Global Veterinaria 2 (2): 73-79, 2008
ISSN 1992-6197

The Effect of Acute and Chronic Stages of *Toxoplasmosis* on the Small Intestine of Female Murine Model

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Abstract: *Toxoplasmosis* is one of the most common parasitic and zoonotic worldwide diseases. The present work was done through evaluation of the dangerously of experimentally induced toxoplasmosis in female mice. The work was carried out on one hundred and fourteen albino female mice. They were divided into *3* main groups: group A, B&C. Morbidity and mortality were remarkably affected in *"group A"* than in *"group B"*. In *group A*, the Impairment of activities was detected especially in young female. In *group A*, highest mortality rate was found in young female. Parasitological examination revealed that young female of both *groups (A&B)* showed the higher number of tachyzoites and PVs, while adult female of both *groups (A&B)* showed lesser number of tachyzoites and PVs. Histopathological study revealed necrosis of intestinal mucosa which was severe in both adult female and young female of *group A*. In *group B*. Adult female showed severe superficial sloughing with mild lymphocytic infiltration. Vigorous inflammatory reaction was noticed in young female with severe oedema and congested blood vessels.So, it can be concluded that female mice were more susceptible to infection with *T. gondii*.

Key words: Rabbits · *T. gondii* · female · pasrasitology · histopathology

INTRODUCTION

Toxoplasmosis is one of the most common parasitic and zoonotic worldwide diseases [1]. A disease caused by infection with an obligate intracellular parasite called *T. gondii* [2]. *T. gondii* is an intestinal coccidium has a wide range of intermediate hosts. Infection is common in many warm blooded animals, including humans [3]. The life cycle of the parasite includes two phases: the intestinal phase & the extra intestinal phase occurs in the intermediate hosts [4]. Most cases of toxoplasmosis in humans are probably acquired by the ingestion of contaminated food. Also, congenital toxoplasmosis results when infection is transmitted transplacentally from primarily infected mother to her fetus [6]. Necrosis in intestinal and mesenteric lymph nodes may occurs before other organs and [3]. As *T. gondii* does not produce toxins, necrosis may be caused by intracellular multiplication of tachyzoites[5]. *T. gondii* cause necrotic areas associated with congestion of cells in examined visceral organs [7]. Also [8] found that degenerative changes and necrosis at lining epithelial in affected areas. It has been reported that, the susceptibility of the host to *gondii* could be related among other factors to age and gender of the host [9]. The present work was planed to evaluate the effect the age of the host in response to *T. gondii* infection.

MATERIALS AND METHODS

The present study was carried out on three groups

Group A: Included 50 mice each mouse was infected with virulent RH strain of *T. gondii*. The strain was supplied from Laboratory of Zoonoses National Research Center Dokki, Cairo Egypt. [10]. This group was divided according to age & weight into two subgroups: supgroup1: Thirty adult female mice (6-8 weeks & 25-30 gm), infected with $2x10^6$ tachyzoites in 0.1 ml sterile saline/mouse and Subgroup 2: Twenty young female mice (2-2.5 weeks and 10-15 gm) infected the same dose.

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Group B: Included 50 mice each one was infected with sporulated oocysts(600 sporulated oocysts/gm body weight) of *T. gondii* perorally by using stomach tube. Oocysts were obtained from Laboratory of Zoonoses National Research Center Dokki, Cairo Egypt [11]. This mice group was divided into: Subgroup 1: Thirty adult female mice. Subgroup2: Twenty young female mice) infected the same dose.

Group C: Included fourteen "age and weight matched" normal non-infected mice. They were served as control group and divided into:-Subgroup 1: seven non infected adult female mice & Subgroup 2: Seven non infected female mice.

Parasitological methods

Maintenance of *Toxoplasma gondii* strains:

Virulent strain: The virulent strain was maintained by repeated intraperitoneal inoculation with approximately, 2×10^6 tachyzoites adjusted in *0.1 ml* sterile saline for each albino mouse every third day [10].

Sporulated oocysts: Non-sporulated oocysts of *Toxoplasma gondii* were collected & prepared according [12]. Then they stored at 4^0 C until used. The sporocysts were washed by saline and counted by using the haemocytometer slides [13].

Study of general condition of mice (Health state): Observation of mice groups throughout the period of the study that extended to 90 days was done.

Collection of small intestine specimens: All mice in *group A* were sacrificed from *3rd* to *6th* days post-infection (PI). Mice in *group C* were sacrificed between *3rd to 11th* days of experiment.

Histopathological methods: A rotatory microtome was used to cut sections of 4-5 micron thickness (50-100 micron distance between sections). Paraffin sections [14] were stained with Haematoxylin and Eosin [15] for histopathological study, while Periodic acid-Schiff reaction "PAS" [16] and Toluidine blue stains [17] used for detection of *T. gondii* parasites.

RESULTS

A.Parasitological Results: The Parasitological results can be summarized in the Table 1 and 2.

Detection of *T. gondii* parasites

Parasitological results of Haematoxylin and Eosin stained sections: Parasitophorous Vacuoles (PVs) as well as scattered parasites were detected in the intestinal sections of group A. The numbers of these vacuoles and

Table 1: General condition in mice infected with acute& chronic strain of *T. gondii* (group A&B)

	Adult female		Young female	
Subgroup	acute	chronic	acute	chronic
Impairment of activity	Moderate	mild	Severe	Moderate
Onset of impairment	From 3rd to 6th days (PI)	From 4th to 11th days (PI)	From 2nd to 4th days (PI)	From 4th to 11th days (PI)

Table 2: Mortality rate in different studied mice groups

Group	Group (A)			Group (B)			Group (C)		
Subgroups		Adult female	Young female		Young female	Young female		Adult female	Young female
Total No of mice	50	30	20	50	30	20	14	7	7
Mortality NO	10	4	6	2	0	2	-	-	-
%	20%	13.3%	30%	4%	0%	10%	0%	0%	0%
Post-infection days		3th-6th	2nd-4th		-	7th-9th		-	-

Table 3:

	Adult male		Young male	
Subgroup	acute	chronic	acute	chronic
Degree of necrosis	Severe	absent	Severe	absent
Lymphocytic infiltration	*absent*	Mild	*absent*	*Absent*
Degree of mucosal sloughing	absent	Severe superficial mucosal sloughing	absent	Severe superficial mucosal sloughing
Mitotic changes	absent	*Present*	absent	*Absent*
Oedema of mucosa	absent	*Massive*+++	absent	*Massive*+++

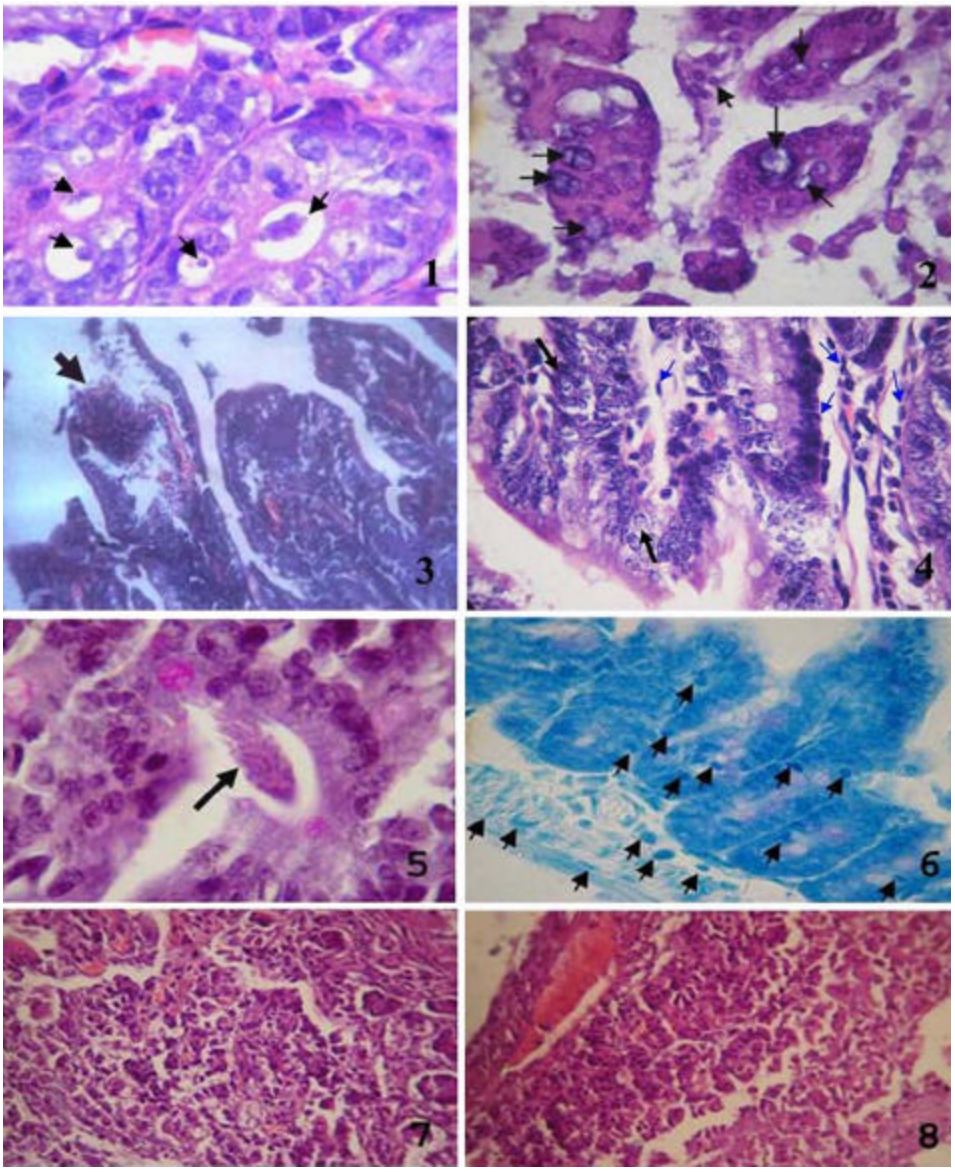

Fig. 1: Section in the ileum of adult female mouse infected with acute *T. gondii* strain, showing multiple ill-localized parasitophorous vacuoles (H&Ex100)

Fig. 2: Section in the ileum of young female mouse infected with acute strain of *T. gondii*. Large numbers of parasitophorous vacuoles can be seen (H&Ex40)

Fig. 3: Section in the ileum of adult female mouse infected with oocysts of *T. gondii*, showing ill-localized parasitophorous vacuoles (H&Ex40)

Fig. 4: Section in the ileum of young female mouse infected with oocysts of *T. gondii*. Scattered tachyzoites in the mucosa (blue arrow) and degenerated parasitophorous vacuoles can be seen (black arrow) (H&Ex40)

Fig. 5: Section in the ileum of adult female mouse infected with acute *T. gondii* strain, showing ill-localized parasitophorous vacuole (PV) containing large number of tachyzoites (PAS x100)

Fig. 6: Section in the ileum of adult female mouse infected with acute *T. gondii* strain, showing aggregations of tachyzoites in the mucosa and submucosa of the villi (Toluidine blue x100)

Fig. 7: Section in the ileum of adult female mouse infected with oocysts of *T. gondii*, showing severe superficial mucosal sloughing (H&Ex10)

Fig. 8: Section in the ileum of young female mouse infected with oocysts of *T. gondii*, showing severe sloughing of superficial layer of the mucosa and. Congested blood vessels (C) and oedema of the mucosa (O) can be detected (H&Ex40)

their outlines varied from one subgroup to another; subgroup 1: showed small number of localized parasitophorous vacuoles in the mucosa of intestinal villi subgroup 2: (adult female mice) showed multiple ill-localized PVs (Fig. 1), subgroup2,: (young female mice) showed large numbers of PVs (well localized, ill-localized and degenerated vacuoles) (Fig. 2).Sections of group B showed lower number of Pvs than the previous group; subgroup 1: (adult female) showed ill-localized PVs (Fig. 3), subgroup 2: (young female mice) showed multiple scattered tachyzoites allover the mucosa with multiple degenerated PVs (Fig. 4).

The results of PAS stained sections: In comparison with the previous stain the parasite was more marked by this stain and the results were nearly similar to that with H&E stain. In group A, Sections showed parasitophorous vacuoles that contain large number of tachyzoites, which are arranged in characteristic rosette shape appearance in subgroup 1 (adult female mice) Multiple PVs were also detected in the other subgroups (subgroup: 2,) (Fig. 5) respectively. In group C: the sections of this group revealed no changes.

The results of Toluidine-blue stained sections: Sections of small intestine of *group A* mice which stained with Toluidine-blue stain showed aggregation of tachyzoites in the mucosa of subgroup aggregation of tachyzoites in the mucosa and submucosa in subgroup *2* (adult female mice) (Fig. 6). Also, scattered tachyzoites can be detected in both subgroups.

B. Histopathological results: The histopathological results of the studied groups varied according to the stage of the parasite and the route used in host infection of mice (Table 2 and Fig. 1-6)

Parasitological results of Haematoxylin and Eosin stained sections: Parasitophorous Vacuoles (PVs) as well as scattered parasites were detected in the intestinal sections of *group A*. The numbers of these vacuoles and their outlines varied from one subgroup to another; *subgroup* 1*:* showed small number of localized parasitophorous vacuoles in the mucosa of intestinal villi *subgroup 1:* (adult female mice) showed multiple ill-localized PVs (Fig. 1), *subgroup2,:* (young female mice) showed large numbers of PVs (well localized, ill-localized and degenerated vacuoles) (Fig. 2). Sections of *group B* showed lower number of PVs than the previous group; *subgroup* 1*:* (adult female) showed ill-localized PVs (Fig. 3), *subgroup* 2: (young female mice) showed multiple scattered tachyzoites allover the mucosa with multiple degenerated PVs (Fig. 4).

The results of PAS stained sections: In comparison with the previous stain the parasite was more marked by this stain and the results were nearly similar to that with H&E stain. In group A, Sections showed parasitophorous vacuoles that contain large number of tachyzoites, which are arranged in characteristic rosette shape appearance in *subgroup 1* (adult female mice). Multiple PVs were also detected in the other subgroups *subgroup 2* (Fig. 5) respectively. In Group B: most sections of this group revealed scattered tachyzoites in intestinal mucosa (Fig. 5).

The results of Toluidine-blue stained sections: Sections of small intestine of *group A* mice which stained with Toluidine-blue stain showed aggregation of tachyzoites in the mucosa of subgroups aggregation of tachyzoites in the mucosa and submucosa in subgroup *2* (adult female mice) (Fig. 6). Also, scattered tachyzoites can be detected in both subgroups.

C. Histopathological results: The histopathological results of the studied groups varied according to the stage of the parasite and the route used in host infection of mice (Table 2 and 3 and Fig. 1-8).

DISCUSSION

Differences in age have been shown to affect susceptibility to infection including those caused by protozoal parasites in human and mice [18]. The present work was planed to study the effect of age of female in the susceptibility to infection with *T. gondii* through evaluation of the general condition, mortality rate and the concomitant parasitological and histopathological changes of the small intestine of murine models. One hundred and fourteen laboratory bred Swiss albino mice were classified into the following groups: group (A) included mice which were infected intraperitoneally with tachyzoites of *T. gondii* (acute RH strain), group (B) included mice which were infected perorally with oocysts of *T. gondii and* group (C) were served as control group. Each group was subsequently divided into subgroups: subgroup subgroup *1* (adult female) and subgroup *2*(young female). In the present work, morbidity and mortality were remarkably affected in intraperitoneally infected mice with acute strain of *T. gondii* (group A) than

in perorally infected mice with oocysts of *T. gondii* (group B). The general condition of infected mice in group A showed mild to moderate impairment of activity, loss of hair and loss of appetite between 3rd to 6th days post-infection (PI) in. subgroup *2* (adult female). While mice in subgroup *2* (young female) showed moderate to severe impairment of activity, loss of hair and loss of appetite from *2nd to 4th* days PI. These changes were evident in female mice. In group B infected mice showed impairment of activity, loss of hair and loss of appetite from *4th to 11th* days PI. These changes were more evident in. subgroup *2* (young female) than in the other two subgroups *1* (adult female mice).Concerning the mortality rate the results revealed that: in group A the highest mortality rate was found in subgroup *4* (young female) *30 %* followed by 13.3% in subgroup *2* (adult female). While, mice members of group B the subgroup *2*(young female), while there were no deaths recorded among mice members in subgroup *2* (adult female) as well as in all members of mice in control group. These results coincide with the report of [19] who revealed that, mortality after acute infection in inbred strain of mice varied according to the route of infection and the disease caused by intraperitoneally inoculated tachyzoites was more severe than that caused by perorally inoculated oocysts of *T. gondii*. As mice infected with the first route showed rapid retardation of the general condition with higher mortality rate than the other group which was infected with the second route. [20] concluded that the age of host playing a role in susceptibility to toxoplasmosis. They found that, mice of any age are susceptible to clinical *T. gondii* infection; whereas adult rats do not become ill although young rats can die of toxoplasmosis. Also adult dogs, like adult rats, are resistant whereas puppies are fully susceptible to clinical toxoplasmosis. Concerning the role of sex and sex hormones on experimental toxoplasmosis, [21] declared that gender of mice markedly affected their response to toxoplasmosis. Also, in accordance with the present results, [22] recorded high mortality rate among female mice infected perorally with oocysts of *T. gondii* in comparison to the male ones infected by the same route. Meanwhile, [23] agreed with this finding as they stated that, the relative pathogenicity of *T. gondii* can be influenced by the route of inoculation, age of the parasite as well as parasitic strain. Characteristic rosettes appearance was occasionally formed when the division of tachyzoites was synchronous [24] Characteristic rosette appearance can be detected in the same subgroup *1*. While other subgroups; adult female (*2*) and young female (2) showed ill-localized PVs that contained large number of tachyzoites. In general, subgroup *2* (young female) of both groups (A&B) showed the highest number of tachyzoites and PVs, while.subgroup *2* (adult female) of both groups (A&B) showed lesser number of tachyzoites and PVs. These findings might be attributed to the fact that the role of sex and sex-associated hormones in toxoplasmosis affected the multiplication of *T. gondii* through modulation of both innate and adaptive immune responses to this parasite. The levels of these hormones not only vary from males to females but also altered with age of the host [25]. The results obtained agree with that of [21] as they found that the number of PVs were significantly higher in the ilea of female mice compared to male which were infected perorally with tissue cysts of *T. gondii*. The more accumulating in the gastrointestinal tract is the first site which contact with intestinal protozoa [2]. The inflammatory reaction appears microscopically in the form of congested blood vessels, oedema in the interstitial space and hydropic changes may be noticed, while the inflammatory cellular infiltration was noticed late in the disease. As epithelium of the small intestine is rapidly regenerated, signs of regeneration may be present as mitotic changes of nuclei (denser irregular or divided nucleus) [26].

Microscopically examination of histopathological stained sections in the present study, revealed that members of group A showed necrosis in their intestinal mucosa. Necrosis was mild severe in both subgroup 1 (adult female) and subgroup *2* (young female). These finding are nearly similar to the results of [27], who reported that mice infected perorally with tissue cysts of *T. gondii* showed severe necrosis, predominantly with the villi of the ileum. This necrosis was observed in most parts of the ilea of female mice. On the other hand the pathological changes in group B mice were well manifested and variable. There was mitotic change in the basal crypts of the villi. The subgroup *2* (adult female) showed severe superficial sloughing with mild lymphocytic infiltration, severe oedema, congested blood vessels and frequent mitotic changes. Vigorous inflammatory reaction with severe oedema and congestion of the blood vessels were noticed in subgroup*1* (young female) that led to severe sloughing of the mucosa, but cellular infiltration could not be detected in this subgroup. The author added that the lower immune responses in subgroups *(1&2)* (adult female) might give chance for tachyzoites to multiply rapidly and cause more pathological damage. In comparison with the previous three subgroups (adult female), the lowest response in mice members of subgroup*2* (young female) led to

fulmination of infection caused by a very high rate of tachyzoites multiplication. This was already demonstrated by [23, 27] who reported that, a well known mechanism of pathogenesis and death during acute infection of mice with virulent strain of *T. gondii* was depended on the inflammatory process and necrosis observed in the small intestine of these mice. Cheng [6] reported that the degree and extent of the pathological changes and damage of the host in toxoplasmosis depend on certain factors. [28] reported that the highly effective resistance induced by *T. gondii* is mediated by *Th1* type cytokines expression pattern. The pathway of *Th1* triggering involves the induction of tumor necrosis factor-α*(TNF-α)* and *IL-12* synthesis from macrophage accessory cells. Then the later cytokines act synergistically to trigger the natural killer *(NK)* cells and interferon gamma *(IFN-γ)* response. Then both *IL-12* and *IFN-γ* promote T-cell differentiation toward the *Th1* phenotype. Therefore the ability of *T. gondii* to trigger *IL-12* release by macrophages may be an important event in the establishment of parasite specific cell mediated immunity during infection. *IL-12* stimulates *IFN-γ* production which activates mononuclear phagocytes and acts directly on T-cells to promote their differentiation and on B-cells to promote switching to the *IgG2* and *IgG3* subclasses. Also, [29] proved that, administration of *IL-12* to chronically infected immunocompromized mice resulted in prolonged survival compared to untreated control ones. From the results obtained in the present study it can be concluded that female mice were more susceptible to infection with *T. gondii* with pronounced pathological lesions This usceptibility is mainly related to difference in sex-associated hormones which modulate the immune responses against *Toxoplasma*.

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(Received: 25/12/2007; Accepted: 23/1/2008)