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Some Diagnostic Studies on Male New Zealand Rabbit Experimentally Infected with *Toxoplasma gondii* Strain

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Abstract: *Toxoplasmosis* is an important opportunistic infection among human caused by *Toxoplasma gondii.* It is one of the most common parasitic zoonotic worldwide diseases. The present study was carried out on 17 mature male New Zealand rabbits divided into 2 groups. The first group was kept as non infected control. The second group was inoculated subcutaneously (s/c) with 150,000 tachyzoites of *Toxoplasma gondii* of RH strain. Blood samples were collected pre-inoculation and at weekly post-inoculation (PI) intervals for two months and sera were separated for investigating some serological parameters relevant to diagnosis and hormonal profile. Tissue specimens were taken for histopathological study. Results indicated that inoculated rabbits showed emaciation and nervous manifestation at the end of experiment. Testosterone level in the serum of infected rabbits was highly significantly decreased in comparison with that of the control group. Anti-toxoplasma antibodies titer was detected markedly in the serum of all *T. gondii* tachyzoites inoculated rabbits on day 14 post inoculation and the positive titer persisted high for about 2 months. Maximum titer (1/1024) was obtained in infected animals. The most pronounced histopathological testicular changes were necrosis and disappearance of epithelial lining of almost all the seminiferous tubules accompanied with intra-tubular multinucleated spermatid giant cells and peritubular C.T. proliferation. Also tissue cysts of bradyzoites were associated with Toxoplasmosis lesions.It could be concluded that Toxoplasma infection could be easily detected by LAT test and it induced testicular dysfunction and had severe adverse effects on the vital organs.

Key words: Rabbits · *T.gondii* · testosterone · Latex Agglutination Test (LAT) histopathology

INTRODUCTION

Toxoplasmosis is an important opportunistic infection among human immunodeficiency syndromes and it is one of the most common parasitic and zoonotic worldwide disease [1]. It is caused by *Toxoplasma gondii* (*T. gondii*) which is an obligate intracellular protozoon causing asymptomatic infection in a wide variety of mammals and birds [2]. It has been reported that *T. gondii* is one of the most important causes of fetal resorption, abortion, stillbirths and neonatal mortalities in small ruminants, resulting in great economic losses [3, 4]. Moreover, cystic ovarian degeneration and chronic endometritis were found in *T. gondii* sero-positive buffalo-cows. [5].

In domestic rabbits toxoplasmosis was first described as early as by [6] in Brazil and since that time there were several studies related to clinical cases of this disease and epizootics of toxoplasmosis in wild and domestic rabbits in many countries such as USA, Scandanavia, Czech Republic, Poland and Spain [7-12]. Severe acute and fatal toxoplasmosis were observed in these outbreaks and many animals died. In Egypt, serological survey indicated that occurrence of *T. gondii* in 25.83% of examined rabbits [13]. It has been established that infected rabbits play important role in the epidemiology of toxoplasmosis in humans [14, 15] and constitute a potential source of toxoplasma infection among agricultural workers [16].

Infection of rabbits with *T. gondii* created public health concern, because the parasite could be transmitted to the human being through contact [14] and during hunting and skinning [15]. In addition, the incidence of toxoplasmosis in rabbits was 20% in Egypt [17]. Also fatal toxoplasmosis might occur after acute illness in domestic rabbits [7].

Diagnosis of toxoplasmosis has traditionally based on the characteristic histopathological changes, serological tests and on isolation of the parasite by mouse inoculation, a technique which is both time consuming and potentially hazardous.

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There is no relevant information about the pathogenesis of *Toxoplasma gondii* on male genital system. Therefore, the aim of the present work is to clarify the pathogenic role of the parasite on testis with special reference to the histopathological and sex-hormonal changes as well as to evaluate the LAT technique for serodiagnosis of early circulating antibodies of toxoplasmosis in male rabbits.

MATERIALS AND METHODS

Experimental animals: A total number of 17 mature male New Zealand rabbit weighing 3-3.5 kg and 5-5.5 months old. in addition to sixty Albino mice, 6-8 weeks old, were used in this experiment. All animals were obtained from Laboratory Animal House, National Research Center, Dokki, Giza. Rabbits were clinically healthy and free from toxoplasma (by using LAT) and coccidia (by using direct fecal smear). Animals were kept under observation for 2 weeks before injection of parasite and also for acclimatization. Rabbits were housed individually in wire cages under good ventilation and hygienic condition and given pelleted food and water *ad libitum*.

Experimental design

Parasite: The parasite was kindly provided by Faculty of Medicine Ein Shams University, Cairo, Egypt. RH strain of *T. gondii* and was propagated by intra-peritoneal injection in albino mice [18].

Rabbits were divided into two groups: The first group formed of five animals and was served as non-infected control. The second group consisted of twelve animals. Each animal was inoculated S/C with $15x10^4$ tachyzoites of *T. gondii*. All animals were kept under daily observation during the experimental period for clinical signs and mortality. Two rabbits from the infected group were slaughtered after 4 days for taking impressions smears and others fifteen animals were slaughtered at 90 days PI for collection of internal organs.

Blood sampling: Blood samples were obtained by ear vein punctures before inoculation and every week for consecutive 8 weeks PI. Serum samples were harvested by centrifugation at 3000 rpm for 10 min. and kept frozen at -20°C till used for serological diagnosis of *T. gondii* as well as evaluation of testosterone level.

Diagnosis of *T. gondii* in experimental inoculated rabbits by impression smears: Another two rabbits were inoculated I/P with *T. gondii* tachyzoites and sacrificed 7 days PI. Impression smears were obtained from liver and kidney. The smears were dried, fixed in methanol and stained with Geimsa stain.

Bio-assay of rabbit-tissue infected with *T. gondii* tachyzoites in mice: Samples from liver and kidney of infected rabbits were ground thoroughly in saline and inoculated i/p in two mice. Three days later, the mice were sacrificed and impression smears were examined for toxoplasma tachyzoites according to[19].

Hormonal assay: Testosterone level was determined by radioimmunoassay (RIA) [20] using kits purchased from Diagnostic Product Corporation (DPC, Los Angles, USA).

Serological examination: Antibodies against *T. gondii* were detected by commercial latex agglutination kit [21-23]. Traditional ELISA antigen (TEA) was prepared by ultrasonication of *T. gondii* RH strain and ELISA based on recombinant antigen 1:20 [24].

Histopathological study: Tissue specimens from testis were collected and fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in benzene and embedded in paraffin wax. Sections of 5μ thickness were prepared and stained with hematoxylin and eosin (H&E), periodic acid Schiff's reagent (PAS) and Giemsa stain then examined microscopically [25].

Statistical analysis: Data were statistically analyzed using Student's t-test according to (26).

RESULTS

Clinical symptoms and post mortem examination: Daily observation of the experimentally infected rabbits revealed that there animals lose their appetite gradually during the first weeks post infection and signs of emaciation appeared in most of infected animals. Also some nervous manifestation appeared at the end of experiment on some model rabbits. Other clinical findings included nasolacrimal discharges and respiratory disorders were noticed. The post mortem examination showed that liver was enlarged in size with white necrotic foci distributed on its surface. In the brain, there was severe congestion of the meningeal blood vessels. Spleen appeared enlarged in size with stretched capsule and rounded border. Lungs were congested and displayed dark red or gray and firm areas of consolidation.

Table 1: Serum testosterone level (ng/dl) in male rabbits inoculated s/c with *T. gondii* tachyzoites (Mean±SE)

Group Week PI	Control	Infected
1	1.759±0.150	0.814**±0.012
2	1.557±0.110	0.826**±0.019
3	1.539±0.183	0.872**±0.021
4	2.598±0.023	0.798**±0.015
5	2.612±0.064	0.701**±0.038
6	2.560±0.143	0.693**±0.028
7	2.710±0.229	0.597**±0.042
8	2.997±0.113	0.564*±0.023

* P<0.05 ** P<0.01

Table 2: Antibodies titer against *T.gondii* in rabbit-using Latex agglutination test

Rabbit number	Days post inoculation								
	0	7	14	21	28	35	42	49	56
1	0	0	32	512	512	512	128	512	32
2	0	0	16	512	512	512	512	512	512
3	0	0	64	1024	512	1024	1024	1024	1024
4	0	0	32	512	512	512	512	512	512
5	0	0	64	1024	1024	1024	1024	1024	1024
6	0	0	16	512	512	512	512	512	64
7	0	0	8	256	1024	1024	1024	1024	512
8	0	0	128	512	512	1024	1024	1024	1024

Impression smears: Microscopically examination showed the presence of individual tachyzoites in the liver, kidney and of infected rabbits at seven days PI (Fig. 1).

Hormonal assay (testosterone level): Table 1 shows testosterone levels in sera of inoculated rabbits with tachyzoites. Levels in the serum of infected rabbits was highly significantly decreased (P<0.01) in comparison with that of the control group from the first week till the end of experiment. The decrease was gradually during the experimental period.

Serological examination: As shown in Table 2 anti-toxoplasma antibodies titer was detected markedly in the serum of all *T. gondii* tachyzoites inoculated rabbits on day 14 PI and the positive titer persisted high for about 2 months. Maximum titer (1/1024) was reached in the rabbit No. 5 on the 3rd week PI and in the other three of inoculated rabbits on day 35 PI. and then begin to decline sharply in most of cases on day 56 PI and remained low till the end of the experiment.

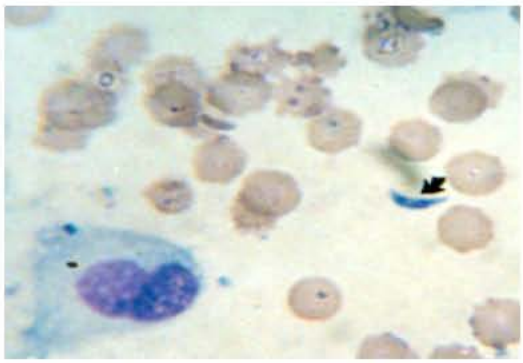

Fig. 1: Impression smear showing tachyzoites

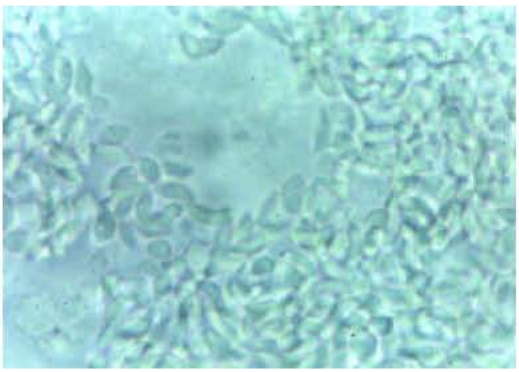

Fig. 2: *Toxoplasma gondii* tachyzoites

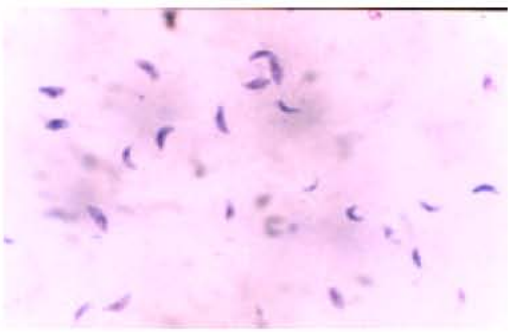

Fig. 3: *Toxoplasma gondii* tachyzoites(gimesa stain)

Pathological study: The testicular histopathological findings were nearly the same in all examined animals but widely differed in the degree and intensity of lesions and were graded as moderate and severe.

In the moderate cases, most of seminiferous tubules appeared relatively small in size, meanwhile, others appeared shrunken, collapsed and irregular in outline and widely separated from each other (Fig. 6). The germinal epithelium of some tubules was single and/or few layers and had vacuolar appearance (Fig. 6). The spermatogonial cells of some tubules showed necrobiotic changes represented by pyknosis and karyorhexis of their nuclei (Fig. 6). Some tubules contained homogenous hyalinized

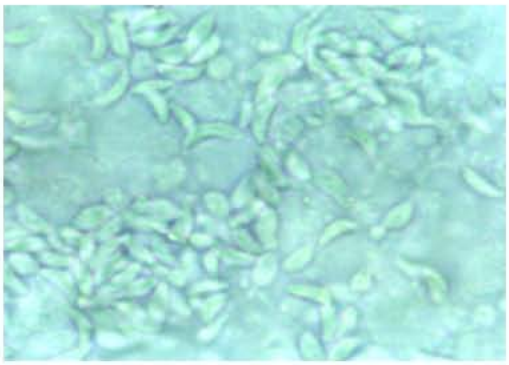

Fig. 4: impression smear from liver and kidney printed film

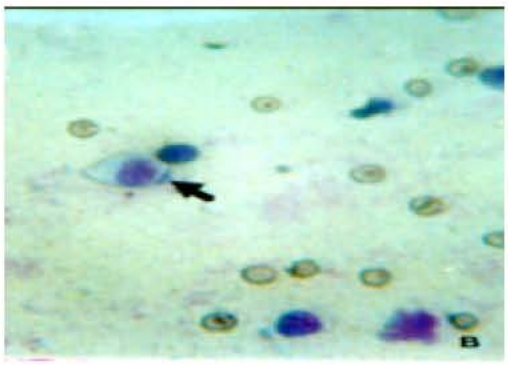

Fig. 5: *Toxoplasma gondii* tachyzoites

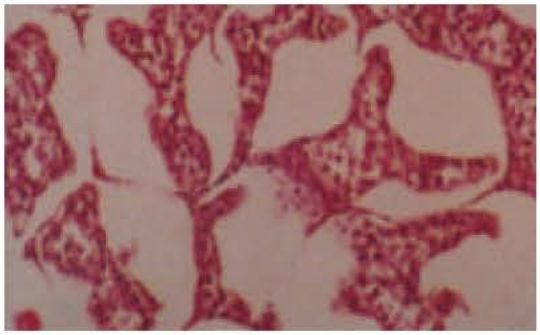

Fig. 6: Testis, showing shrunken and collapsed seminiferous tubules (H&E, X100)

eosinophilic material with no sperms within the lumen (Fig. 9). In addition to the above mentioned lesions, spermatogenesis was arrested. (Fig. 7). Numerous multinucleated giant cells, desquamated spermatogenic cells and cellular debris were seen within the luminae of some tubules (Fig. 8). Leydig cells appeared vacuolated and proportionally increased in the number (Fig. 9). There was prominent interstitial oedema together with congestion of blood vessels.

In severe cases, there was marked hyperplasia of C.T. of tunica albugenia. Most of tubules revealed complete

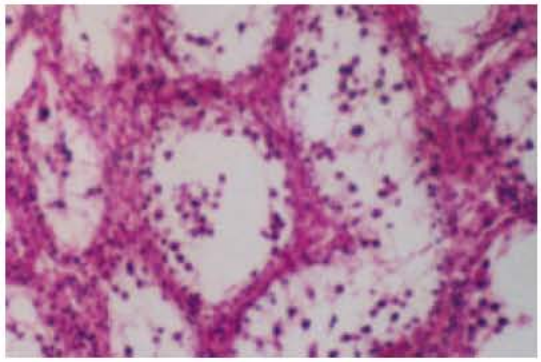

Fig. 7: Testis, seminiferous tubules showing thickening of the basement membrane associated with proliferation of peritubular C.T. in addition to necrosis and disappearance of most epithelial lining (H&E, X200)

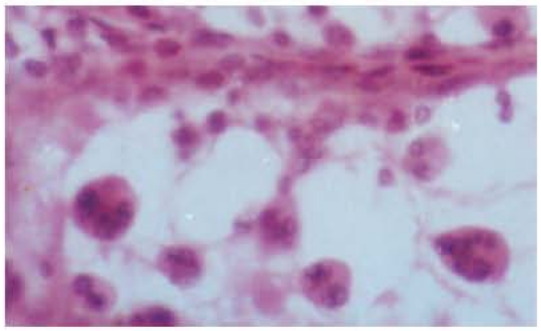

Fig. 8: Testis, showing seminiferous tubule contains several multinucleated giant cell, cellular debris and exfoliated cells within the lumen (H&E, X400)

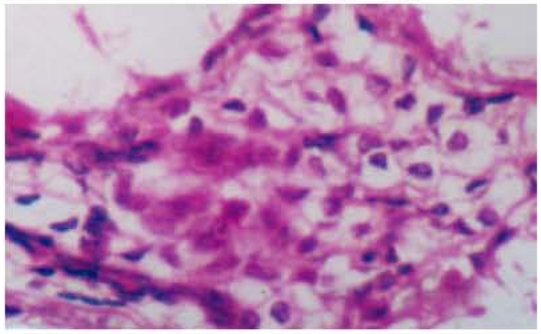

Fig. 9: Testis, showing vacuolar degeneration associated with moderate proliferation of Leydig cells (H&E, X400)

depletion of spermatogenic epithelium lining (Fig. 7) The basement membrane of some tubules was greatly thickened, oedematous and associated with marked increase in the peritubular C.T. Most of the epithelial lining of such tubules was severely necrosed,

disappeared and the remnants were vacuolated and exfoliated into the lumen. The tubules were lined by Sertoli cells and few.

DISCUSSION

Toxoplasma gondii is one of the most widely distributed intracellular parasites and is of veterinary and medical importance.It is recognized as major cause of abortion and congenital disease in its intermediate hosts [1]. In the present study, the s/c inoculated rabbits with *T. gondii* tachyzoites showed emaciation and moderate nervous manifestation at the end of the experimental period. Such signs were confirmed by observations reported by [13, 27, 28]. Brain is one of the organs most frequently parasitized with *T. gondii* which regarded by some as neurotropic because encephalitis is a prominent symptoms of clinical toxoplasmosis in human [29] and *Toxoplasma gondii* encysts more often in brain than in other organs of mice [30]. Moreover, the outcome of infection is determined by a number of factors including the number and strain of toxoplasma in the infective dose, species, age and immune status of the host [31]. Testosterone level was significantly decreased in the serum of infected rabbits. The condition might be due to interference with gonadotropin hormones (FSH and LH) and their effects on the testosterone production by Leydig cell. Such interference could be due to infection, which induced pathological changes in pituitary and/or hypothalamus [32], whereas, tissue cysts were observed in brain tissue. At the same time, the decline of serum testosterone level was confirmed with the accompanied histopathological findings in testis. In the current work, LAT was evaluated as a highly specific and sensitive screening serological test to detect IgG and IgM- type of *T.gondii* in animals [21-23]. The high titer of *T.gondii* antibodies detected by using LAT during days 14-63 PI means that the humoral immune response of rabbits was strong [10]. In this instance [33] reported that *T. gondii* antigen becomes demonstrable in the circulation 3 days after subcutaneous inoculation of rabbits with RH strain and before emergence of antibody in serum and development of parasitemia. The highest titer occurs at 35 days PI and still high at 150 days [34]. It was found [9] that the production of IgM and IgG antibodies against *T.gondii* developed one week post infection in rabbits, which support the detection of antibodies after two weeks post inoculation in the present study. The histopathological findings of infected rabbit were mainly characterized by multiple necrotic areas, congestion and haemorrhage together with inflammatory mononuclear cell reaction. The above mentioned toxoplasmic lesions were associated with the presence of tissue cysts of bradyzoites and/or multiplying colonies of bradyzoites. Similar lesions were observed by [7, 8, 35-37]. During the early phases of infection, *T. gondii* tachyzoites actively have penetrated and rapidly multiplied in a wide variety of host cells and eventually caused lysis which resulted in foci of necrosis [3]. Also [31] concluded that necrosis was a feature in organs heavily infected with tachyzoites and [38] reported that tachyzoites were the principal pathogenic form of toxoplasma, they actively invaded host cells by means of an anterior organelle and released of proteolytic enzymes causing local disruption of host cell plasmalemma. The invading organism became surrounded by parasitophorous vacuole predominantly formed by invagination of host cell plasma membrane [39]. The ability of toxoplasma to survive in the intracellular environment was apparently due to failure of fusion of lysosomal membranes with membranes of parasitophorous vacuole [31] and that could be due to absence of host protein markers in the parasitophorous vacuole results from its unique non fusigenic properties [40]. Regarding the histopathological changes in testis of infected rabbits, the outstanding picture of testicular lesions was small and shrunken seminiferous tubules with thickened basement membrane, peritubular C.T. proliferation, necrosis and disappearance of most epithelial lining the tubules, intratubular multinucleated giant cells and vacuolar degeneration of Leydig cells. The above mentioned changes were in harmony with the significant decrease in serum testosterone level of the present study. In this respect no available literature could be traced however, the condition could be attributed to pituitary gonadotropins insufficiency resulting from interference with release of GnRH from hypothalamus [41]. Whereas, tissue cyst of bradyzoites was detected in the examined brain tissue, therefore, hypothalamus-pituitary-gonadal reproductive axis was impaired.

It could be concluded that toxoplasma infection induced testicular dysfunction as indicated by hormonal and pathological changes and had adverse effect on the vital organs. Also, LAT is highly sensitive technique for early serodiagnosis of toxoplasma infection.

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