the Ministry's approval to Paul Isaac Ojodale, MSc. student of ABU - Zaria. Topic: **DETECTION OF TRICHINELLOSIS IN HUMANS & SLAUGHTERED PIGS IN KADUNA METROPOLIS.**

It is expected that necessary assistance be accorded to him in the process of his research, please.

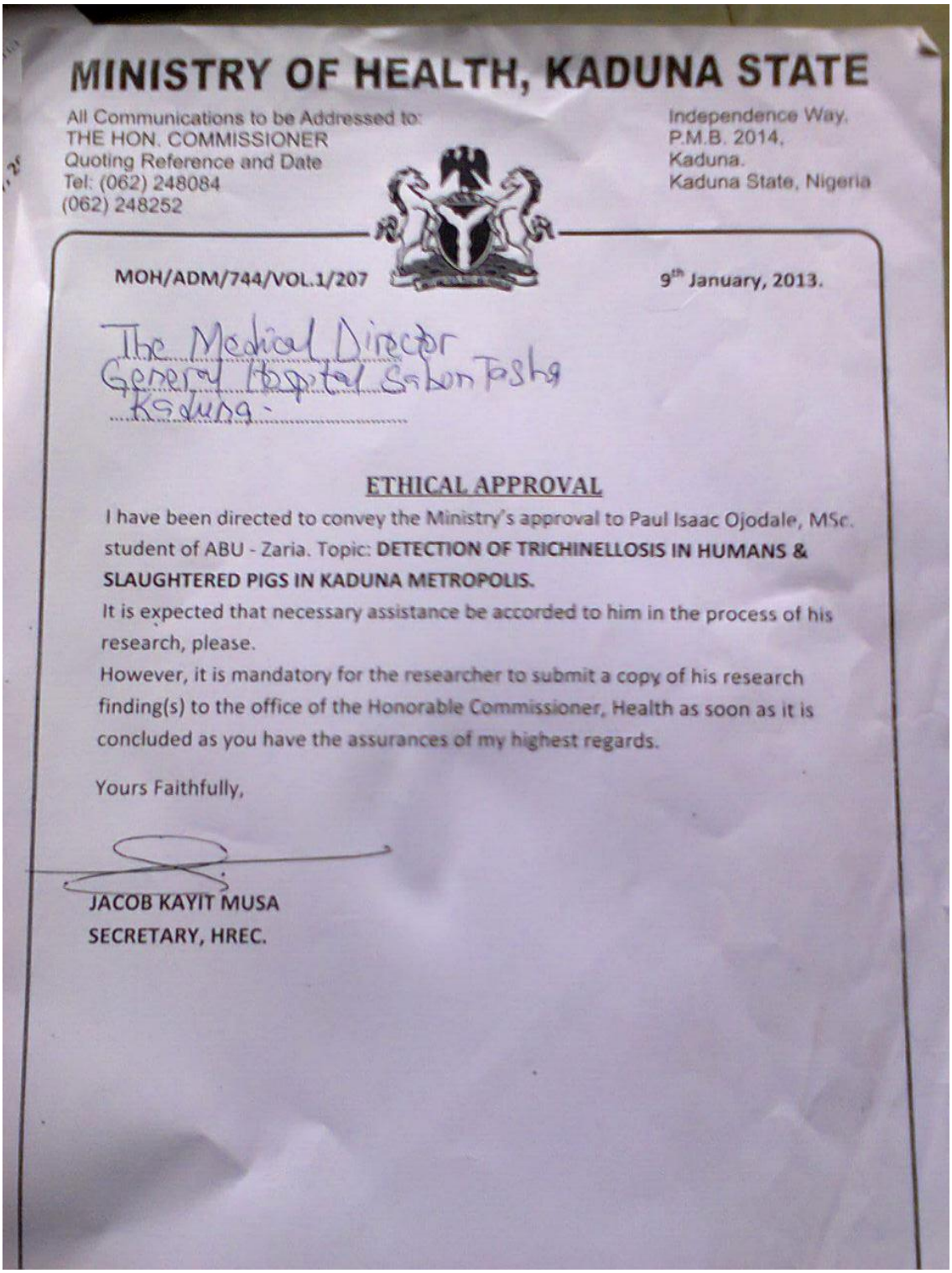
However, it is mandatory for the researcher to submit a copy of his research finding(s) to the office of the Honorable Commissioner, Health as soon as it is concluded as you have the assurances of my highest regards.

Yours Faithfully,

JACOB KAYIT MUSA
SECRETARY, HREC.

HOD Lab
Please afford the researcher,
Kindly
12/3/13

APPENDIX VI: Ethical approval letter to General Hospital Sabon Tasha



MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 248084
(062) 248252

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

MOH/ADM/744/VOL.1/207

9th January, 2013.

The Medical Director
General Hospital Sabon Tasha
Kaduna.

**ETHICAL APPROVAL**

I have been directed to convey the Ministry's approval to Paul Isaac Ojodale, MSc. student of ABU - Zaria. Topic: **DETECTION OF TRICHINELLOSIS IN HUMANS & SLAUGHTERED PIGS IN KADUNA METROPOLIS.**

It is expected that necessary assistance be accorded to him in the process of his research, please.

However, it is mandatory for the researcher to submit a copy of his research finding(s) to the office of the Honorable Commissioner, Health as soon as it is concluded as you have the assurances of my highest regards.

Yours Faithfully,

JACOB KAYIT MUSA
SECRETARY, HREC.