

PREVALENCE AND HISTOPATHOLOGY OF *TRICHINELLA SPIRALIS* LARVAE OF SLAUGHTERED PIGS IN CAIRO GOVERNORATE, EGYPT

By

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Abstract

The present work was planned to study the prevalence and the pathological lesions of *Trichinella spiralis* larva of slaughtered pigs in Cairo Governorate by using Trichinoscopic examination in slaughterhouse and confirmatory diagnosis by histopathological examination. For this purpose 184 muscle samples of pigs were collected from Al-Basatin Slaughterhouse, Cairo Governorate. The overall prevalence of *Trichinella spiralis* infection was 1.08% of examined pig's carcasses.

Key words: *Trichinella spiralis*, Pig, Trichinoscopy, Histopathology.

Introduction

Trichinellosis is one of the most widely distributed parasitic zoonosis worldwide and is caused by infection with nematodes of the genus *Trichinella* (Conlan *et al*, 2014). *Trichinella spiralis* infect a broad variety of mammals, birds and reptiles and are distributed worldwide. Human infections occur after ingestion of raw or inadequately cooked meat containing parasite larvae. Pigs represent a major source of human infection (Pannwitz *et al*, 2010). It can be a serious disease, particularly in the elderly, where severe complications such as myocarditis or encephalitis can lead to death (Djurkovic *et al*, 2013)

The prevalence of the parasite in swine in some regions of the world is a public health concern (Gajadhar *et al*, 1997). Also represents an economic problem in porcine animal production and food safety (Gottstein *et al*, 2009). When the role of pigs in the trichinellosis cycle was definitively established in the 1860s, it became imperative to monitor the development of the disease in both animals and humans (Blancou, 2001). *Trichinella* infection rarely causes clinical signs in the parasite's natural hosts, unless they are infected with a very large number of larvae (Bruschi and Murrell, 2002). *T. spiralis* is diagnosed by detecting *T. spiralis* larvae in muscle samples or by testing for the presence of *Trichinella* antibodies in the serum or in the meat juice

(Gottstein *et al*, 2009). In pigs, establishment of the larvae of *T. spiralis* results in extensive disturbances at the cellular level and consequent pathological changes in skeletal muscles and organs (Ribicich *et al*, 2007). This study aimed to assess the percentage of infection and pathological lesions of *Trichinella spiralis* larvae in pig slaughtered in Governom- ental Slaughterhouse, Cairo Governorate.

Materials and Methods

Study design and sampling: A total of 184 muscle samples (mainly diaphragm and skeletal muscle) were collected over a period of 6 months on days of slaughtering pigs from December 2018 to May 2019, in Al-Basatin Slaughterhouse, Cairo Governorate. Egypt.

I- Postmortem examination: 1- Trichinoscopy or compression method: Traditional way of analyzing meat for *Trichinella* larvae in slaughterhouse, is to use a compressorium or trichinoscopy and performed as follow; One gram of diaphragm or m. masseter is cut into small pieces. After being placed between the glass plates of the compressorium, the plates were screwed tightly together, so the meat was pressed into a thin, transparent layer. The compression was examined directly in the dissection binocular microscope (Roepstorff and Nansen, 1998).

2-Histopathological examination: Specimens from muscle of infested pigs were recovered and fixed in 10% formaline solution

for histopathological examination. After ensuring the complete fixation, the specimens were washed with tap water, dehydrated in ascending series of ethanol up to absolute alcohol, cleared in xylene and embedded in paraffin. Section of 5 micron thickness were prepared and stained with Hematoxylin & Eosin (Hassanin *et al.*, 2013).

II- Experimental animals: 1- Laboratory-bred parasite-free male Swiss Albino mice, 6-8 weeks old, weighing 25-30g each were used. The animals were purchased from (Animal House, Faculty of Medicine, Assiut University, Egypt).

2- Experimental infection of mice: The larvae of *T. spiralis* were isolated from the infected pork meat obtained from the muscle samples (mainly the diaphragm and skeletal muscles) from El-Basatin Slaughterhouse, Cairo Governorate. The infected samples were put in the digesting fluid (1% pepsin and 1% HCL in 200ml distilled water). The mixture was incubated at 37°C for an hour under continuous agitation using an electric stirrer. The outcome digested product was passed through a 50-mesh/inch sieve to remove the coarse particles. Encysted larvae were collected on a 200-mesh/inch silver sieve, washed twice with tap water and then suspended in 150ml of tap water in a conical flask. The supernatant fluid was discarded and the larvae were collected by Pasteur pipettes. Mice were infected with *T. spiralis* larvae orally and were housed in appropriate insect proof-cages and fed in the accordance with the institutional and national guidelines. They were kept under close observation. On the 35th day post infection, the infected mice's were sacrificed by their neck dislocation.

The small intestine of each mouse was removed, dissected longitudinally, washed and the *T. spiralis* adult worms were detected and their muscles were digested for larval detection the sediment reconstituted in 3-5 drops of saline, and then examined drop by drop at a magnification of 20X (Yang *et al.*, 2014).

Results

Prevalence of trichinellosis: The results showed that the prevalence of *T. spiralis* larvae infection in the slaughtered pigs, from El-Basatin Slaughterhouse was 2 out of 184 (1.08%).

Histopathology examination; encysted larvae were replacing in the muscle fibers and inflammation was minimal to absent consisting of few lymphocytes.

The details were given in figures (1, 2, 3, 4, 5, 6 & 7).

Discussion

Prevalence: However, domestic trichinellosis (*Trichinella spiralis*) is present in Egypt. Few reports of *T. spiralis* infection in fresh and processed pork in Egypt are available (Youssef and Uga, 2014). The present study revealed that the prevalence of *Trichinella spiralis* larvae infection in slaughtered pigs, in Albasatin slaughterhouse in Cairo Governorate (Egypt), was 1.08% (2/184), the lowest infection rate in this study agree with mentioned by Abdel-hafeez *et al.* (2015) in El-Minia (Egypt), who concluded that, no pig was infected with *T. spiralis* by using the different techniques throughout the governorate. The results were lower than that of Sayed *et al.* (2010) in Upper Egypt, who detected the infection rate of 5% & 2% in Assiut and Sohage Governorates, respectively. Conlan *et al.* (2014) in Laos (Australia) reported trichinellosis in 2.1% of examined pigs. The low prevalence rate of infection with trichinellosis in pigs might be related to hygienic conditions for rearing pigs in private farms or large farm and rising of the Egyptian pigs is mainly indoors away from the sources of infection. No doubt, lack of accuracy in slaughterhouses during examination of pig carcasses depending on trichinoscopy, which is of more or less not dependable accuracy and need well a trained technician. Djurkovic *et al.* (2013) reported that the trichinoscopy gave a sensitivity of around one larva/ gram, which was not accepted to exclude trichinosis infection.

In the present study, histopathological examination revealed that, encysted larvae were replacing the muscle fibers and inflammation is minimal to absent minimal inflammation consisting of few lymphocytes. This result agreed with Zachary and McGavin (2012) who reported that after the cyst formation, the larvae are protected from the hosts' immune response and inflammation is minimal to absent other than focal inflammation consisting of eosinophils, neutrophils, and lymphocytes occur associated with invasion of muscle by *Trichinella* larvae. Wu *et al.* (2008) found that *Trichinella spiralis* larvae build their own home in the infected muscles. The home is a capsule composed of a collagenous wall and cellular components. The wall provides some protection to the parasite and the cellular component that takes care of the parasites in terms of metabolism. Because of its function, the name "nurse cell" has been given to the cellular components. A series of recent studies provide more evidence to strengthen their ideas that *Trichinella* utilizes a repairing process of muscles cells to construct the capsule. In other words, after injury induced by parasite invasion, muscle cells start going through the process of repair, just like after any trauma. *Trichinella sp.* larva borrows only the initial part of this repair process to construct its own home. Since the satellite cell is a progenitor cell located within the capsule wall, a new cell can be continuously supplied from the myoblast, even if the present nurse cell dies. This explains why the nurse cell looks intact and active for years in spite of intracellular parasitism. Sometimes, the presence of nurse cell-parasite complex indicates the ongoing infection. A mature capsule and a coiled parasite indicate an older infection (Capo and Despommier, 1996).

Generally, worldwide about 10,000 trichinosis infections occur a year. At least 55 countries including the United States, China, Argentina, and Russia have had been reported cases (CDC, 2015). Trichinosis occurs in the tropics is less common (Jeremy,

2013). Trichinosis rates in USA decreased from 400 cases/year in 1940s to 20 or fewer /year in 2000s (CDC, 2016).

Conclusion

Pigs are the main reservoir of trichinellosis, but their role depends on their breeding. In large farms infested rodent and did not apply hygienic programmes, pig meat must be thoroughly examined in slaughterhouse and properly cooked before consumption.

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Explanation of figures

Fig. 1: Encysted larva of *Trichinella sp.* in muscle tissue of pig with minimal inflammation consisting of few lymphocyte and degeneration in muscle showing loss of cross stration and become more eosinophilia (arrow) (H&E stain x400).

Fig. 2: Muscle biopsy from pig infected with trichinellosis a) minimal Cellular infiltrates; b) Collagen capsule of nurse cell; c) Intersected muscle larva (H&E stain x200).

Fig. 3: Calcified cyst within muscle tissue of pigs (H&E stain x100).

Fig. 4: *Trichinella spiralis* larvae encysted in muscle (after inflammatory cell reaction subsided). (H&E stain x100).

Fig. 5: Encysted larva within muscle fibers of pig infected with trichinellosis. (after inflammatory cell reaction subsided) (H&E stain x200).

Fig. 7: Adult *T. spiralis* obtained from small intestine of mice infected experimentally with larva of *T. spiralis* (Unstained specimen).

