Detection of *Toxoplasma gondii* infection in domestic rabbits by using multiple techniques

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Abstract

Fifty local breed rabbits were purchased from commercial markets in Mosul city for *Toxoplasma gondii* detection during the period from April 2007 to October 2007, using five techniques. Of these, impression smear made from different body organs were positive in 82% of the tested samples. Trypsin digestion technique was positive in 68% of the samples, while pepsin digestion in 82% of them. Serological techniques (latex agglutination test and modified latex agglutination test) were positive in 86% of the tested samples, with IgG titer of 1/64. Biological assay in mice was performed by intraperitoneal inoculation of 0.1 ml digested organs suspension, (lungs, heart, liver, spleen, kidney, muscles, uterus, testes and brain). Brain tissue shows the highest rate of tissue cysts detection among all other organs examined of scarified mice post one month of inoculation.

Keywords: Toxoplasmosis; Domestic rabbits; Latex agglutination test.
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الكشف عن خمج الأرانب الأليفة بمقوسات كوندي

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الخلاصة

استخدمت في هذه الدراسة خمسون من الارانب المحلية تم شراؤها من الاسواق في في مدينة الموصل للكشف عن الاصابة بمقوسات كوندي خلال الفترة من نيسان ٢٠٠٧ الى تشرين الاول ٢٠٠٧، استخدمت خمسة تقنيات لهذا الغرض وهي تقنية الطبعة النسيجية التي سجلت نسبة مئوية موجبة وصلت الى ٨٢% والهضم بالتربسين اعطت نسبة موجبة هي ٦٨% بينما بلغت ٨٢% عند استخدام الهضم بالتريسين. الطرق السيرولوجية المستخدمة اختبار تلازن اللاتكس واختبار التلازن المحور حيث بلغت نسبة الايجابية فيهما ٨٦% وسجل المعيار ١/ ٦٤ اعلى نسبة تواجد للضد IgG. تم التاكد من الاصابة بايولوجيا بحقن الفئران في الخلب ٠,١ عالق الاعضاء التي تم هضمها بالببسين (الرئة الكبد القلب الطحال الكليتين العضلات الرحم والخصيتين وكذلك الدماغ). اظهر نسيج الدماغ اعلى تواجد للاكياس النسيجية مقارنة بالاعضاء الاخرى بعد شهر واحد من حقن الفئران.

Introduction

Infection by *Toxoplasma gondii* is widely prevalent in humans and many species of warmblooded animals, including domestic rabbits (1,2). Splendore (3) was the first who described toxoplasmosis in domestic rabbits (*Oryctolagus cuniculus*) in Brazil, since then clinical cases of this disease among rabbits have been reported by many authors in various countries such as England (4) Argentina (5), Italy (6), Scandinavian countries (7,8) and the U.S.A (9). The last author reported fatal toxoplasmosis in three domestic rabbits in USA and the most striking lesions in all three rabbits was necrotic foci in the spleen and liver associated with massive presence of multiplying *Toxoplasma gondii* tachyzoites. The affected rabbits with toxoplasmosis showing different clinical findings include

loss of appetite, fever, anorexia, lethargia, nasolacrimal discharge and respiratory, disorders, tremors, uncoordinated gait, voluminous, hair coat, and diarrhea. The pathological lesions of toxoplasmosis were mainly observed in the liver and spleen (10).

In general, severe acute toxoplasmosis many animals died and most outbreaks occurred in winter months (January and February) and few in summer months (June and July), on the other hand, acute systemic toxoplasmosis is seen especially in young and immunesuppressed animals (1,11).

The positive serological response of rabbits to *Toxoplasma* antigen is variable from 2.4-8.3% in China, (12,13) to 53% in Germany (14) and 48.4-57.9% in the Czech republic (15). The role of domestic rabbit in epidemiology of toxoplasmosis in humans has not been established in detail, but is probably important although some authors treat this role marginally (1) others place the rabbit among the animal species posing a major source of infection from man (16,17). Ishikawa *et al.* (18) described the case of cervical toxoplasmosis transmitted from rabbit to man. (16) found very high liters of anti-*Toxoplasma* antibodies in rabbit hunters. Sroka *et al.* (19) also found positive *Toxoplasma* in workers in rabbit farms, therefore suggested that the rabbits should be considered as a potential source of *Toxoplasma* infection among agricultural workers. No studies on *T. gondii* in rabbits are reported in Iraq, therefore the aim of this study was to determine the natural infection with *Toxoplasnni gondii* in domestic rabbits by using different diagnostic techniques.

Materials and methods

Animals

Fifty local breed rabbits were purchased from commercial markets in Mosul city (Iraq) during the period from April 2007 to October 2007. They were 25 females and 25 males, weighted 1000-2500 grams, and were put in cages (1/2 m x 1 m) until scarifying..

Diagnosistic techniques

Toxoplasma *gondii* was diagnosed by; Impression smears: These were made from various body organs (brain, lungs, heart, liver, spleen, kidney, muscles, uterus and testes) for detection of tissue (20). Trypsin digestion: Tested organs were digested by trypsin as described by (21). Pepsin digestion: Tested organs were digested by pepsin as described by (22). Serological tests: Blood samples (5 ml) were collected from jugular vein of individual animal, and stored under 4°C temperature over night. Sera were isolated by centrifugation of blood samples at a rate of 2500 rpm for 10-15 minutes. Serum samples were tested for reactive animals using latex agglutination test (Biokit, Spain) and Modified agglutination test (2-ME) (23). Biological assay: white Suis-mice (25 gr.) 4-6 weeks age, negative for *T. gondii* were inoculated intraperitoneally with 0.1 ml suspension of digested organs (lungs, heart, liver spleen, kidneys, muscles, uterus, testes and brain) for confirmation of *T.gondii* infection of tested rabbits.

Results

Results of different techniques used for detection of *T. gondii* are presented in Table (1). From this table it is evident that serological examinations (Latex agglutination test) were positive in 86% of the tested rabbits. To a lower extent 82% were the results obtained by using both pepsin digestion and impression smears of the examined rabbits. The lowest result 68% was obtained by using trypsin digestion technique.

The intensity of tissue cysts in different organs of tested rabbits using impression smears are shown in Table (2), which reveled a wide variation of tissue cysts distribution in different organs noticed in examined rabbits, being highest in brain (> 1000) in which severe infection by *T.gondii* tissue cysts was detected (Figure 1). In the second order were heart, lungs, liver, and spleen, where moderate (500-1000) tissue cysts were observed. In the third order kidneys, testes and uterus with a relatively moderate presence of tissue cysts (100-500). Less than 100 tissue cysts were observed in muscle tissues.

Table 1: Percentages of Rabbits infected with *Toxoplasma gondii* by using different diagnostic techniques.

Technique	No. of positive rabbits	%
Impression smears	41	82
Trypsin digestion	34	68
Pepsin digestion	41	82
Serodiagnosis	43	86

Table 2: Scores of *Toxoplasma gondii* tissue cysts detected in different organs using impression smear method.

Name of organ	Score of tissue cyst detected
Brain	+ + + + *
Heart	+ +
Lungs	+ + +
Liver	+ + +
Spleen	+ + +
Kidneys	+ +
Testes	+ +
Uterus	+ +
Muscles	+

* (+ + + +)>1000, (+ + +) 500-1000, (++) 100-500, (+) < 100.

The positive male and female rabbits to *Toxoplasma gondii* are represented in Table (3). It is clear that females were infected more than males with *T. gondii* with a percentage of 92% for females versus 72% for males and with a ratio of 0.56 for females and 0.43 for males.

Table 3: The percentage of infection with *T. gondii* according to sex by impression smears.

Sex of animals	No. of the examined rabbits	No. of positive rabbits	%	Ratio
Males	25	18	72	M/T=0.43**
Females	25	23	92	F/T=0.56
Total	50	41	82	

** M=male, F= female, T= total.

Serological examination

Forty three rabbits showed positive results for *T. gondii using* Latex agglutination and modified agglutination test (2-ME) tests. Titers were occurred between 1:4 to l:512. Deccendically 23.25% of test serum samples were occurred with titer of 1:64. In the second range 16.27% of the samples were noticed in the titers 1:128 and 1:256. Three titers 1:4, 1:16 and 1:512 had a percentage of 11.6%. Titers of 1:32 and 1:8 showed a percentage of 4.65% and 2.32% (Figure 2).

Biological assay

Typical tissue cysts were found in the brain of the inoculated mice with suspension of brain and digested organs (brain, heart, lungs, liver, spleen, kidney, muscles, uterus and testes). Others organs found to be infected with tissue cyst but to a lesser extent than brain (Figure3).

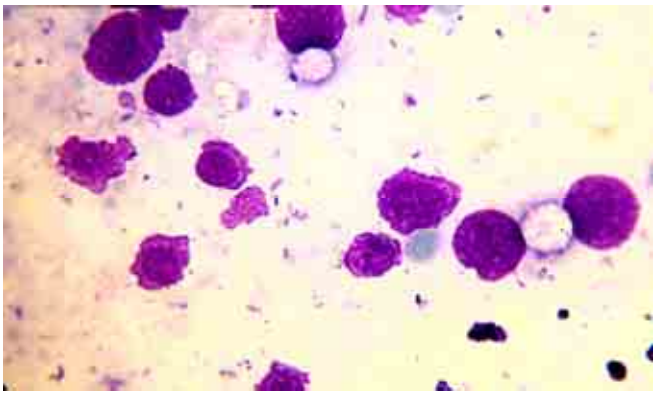

Figure 1: Cysts of *T.gondii* in the brain of the natural infected rabbits.

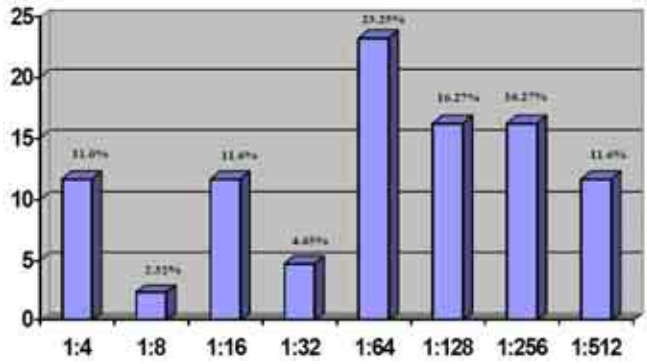

Figure (2): The antibody titers and the percentage of infection with *T.gondii* in the infected rabbits.

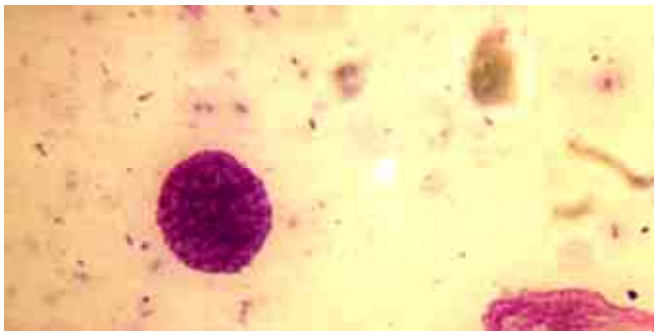

Figure (3): Cysts of *T.gondii* appeared in brain of mice inoculated with suspension of digested organs of rabbits.

Discussion

Diagnosis of toxoplasmosis in rabbit in this study was depended upon organs impression smear, tissue trypsin digestion, pepsin digestion, serological test and mice bioassay.

The serological latex agglutination test was useful in exploring 86% positive exposing rabbits to *Toxoplasma gondii* infection through either contaminated feed or water with parasite oocysts, or by maternally acquired infection (2,9,19). In Ninevah governorate (north of Iraq) with more rainy seasons, a location that may provide a suitable environmental conditions for *Toxoplasma* infection, a case which was reported in aborted women (24) and aborted ewes (25) in Ninevah governorate through the recent years.

These results revealed that there was a difference in the ratio of seropositivity in males versus females, this is inconsistent with (26) who reported that there was no effect of age, sex and season on the prevalence of toxoplasmosis.

The positive modified latex agglutination test, means that IgG antibodies superimpose on IgM antibodies and that the infection was a chronic one. This suggestion could be traced by the purchased rabbit, show no symptoms of

infection, and so may acquired their infection from their infected does, which pass their IgG antibodies through placenta and suppressing IgM antibody response in their off springs (27).

The moderate titers of antibodies (up to 512) of the tested rabbits may also explain the chronicity of infection.

Our serological results of 86% positivity were higher than those reported in China (2.4-3.8%) (12,13), in Germany (53%) (14) and in the Czeck republic 48.4-57.9% (15). Impression smears taken from different tissue organs, revealed positive results in brain, lungs, heart, liver, spleen, kidneys, muscles, uterus and testes. The presences of *T.gondii* cysts in these organs also reported by other investigators (9,10,28,29). It is not surprising to find parasitemia to occur spontaneously in rabbits chronically infected with certain strains of *Toxoplasma* and persist in the presence of high antibody triers (30).

The presence of *Toxoplasma* cysts in brain tissue reported here was in accordance with (31) who was the first one to describe its presence in rabbits brain tissue and followed by (14). Anyhow, (32) reported no *Toxoplasma* cysts in histological examination of brain tissue from 51 wild rabbits, while the work of (19), confirms the possibility of spontaneous development of *T. gondii* in rabbits brain.

The absence of gross lesions in necropsed rabbits give an additional confirmation of chronicity of infection (2).

The presence of bradyzoites in different tissue impression smears especially in brain which may be of focal brain lesions of chronic infection (FBL), indeed supports the serological diagnosis (33) and rabbits in the same time is regarded as a good model of toxoplasmosis of the CNS (34).

The presence for example of bradyzoites in brain was not accompanied by posterior paralysis and death of these rabbits. This may be due to the inadvancement of the case to that stage, and even fatal toxoplamosis could occur even without symptoms in rabbits (19). The bioassay positive results in mice through their inoculation with 1 ml suspension of pooled digested organs and the recovery of bradyzoites after 1 month necropsy in different organs, confirms the infectivity of rabbits by *T. gondii*. The importance of these findings is that these animals could be a major source of infection to human (19,15) especially in those suffering from acquired immunodeficiency syndrome (AIDS) (34), although there is lack of controlled epidemiological studies regarding the degree of correlation between the prevalence of toxoplasmosis in rabbits and in humans in contact with these animals (35).

Conclusion

Toxoplasma gondii was isolated from local rabbit bread in Mosul city-Iraq. Diagnosis was accomplished by different diagnostic methods. Confirmation of results was ascertained by mice bioassay.

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