

# Seroprevalence of Porcine Cysticercosis and Associated Risk Factors in Pigs Slaughtered in Abattoirs in Thika, Kiambu County, Kenya

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**Abstract:** *Taenia solium* is an important food-borne pathogen worldwide and is emerging as a serious public health risk in both rural and urban communities where pigs are raised and consumed. Adult tapeworms are found in the intestines of humans while the developmental larval forms occur in the muscles and organs constituting cysticercosis of pigs and humans. Cysticercosis has a worldwide distribution, mainly related to poor hygiene and sanitation and consumption of infected pork. Pigs get infected through consumption of food and contaminated with human faeces containing eggs. In recent years pork consumption has increased with the opening up of pork eating centres. Porcine cysticercosis has previously been reported in Kenya, however, there are scarce data on the occurrence of the disease, as well as on the risk factors for transmission, in key production and consumption areas including Thika. The purpose of the study was to determine the seroprevalence of porcine cysticercosis in slaughtered pigs and associated risk factors for occurrence of the disease in selected abattoirs in Thika. Systematic random sampling was used to select a total of 276 pigs. The source of the slaughter pigs was derived from the movement permits, the breed, sex and estimation of age was done at ante mortem examination. The slaughter and meat inspection processes were carried out by the slaughter house personnel and the investigators only observed and received the outcome of the inspection. Blood samples were collected from each identified pig at slaughter, processed and analyzed using purified *Taenia solium* antigen ELISA commercial kit. The results meat inspection showed that none of the pigs in this study had any visible cysts whereas 4.35% of the pigs were seropositive which poses public health risk.

**Key words:** Abattoir surveillance, antigen ELISA, pork tapeworm, zoonosis.

## 1. Introduction

Cysticercosis remains a major neglected tropical disease of humanity in many regions, especially in sub-Saharan Africa, Central America and elsewhere where pigs are raised and consumed. Among food borne diseases in Eastern and Southern Africa, porcine cysticercosis is ranked low except in South Africa [1-3].

Pig production has increased significantly in the ESA (Eastern and Southern Africa) region during the past decade, especially in rural, resource-poor, smallholder communities. Concurrent with the

increase in smallholder pig keeping and pork consumption, there have been increasing reports of porcine cysticercosis in the ESA region [4]. Pig farming in Kenya is important with various production systems from free-range to intensive keeping in confinement. Some of the production systems may increase the risk of disease transmission. Previous studies in Homa Bay and Busia reported presence of disease at prevalences of 32.8% and 6.5% [5, 6] respectively. Serological survey of porcine cysticercosis and associated risk factor in pigs slaughtered at Ndumbuini abattoir in Nairobi reported prevalence rates of 43% and source of slaughter pigs as the highest risk factor [7] while the pig production system was also a risk [8, 9]. With the

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increase in small holder pig production and pig consumption there has been an increase in number of reports of cysticercosis and some of these reports from Thika, Kenya while no study has been done in this region.

The community-based studies on porcine cysticercosis indicate a prevalence of 5 to 35. In Kenya recent surveys in the southwestern part of the country where smallholder pig keeping is popular indicate that 10%-14% of pigs are positive for cysticercosis by lingual examination [3]. In Zambia, abattoir records reported porcine cysticercosis in six of the nine provinces. Routine meat inspection of 1,316 pigs at a slaughter slab in Lusaka showed that 20.6% of the pigs had cysticercosis whereas serological testing of 874 pigs at the same abattoir indicated that 56.6% were found to have circulating antigens of *Taenia solium* [4]. In Uganda, Kisakye and Masada [10] found a prevalence of 9.4% while Mwape et al. [11] recorded a seroprevalence of 21.65% in Democratic Republic of Congo using Ag-ELISA (antigen-ELISA). A study on the seroprevalence of porcine cysticercosis measuring the antibody responses and CCA (circulating cysticerci antigen) responses by Ab-ELISA (antibody-ELISA) and antigen ELISA (Ag-ELISA), respectively was conducted [12]. Positive correlations were observed between antibodies, CCA and the total number of cysticerci enumerated at necropsy.

In recognition that some serious human infections can be transmitted through meat consumption, the Government of Kenya through the Meat Control Act Chapter 356 of 1977 requires that all meat used for human consumption be inspected by qualified personnel [13]. Meat inspection prevents transmission of such diseases by detecting sick animals through identification of macroscopic lesions in specific organs during carcass inspection and prevents infected meat from reaching the market for consumption through confiscation of infected carcasses [14]. Despite this, *Taenia solium* infections remain an

active threat to public health and incomes in many parts of the world, especially third world countries [15]. High consumption of pork in Thika region, coupled with modern trends in human movement, industrialized food processing and distribution, pose new threat of widespread re-emergence of these diseases with possible serious public health consequences unless the measures of control that are already in place are highly effective. This creates a need to evaluate the effectiveness of routine meat inspection in cutting down supply of infected pork into the human consumption market. Development of a serologic assay that can be used in diagnosis of porcine cysticercosis pre-slaughter is also highly desirable for pig farmers, to enable them to avoid losses incurred from condemned carcasses. This study evaluated the seroprevalence of pigs at point of slaughter.

## **2. Materials and Methods**

### *2.1 Study Location*

This study was done in two slaughterhouses (Thika Turi and Kabati Kenol) located in Thika and Muranga, respectively. Thika is an industrial town, which is administratively in Kiambu County and neighbours Muranga County.

### *2.2 Study Design*

Cross sectional study was conducted in the two pig slaughterhouses that supply meat to Thika Town and its environs.

### *2.3 Study Animals*

The slaughterhouses receive pigs mainly from small scaleholder farmers in Kiambu and Muranga Counties and occasionally from as far as Kirinyaga, Meru and Nyeri Counties. Kabati Kenol slaughterhouse has an average throughput of 40 pigs per day while Thika Turi slaughters approximately 15 pigs daily. Mature market ready pigs were presented for slaughter, both males and females and predominantly of large white

breed. Systematic random selection of every 5th pig presented for slaughter at abattoirs was done until 276 pigs were selected.

#### *2.4 Meat Inspection Procedure*

Inspection of pigs at slaughter was conducted as per the Kenya Meat Control Act, Chap 356 [16] by the inspector in charge of the abattoir. Inspection included palpation and incision of the tongue, visual inspection and incision of the heart and visual inspection of all exposed muscles, especially the neck, loin, ham and fleshy part of the diaphragm for presence of cysts and as described by Boa et al. [17]. Meat inspection results were obtained from the report of the meat inspector on duty in the slaughter facility at the end of each day on all pigs slaughtered. The investigators did not interfere with the process of pig slaughter or meat inspection in order to obtain a true reflection of the activities carried out.

#### *2.5 Collection of Blood Samples at the Slaughterhouse*

Blood samples were drawn from the cava vein and processed. Venous blood was obtained from 276 pigs slaughtered during the study period. Anterior vena cava blood samples were collected in BD Vacutainer® 10 mL plain tubes from freshly slaughtered pigs and transported to the University of Nairobi, Clinical Studies Department research laboratory on ice. The blood was allowed to clot at room temperature, placed in refrigerator at 4 °C overnight for maximum yield of sera, and then centrifuged at 3,000 rpm for 20 min at room temperature. Sera was separated into two aliquots in 2 mL labeled cryovials and stored at -20 °C prior to laboratory analysis.

#### *2.6 Cysticercosis Ag-ELISA Assay*

Purified *Taenia solium* antigen ELISA commercial kit as per manufacturers' instructions was used to determine the seroprevalence of porcine cysticercosis in pigs at slaughter. The Ag-ELISA was performed as

described by Brandt and others [18] and modified by Dorny and others [19]. The serum samples and controls (positive and negative) were pre-treated using TCA (trichloroacetic acid) and used in ELISA at a final dilution of 1/4. Two MoAbs (monoclonal antibodies) were used in a sandwich ELISA, B158C11A10 (apDia, Ref 650510, IVD, Turnhout, Belgium) diluted at 5 mg/mL in carbonate buffer (0.06 M/pH 9.6) for coating and a biotinylated MoAb B60H8A4 (apDia, Ref 650510, IVD, Turnhout, Belgium) diluted at 1.25 mg/mL in phosphate buffered saline-Tween 20 (PBS-T20)+1% NBCS (new born calf serum) as detector antibody and incubated at 37 °C on a shaker for 30 min for the coating of the first MoAb and for 15 min for all subsequent steps. A solution consisting of OPD (ortho phenylenediamine) and H<sub>2</sub>O<sub>2</sub> was added and incubated without shaking at 30 °C for 15 min as a substrate. And 50 mL of H<sub>2</sub>SO<sub>4</sub> (4 M) was added to each well to stop the reaction. The plates were read using an ELISA reader at 630 nm. To determine the cut-off, the mean OD<sub>negative</sub> was multiplied by 3.5 while the calculation of Ag (antigen) Index was divided the mean OD<sub>sample</sub> by the cut-off value.

#### *2.7 Data Analysis*

The measure of consistency for the level of agreement between the detection of circulating antigens as determined using ELISA and meat inspection findings was used. Absolute values for optical densities and calculation of the means in paired samples were done. Through search for cysts in different organs, muscle tissues were done during the routine meat inspection procedure. Interpretation of the results for the detection of cysticercosis antigen in porcine specimens: a positive reaction corresponded to an Ag-Index of above or equal to 1.3, a negative reaction corresponded to an Ag-Index below or equal to 0.8 while samples with Ag-Index between 0.8 and 1.3 were considered doubtful and would require further evaluation.

### 2.8 Ethical and Logistical Considerations

Permission to carry out the research was sought and granted from National Council of Science, Technology and Innovation through the office of the Principal Secretary, Education, County Government of Kiambu, and the County Director of Veterinary Services, Kiambu County.

## 3. Results

### 3.1 Characteristics of the Study Animals

Of the 276 pigs selected 76% were females while 24% were males, the age range was reported to be 6 to 12 months with mean age of 8 months. A majority (96%) of the pigs were sourced from farms close to the slaughterhouses in Kiambu and Muranga counties while 4% of the pigs in Kabati Kenol slaughterhouse were from Kirinyaga and Meru.

### 3.2 Meat Inspection Reports

Pigs were slaughtered everyday from Monday to Saturday throughout the study period which was over 2 months, routine meat inspection was carried out on all the pigs but none of the pigs were positive for *Taenia solium* cysts on visual inspection of the organs and tissues incised. The pigs selected for seroprevalence evaluation were also negative for *T. solium* cysts.

### 3.3 Seroprevalence

Positive antigen responses were recorded in 12 out of 276 slaughter pigs which were seropositive of

4.35% (Table 1). Equal numbers of males and females were positive and were all aged 9 months. As the blood samples were collected at time of slaughter no further evaluation was carried out on the three doubtful results. The positive pigs were from Kiganjo, Muguga and Kimorori Wards.

## 4. Discussion

Cysticercosis, although normally clinically insignificant in pigs, is associated with significant economic losses due to carcass condemnation and decreased value of pigs, and causes a major source of infection in humans [20].

Ag-ELISAs do have use in field-based epidemiological studies for indicating transmission and efficacy of treatments. Sikasunge et al. [12] used correlations between circulating Cysticercosis Antigens and the total number of cysticerci enumerated at necropsy to indicate treatment efficacy as they disappeared early after treatment. The detection of viable infections in pigs as was the case in the current study could indicate point sources of infection, season of transmission and age of animals at risk. The infections were observed in three specific sites in pig aged 9 months. These sites would be considered for further study to elucidate transmission model in the area.

The seroprevalence of porcine cysticercosis in the current study was low as compared to other studies, in Kenya [7] (43%) and [8] (14%), in Uganda [10] (9.4%) and in Democratic Republic of Congo [11] (21.65%). While all these studies consistently report the presence

**Table 1 The circulating antigen responses reported in seroprevalence evaluation of porcine cysticercosis in slaughter pigs in two abattoirs in Thika, Kenya in 2016.**

Characteristic feature	ELISA Plate 1	ELISA Plate 2	ELISA Plate 3	Total (N)	% response	Remarks
Mean OD Negative	0.322	0.055	0.213	-	-	
Mean OD positive	2.12	2.32	2.31	-	-	
Cut off Value	1.13	0.195	0.744	-	-	
Negative Results	84	90	87	261	94.6	Ag Index < 0.8
Positive Results	7	1	4	12	4.35	Ag Index ≥ 1.3
Doubtful results	1	1	1	3	1.08	Ag Index 0.8 to 1.3
Total	92	92	92	276	100	

N is number, OD is Optical Density.

of the infection, the prevalence levels may differ in relation to source of the slaughter pigs and pig production system. Majority of the pigs were sourced from Kiambu and Muranga where the farmers raise their pigs in small holder zero grazed units as opposed to free range grazed pigs [8].

The finding on assessment of routine meat inspection procedure is in agreement with the work done by Phiri et al. [21] in Zambia where the current practice is inadequate cysts where the infections are of low intensity. Non observance of cysts in the slaughter pigs led to the all the pig meat being approved for human consumption and hence entering the food chain which would be a risk as reported by Kariuki et al. [22]. However, the positive antigen responses as observed in the study indicate presence of slaughter pigs in the area and further demonstrate the low sensitivity of current meat inspection procedure. Further investigations are required to establish the relationship between positive cysticerci responses and the period of occurrence of the cysts in the muscles and tissues.

In conclusion, though none of the pigs examined at the slaughterhouses during the study period had cysts either in the mouth or tissues following routine meat inspection, the seroprevalence of porcine cysticercosis recorded in pigs slaughtered in Thika indicates presence of disease and this may pose a risk to public health.

### **Acknowledgements**

This work was supported by funds from the Kenyatta University Vice Chancellor's 2015 Research Grant.

Our appreciation goes to the County Government of Kiambu-Department of Veterinary Services and Medical Services for permission to carry out research in their facilities.

Research Team and Collaborators are grateful for the Technical assistance (meat inspectors) for data collection and Laboratory analysis of the serum

samples. We also wish to thank the proprietors and management of the two pig slaughterhouses for allowing us to carry out the work in their premises.

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