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Histopathological changes of the blood vessels in the renal interstitium of infected mice by *Trichinella spiralis* model.

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Abstract

Trichinellosis is a zoonotic disease caused by consumption uncooked meat containing by first stage larvae of *Trichinella spiralis* which are still an important problem in global public health, besides cardiac and neurological complications of trichinellosis, renal involvement is one of the most important serious complications lead to the cause of renal failure 8.7%. Aims of this study were focused on the alteration of the blood vessels in the renal interstitium of infected mice by *T. spiralis* model and performed by 132 female ICR mice, 8-12 week-old, and divided into 2 groups as control group without infection and experimental group with infected with 450 *T. spiralis* larvae. All 132 mice in each group were euthanized via CO_2 inhalation until definitely dead on day 5th, 10th, 14th, 21st, 35th and 45th days post infection (DPI) as study period and followed by gross examination dissection and histological study of the kidneys tissues. Severity grading and area of an inflammatory reaction of blood vessels in renal interstitium was evaluated histopathological changes with Hematoxylin and Eosin staining (H&E staining) technique under light microscope and software image flame work. The results of present study of a total numbers of infected mice showed acute severe perivasculitis in the renal interstitium on day 21st DPI 36.36% with statistical significance ($P<0.05$) and an area of perivasculitis $4.65x10^4$ –$36.37x10^4$ μm^2 (0.050%–0.365%) meanwhile acute vasculitis was observed on 35th DPI 9.09% no significance ($P>0.05$) and area of vasculitis showed $5.67x10^4$ μm^2 (0.089%) and also perivasculitis was observed on 35th and 45th DPI. In conclusion, this study demonstrated that an inflammatory reaction of blood vessels after infected mice with *T. spiralis* 450 larvae could induce an inflammatory reaction of the renal blood vessels to represent as vasculitis and perivasculitis in the early third week after infection. Therefore post infection should be a concern and awareness of renal involvement and these complications with possible renal failure.

Keywords: vasculitis, perivasculitis, *Trichinella spiralis*, Inflammatory reactions, Histology, Hematoxylin and Eosin staining (H&E staining).

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1. Introduction

Trichinellosis is a zoonosis caused by consumption uncooked meat by first stage larvae with *Trichinella spiralis* is an important problem global public health and the most common species which cause disease in worldwide and high infection in human have been identified as *T. spiralis* (Kaewpitoon et al., 2008). *T. spiralis* larvae have four cycle of molt to become adult stage and then penetrate into the epithelium of mucosal layer of small intestine. Incorporation mating have occurred within 36 hours and shedding larvae by female adult within 4-7 days after infection. *T. spiralis* larvae invade into mucosal and submucosal lymphovascular which are disperse to internal organ such as heart, brain, liver, muscle and then they are forming nurse cell complexes in tissues (Anunnatsiri et al., 2004). *T. spiralis* larvae be survived for year (up to 40 years in humans and over 20 year, e.g., polar bears) (Gottstein et al., 2009). The clinical pathology is characterized by an inflammatory reaction response between host and parasite reaction (immune response) in tissues and internal organs, which causes the signs and symptoms, such as high grade fever, diarrhea, myalgia, periorbital swelling, renal involvement and serious complications (myocarditis 26% neurological 0.2-52% and renal failure 8.7%) (Neghina et al., 2011). Based on reviewed literatures in trichinellosis renal involvement (Neghina et al., 2011) mentioned as fatality rate was 26.1%. Proteinuria was detected in 84.8% of cases, in addition, mild or transient proteinuria was reported to be associated with trichinosis (Barsoum, 1997), hematuria in 30.4%, and casts were observed in urine specimens from 23.9% of patients and renal lesions were found by biopsy or necropsy in 43.5% of cases (Neghina et al., 2011). Trichinosis nephritis was first characterized by inflammatory infiltrations and dystrophic alterations of the tubular epithelium (Pambuccian and Cironeanu, 1961). Distinct opacity of the kidney cortex and swelling of the intracapsular, intratubular and interstitial locations were observed in man (Gould, 1945). Focal hemorrhages and infarction were also seen. Therefore, the present study focused on an inflammatory reaction of the kidneys in infected mice model.

2. Materials and methods

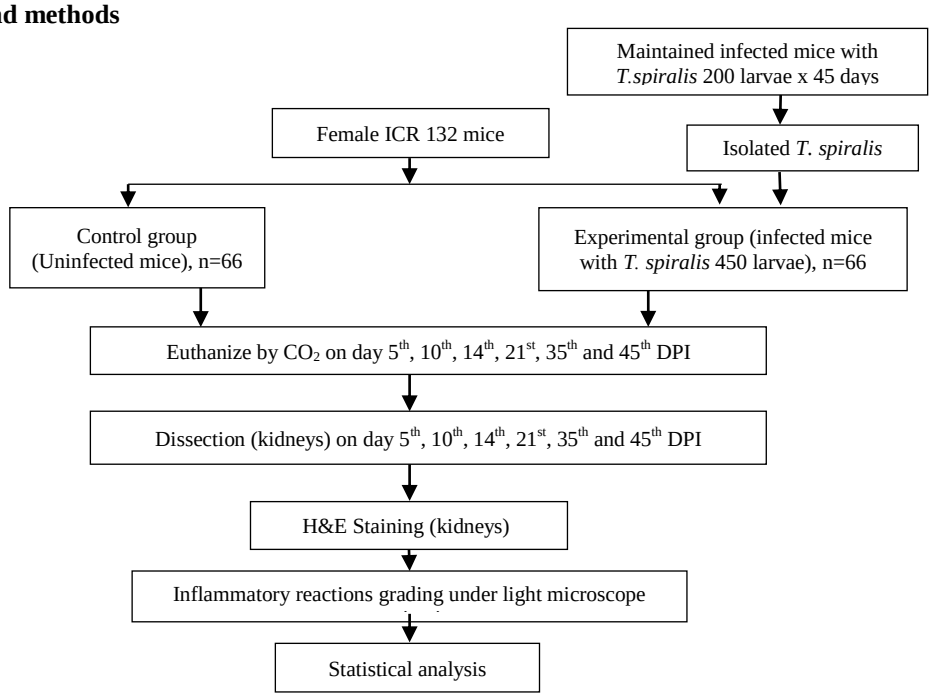

Figure 1. The study design diagram

2.1 Sample size calculation

The sample size was calculated according to the formula as below. The power of the test was 90%, with signification level of 5. The appropriate standard deviation and mean were obtained from a review of the public literature (Glahan et al., 2014). The sample size was calculated "Comparison of two mean" formula (Riffenburgh., 1999 and Sakpal, 2010) n= total sample size per group for each case, u = power 90%, v= signification level =5%, μ_1 =means of experimental groups from reviewed literature, μ0= means of control groups from reviewed literature, σ_1= standard deviation of experimental groups from reviewed literature, σ_2 = standard deviation of control groups from review literature.

$$n = \frac{(u+v)^2(\sigma 1+\sigma 0)^2}{(\mu 1-\mu 0)^2}$$

A total number of mice in this study design was 132 mice and described as the followings: the sample size per group as 9 mice per group of each DPI, plus 20% increase for unexpected sample loss during the experimental period, resulting in 11 mice per group of each days post infection (DPI), therefore a total number of mice in control group was 66 mice and a total number of mice in experimental group was 66 mice (Figure 1).

2.2 Preparation of parasite and infection

The study design was performed by female ICR mice 8-12 weeks old, and weight 25-40 grams obtained from the Nomura Siam Internalional Co.,Ltd. The ethic clearance number FTM-ACUC 021/2017 was approved by Faculty of Tropical Medicine Animal Care Mahidol University. The maintained parasites in ICR mice 8–12 weeks old with 200 *T. spiralis* larvae per mouse and care under conventional conditions for 45 days until performing experiment. All infected mice were euthanized via CO_2 inhalation and definitely dead. After that all infected mice were generalized gross examination and dissection for detecting encysted larvae in striated muscle by crushing technique under standard protocol (Gail et al., 2013). Selected positive encysted larvae muscle for digestion with pepsin solution (pepsin 1g/ HCl 1 ml/ distilled water to 100 ml) at 37 ºC for 12-17 hours (Siriyasatien et al,. 2003) for recovery and counting *T.spiralis* larvae under stereomicroscope, and then all selected live larvae were washed by PBS and kept into NSS in container for further experiment.

For parasite infection, the experimental group was composed of sixty-six infected mice with 450 *T. spiralis* live larvae per mouse. Oral feeding of *T. spiralis* was performed by stainless curved nasogastric-gavage no.18. For control group, sixty-six mice with no infection with parasites was performed. All 132 mice were euthanized via CO_2 inhalation until dead on day 5^{th}, 10^{th}, 14^{th}, 21^{st}, 35^{th} and 45^{th} DPI (Gail et al., 2013) followed by dissection, gross examination and histopathological study of the head and kidneys tissues.

2.3 Histopathology H&E Staining

The selected kidneys have been processed histopathology with H&E staining. The kidneys was serial section (~2 cm); fixed for 24-48 hours in 10% neutral buffered formalin; dehydrated in ascending grades of alcohols (70% - 95%) (Geetika, 2017); cleared in xylene; embedded in paraffin blocks then underwent paraffin embedding; sectioned at 5 μm in thickness by microtome and H&E staining at the Department of Pathology, Chulalongkorn University, Bangkok, Thailand.

2.4 The inflammatory reactions grading criteria of the renal blood vessel and interpretation.

The modified criteria grading of inflammatory reactions of the renal blood vessels (Fenoy, 2012) (Table 1) the interpretation of inflammatory reactions grading was performed by microscopic examination in consecutive pattern with 400X objective (high power field) in all serial sectioned of kidneys tissues at least 70 fields. An area of inflammation lesion was evaluated under image frame work software version: 3.84.0.68 update on 3rd March 2019 (μm^2). The grading of inflammatory reaction is as follows; 0=normal, 1=mild, 2 =moderate and 3=severe.

Table 1. Histological inflammatory reaction grading of the vessels.

Grading	Inflammatory cell infiltrate in blood vessel	Inflammatory cell infiltrate in Perivascular area
Normal (0)	No inflammatory cell infiltrated in vascular or perivascular	No inflammatory
Mild (1)	< 4 cell diameter thickness	occasional cuffing with inflammatory cells
Moderate (2)	4-10 cells diameter thickness	surrounded by a thin layer (one to five cells thick of inflammatory cells)
Severe (3)	>10 cells diameter thickness	Surrounded by a thick layer (more than five cells thick of inflammatory cells)

2.5 Statistical analysis

Mann-Whitney U-Test was used to compare the inflammatory reactions grading between experimental group and control group. Ordinal logistic regression (date by date) was used to compare the grading inflammatory reactions between experimental groups required. The test different of inflammatory reactions among groups were considered as statistically significant when p-value < 0.05.

3. Result

The result of inflammatory reaction in the blood vessels of renal interstitium were evaluated by histology (H&E staining) under light microscopic examination. The experimental groups showed acute mild to severe perivasculitis in the renal interstitium and a total number of mice in infected mice on day 21st, 35th and 45th DPI (36.36%, 18.18% and 27.72%) with statistical analysis in the perivascular grading of the experimental group compared control group with was Mann-Whitney U-Test was showed significance (P<0.05) on day 21st DPI (Table 3.) and characterized by vessel cuffing with inflammatory cells or surrounded by a thick layer of inflammatory cell , on day 5th, 10th, 14th DPI of experimental groups and control groups were showed normally architecture and no inflammatory. Meanwhile, on day 21st, 35th and 45th DPI showed an area of perivasculitis $2.51x10^4$-$7.25x10^4$ μm^2 (0.050-0.974%) (Table 7-9.).

On day 35th DPI showed mild acute vasculitis 9.09% (Table 2.) characterized by lymphoplasmacytic cells infiltration in wall of the vessel but no statically significance (P>0.05) and an area of vasculitis 5.67x104μm^2 (0.089%) (Table 8.), on day 5th, 10th, 14th, 21st and 45th DPI of experimental groups and control groups were showed normally architecture and no inflammatory characterized by infiltrated in wall blood vessel.

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Table 2. The inflammatory reaction grading of the vessels in the renal interstitium of mice in the control group and the experimental group during study periods on day 5th, 10th, 14th, 21st , 35th and 45th DPI

Inflammatory reaction	Day post infection	Group	N	A number of mice(%) with inflammatory reaction grading of the renal interstitium				(P-value)
				Normal (0)	Mild (1)	Moderate (2)	Severe (3)	
Vasculitis	5	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	
	10	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	
	14	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	
	21	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	
	35	Experiment	11	10	1(9.09)	0	0	0.998
		Control	11	11	0	0	0	
	45	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	

Note: Asterisks (*) denote; the significant P-value < 0.05; the data show the number of mice (%) and inflammation reactions grading; N= number of mice per group; DPI= Day Post Infection.

Table 3. The inflammatory reaction grading of the vessels in the renal interstitium of mice in the control group and the experimental group during study periods on day 5th, 10th, 14th, 21st , 35th and 45th DPI.

Inflammatory reaction	Day post infection	Group	N	A number of mice(%) with inflammatory reaction grading of the renal interstitium				(P-value)
				Normal (0)	Mild (1)	Moderate (2)	Severe (3)	
Perivasculitis	5	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	
	10	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	
	14	Experiment	11	11	0	0	0	1.000
		Control	11	11	0	0	0	
	21	Experiment	11	7	2(18.18)	0	2(18.18)	0.032*
		Control	11	11	0	0	0	
	35	Experiment	11	9	0	1(9.09)	1(9.09)	0.148
		Control	11	11	0	0	0	
	45	Experiment	11	8	3(27.27)	0	0	0.069
		Control	11	11	0	0	0	

Note: Asterisks (*) denote; the significant P-value < 0.05; the data show the number of mice (%) and inflammation reactions grading; N= number of mice per group; DPI= Day Post Infection.

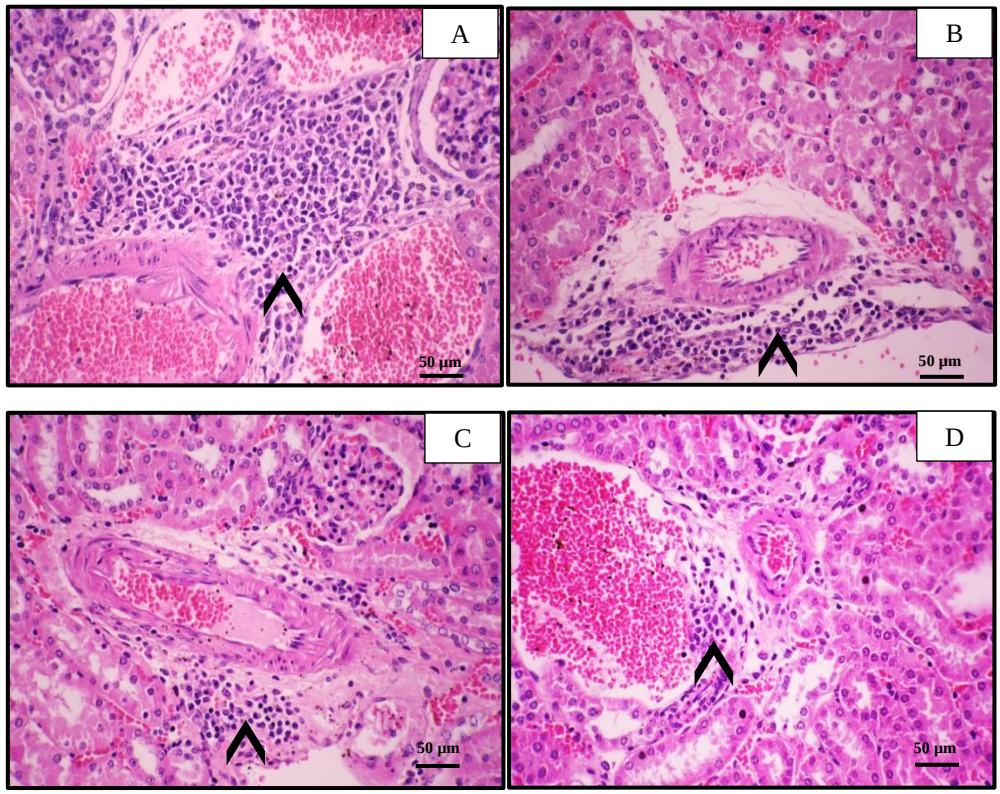

Figure 2. Photomicrographs of histopathology (400X) by H&E staining in the blood vessels in the renal interstitium of infected mice by *T.spiralis* on day 21st, 35th, 45th DPI; (A) experimental group showed severe perivasculitis (arrowhead) on day 21st DPI; (B) Moderate perivasculitis (arrowhead) on day 35th DPI, (C) mild perivasculitis (arrowhead) on day 45thDPI, characterized by inflammatory cells infiltrated in perivascular area. (D) Showed vasculitis (arrowhead) characterized by infiltrated in wall blood vessel were observe on day 35th DPI.

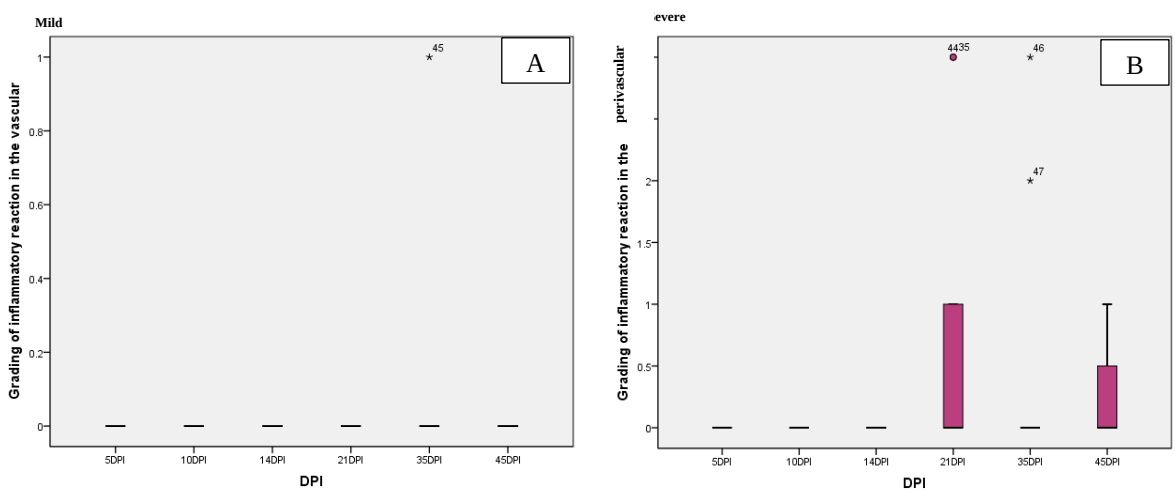

Figure 3. The graph shows an inflammatory reaction grading* of the renal blood vessels in infected mice by *T. spiralis* on day 5th, 10th, 14th, 21st, 35th and 45th DPI. (A) There is a number 45th infected mice by *T. spiralis* showed acute mild vasculitis on day 21st DPI, (B) There is a number of 35th and 44th infected mice with *T. spiralis* showed acute severe inflammatory reaction in perivascular area and acute inflammatory reaction in perivascular area on day 21st, a number 46th showed acute severe inflammatory reaction in perivascular area and a number 47th showed acute moderate inflammatory reaction in perivascular area on day 35th, on day 45th showed acute mild inflammatory reaction in perivascular area . Note* inflammatory reaction grading: normal = 0 mild, moderate =2, severe = 3.

Table 4. The total area of inflammatory reaction (%) in the renal interstitium of mice in the control group and the experimental group during study during periods on day 5th DPI.

Number of mice	Group	Total area of renal(μm^2)	Area of vasculitis μm^2 (%)	Grading of vasculitis	Area of perivasculitis μm^2 (%)	Grading of perivasculitis
1	Control	9.44×10^7	0	0	0	0
	Experiment	7.71×10^7	0	0	0	0
2	Control	8.74×10^7	0	0	0	0
	Experiment	9.01×10^7	0	0	0	0
3	Control	9.00×10^7	0	0	0	0
	Experiment	11.66×10^7	0	0	0	0
4	Control	7.89×10^7	0	0	0	0
	Experiment	8.94×10^7	0	0	0	0
5	Control	8.90×10^7	0	0	0	0
	Experiment	7.74×10^7	0	0	0	0
6	Control	11.23×10^7	0	0	0	0
	Experiment	9.04×10^7	0	0	0	0
7	Control	7.78×10^7	0	0	0	0
	Experiment	8.48×10^7	0	0	0	0
8	Control	9.83×10^7	0	0	0	0
	Experiment	11.23×10^7	0	0	0	0
9	Control	9.84×10^7	0	0	0	0
	Experiment	8.85×10^7	0	0	0	0
10	Control	9.02×10^7	0	0	0	0
	Experiment	11.53×10^7	0	0	0	0
11	Control	7.04×10^7	0	0	0	0
	Experiment	9.00×10^7	0	0	0	0

Table 5. The total area of inflammatory reaction (%) of blood vessels in the renal interstitium of mice in the control group and the experimental group during study during periods on day 10th DPI.

Number of mice	Group	Total area of renal(μm^2)	Area of vasculitis μm^2 (%)	Grading of vasculitis	Area of perivasculitis μm^2 (%)	Grading of perivasculitis
1	Control	8.99×10^7	0	0	0	0
	Experiment	11.45×10^7	0	0	0	0
2	Control	8.70×10^7	0	0	0	0
	Experiment	9.94×10^7	0	0	0	0
3	Control	11.20×10^7	0	0	0	0
	Experiment	8.94×10^7	0	0	0	0
4	Control	8.98×10^7	0	0	0	0
	Experiment	10.04×10^7	0	0	0	0
5	Control	10.05×10^7	0	0	0	0
	Experiment	8.93×10^7	0	0	0	0
6	Control	8.90×10^7	0	0	0	0
	Experiment	7.87×10^7	0	0	0	0
7	Control	10.04×10^7	0	0	0	0
	Experiment	7.16×10^7	0	0	0	0
8	Control	9.02×10^7	0	0	0	0
	Experiment	9.50×10^7	0	0	0	0
9	Control	11.13×10^7	0	0	0	0
	Experiment	8.93×10^7	0	0	0	0
10	Control	8.89×10^7	0	0	0	0
	Experiment	11.34×10^7	0	0	0	0
11	Control	8.97×10^7	0	0	0	0
	Experiment	9.01×10^7	0	0	0	0

Table 6. The total area of inflammatory reaction (%) in the renal interstitium of mice in the control group and the experimental group during study during periods on day 14th DPI.

Number of mice	Group	Total area of renal(μm^2)	Area of vasculitis μm^2 (%)	Grading of vasculitis	Area of perivasculitis μm^2 (%)	Grading of perivasculitis
1	Control	$8.08x10^7$	0(0)	0	0(0)	0
	Experiment	$6.70 x10^7$	0(0)	0	0(0)	0
2	Control	$9.06 x10^7$	0(0)	0	0(0)	0
	Experiment	$10.03 x10^7$	0(0)	0	0(0)	0
3	Control	$11.00 x10^7$	0(0)	0	0(0)	0
	Experiment	$7.89 x10^7$	0(0)	0	0(0)	0
4	Control	$9.04 x10^7$	0(0)	0	0(0)	0
	Experiment	$8.89 x10^7$	0(0)	0	0(0)	0
5	Control	$7.70 x10^7$	0(0)	0	0(0)	0
	Experiment	$9.04 x10^7$	0(0)	0	0(0)	0
6	Control	$9.80 x10^7$	0(0)	0	0(0)	0
	Experiment	$10.03 x10^7$	0(0)	0	0(0)	0
7	Control	$9.79 x10^7$	0(0)	0	0(0)	0
	Experiment	$11.64 x10^7$	0(0)	0	0(0)	0
8	Control	$8.90 x10^7$	0(0)	0	0(0)	0
	Experiment	$9.96 x10^7$	0(0)	0	0(0)	0
9	Control	$9.89 x10^7$	0(0)	0	0(0)	0
	Experiment	$8.94 x10^7$	0(0)	0	0(0)	0
10	Control	$8.90 x10^7$	0(0)	0	0(0)	0
	Experiment	$7.20 x10^7$	0(0)	0	0(0)	0
11	Control	$7.08 x10^7$	0(0)	0	0(0)	0
	Experiment	$6.99 x10^7$	0(0)	0	0(0)	0

Table 7. The total area of inflammatory reaction (%) in the renal interstitium of mice in the control group and the experimental group during study during periods on day 21st DPI.

Number of mice	Group	Total area of renal(μm^2)	Area of vasculitis μm^2 (%)	Grading of vasculitis	Area of perivasculitis μm^2 (%)	Grading of perivasculitis
1	Control	$10.04x10^7$	0(0)	0	0(0)	0
	Experiment	$8.90x10^7$	0(0)	0	0(0)	0
2	Control	$9.00 x10^7$	0(0)	0	0(0)	0
	Experiment	$8.34 x10^7$	0(0)	0	$8.01x10^4$(0.134)	3
3	Control	$9.00 x10^7$	0(0)	0	0(0)	0
	Experiment	$11.05 x10^7$	0(0)	0	0(0)	0
4	Control	$10.04 x10^7$	0(0)	0	0(0)	0
	Experiment	$9.98 x10^7$	0(0)	0	0(0)	0
5	Control	$8.50 x10^7$	0(0)	0	0(0)	0
	Experiment	$8.96 x10^7$	0(0)	0	0(0)	0
6	Control	$9.67 x10^7$	0(0)	0	0(0)	0
	Experiment	$8.77 x10^7$	0(0)	0	$27.15x10^4$(0.310)	1
7	Control	$11.70 x10^7$	0(0)	0	0(0)	0
	Experiment	$8.98 x10^7$	0(0)	0	0(0)	0
8	Control	$7.80 x10^7$	0(0)	0	0(0)	0
	Experiment	$9.04 x10^7$	0(0)	0	0(0)	0
9	Control	$9.00 x10^7$	0(0)	0	0(0)	0
	Experiment	$7.85 x10^7$	0(0)	0	0(0)	0
10	Control	$7.72 x10^7$	0(0)	0	0(0)	0
	Experiment	$9.94 x10^7$	0(0)	0	$36.37x10^4$(0.365)	1
11	Control	$8.51 x10^7$	0(0)	0	0(0)	0
	Experiment	$9.40 x10^7$	0(0)	0	$4.65x10^4$(0.050)	3

Table 8. The total area of inflammatory reaction (%) in the renal interstitium of mice in the control group and the experimental group during study during periods on day 35th DPI.

Number of mice	Group	Total area of renal(μm^2)	Area of vasculitis μm^2 (%)	Grading of vasculitis	Area of perivasculitis μm^2 (%)	Grading of perivasculitis
1	Control	$10.03x10^7$	0(0)	0	0(0)	0
	Experiment	$8.94x10^7$	0(0)	0	0(0)	0
2	Control	$9.80x10^7$	0(0)	0	0(0)	0
	Experiment	$10.72x10^7$	0(0)	0	$72.59x10^4$(0.974)	3
3	Control	$9.05x10^7$	0(0)	0	0(0)	0
	Experiment	$11.54x10^7$	0(0)	0	$7.04x10^4$(0.622)	2
4	Control	$8.00x10^7$	0(0)	0	0(0)	0
	Experiment	$7.75x10^7$	0(0)	0	0(0)	0
5	Control	$7.80x10^7$	0(0)	0	0(0)	0
	Experiment	$6.40x10^7$	$5.6x10^4$(0.089)	1	0(0)	0
6	Control	$10.20x10^7$	0(0)	0	0(0)	0
	Experiment	$8.73x10^7$	0(0)	0	0(0)	0
7	Control	$9.85x10^7$	0(0)	0	0(0)	0
	Experiment	$9.51x10^7$	0(0)	0	0(0)	0
8	Control	$9.90x10^7$	0(0)	0	0(0)	0
	Experiment	$11.04x10^7$	0(0)	0	0(0)	0
9	Control	$7.96x10^7$	0(0)	0	0(0)	0
	Experiment	$8.79x10^7$	0(0)	0	0(0)	0
10	Control	$9.80x10^7$	0(0)	0	0(0)	0
	Experiment	$10.45x10^7$	0(0)	0	0(0)	0
11	Control	$9.12x10^7$	0(0)	0	0(0)	0
	Experiment	$9.12x10^7$	0(0)	0	0(0)	0

Table 9. The total area of inflammatory reaction (%) in the renal interstitium of mice in the control group and the experimental group during study during periods on day 45th DPI.

Number of mice	Group	Total area of renal(μm^2)	Area of vasculitis μm^2 (%)	Grading of vasculitis	Area of perivasculitis μm^2 (%)	Grading of perivasculitis
1	Control	$8.96x10^7$	0(0)	0	0(0)	0
	Experiment	$9.61x10^7$	0(0)	0	0(0)	0
2	Control	$8.90x10^7$	0(0)	0	0(0)	0
	Experiment	$11.25x10^7$	0(0)	0	0(0)	0
3	Control	$9.05x10^7$	0(0)	0	0(0)	0
	Experiment	$7.88x10^7$	0(0)	0	0(0)	0
4	Control	$11.20x10^7$	0(0)	0	0(0)	0
	Experiment	$8.95x10^7$	0(0)	0	0(0)	0
5	Control	$9.00x10^7$	0(0)	0	0(0)	0
	Experiment	$8.78x10^7$	0(0)	0	$2.51x10^4$(0.029)	1
6	Control	$8.90x10^7$	0(0)	0	0(0)	0
	Experiment	$7.17x10^7$	0(0)	0	$24.20x10^4$(0.337)	1
7	Control	$8.70x10^7$	0(0)	0	0(0)	0
	Experiment	$9.07x10^7$	0(0)	0	0(0)	0
8	Control	$8.99x10^7$	0(0)	0	0(0)	0
	Experiment	$7.88x10^7$	0(0)	0	0(0)	0
9	Control	$9.93x10^7$	0(0)	0	0(0)	0
	Experiment	$10.05x10^7$	0(0)	0	0(0)	0
10	Control	$9.57x10^7$	0(0)	0	0(0)	0
	Experiment	$11.68x10^7$	0(0)	0	0(0)	0
11	Control	$8.90x10^7$	0(0)	0	0(0)	0
	Experiment	$11.31x10^7$	0(0)	0	$34.58x10^4$(0.305)	1

4. Discussion

The present study shows post infected with *T. spiralis* in mice model on study period 5th,10th, 14th, 21st, 35th and 45th DPI could induce inflammatory reaction of the blood vessels in the renal interstitium represent as acute vasculitis and perivasculitis that occurring on 21st, 35th and 45th DPI. This phenomenon could explain based on theoretically of *T. spiralis* life cycle and previously reviewed literatures concerned to trichinellosis and renal involvement. This period occurring within the parenteral phase during *T. spiralis* life cycle that the parasites spread and invade into various internal organs and tissue via lymphovascular tract and induce immune response between host and parasite interaction and in consequence of myocarditis, pneumonia, encephalitis, glomerulonephritis and renal failure. According to the present study was identified an inflammatory reaction in the blood vessels in the renal interstitium represent as vasculitis and perivasculitis 21st, 35th and 45th DPI. That conform to previously reviewed published of Neghina et al., 2011 as Reviews on Trichinellosis(I); Renal involvement dedicated that fatality rate was 26.1%, proteinuria was detected in 84.8% of cases, hematuria in 30.4%, and casts were observed in urine specimens from 23.9% of patients. Renal failure was evidenced in 8.7% of patients, whereas renal lesions were found by biopsy or necropsy in 43.5% of cases, vascular modifications on disseminative phase by increased cellularity in the vascular tuft. Also, the presence of oedema was evident at the interstitial tissue, being moderate on day 5th DPI, stronger from day 8th to day 30th DPI. After day 45th DPI, the oedema was replaced by a lymphoplasmocellular infiltration, showing an interstitial nephritis, which increased until the end of the experiment (Reina, 2000). Because lesions developed late in the course of infection and no larvae were detected, the possibility of an immune reaction was considered. This hypothesis could also be supported by Welt, 1941. Therefore phenomenon in this present study with acute vasculitis and perivasculitis in the renal interstitium demonstrated that conform to published of Neghina et al., 2011 and Reina, 2000. In term of this study showed acute perivasculitis in the renal interstitium in the 35th and 45th DPI less than in the 21st DPI. This phenomenon could explain based on theoretically of *T. spiralis* life cycle as follow: firstly this period is occurring in the muscle phase (parenteral phase) (Anunnatsiri et al., 2004), these new born larvae migrate into cell leaving capillaries and induce immune response host and parasite interactions resulting inflammatory reaction represent as vasculitis and perivasculitis on days 35th and 45th that could be explained and supported by Goutam and Saikat, 2014 with the mechanism of angiogenesis in nurse cell formation and maintenance vascular endothelial growth (VEGF) mRNA by in situ hybridization in the cytoplasm of the developing nurse cell beginning on day 7, up to eight months after initial infection of the muscle cell. The presence of VEGF peptide was observed shortly thereafter, beginning on day 9 using immunohistochemical methods, and was demonstrable within the nurse cell from that point on. Thus, the VEGF gene remains upregulated throughout the infection period, while the mRNA signal appears to be strongest at day 15. A constant, low level of production of VEGF peptide (also known as vascular permeability factor) after circulatory rete formation is complete implies a permanently heightened state of vascular permeability, and would present obvious advantages to the parasite for maintaining itself within the host for long periods of time. The vessels of the circulatory rete are now known to be derived from adjacent venules, not arterioles as was thought previously, and they have the diameter of sinusoids, thus facilitating the rapid flow of formed elements through them. The large diameter of the vessels, compared with capillaries, also favours rapid exchange of nutrients and wastes, but offers less than optimal conditions for the efficient exchange of gasses between the nurse cell and the red blood cells that circulate past it. These observations are consistent with data collected from a variety of experimental approaches indicating that larval and nurse cell energy metabolism are anaerobic. This metabolic strategy explains how the parasite remains infectious for another host (ie. scavengers) from days up to weeks after the death of the infected host (depending upon the ambient temperature) in its decaying muscle tissue the ultimate in anaerobic environment according to this phenomenon that support result of perivasculitis on 35th and 45th DPI yield less than on day 21st DPI. After invasion, they induce a remarkable series of cell physiological changes causing the fully differentiated muscle cell to transform into one that supports the growth and development of the larva. This process is termed “Nurse cell” formation and the structure conferred by the tyvelose moiety creates an antibody epitope, which occurs on multiple excretory-secretory proteins of *T. spiralis* muscle stage larvae. From an immunological perspective, antibodies against his epitope can protect against intestinal invasion by the parasite (Goutam and Saikat, 2014).

In conclusion, this study demonstrated after infected mice with *T. spiralis* 450 larvae could induce an inflammatory reactions of the blood vessel in the renal interstitium in early third week after infection and represent as vasculitis (9.09%) and perivasculitis (36.36%). Therefore post infection should be concern and awareness of renal involvement with alteration of renal function and possible leading to renal failure.

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