**Sedimentation and wet mount recoveries of *Taenia solium* eggs in faeces of pigs in Wukari, Southern Taraba, Nigeria**

**Running title:** Recoveries of *Taenia solium* eggs in faeces of pigs in Wukari, Taraba State,

Nigeria

**ABSTRACT**

*Taenia solium* (*T. solium*), also known as pork tapeworm, is a segmented intestinal parasite of humans and pigs that is endemic in many developing countries. This study was carried out to recover *T. solium* eggs in feacal droppings of one hundred (100) unrestrained pigs in different sampling areas of Wapan Nghaku and Mission Quarters in Wukari, Southern Taraba State, Nigeria, using the formol-ether-sedimentation (FES) and direct wet mount (DWM) techniques. The detection of eggs in both recovery methods was compared using percentages. The recovery of *T. solium* eggs using the FES and DWM technique was 48% and 32% respectively. In both diagnostic isolation methods, the recovery rate was highest in the Wapan Nghaku (FES: 56%; DWM: 40%) than in the Mission Quarters areas (FES: 40%; DWM: 20%). This study which describes for the first time, the study on *T. solium* in nomadic pigs and techniques to recover their eggs in faeces in Wukari, has improved data on the epidemiology of *T. solium* by showing that nomadic pig farming in Wukari predisposes transmission of taeniasis in the study population of pigs that serve as reservoirs of *T. solium* eggs. Furthermore, these eggs can be isolated with more accuracy using the FES technique which is simple. Whilst a robust surveillance data is advocated, there is need to adopt a quality intensive system of pig management to maintain good hygienic environment and disease free pork for consumption in the study areas.

Keywords: Formol-ether-sedimentation, wet mount, *Taenia* *solium*, faeces, pigs

# INTRODUCTION

*T. solium* is a zoonotic pork tapeworm of the phylum plathyhelminthes and class Cestoda (Otubanjo, 2013; Weka et al. 2020). It is cosmopolitan in distribution but endemic in many developing countries of Latin America, Africa and South East Asia (Zammarchi et al. 2013; Okello et al. 2014; Rodriguez-Morales et al. 2018; Alarakol et al. 2021). Nigeria is among the significant pig rearing countries in Africa (Robinson et al. 2014). *T. solium* taeniasis/cysticercosis/neurocysticercosis is an important but neglected public health problem and serious social-economic obstacle for pig breeders in many African countries including Nigeria (Igbokwe and Maduka, 2018; Melki et al. 2018). The number of live pigs in Nigeria was reported to increase from 8,005 thousand heads in 2011 to 8,092 heads in 2021, growing at an average annual rate of 4.09% (Sasu, 2023).

Infection with *T. solium* tapeworm (taeniasis) occurs when a person eats raw or undercooked infected pork containing the parasites’ larval cysts or cysticerci (Otubanjo, 2013). Within four months, the larvae evaginate in the stomach and duodenum, attach to the intestinal wall and develop into adult tapeworms with gravid segments. Upon maturity, a single tapeworm can shed as many as 1000-2000 eggs per day. Each egg encloses an invasive hexacanth, or onchosphere which along with the distal gravid proglottids, are shed sporadically into the environment in the faeces of the tapeworm carrier. Although many eggs are discharged from the proglottids through an anterior pore, some remain within the uteri and remain viable for many months protected by the environmental factors and faeces and, from where they can act as sources of zoonoses. In unsanitary habitats and practices, *T. solium* eggs may also infect humans via ingestion of contaminated food or water. Ingested eggs develop into viable larvae (cysticerci) in tissues causing human cysticercosis. Autoinfection involves the retrograde transmission of proglottids from the intestines into the stomach with subsequent release of *T. solium* eggs into the human gut (Otubanjo, 2013; Zammarchi et al.2013; WHO, 2023).

Pig rearing is an important business globally (Robinson et al. 2014). However, cysticercosis reduces the market value of pigs and makes pork unsafe to eat (WHO, 2023). Socioeconomic conditions such as poor environmental hygiene and management practices are huge contributory risk factors of pig infections with parasites (Kungu et al. 2015; WHO, 2023).

In Nigeria, pigs have been found to be reared in small holder areas as scavengers of refuse dumps (Tidi et al. 2011). In areas where open defecation is still practiced, pigs freely forage human refuse and contaminated water or food. The pigs raised in this way are sold in the local market either directly to butchers or via traders who travel from one farm to another to purchase pigs thereby perpetuating the transmission of *T. solium* (Tidi et al. 2011; Karshima et al. 2013; Bernard et al. 2015). Investigations have shown varied prevalences of parasitosis in Nigeria. For example, Olaniyi (2014) reported a rate greater than 17% in pigs in Kwara State, Nigeria while Bernard et al. (2015) recorded a prevalence of 32.5% amongst pigs in Pankshin, Plateau State of Nigeria,

## There is no existing research that either investigated *T. solium* in nomadic pigs or techniques to recover their eggs in faeces in Wukari. Pigs are in abundance in Wukari and roam the entire neighborhood where they are raised under poor sanitary conditions for human consumption. The present study was undertaken to recover *T. solium* eggs in faecal droppings of free or unrestricted pigs using the formol ether sedimentation and direct mount techniques.

**MATERIALS AND METHODS**

**Study Area:**

The study was conducted in Wukari town in Wukari Local Government Area (LGA) of Taraba State, Nigeria. Wukari is geographically situated between Latitude 7053 42 North and Longitude 9047 59 East. It has an area of 4,308 km2 and a population of 241, 546 people made up of ethnic Jukun, Kuteb, Fulani, Hausa, Shombo and Tiv. Prominently, the Jukuns and other tribes in their minorities that inhabit Wukari are involved in occupations such as agriculture, trading and hunting (Oruonye and Abbas, 2011).

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### Ethical approval and faecal dropping collection

Approval for this research project was obtained from the Ethical Board of the Department of Microbiology, Federal University Wukari. Twenty-five pigs each from each sampling area were randomly selected and monitored during sample collection. Different droves were painstakingly and patiently followed as they wandered about and care was taken to ensure that droppings were collected immediately they were passed out and the sampled pig ink marked thereafter. The faecal dropping of each pig passed out in soil was collected as the drove roamed the roadsides, backyards, fields and home surroundings of four (4) areas in Wukari town (Wapan Nghaku, Ken Kisu, East and Mission Quarters). Thus, a total of one hundred (100) droppings from 100 pigs were collected. A separate disposable glove and clean pre-labelled specimen bottle was used for each faecal dropping. The sample bottles were well sealed and transported to the laboratory for analysis within 24 hours.

### Laboratory examination of droppings

The faecal droppings were first examined macroscopically for the presence of whole worms or segments. Thereafter, the droppings were processed for microscopy using the direct wet mount (DWM) and formol-ether sedimentation (FES) techniques described below (Cheesbrough, 2006).

To prepare a direct saline wet mount, one drop of physiological saline solution (0.85% w/v sodium chloride) was placed on to a clean and grease-free microscope slide. Thereafter, with the aid of a tooth pick, a small amount of the faecal dropping corresponding to a match stick head was added to it and mixed thoroughly. Finally, a cover slip (22mm by 22mm) was applied over this uniform suspension and microscopically examined using the x10 and x40 objectives. The goal of this method was to increase the translucency of the sample and enhance the detection of *T. solium* eggs.

In order to detect and isolate *T. solium* eggs in the faecal droppings, a small quantity of the sample is transferred into a 50mL beaker containing 10 mL of physiological saline. The solution was thoroughly vortexed with a glass rod and the emulsion filtered through fine mesh gauze into a 15 mL conical centrifuge tube. The suspension was centrifuged at a relative centrifugal force of 600g (2000 revolution per minute, rpm) for 10 minutes to yield about 0.75 mL of sediment. The supernatant was decanted and the sediment washed with 10 mL of saline solution. This was centrifuged and washed again until a clear supernatant was obtained. After the last wash, the supernatant was decanted and 10 mL of 10% buffered formalin was added to the sediment, mixed and left to stand for 5 minutes for fixation to occur. Thereafter, 4 mL of diethyl ether was added to the sediment and the tube was stoppered, contents vigourously shaken and centrifuged at 1500 rpm for 10 minutes. Resultantly, four layers consisting of top layers of diethyl ether and debris plug, layers of formalin and sediments were obtained. The debris plug was carefully freed from the side of the centrifuge tube by ringing with an applicator stick while the top three layers were decanted. The remaining sediment was mixed with a pipette and one drop each transferred to a drop of saline and iodine on a glass slide and mixed. The two drops were covered with cover slips and microscopically examined for the presence of *T. solium* eggs using the x10 and x40 objectives

**RESULTS**

The result of the formol-ether sedimentation test showed that out of one hundred (100) faecal droppings examined for the presence of *T. solium* eggs, the percentage recovery of positive droppings was 48% (Table 1). The highest recovery of eggs (56%) was from the Wapan Nghaku droppings while the least egg recovery (40%) was from Mission Quarters droppings. The result also showed equal recovery rates of *T. solium* eggs (48%) from the Ken Kisu and East area droppings.

Using the direct wet mount technique, the percentage recovery of *T. solium* eggs was highest in the droppings collected from the Wapan Nghaku area (40%) while the droppings collected from the Mission Quarters contained the least eggs (20%). The recovery of eggs in Ken Kisu and East droppings was 32% and 36% respectively (Table 1).

Comparatively, in each of the areas sampled, the percentage detection of *T. solium* eggs was highest in the faecal droppings recovered using the FEC technique and least in those detected with the direct wet mount technique (Table 1).

**Table 1: Recovery of *T. solium* eggs in faecal droppings using the formol-ether sedimentation**

**and direct wet mount techniques**

|  |  |  |  |
| --- | --- | --- | --- |
| Sample area | Number examined | Number of positive samples  Formol-ether sedimentation Direct wet mount | |
| Wapan Nghaku | 25 | 14(56.0) | 10(40.0) |
| Ken Kisu | 25 | 12(48.0) | 8(32.0) |
| East | 25 | 12(48.0) | 9(36.0) |
| Mission Quarters | 25 | 10(40.0) | 5(20.0) |
| Total | 100 | 48(48.0) | 32(32.0) |

Figures in parentheses represent percentages

# DISCUSSION

Mature eggs that are shed into the environment might remain active for months under favourable environmental conditions (WHO, 2023). It is probable that this attribute might have contributed to the prolonged viability of *T. solium* eggs in the soil. The recovery of *T. solium* eggs using the FES method was higher than the DWM technique because the number of helminthic eggs is often too low to be observed microscopically in DWM and the use of FES increases the percentage detection of the copromicroscopic technique. This implies that the FES method concentrated the helminth eggs by taking advantage of their high specific gravity compared to water. The natural inclination of these eggs to settle (sediment) in aqueous solutions is accelerated through centrifugation. Formalin fixed the eggs rendering them non-infectious and preserved their morphology while ether was used to extract debris and fat from the faeces thus enhancing parasite recovery and identification (Cheesbrough, 2006).

Just as Saelens et al. (2022) observed, several techniques that have been employed by researchers to detect taeniid eggs seldom have standardization, performance evaluation and viability assessment significance and hinder understudy comparisons even as prospective investigators find it difficult to comprehend and decide the best method to use to determine environmental contamination by eggs of *Taenia* sp. No supporting and appropriate reference article as far as could be ascertained, was found to enable comparative studies to be made for FEC better than DWM in detecting *T. solium* eggs in contaminated soils. Most of published literature usually utilize a single diagnostic procedure to detect taeniid eggs in animal, water, food and soil environments (Adenusi et al. 2015; Bernard et al. 2015; Guggisberg et al. 2020) and where two or more methods have been used, the objectives were not to compare the detection rates based on recovery methods (Satchwell, 1986; Maikai et al. 2012; Jimenez et al. 2016; Aghaindum et al. 2019). It is therefore worthy of note that the present finding was successful at evaluating the performance of FES and DWM and advocates the use of the latter in *T. solium* egg recovery and detection.

Generally, the egg positivity in the samples detected using either of the two diagnostic techniques was high in the study area due to the increased risk of parasite transmission arising from the large population of freely roaming pigs, their feeding habit and circumstances of raising them under this free range system (Kungu et al. 2015). In the areas they roamed, some households had no toilets and most of the children defecate on near-by shrubby areas or in the bushes, a high risk factor for contamination and transmission (Jansen, 2021). Under these conditions, roaming pigs are likely to feed on faecal materials that might contain *T. solium* eggs. This may be the reason egg recovery was least in Mission Quarter as the living standards especially toilet facilities and wastes disposal practices were observed to be better than in the other areas.

**CONCLUSION**

It is evident in this study that pigs are important sources of *T. solium* eggs. Therefore, early detection, using the FES as a diagnostic means, is recommended. To reduce the risk of transmission to humans, local breeders are advised to vaccinate and/or de-worm their pigs as core “rapid impact” intervention schemes. Open defecation and dumping of faecal refuse should be discouraged through vigourous community health education as supporting measures. Fundamental societal changes such as thorough cooking of meat before consumption, improved husbandry management and meat inspection practices, are hereby advocated since pork meat is a widely accepted delicacy in Wukari.

**CONFLICT OF INTEREST AND FUNDING STATEMENT**

The authors declare no conflict of interest. They also did not receive any funding whatsoever in the course of this research.

**STATEMENTS AND DECLARATIONS**

The authors declare that this work has neither been published before nor under consideration for publication anywhere else. The manuscript has also been read and approved by all authors.

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