2021, Scienceline Publication
World's Veterinary Journal
World Vet J, 11(4): 594-601, December 25, 2021
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2021.wvj75

The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection

Nermeen Makram Louis Malak[1] and Shimaa Abdel-Radi[2*]

[1]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza 12613, Egypt*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Giza 12613, Egypt*

*Corresponding author's email address; Shimaa.abdelradi@cu.edu.eg; ORCID: 0000-0001-7856-8147

ORIGINAL ARTICLE
pii: S232245682100075-11
Received: 29 September 2021
Accepted: 24 November 2021

ABSTRACT

Trichinellosis is a worldwide zoonotic food-borne disease that causes public health problems. The present study investigated the role of domestic ducks (*Muscovy*) and catfish (*Clarias lazera*) in transmitting *Trichinella spiralis* infection in Egypt. Ducks, fish, and rats were inoculated by high doses of *Trichinella spiralis* (*T. Spiralis*) larvae in naturally infected muscles of pigs and by free larvae extracted from artificial digestion. Both methods failed to develop an infection in ducks. No worms or cysts could be detected in ducks slaughtered 10, 25, and 45 days after inoculation. Moreover, there was no significant increase in the mean ELISA optical density (OD) value, compared to the control non-inoculated ducks**.** Inoculation of fish resulted in a significant increase in the mean ELISA OD value, compared to the control non-inoculated fish. This elevation was associated with the temporary detection of a few adult worms in the intestine of these fish which decreased with time till disappeared 45 days after inoculation but a non-significant increase was observed, compared to that obtained in the inoculated rats at the same stages. A large number of *T. spiralis* adult worms and cysts associated with a significant increase in the mean ELISA OD were recorded in the inoculated rats. Infection of rats (xenodiagnosis) by muscles collected from the inoculated ducks or fish failed to induce infection or elevation in the level of anti-*T. spiralis* antibodies (ATs-Ab) in their sera. In conclusion, *Muscovy* ducks and *Clarias lazera* could not be infected by *T. spiralis* in their muscles and the consumption of their meat could not transmit this infection to consumers in Egypt. However, proper cooking of meat is still necessary to avoid infection with other species.

Keywords: *Clarias lazera*, Ducks, ELISA, *Trichinella spiralis*

INTRODUCTION

Trichinellosis is a worldwide zoonotic food-borne disease causing economic losses, public health problems, and even mortalities in humans. This disease is caused due to ingestion of inadequately cooked or raw meat containing the infective *Trichinella species* larvae (Djurković-Djaković et al., 2013; Noeckler et al., 2019). There are 8 identified species and 12 genotypes belonging to the family Trichinellidae (Conlan et al., 2014)**.** *Trichinella* species that infect mammals are subdivided into two groups. The first group is encapsulated in host muscles to form cysts (such as *T. spiralis*, *T. nelson*, *T. native*, and *T. murrelli*), however, the other group does not form a cyst in the body of the infected hosts, such as *T. pseudospiralis*, *T. zimbabwensis*, and *T. papuae* (La Rosa et al., 2003). The encapsulated *Trichinella* species could complete their life cycle in mammals as they require a body temperature ranging from 37-40ºC. While the non-encapsulated species, such as *T. pseudospiralis*, possess an increased host range, as they can be developed in mammals and birds with a body temperature of 37-42ºC. Species, such as *T. zimbabwensis* and *T. papuae*, can infect both mammals and reptiles as they require host body temperature ranging from 25 to 40ºC (Pozio, 2019).

Diagnosis of *Trichinella* by trichinoscope is a less accurate technique with low sensitivity and needs experience (Djurković-Djaković et al., 2013). On the other hand, a serological test, such as ELISA, is one of the most common diagnostic tests and a useful tool for the diagnosis of *Trichinella* infection (Hewitson et al., 2009; Franssen et al., 2011) which is easily applicable and suitable for simultaneous evaluation of a large number of samples. *T. spiralis* excretory-secretory antigens (ESAg) of the first stage larvae (TSL1-ESAg) is abundant and stage-specific antigens, excreted from the stichocytes present in the anterior part of the first stage larvae. Moreover, it is present in its cuticular surface. This TSL1-ESAg contains an immunodominant carbohydrate epitope, which is specific to *Trichinella* (Taher et al., 2017; Bruschi et al., 2019). These antigens contain the most sensitive and specific protein fraction which is valuable for the diagnosis of infection in suspected cases (Ludovisi et al., 2008).

Pigs are considered the main representative host of the domestic cycle of *T. spiralis* and the primary cause of transmission of *Trichinella* infection in humans (Franssen et al., 2011). Furthermore, the parasite can infect a wide range of hosts, including humans, wild and domestic animals, reptiles, and some birds. Each of these hosts serves as a

To cite this paper: Malak NML and Abdel-Radi S (2021). The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection. *World Vet. J.*, 11 (4): 594-601. DOI: https://dx.doi.org/10.54203/scil.2021.wvj75

definitive (containing the adult worm), and as an intermediate host that contains the larval stage (infective stage of the parasite) (Pannwitz et al., 2010; Pozio, 2019).

In Egypt, pigs are usually raised in barns with poor hygienic conditions where some domestic birds, such as ducks, geese, and chickens are also usually raised with pigs. Moreover, rodents and other reservoirs can be easily accessed by these barns. Consequently, these hosts may be infected by *Trichinella* from pigs. Rats are revealed to be a potential reservoir host species of *Trichinella* and play an important role in the transmission of *T. spiralis* from and to pigs, especially in the low housing sanitation level (Bilska-Zając et al., 2018). Moreover, the incidence of *T. spiralis* infection reached 13.3% in rodents (Youssef and Uga, 2014.). In addition, a large number of fish farms feed their fishes on bone, meat meals, wastes of some domestic birds, and slaughtered pigs' offals that may facilitate the delivery of *T. spiralis* to carnivorous and omnivorous fish species. As a result, these ducks or fish meat may incriminate as a source of infection to humans.

Some researchers studied the susceptibility of different hosts, such as sheep, pigeons, cattle, Guinea pigs, camels, monkeys, horses, and reptiles to *T. spiralis* (Smith and Snowdon, 1989; Yacoub et al., 1993; Asatrian et al., 2001). However, studying the role of ducks and fish in transmitting *T. spiralis* infection is neglected in Egypt. For these reasons, the present study focused on the role of domestic ducks (*Muscovy*) and catfish (*Clarias lazera* as a scavenger fish) in the transmission of *T. spiralis* infection.

MATERIALS AND METHODS

Ethical approval

All procedures of the study were performed regarding the instructions listed by the Institutional Animal Care and Use Ethical Committee (CU-IACUC) of Cairo University, Giza, Egypt.

Preparation and collection of *Trichinella spiralis* larvae

Trichinella spiralis larvae were determined after the trichinoscopic examination of naturally infected diaphragmatic muscles of freshly slaughtered pigs in Cairo abattoir, Egypt, according to the method described by Vignau et al. (1997) where one gram of these muscles adjacent to the tendinous part of the diaphragm was sub-cut into small pieces then compressed between two glass plates of the trichinoscope and examined under 40× magnification of a binocular light microscope (LABOMED, Labo America, Inc. U.S.A). An amount of 50 g from these muscles was dissected into very small pieces. Larvae in 10 g from the heavily infected samples were extracted after artificial digestion in 0.3% (w/v) pepsin in 0.06 N HCI at 37°C for 2-3 hours, supported with a magnetic stirrer. The obtained larvae were counted using McMaster counting chambers according to IQACA (2012). The number of larvae per gram of the infected muscles was calculated after compression between two glass slides where one of these slides was divided into equal counting chambers (squares each of 1cm × 1 cm). A dose of 1000 *T. spiralis* larvae either after extraction by digestion or those present in muscle cysts were used for the experimental infection of ducks, fish, and albino rats.

Parasite identification

Trichinella spiralis cysts and larvae which were collected at the Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Egypt from muscles of the naturally infected pigs slaughtered in Cairo abattoir, Egypt, as well as the adult worms produced after experimental infection in this study, were identified according to Faubert and Tanner (1971) and Walden (2013).

Protocol of the experimental infection

Experimental infection of Muscovy ducks

Muscovy ducks of five weeks old were purchased from a private poultry production farm. These ducks were kept under observation in the Faculty of Veterinary Medicine, Cairo University for another three weeks. During this period, blood and fecal samples were weekly collected for parasitological examination. At the age of eight weeks old, a total of 45 *Muscovy* ducks free from parasitic infections were selected for the study. Thirteen selected ducks were allocated into two groups each of 15. The first group received 1000 free larvae extracted from the digested infected pigs' muscles. While the second group was orally inoculated by known grams of dissected muscles containing 1000 larvae counted as before. At the same time, a group of 15 non-inoculated ducks was kept as a control group.

Experimental infection of catfish (Clarias lazera)

Living active 45 *Clarias Lazera* fish (25-30 cm) were collected from a private fish farm that follows regular hygienic and sanitary conditions. They were divided into three groups each of 15. The first and second groups (30 fish) were inoculated similarly to ducks by 1000 larvae as free larvae and larvae in muscles cysts, respectively. While the last group consists of 15 fish used as a control non-inoculated group.

To cite this paper: Malak NML and Abdel-Radi S (2021). The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection. *World Vet. J.*, 11 (4): 594-601. DOI: https://dx.doi.org/10.54203/scil.2021.wvj75

Experimental infection of rats

In the present study, rats were used for two separate purposes; the first one was infecting them by infected pig's muscles or free larvae to be used as a reference control for *T. spiralis* experimental infection of ducks and fish. While the second purpose was to use rats for xenodiagnosis to evaluate the success or failure of the experimental infection of ducks and catfish by following up the development of infective *T. spiralis* cysts in the muscles of these inoculated hosts.

Thirty white albino-female rats (125 gm each) were allocated into two groups each of 15, one group inoculated with muscles containing cysts and the other group inoculated with free active extracted larvae as the same was done for ducks and fish, while at the same time, a group of 15 rats was kept as control non-inoculated.

In addition, 60 female rats were used for xenodiagnosis(five rats for each group); they were fed on pooled muscles samples collected from the larvae or cysts inoculated ducks and catfish after each slaughtering time of these hosts(10, 25, and 45 dpi). These rats were sacrificed after 45 days of inoculation. Besides, 15 rats were kept as control non-inoculated (three groups each of five corresponding to the three slaughtering times).

The inoculated hosts were sacrificed by cervical dislocation, and their blood was collected at 10, 25, and 45 dpi. After each slaughtering time, serum samples were separated after centrifugation of the clotted blood samples at 3000 ×x g for 5 minutes undercooling. Anti-*T. spiralis* antibodies (ATs-Ab) were evaluated in the collected sera from ducks, fish, and rats using indirect Enzyme-linked- Immuno-sorbent assay (ELISA). Moreover, the intestine in each case was removed and dissected into equal segments, then opened longitudinally, scraped by scalpel, and washed in warm saline to remove and collect the available worms. A suitable amount of muscles of all inoculated hosts were also examined for *T. spiralis* cysts using Trichinoscopic examination according to the technique described by Roepstorff and Nansen (1998) and the digestion technique (Pozio, 2005). In addition, muscles from the inoculated ducks and fish were collected after each slaughtering time, dissected, and mixed then fed to the rats' groups assigned for xenodiagnosis. Infection in these rats was inspected at 45 dpi as described before.

Serological diagnosis

Preparation of Trichinella spiralis larvae antigen

Trichinella spiralis first larvae excretory-secretary antigens (TsL1-ESAg) were prepared from active motile first larval stages according to Gómez-Morales et al. (2008). About 3000 active larvae collected after digestion were washed several times by centrifugation and sedimentation in Phosphate Buffer Saline (PBS) pH 7.4 containing 1,000,000 I.U. of penicillin G sodium and 1 g of streptomycin sulphate /liter. The clean sedimented larvae were used for the production of TsL1-ESAg after cultivation in RPMI 1640 Medium as detailed described by Taher et al. (2017)**.** The produced ES products were collected by centrifugation, concentrated by dialysis versus polyethyleneglycol in molecular porous membrane tubing 6-8 M.W. according to Abdel-Rahman and Abdel-Radi (2021). After estimating its protein contents using the Bradford method (Bradford, 1976), the concentrated antigen was collected in a 1 ml vial and stored at -20^{o}C until use.

Indirect ELISA

Deviations in the level of specific ATs-IgG Ab in sera of all inoculated hosts in the current study were estimated at the beginning day and at 10, 25, and 45 dpi using ELISA. The test was adjusted after checkerboard titration, and performed according to the protocol of Mahdy et al. (2017) with little modifications. The microtiter ELISA plates were coated with TsL1-ESAg; 100 ml/well (4 mg/ml) in carbonate-buffer (pH 9.6) and incubated for one hour at 37^{o}C. After three times of washing using washing buffer (0.5% Tween 20 in PBS pH7.3 ± 0.2). All wells were blocked with 200 ml of 0.5% bovine serum albumin (BSA), in PBS-0.05% Tween 20 (PBST) then incubated for one hour at 37^{o}C as before. After washing, 100 ml/well from the following reagents were sequentially added and incubated as before; 1:100 diluted sera in PBST, then horseradish peroxidase HRP-conjugated anti- Rat IgG (Sigma, USA), Rabbit anti-Duck IgG (H+L) (ARIGO 66303) and Anti- Fish IgG (Cedarlane, CL50171AP-T) diluted 1:2000. The substrate O-phenylene diamine dihydrochloride (OPD), (Sigma, USA) plus H2O2 were used for detection of the reaction (100 µl/ well). The reactions were stopped by the addition of 50 ml/well of 1 N H2SO4. Optical density (OD) values at 450 nm were measured using a microplate reader (Titer teck multi-scan ELISA reader). The cut-off point for positive results was calculated based on the double of the mean OD value of negative control sera. As double of the negative control value was considered to be positive for the same plate. Serum samples of all inoculated hosts were tested in duplicate associated with three positive and three negative reference serum samples per plate.

Statistical analysis

Statistical analysis was performed using SPSS statistics 17.0 for windows. All data are presented as the mean ± standard error of the means (SE). Post hoc ANOVA (LSD test) was used to analyze the differences and similarities between experimental groups. $P < 0.05$ was regarded as statistically significant.

To cite this paper: Malak NML and Abdel-Radi S (2021). The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection. *World Vet. J.*, 11 (4): 594-601. DOI: https://dx.doi.org/10.54203/scil.2021.wvj75

RESULTS

According to Table 1, *T. spiralis* heavily infected pigs' muscles containing cysts as well as active larvae extracted after artificial digestion failed to be developed to worms in the intestine of the experimentally inoculated *Muscovy* ducks when they were slaughtered at 10, 25, and 45 dpi. Furthermore, no cysts were detected in the muscles of these inoculated ducks. Additionally, inoculation of ducks by free active larvae or muscles containing cysts did not result in significant ($p > 0.05$) elevation for the level of ATs-Ab in their sera as it displayed negative ELISA OD values in comparison with the control non-inoculated ducks**.** The recorded mean of ELISA OD value was 0.11 ± 0.02, 0.14 ± 0.15, and 0.12 ± 02 for ducks inoculated with larvae, 0.10 ± 0.02, 0.12 ± 0.02, and 0.11 ± 0.02 for those inoculated with infected muscles and 0.09 ± 0.01, 0.11 ± 0.01, and 0.10 ± 0.01 for the control non-inoculated ducks at 10, 25, and 45 dpi, respectively.

At the same time, the inoculation of catfish (*Clarias Lazera*) with *T. spiralis* free larvae or naturally infected pig's muscles resulted in a significant increase in mean ELISA OD value in comparison with the control non-inoculated fish group ($p < 0.05$). This increase was respectively 0.48 ± 0.02 and 0.31 ± 0.05 at 25 dpi and 0.21 ± 0.02 and 0.18 ± 0.03 at 45 dpi for fish groups inoculated by free larvae and muscles cysts, compared to 0.11 ± 0.00 and 0.09 ± 0.00 for the control non-inoculated fish group at 25 and 45 dpi, respectively. However, there were non-significant differences in the level of ATs-Ab of fish groups at 10 dpi ($p > 0.05$). In addition, few worms were collected after evisceration of the intestine of these fish. The mean number of these worms per fish decreased with the time elapsing post-inoculation as it was 1.92 ± 0.31 at 10 dpi then decreased to 1.00 ± 0.45 fish at 25 dpi and disappeared at 45 dpi in the fish group inoculated by free larvae. Moreover, the mean number of worms in the intestine of the fish group inoculated by infected pigs' muscles was 1.60 ± 0.25 and 1.00 ± 0.00/fish at 10 and 25 dpi, respectively while no worms were detected in fish slaughtered at 45 dpi (Table 1).

On the other hand, inoculation of albino rats (as a normal host) by free larvae or infected pigs' muscles revealed a high number of worms in their intestine. The mean number of these extracted worms reached 37.13 ± 0.34, 32.80 ± 0.38, and 18.60 ± 0.25 for rats inoculated with free larvae and 32.20 ± 0.20, 30.60 ± 0.40, 15.40 ± 0.25 for those inoculated with pigs' muscles containing cysts at 10, 25, and 45 dpi, respectively. This was associated with a significant increase of the ATs-Ab titer in their sera ($p < 0.05$), compared to the control non-infected rats. The mean ELISA OD in rats infected either by free larvae or muscles containing cysts showed a significant increase ($p < 0.05$) for rats inoculated with free larvae (0.85 ± 0.05, 0.69 ± 0.02) and for those inoculated by muscles' cysts (0.72 ± 0.09, 0.61 ± 0.1) at 25 dpi and 45 dpi, respectively. Moreover, there was a gradual increase (reached 100/inch) in *T. spiralis* larvae which were diagnosed microscopically in the diaphragm of all these rats when they were inspected from the 10 to 45 dpi (Table 1).

Table 1. Mean ELISA optical density values and number of collected worms from the inoculated hosts and control groups at different times post-inoculation

Hosts	Groups	Mean ELISA OD values and number of collected worms/hosts					
		10 dpi		25 dpi		45 dpi	
		Mean ELISA OD Value	Mean No. of worms /host ($N = 5$)	Mean ELISA OD Value	Mean No. of worms /host ($N = 5$)	Mean ELISA OD Value	Mean No. of worms /host ($N = 5$)
Ducks	Control	0.09 ± 0.01^{a}	0.00 ± 0.00^{a}	0.11 ± 0.01^{a}	0.00 ± 0.00^{a}	0.10 ± 0.01^{a}	0.00 ± 0.00^{a}
	Larvae	0.11 ± 0.02^{a}	0.00 ± 0.00^{a}	0.14 ± 0.15^{a}	0.00 ± 0.00^{a}	0.12 ± 0.02^{a}	0.00 ± 0.00^{a}
	Cysts	0.10 ± 0.02^{a}	0.00 ± 0.00^{a}	0.12 ± 0.02^{a}	0.00 ± 0.00^{a}	0.11 ± 0.02^{a}	0.00 ± 0.00^{a}
Fish	Control	0.11 ± 0.03^{a}	0.00 ± 0.00^{c}	0.11±0.00^{b}	0.00 ± 0.00^{c}	0.09 ± 0.00^{b}	0.00 ± 0.00^{a}
	Larvae	0.13 ± 0.02^{a}	1.92 ± 0.31^{a}	0.48 ± 0.02^{a}	1.00^{a} ± 0.45	0.21 ± 0.02^{a}	0.00 ± 0.00^{a}
	Cysts	0.12 ± 0.00^{a}	1.60 ± 0.25^{b}	0.31 ± 0.05^{a}	1.00^{a}±0.00	0.18 ± 0.03^{a}	0.00 ± 0.00^{a}
Rats	Control	0.11 ± 0.00^{a}	0.00 ± 0.00^{c}	0.12 ± 0.00^{b}	0.00 ± 0.00^{c}	0.12 ± 0.03^{b}	0.00 ± 0.00^{c}
	Larvae	0.16 ± 0.02^{a}	37.13 ± o.34^{a}	0.85 ± 0.05^{a}	32.80 ± 0.38^{a}	0.69 ± 0.02^{a}	18.60 ± 0.25^{a}
	Cysts	0.14 ± 0.02^{a}	32.20 ± 0.20^{b}	0.72 ± 0.09^{a}	30.60 ± 0.40^{b}	0.61 ± 0.10^{a}	15.40 ± 0.25^{b}

*$^{a-c}$ Means with different superscripts within the same column are significantly ($p < 0.05$) different. Values represent the mean of 3 independent replicates ± Standard error. dpi: Day post-inoculation, OD: Optical density, No: Number.

The ANOVA analysis for the variations in the level of ATs-Ab in sera of ducks, fish, and rats inoculated by *T. spiralis* free larvae at different times after inoculation revealed that there was no significant ($p > 0.05$) difference between the three hosts at 10 dpi. On the other hand, the mean ELISA OD value (0.14 ± 0.15) in sera of the inoculated ducks was significantly ($p < 0.05$) lower than that in rats (0.85 ± 0.05) and fish (0.48 ± 0.02) sera at 25 dpi. While there was a significant ($p < 0.05$) increase in this value in the rats' sera compared with ducks and fish at 45 dpi (Table 2). Concerning the corresponding groups of the three hosts inoculated by pigs' muscles containing *T. spiralis* cysts (Table 3), it was observed that there was no significant ($p > 0.05$) difference among the three inoculated hosts at 10 dpi regarding the level of ATs-Ab. However, a significant ($p < 0.05$) difference was recorded at 25 dpi, as the highest level

To cite this paper: Malak NML and Abdel-Radi S (2021). The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection. *World Vet. J.*, 11 (4): 594-601. DOI: https://dx.doi.org/10.54203/scil.2021.wvj75

was in rats (0.72 ± 0.09) followed by fish (0.31 ± 0.05) then ducks (0.12 ± 0.02). Inoculation of rats by muscles collected from experimentally inoculated ducks and fish revealed no worms or cysts or even elevation in the mean ELISA OD values in the inoculated rats in comparison to the Control non-inoculated one (Table 4).

Table 2. Comparison between the changes in mean ELISA optical density values of the three hosts after inoculation by *Trichinella spiralis* free larvae at different times post-inoculation.

Hosts \ Slaughtering days after inoculation	10 dpi	25 dpi	45 dpi
Ducks	0.11 ± 0.02[a]	0.14 ± 0.15[c]	0.12 ± 0.02[b]
Fish	0.13 ± 0.02[a]	0.48 ± 0.02[b]	0.21 ± 0.02[b]
Rats	0.16 ± 0.02[a]	0.85 ± 0.05[a]	0.69 ± 0.02[a]

*[a-c] Means with different superscripts within the same column are significantly ($p < 0.05$) different. Values represent the mean of 3 independent replicates ± Standard error. dpi: Day post-inoculation.

Table 3. Comparison between the changes in mean ELISA optical density values of the three hosts after inoculation by pig's muscles containing *Trichinella spiralis* cysts (ANOVA analysis)

Hosts \ Slaughtering days after inoculation	10 dpi	25 dpi	45 dpi
Ducks	0.10 ± 0.02[a]	0.12 ± 0.02[c]	0.11 ± 0.02[b]
Fish	0.12 ± 0.00[a]	0.31 ± 0.05[b]	0.18 ± 0.03[b]
Rats	0.14 ± 0.02[a]	0.72 ± 0.09[a]	0.61 ± 0.10[a]

*[a-c] Means with different superscripts within the same column are significantly ($p < 0.05$) different. Values represent the mean of 3 independent replicates ± Standard error. dpi: Day post-inoculation.

Table 4. Results of the feeding of rats with the muscles of the experimentally inoculated ducks and catfish after slaughtering (xenodiagnosis)

Rats' groups	Rats fed on muscles collected from experimentally inoculated hosts	No. of collected worms	Range of ELISA OD value	Mean ELISA OD Value ± SE
G-1	Ducks inoculated by *T. spiralis* infected pigs' muscles	0.00	0.07 – 0.10	0.036 ± 0.00[a]
G-2	Ducks inoculated by *T. spiralis* larvae	0.00	0.08 – 0.10	0.045 ± 0.01[a]
G-3	Fish inoculated by *T. spiralis* infected pigs' muscles	0.00	0.09 – 0.11	0.040 ± 0.02 [a]
G-4	Fish inoculated by *T. spiralis* larvae	0.00	0.07 – 0.11	0.042 ± 0.00 [a]
G-5	Control non-inoculated rats	0.00	0.09 – 0.12	0.041 ± 0.00 [a]

[a] Means with different superscripts within the same column are significantly ($p < 0.05$) different. Values represent the mean of 3 independent replicates ± Standard error. OD: Optical density, No: Number.

DISCUSSION

Trichinella species has a wide host range and can infect mammals, birds, and reptiles. Infection with this parasite causes public health problems. Pigs are considered the primary cause of *Trichinella* infection in humans (Noeckler et al., 2019). In Egypt, pigs are usually raised in barns with poor hygienic conditions in association with some domestic birds at the same place. Moreover, rodents and other reservoirs can be easily available in these barns. These hosts are under continuous exposure and contact to offals, garbage, and other wastes of pigs that may have *Trichinella* infection. Consequently, they may get infected and act as a general reservoir. In this respect, Youssef and Uga (2014) presented an increase in the infection rate of *T. spiralis* (13.3%) in rodents that were detected in overcrowded pigs' barns of low sanitary conditions. Dyab et al. (2019) detected *T. spiralis* infection in pigs in Egypt which mainly was in pigs' farms with poor hygiene and infested with the rodent. In addition, most fish farms use meals made from birds and pigs' meats and wastes, that facilitate the arrival of *T. spiralis* infection to the farmed fish. The previous explanations can direct the attention to the possible role of domestic ducks or fish meat as a source of infection by *T. spiralis* to the consumers in Egypt.

Therefore, the present study investigated the possibility of developing *T. spiralis* infection in *Muscovy* ducks and catfish (*Clarias lazera*) and their role in transmitting this infection to ducks and fish meat which may infect consumers in Egypt.

In the present study, the failure of *T. spiralis* heavily infected pigs' muscles containing cysts as well as the extracted active free larvae to induce infection or even elevate the level of ATs-Ab in the inoculated *Muscovy* ducks was recorded. Moreover, no successful infection could be diagnosed in rats fed on muscles of different inoculated ducks. This was in

To cite this paper: Malak NML and Abdel-Radi S (2021). The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection. *World Vet. J.*, 11 (4): 594-601. DOI: https://dx.doi.org/10.54203/scil.2021.wvj75

agreement with Pozio (2005) who demonstrated that the encapsulated *Trichinella* species forming cysts, such as *T. spiralis*, can complete their life cycle in warm-blooded mammals only which have body temperature ranging from 37-40ºC. Moreover, Yacoub et al. (1993) reported that their trial to infect chickens failed and they could not detect *T. spiralis* larvae in different tissues of the inoculated chickens since they were considered as non-specific hosts.

The condition was different after inoculation of catfish either with *T. spiralis* free larvae or naturally infected pig's muscles. A very low elevation in the ELISA OD was recorded, this may refer to the attempts of the detected small numbers of worms in the intestine of the inoculated fish to penetrate the intestine and stimulate the immune mechanism, causing anti-parasite Ab production. However, this elevation is non-significant compared to that recorded after inoculation of rats. Moreover, no cysts were formed in the muscles of these inoculated fish. This may be due to the failure of these worms to survive after a period under the intestinal condition of fish which explains the decrease in the number of worms detected in the inoculated fish intestine until their disappearance at 45 dpi. Consequently, these worms failed to reach maturity or migrate and form cysts in the fish muscles, which may refer to the correlation between the development, reproduction, and metabolic rate of a parasite and the host body temperature (Skorping, 1981; Gelnar, 1987; Pozio, 2005). Moreover, Watson et al. (1998) noticed that the fish body temperature influences the pathogen development and the host immune response. In addition, the failure of catfish to get *T. spiralis* infection in the present study was confirmed by the failure of infection of rats used for xenodiagnosis and fed on muscles of these inoculated fish. This was in agreement with Pozio and Murrell (2006) who recorded the failure of the attempts to infect fish with *T. spiralis*.

In the current study, the high recorded ATs-Ab titer diagnosed by ELISA OD and the high number of worms in rats inoculated by infected pigs' muscles or by the free larvae demonstrate the ability of these larvae to develop in their normal mammalian host in comparison with the other foreign hosts such as ducks and fish. Moreover, this proves the infectivity of the used pigs' muscles and larvae for the experimental inoculation in the present study. These results are in harmony with Youssef and Uga (2014) who mentioned that *T. spiralis* could infect rodents with an incidence of 13.3%. Moreover, (Bilska-Zając et al., 2018) stated that rats were considered to be a potential reservoir host s of *Trichinella* and play an essential role in the transmission of *T. spiralis* from and to pigs and other infected hosts. In addition, (Franssen et al., 2011) recorded a high number of muscles cysts, antibodies levels and immune response in rats experimentally infected with *T. spiralis* larvae as well as they reported that there was a positive correlation between the number of recovered muscles cysts of the infected rats and the level of serum antibody. However, there was no elevation in mean ELISA OD values in hosts slaughtered at 10 dpi and this is considered to be acceptable as (Bruschi et al., 2019; Ramadan et al., 2021) stated that the elevation in the level of anti-parasite Ab in sera could not be recorded before 2-3 weeks post-infection.

CONCLUSION

Regarding the obtained results, it could be concluded that there is no development of infective *T. spiralis* cysts in the muscles of domestic ducks (*Muscovy*) and catfish (*Clarias lazera*) either inoculated by high doses of *T. spiralis* cysts or free larvae extracted from infected pig's muscles. This means that the consumption of meat of these hosts cannot be considered as a source for transmission of *T. spiralis* infection to the consumers in Egypt. However, proper cooking of meat is necessary to avoid infection with other species. In addition, improving the hygienic housing conditions is recommended for pigs, ducks, and fish farms.

DECLARATIONS

Authors' contribution

Nermeen M.L. Malak collected the muscles from the naturally infected freshly slaughtered pigs, contributed to the experimental inoculation of fish, performed the data statistical analysis, and contributed in writing the introduction part in the subsequent draft. Shimaa Abdel-Radi carried out the parasitological examination of the collected' muscles of all animals used throughout the study, collection, identification, and preparation of the parasite larvae, carried out the protocol of the experimental inoculation of ducks, fish, and rats as well as the serological diagnosis. Moreover, Shimaa Abdel-Radi contributed in writing the introduction part and wrote the methodology, results, discussion and references in the subsequent draft. All authors approved and revised the final submitted version.

Competing interests

The authors declare that there are no competing interests regarding this study.

Acknowledgments

The authors are grateful to the Veterinary technical staff or assistance during the collection of samples.

To cite this paper: Malak NML and Abdel-Radi S (2021). The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection. *World Vet. J.*, 11 (4): 594-601. DOI: https://dx.doi.org/10.54203/scil.2021.wvj75

Ethical consideration

Ethical issue including plagiarism, consent to public misconduct, data fabrication and⁄ or falsification, double publication and⁄or submission, redundancy has been checked by the authors.

REFERENCES

Abdel-Rahman GH, and Abdel-Radi S (2021). Synergistic action of Viteselen with anti-Fasciola drug as a tool for improving fertility and hemato-biochemical biomarkers in *Fasciola* infected sheep. Journal of Parasitic Diseases, DOI: https://www.doi.org/10.1007/s12639-021-01423-9

Asatrian A, Movsessian S, and Gevorkian A (2001). Experimental infection of some reptiles with *T. spiralis* and *T. pseudospiralis*. Abstract Book of the 10th International Conference on Trichinellosis, 20-24 August, 2000. Fontainebleau, France. Available at: https://pubmed.ncbi.nlm.nih.gov/11484387/

Bilska-Zając E, Ro´życki M, Antolak E, Bełcik A, Grądziel-Krukowska K, Karamon J, Sroka J, Zdybel J, and CenceK T (2018). Occurrence of *Trichinella* spp. in rats on pig farms. Annals of agricultural and environmental medicine, 25 (4): 698-700. DOI: https://www.doi.org/10.26444/aaem/99555

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254. DOI: https://www.doi.org/10.1016/0003-2697(76)90527-3

Bruschi F, Gómez-Morales MA, Dolores E, and Hill DE (2019). International commission on trichinellosis: Recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and humans. Food and Waterborne Parasitology, 14: e00032. DOI: https://www.doi.org/10.1016/j.fawpar.2018.e00032

Conlan JV, Vongxay K, Khamlome B, Gomez-Morales MA, Pozio E, Blacksell SD, Fenwick S, and Thompson RCA (2014). Patterns and risks of *Trichinella* infection in humans and pigs in northern Laos. PLoS Neglected Tropical Diseases, 8(7): e3034. DOI: https://www.doi.org/10.1371/journal.pntd.0003034

Dyab AK, Ahmed MA, and Abdelazeem AG (2019). Prevalence and histopathology of *Trichinella spiralis* larvae of slaughtered pigs in Cairo governorate, Egypt. Journal of the Egyptian Society of Parasitology, 49(2): 439-442. DOI: https://www.doi.org/10.21608/JESP.2019.68187

Djurković-Djaković O, Bobić B, Nikolić A, Klun I, and Dupouy-Camet J (2013). Pork as a source of human parasitic infection. Clinical Microbiology and Infection, 19(7): 586-594. DOI: https://www.doi.org/10.1111/1469-0691.12162

Faubert G, and Tanner CE (1971). *Trichinella spiralis*: Inhibition of sheep hemagglutinins in mice. Experimental Parasitology, 30(1): 120-123. DOI: https://www.doi.org/10.1016/0014-4894(71)90077-4

Franssen FFJ, Fonville M, Takumi k, Vallée I, Grasset A, Koedam MA, Wester PW, Boireau P, and Giessen JWB (2011). Antibody response against *Trichinella spiralis* in experimentally infected rats is dose dependent. Veterinary Research, 42: 113. DOI: https://www.doi.org/10.1186/1297-9716-42-113

Gelnar M (1987). Experimental verification of the effect of water temperature on micropopulation growth of Gyrodactylus kathahneriMalmberg, 1964 (Monogenea) parasitizing carp fry (*Cyprinuscarpio* L.). Folia Parasitologica, 34: 19-23. Available at: https://www.cabdirect.org/cabdirect/abstract/19870841909

Gómez-Morales MA, Ludovisi A, Amati M, Cherchi S, Pezzotti P, and Pozio E (2008). Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis. Clinical and Vaccine Immunology, 15(11): 1723-1729. DOI: https://www.doi.org/10.1128/CVI.00257-08

Hewitson JP, Grainger JR, and Maizels RM (2009). Helminth immunoregulation: The role of parasite secreted proteins in modulating host immunity. Molecular and Biochemical Parasitology, 167(1): 1-11. DOI: https://www.doi.org/10.1016/j.molbiopara.2009.04.008

Internal Quality Assurance Committee Auditor (IQACA) (2012). Essential quality assurance standards for *Trichinella* digestion assays. ICT QualityAssururance Committee. Available at: http://www.trichinellosis.org/Guidelines.html

La Rosa G, Marucci G, Zarlenga DS, Casulli A, Zarnke RL, and Pozio E (2003). Molecular identification of natural hybrids between *Trichinella nativa* and *Trichinella*T6 provides evidence of gene flow and ongoing genetic divergence. International Journal for Parasitology, 33(2): 209-216. DOI: https://www.doi.org/10.1016/S0020-7519(02)00258-8

Ludovisi A, Amati M, Simona Cherchi S, Pezzotti P, and Pozio E (2008). Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis. Clinical and Vaccine Immunology, 11(15): 1723-1729. DOI: https://www.doi.org/10.1128/CVI.00257-08

Mahdy OA, Mousa WM, Abdel-Maogood SZ, Abdel-Radi S, and El-Bahy MM (2017). Characterization of immunogenic protein fractions of sheep cysticercosis in Cairo, Egypt. Journal of the Hellenic Veterinary Medical Societ, 68: 291-298. DOI: https://www.doi.org/10.12681/jhvms.15471

Noeckler K, Pozio E, van der Giessen J, Hill DE, and Gamble HR (2019). International commission on trichinellosis: recommendations on post-harvest control of *Trichinella* in food animals. Food and Waterborne Parasitology, 14. DOI: https://www.doi.org/10.1016/j.fawpar.2019.e00041

Pannwitz G, Mayer-Scholl A, Balicka-Ramisz A, and Nöckler K (2010). Increased prevalence of *Trichinella* spp. northeastern Germany, 2008. Emerging Infectious Diseases, 16(6): 936. DOI: https://www.doi.org/10.3201/eid1606.091629

Pozio E (2005). The broad spectrum of *Trichinella* hosts: from cold-to warm-blooded animals. Veterinary Parasitology, 132: 3-11. DOI: https://www.doi.org/10.1016/j.vetpar.2005.05.024

Pozio E (2019). *Trichinella* and trichinellosis in Europe. Veterinarski Glasnik, 73(2): 65-84. DOI: https://www.doi.org/10.2298/VETGL190411017P

Pozio E, and Murrell KD (2006). Systematics and epidemiology of *Trichinella*. Advances in Parasitology, 63: 367-439. DOI: https://www.doi.org/10.1016/S0065-308X(06)63005-4

Ramadan RM, Khalifa MM, El-Akkad, Abdel-Wahab AM, and El-Bahy MM (2021). Animal hydatid cyst genotypes as a potential substitute for human hydatid cyst as a source of antigen for diagnosis of zoonotic hydatidosis. Journal of Parasitic Diseases, 45(2): 424-434. DOI: https://www.doi.org/10.1007/s12639-020-01309-2

Roepstorff A, and Nansen P (1998). Epidemiology, diagnosis and control of helminthic parasites of swine. In: Animal Health Manual, 3rd edition. FAO, WHO. Available at: http://www.fao.org/3/x0520e/X0520E.pdf

Skorping A (1981). Seasonal dynamics in abundance, development, and pattern of infection of Bunoderaluciopercae (Miiller) in pe'rch, PercaJluviatilis L. from an oligotrophic lake in Norway. Journal of Fish Biology, 18: 401-410. DOI: https://www.doi.org/10.1111/j.1095-8649.1981.tb03781.x

Smith HJ, and Snowdon KE (1989). Comparative assessment of a double antibody enzyme immunoassay test kit and a triple antibody enzyme immunoassay for the diagnosis of *Trichinella spiralis spiralis* and *Trichinella spiralis nativa* infections in swine. Canadian Journal of Veterinary Research, 53(4): 497-499. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1255584/pdf/cjvetres00052-0129.pdf

Taher EE, Méabed EM, El Akkad DM, Kamel NO, and Sabry MA (2017). Modified dot-ELISA for diagnosis of human trichinellosis. Experimental Parasitology, 177: 40-46. DOI: https://www.doi.org/10.1016/j.exppara.2017.04.002

Vignau ML, Guardis MDV, Risso MA, and Eiras DF (1997). Comparison between Two Methods for Diagnosis of Trichinellosis: Trichinoscopy and Artificial Digestion. Memórias do Instituto Oswaldo Cruz, 92(5): 585-587. DOI: https://www.doi.org/10.1590/S0074-02761997000500004

Walden HDS (2013). Food born infections and intoxications, 4th edition. Food Science and Technology, pp. 343-351. DOI: https://www.doi.org/10.1016/B978-0-12-416041-5.00025-1

Watson LR, Milani A, and Hedrick RP (1998). Effect of water temperature on experimentally-induced infections of juvenile white sturgeon (Acipenseriransmontanus) with the white sturgeon irido virus (WSIV). Journal of Aquaculture, 166: 213-228. DOI: https://www.doi.org/10.1016/S0044-8486(98)00283-X

Yacoub RS, Salem GH, Nessiem MG, and Siam MA (1993). The possible role of non-specific hosts (chickens and rabbits) in the epidemiology of *Trichinella spiralis*. Journal of Veterinary Medicine, 47: 27-31. Available at: https://agris.fao.org/agris-search/search.do?recordID=EG9401637

Youssef AI, and Uga S (2014). Review of parasitic zoonoses in Egypt. Tropical Medicine and Health, 42: 3-14. DOI: https://www.doi.org/10.2149/tmh.2013-23

To cite this paper: Malak NML and Abdel-Radi S (2021). The Role of Ducks (*Muscovy*) and Catfish (*Clarias Lazera*) Meat in Transmitting *Trichinella Spiralis* Infection. *World Vet. J.*, 11 (4): 594-601. DOI: https://dx.doi.org/10.54203/scil.2021.wvj75