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

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Abstract: Toxoplasma gondii is an important opportunistic infection among human. The present work was planned to study the susceptibility of male to infection with T.gondii. This was done through evaluation of the general condition, morality rate, parasitological and the concomitant histopathological changes in the small intestine of experimentally induced toxoplasmosis in mice. The work was carried out on one hundred and fourteen albino mice. They were divided into 3 main groups: group A, infected intraperitoneally with tachyzoites RH strain of *T. gondii.* Group B, infected peroraly with oocysts of T.gondii. Morbidity and mortality were remarkably affected in "group A" than in "group B". In group A, the Impairment of activities were detected in adult male, moderate to severe in young male. In group A, highest mortality rate was found in young male (20%). While, adult male showed the lowest mortality rate (6.7%) in this group. In group B young male showed mortality rate equal to 10%. Parasitological examination revealed that; group A mice showed higher number of parasite (parasitophorous vacuoles"PVs"& tachyzoites) than group B mice. Histopathological study revealed necrosis of intestinal mucosa which was mild in adult male, moderate in young male of group A. In group B; mild oedema and congested blood vessels were detected in adult male. While young male showed moderate superficial sloughing with mild inflammatory cellular infiltration and congested blood vessels could be noticed in this subgroups. So, it can be concluded that young mice were more affected than adult ones especially in using virulent strain.

Key words: Rabbits · male · *T. gondii* · parasitology and histopathology

INTRODUCTION

Toxoplasmosis is a disease caused by infection with an obligate intracellular parasite called *Toxoplasma gondii.* [1]. *T.gondii* is an intestinal coccidium that parasitized members of the cat family [2]. The life cycle of the parasite includes two phases. The extra intestinal phase occurs in the intermediate hosts man and produces tachyzoites [3]. Most cases of toxoplasmosis in humans are probably acquired by the ingestion of either tissue cysts or oocysts in food. [4]. Bradyzoites from the tissue cysts or sporozoites released from oocysts penetrate the intestinal epithelial cells and multiply in the intestine [5]. *T. gondii* may spread both locally to mesenteric lymph nodes and to distant organs by invading the lymphatics and blood vessels [1]. Necrosis in intestinal and mesenteric lymph nodes may occurs before other organs and become severely damaged [2]. It has been reported that, the susceptibility of the host to *T. gondii* could be related among other factors to age and gender of the host [6,7]. The present work was planed to evaluate the effect of 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. [8]. This group was divided according to age and weight into two subgroups:supgroup1:Thirty adult male mice(6-8 weeks&25-30gm),infected with $2x10^6$ tachyzoites in 0.1 ml sterile saline / mouse & Subgroup 2: Twenty young male mice(2-2.5weeks&10-15gm) 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 [9]. This mice group was divided into: Subgroup 1: Thirty adult male mice. Subgroup2:Twenty young male mice.

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 male mice and Subgroup 2:Seven non infected male mice.

Parasitological methods

Maintenance of *Toxoplasma gondii* strains

Virulent strain: The virulent strain was maintained according [8].

Sporulated oocysts: Non-sporulated oocysts of Toxoplasma gondii were collected according [10]. Sporulated oocysts were prepared according [11].

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). All mice in-group B were sacrificed from 4th to 11th days post-infection.

Histopathological methods: A rotatory microtome was used to cut sections of 4-5 micron thickness (50-100 micron distance between sections). Paraffin sections [12] were stained with Haematoxylin and Eosin [13] for histopathological study, while Periodic acid-Schiff reaction "PAS" and Toluidine blue stains used for detection of T. gondii parasites [14].

RESULTS

A. Parasitological results: The Parasitological results can be summarized in the following Table 1-3.

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 their outlines varied from one subgroup to another; subgroup 1: (adult male mice) showed small number of localized parasitophorous vacuoles in the mucosa of

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

	Adult male		*Young male*	
Subgroup	*acute*	*chronic*	*acute*	*chronic*
Impairment of activity	mild	mild	moderate	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 male	Young male		Young female	Young male		Adult male	Young male
Total No of mice	50	30	20	50	30	20	14	7	7
Mortality NO	6	2	4	2	-	2	-	-	-
%	3%	6.7%	20%	4%	0%	10%	0%	0%	0%
Post-infection days		4th-6th	2nd-4th		-	9th-11th		-	-

Table 3: Pathological finding in mice infected with acute&chronic strain of T. gondii (group A)

	Adult male		Young male	
Subgroup	Acute	Chronic	Acute	Chronic
Degree of necrosis	mild	absent	moderate	absent
Lymphocytic infiltration	present	dense	absent	mild
Degree of mucosal sloughing	absent	Nearly normal mucosal pattern	absent	Moderate superfacial mucosal sloughing
Mitotic changes	absent	present	absent	absent
Oedema of mucosa	absent	Mild+	absent	Moderate++

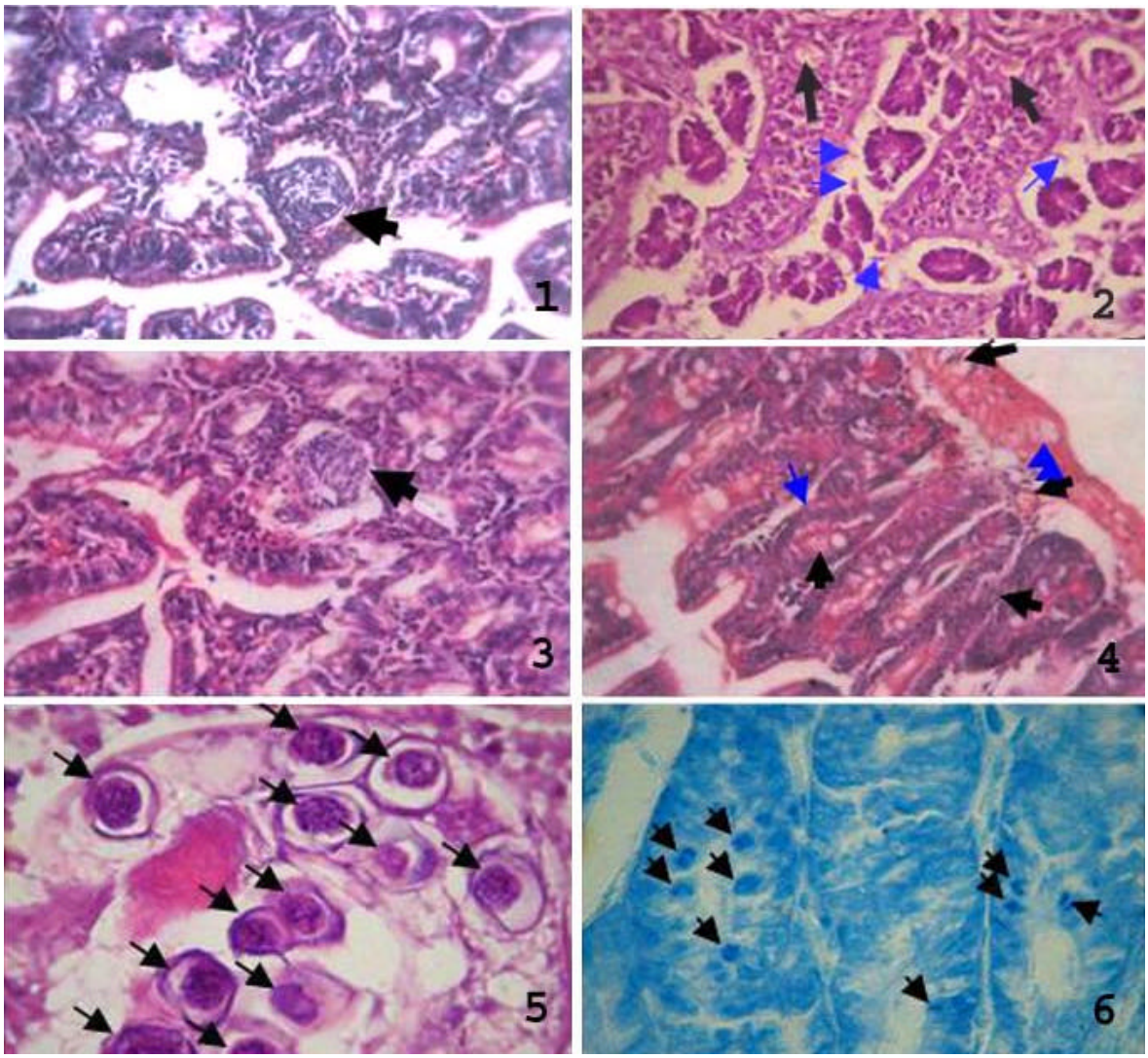

Fig. 1*:* Section in the ileum of adult male mouse infected with acute *T. gondii* strain, showing localized parasitophorous vacuole (H&Ex40)

Fig. 2*:* Section in the ileum of young male mouse infected with acute strain of *T. gondii*, showing multiple parasitophorous vacuoles (PVs) (black arrow) and scattered tachyzoites all over the mucosa (blue arrow) (H&Ex40)

Fig. 3: Section in the ileum of adult male mouse infected with oocysts of *T. gondii*, showing well localized parasitophorous vacuole (H&Ex40)

Fig. 4: Section in the ileum of young male mouse infected with oocysts of *T. gondii*. Scattered tachyzoites of *T. gondii* appear in the mucosa (black arrow) and submucosa of the villi (blue arrow) (H&Ex40)

Fig. 5: Section in the ileum of young male mouse infected with acute *T. gondii* strain, showing multiple well localized parasitophorous vacuoles (PVs) (PASx40)

Fig. 6*:* Section in the ileum of adult male mouse infected with acute *T. gondii* strain, showing aggregations of parasites in the intestinal mucosa (Toluidine bluex100)

intestinal villi (Fig. 1), subgroup 1: (adult male mice) showed small number of well localized PVs (Fig. 2).

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 male mice) (Fig. 3).

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.

Multiple PVs were also detected in the other subgroups (subgroup: A1 and subgroup: A2) (Fig. 5) respectively. In group B: most sections of this group revealed scattered tachyzoites in intestinal mucosa (Fig. 5). 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 1 (adult male mice) (Fig. 6) and aggregation of tachyzoites in the mucosa.. 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 of infection and age of mice (Tables 1-3).

DISCUSSION

Differences in age and gender have been shown to affect susceptibility to infection including those caused by protozoal parasites in human and mice [15]. With regard to infection with *Toxoplasma gondii,* gender related differences in susceptibility to infection have been reported [6].

The present work was planed to study the effect of acute and chronic infections 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 with *T. gondii* tachyzoites, group (B) included mice which were infected perorally with oocysts of *T. gondii and* group (C) included normal un-infected mice. In the present work, morbidity and mortality were remarkably affected in mice with acute toxoplasmosis (group A) than chronic toxoplasmosis (group B). The general condition of acute stage showed mild to moderate impairment of activity, loss of hair and loss of appetite between 3rd to 6th days Post-infection (PI) in both subgroup 1 (adult male). While mice in subgroup 2 (young male) showed moderate to severe impairment of activity, loss of hair and loss of appetite from 2nd to 4th days PI. 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 male) than in the other subgroup 1(adult male mice). Concerning the mortality rate the results revealed that: in group A the highest mortality rate was found in subgroup20 % in young male, while in adult male showed the lowest mortality rate (6.7 %). In group B the young male showed mortality rate equal to *10 %* & no mortalities in adult male as well as in all members of mice in control group. These results coincide with the report of [16] 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. [17] concluded that the age of host playing a role in susceptibility to toxoplasmosis. They found that adult rats do not become ill although young rats can die of toxoplasmosis. [22] agreed with this finding as they stated that, the relative pathogenicity of *T. gondii* can be influenced by the route of inoculation, age and gender. When *T. gondii* parasite penetrated the intestinal mucosa either through blood or lymphatics from peritoneal cavity or through mouth, it multiplied rapidly as actively proliferating tachyzoites in the intestinal epithelial cells to form the parasitophorous vacuoles [18]. In the present study, collected samples of small intestine were stained with H&E, PAS and Toluidine blue stains to detect the parasite and evaluate the histopathological changes in infected mice. The results obtained revealed that group A mice showed higher number of parasitophorous vacuoles and tachyzoites than group B mice. The outer lining of these PVs were well localized in subgroup 1 (adult male mice) in both groups (A&B) "indicate good host response to parasite". Also, characteristic rosette appearance can be detected in the same subgroup 1. In general, subgroup 2 (young male) showed lesser number of tachyzoites and PVs. In comparison with the previous *2* subgroups the

lowest number of the parasite was recorded in subgroup 1 (adult male) of both groups (A&B). These findings might be attributed to the fact that the role of 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 [7]. The results obtained agree with that of [19] as they found that the number of PVs were significantly higher in the ilea of female mice compared to male. And they also found that female mice treated with testosterone before infection with *T. gondii* showed lower number of PVs than control group (non-treated female mice). [20] concluded that although it is generally believed that male hosts are more susceptible to parasitic infections. Increasing evidence is being accumulated that the gastrointestinal tract is the first site which contact with intestinal protozoa [1]. While the inflammatory cellular infiltration was noticed late in the disease [21]. 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 in subgroup *1* (adult male), moderate in subgroup *2* (young male). These finding are nearly similar to the results of [19], who reported that mice infected perorally with tissue cysts of *T. gondii* showed severe necrosis, predominantly with the villi of the ileum. On the other hand the pathological changes in group B mice were well manifested and variable. The subgroup 1 (adult male) showed nearly normal mucosal pattern with mild oedema. While the subgroup 2 (young male) showed moderate superficial sloughing with mild inflammatory cellular infiltration, moderate oedema and congested blood vessels. These results might be attributed to a good immune response of adult male mice subgroup 1 against the parasite which make it capable to reduce multiplication of tachyzoites. The mitotic changes in this subgroup (adult male) denote regeneration and repair of the mucosa. The author added that the lower immune responses in subgroup *2* (young male) than the previous subgroup *1* might give chance for tachyzoites to multiply rapidly and cause more pathological damage. This was already demonstrated by [19] and [22] 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. [5] reported that the degree and extent of the pathological changes depend on certain factors: age of the host; as older hosts being more resistant to the disease, virulence of the strain of *T. gondii* and the degree of acquired immunity of the host. [23] concluded that *T. gondii* strongly and persistently stimulates cell mediated immune response which protects the host against tachyzoites. Meanwhile, [24] reported that the highly effective resistance induced by *T. gondii* is mediated by Th1 type cytokines expression pattern. Also, [25] 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 young mice were more affected than adult ones especially in using virulent strain.

REFERENCES

1. Kasper, L.H., 1998. Toxoplasma Infection, Harrison's Principles of Internal Medicine, Textbook, 14th Edn.
2. Gillespie, S. and R.D. Pearson, 2001. Principles and Practice of Clinical Parasitology, Gohn Wiley and Sons Ltd, Baffins lane, Chichester west sussex. 19 Iup, England, pp: 113-138.
3. Parija, S.C., 2004. Text Books of Medical Parasitology, Protozoology and Helminthology: 2nd Edn. Medical Books Publishers Chennai, New Delhi, pp: 172-183.
4. Paniker, C.K.J., 2002. Textbook of Medical Parasitology 5th Edn. Miscellaneous sporozoa and microspora. Jaypee Brother: Medical publishers (P) LTD New Delhi., 89-96.
5. Cheng, T.C., 2006. General parasitology, Elsevier India., phylum Apicomplexa 2nd Edn., pp: 189-192.
6. Walker, W., C.W. Roberts, D.J.P. Ferguson, H. Jebbari and J. Alexander, 1997. Innate immunity to Toxoplasma gondii is influenced by gender and is associated with differences in interleukin-12 and gamma interferon production. Infectious Immunology, 65: 1119-1121.
7. Roberts, C.W., W. Walker and J. Alexander, 2001. Sex-Associated hormones and immunity to protozoan parasites. Am. Soc. Microbiol., 14: 476-488.
8. Rougier, D. and A.P. Thomas, 1985. Detection of toxoplasmic immunity by multipuncture skin test with excretory-secretory antigen. Lancet, 11: 121-123.
9. Fayed, H.M., K.A.M. Allam and N.S.Ali, 2004. Merogony of Toxoplasma gondii (Apicomplexa: Coccidia) and its effect on the mortality and histopathology in the house mouse Mus Musculus. J. Egypt. Soc. Parasitol., 34: 45-65.

10. Long, P.L., B.J. Millared, L.P. Joyner and C. Norton, 1976. A Guide to Laboratory Techniques Used in the Study and Diagnosis of Avian Coccidiosis. Folia Veteterinaria Let, 6: 201-207.
11. Mehlhorn, H., 1988. Morphology In: Parasitology in Focus: Facts and Trends. Mehlhorn, H. (ED.) 1st Edn. Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo.
12. Hollands, B.C.S., 1962. In: Progress in Medical Laboratory Technique, Baker, F.J., Butter worths, London, Vol: 1.
13. Bancroft, J.D. and A. Stevens, 1982. Theory and Practice of Histological Techniques. 2nd Edn. Churchill Livingstone, London, Melbourne and New York, 49.
14. Careton, H.M., 1980. Carleton's Histological Technique. 5th Edn. Oxford University Press, Oxford, New York, Toronto.
15. Cross, C.E. and J. Langorne, 1998. Plasmodim chabaudi, inflammatory cytokines and pathology. Erythrocytic stage infection in mice. Exper. Parasitol., 90: 220-222.
16. Blackwell, J.M., C.W. Roberts and J. Alexander, 1993. Influence of genes within the MHC on mortality and brain cysts development in mice infected with Toxoplasma gondii; kinetics of immune regulation in BALB/CH-2 congenic mice. Parasitological Immunol., 15: 317-324.
17. Dubey, J.P., D.S. Lindsey and C.A. Speer, 1998. Structures of T. gondii tachyzoites, bradyzoites and sporozoites and biology and development of tissue cysts. Clin. Microbiol. Rev., 11: 267-299.
18. Roos, D.S., R.G.K. Donald, N.S. Morrissette and A.L.C. Moulton, 1994. Molecular tools for genetic dissection of the protozoan parasite Toxoplasma gondii. Methods Cell Biol., 45: 27-63.
19. Liesenfeld, O., T. Anhnguyen, C. Phark and Y. Suzuki, 2001. Importance of gender and sex hormones in regulation of susceptibility of the small intestine to peroral infection with *Toxoplasma gondii* tissue cysts. J. Parasitol., 87: 1491-1493.
20. Klein, S.L. and J. Hopkins, 2004. Hormonal and Immunological Mechanisms Mediating Sex Differences in Parasite Infection. Blackwell Publishing Ltd.
21. Robbin, S.l., V. Kumar and R.S. Cotran, 2001. Pathologic Basis of Disease 7th Ed., 2: 20-23.
22. Fux, B., C.V. Rodeigues, R.W. Portela, S.U. Nei, D. Sibley, R.W.A. Vitor and R.T. Gazzinell, 2003. Role of cytokines and major histocompatibility complex on the mouse resistance to infection with a natural Recombinant types I and II of Toxoplasma gondii. Infection and immunity. Am. Soc. Microbiol.,
23. Gazzinelli, R.T., S. Hieny, T.A. Wynn, S. Wolf and A. Sher, 1993. Interleukin-12 is required for the T-lymphocyte-independent induction of interferon-ã by an intracellular parasite and induces resistance in T-cell deficient host. Proceding of the National. Academic Science USA, 90: 6115.
24. Kasper, L.H., T. Matsuura and I.A. Khan, 1995. IL-7 stimulates protective immunity in mice against the intracellular pathogen, *Toxoplasma gondii*. Immunol., 155: 4798-4804.
25. Tawfeek, G.M., N.M. Oteifa and M.A. Mustaf, 2001. Prophylaction efficacy of recombinant IL-12, clindamycin alone or in combination against experimental reactivated toxoplasmosis. J. Egypt. Soc. Parasitol., 31: 853-865.

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