Protection Against Toxoplasmosis in Swiss Albino Mice Immunized with Attenuated *Toxoplasma Gondii*

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Abstract

The present study aimed at investigation on developing the immune response against Toxoplasmosis in Swiss Albino mice of the species *Mus musculus*, BALB/c strain, which were infected, experimentally, with *Toxoplasma gondii* in order to render the host able to resist any infection with this disease in the future. To achieve this, *Toxoplasma gondii* tissue cysts, collected from placenta of aborted women, they attenuated by irradiation with two absorbed doses of X-Ray irradiation 0.4 and 0.8 KGy.

Mice were injected, intraperitoneally with tissue cysts at a rate of 100 cysts/mice single or double doses. In addition, groups of mice were injected with non-irradiated tissue cysts, as a positive control groups. The criteria used in the present study was humoral immunity, represented by IgM using Enzyme–Linked Immunosorbent Assay (ELISA IgM) to demonstrate the differentiated in IgM level between mice inoculated with X-Ray irradiated tissue cysts and positive control groups, which infected with non-irradiated tissue cysts.

The result shown that the immunization with X-Ray irradiated *Toxoplasma gondii* tissue cysts could partially protect the mice from *Toxoplasma gondii* infection, and produce specific IgM antibody.

Introduction

Toxoplasmosis is widespread in human beings and many other warm-blooded animals (Dubey & Beattie, 1988). *Toxoplasma gondii* is an obligate intracellular parasite. The three means by which it is mainly spread are transplacental transmission, ingestion of infective tissue, and ingestion of food or water contaminated with infective feces (Dubey, 1994).

Cats, including wild felidae, are the only definitive host. Cats excrete *Toxoplasma gondii* oocysts in their feces. Excreted oocysts are non-sporulated and, therefore are, non-infective. After defecation, sporulation requires 1 to 5 days and is dependent on environmental conditions. Oocysts can survive for several months to a year during unfavorable environmental conditions (Dubey & Beattie, 1988). Infection consists of a transient acute phase caused by proliferative tachyzoites followed by the formation of dormant tissue cysts containing bradyzoites. The disease can be serious if acquired congenitally (Wong & Remington, 1994) or in immunosuppressed individuals, particularly patients with Acquired Immunodeficiency

Syndrome (AIDS) (Luft *et al*., 1993).

Toxoplasmosis, in an immunocompetent host, leads to the induction of a life- long protective immunity against illness due to re-infection. These privileged protective antigens are candidates for the development of a vaccine strategy (Alexander *et al*., 1993).

Vaccination attempts with live, attenuated, killed or lysed parasites, as well as, different antigenic fractions of the parasite, have been conducted with varying success (Araujo, 1994). Vaccines with live organisms are currently in use (Buxton, 1993; Dubey *et al*., 1994). The development of vaccines in the future will not only have to take into account all life-cycle stages that need to be targeted but will also have to consider which immune responses need to be generated and in which tissue sites (Elsaid *et al*., 1999). As a consequence, there has been an interest in defining how the immune system controls these organisms, with the hope that this would lead to the design of effective vaccines. In the last 30 years, there has been significant progress in defining the role of different immune cell types in resistance or susceptibility to many of these parasites (Donald & Roos, 1993; Soldati & Boothroyd, 1993). Humoral immunity was studied because administration of *Toxoplasma* antigens enhanced survival after parenteral *Toxoplasma* challenge in the presence of specific *Toxoplasma* antibody (Krahenbuhl *et al*., 1972; Sharma *et al*., 1984).

Attenuated *Toxoplasma gondii* with different source has been used in number of studies (Duarte *et al*., 2002). On the other hand irradiated *Toxoplasma gondii* with cobalt 60 irradiation has been used to vaccinate cats and mice against *Toxoplasma* infection (Omata *et al*., 1996). Killing of *Toxoplasma gondii* oocysts (Dubey *et al*., 1996) and tissue cysts (Dubey and Thayer, 1994) by 137 Cs irradiation and protective immunity induced by vaccination with irradiated *Toxoplasma gondii* (Lin *et al*., 1999).

The purpose of the present study was to administrate X-ray irradiated *Toxoplasma* tissue cysts, which activates IgM antibody to inhibit or kill *Toxoplasma gondii*, contribute to protection against any infection in the future.

Materials and methods

Mice: Six - eight weeks old female were divided in ten groups of thirty animals.

Parasites: Tissue cysts were isolated from the placenta tissue of aborted women (Sharma & Dubey, 1981; Dubey *et al*., 1986.

Irradiation: X-Ray source was used to irradiation *Toxoplasma* tissue cysts (Dubey *et al*., 1986 and Song *et al*., 1993) to absorbed doses of 0.4 and 0.8 KGy (Dubey *et al*., 1998).

Experiment Design: Animals were injected intraperitoneally (Derouin *et al*., 1987; Freyre, 1995 and Freyre *et al*., 1999) with one - two inoculations at 15 days intervals using non-irradiated and irradiated *Toxoplasma* tissue cysts to absorbed doses of 0.4 and 0.8 KGy with X-ray irradiation source(Omata *et al*., 1996) as following:

Group 1: 30 mice were inoculated only once with irradiated tissue cysts at 0.4 KGy.

Group 2: 30 mice were inoculated twice with irradiated tissue cysts at 0.4 KGy, at 15 days intervals.

Group 3: 30 mice were inoculated firstly with non-irradiated tissue cysts, and after 15 days inoculated with irradiated tissue cysts at 0.4 KGy.

Group 4: 30 mice were inoculated with irradiated tissue cysts at 0.4 KGy, and after 15 days infected with non-irradiated tissue cysts as a challenge dose.

Group 5: 30 mice were inoculated only once with irradiated tissue cysts at 0.8 KGy.

Group 6: 30 mice were inoculated twice with irradiated tissue cysts at 0.8 KGy, at 15 days intervals.

Group 7: 30 mice were inoculated firstly with non-irradiated tissue cysts, and after 15 days inoculated with irradiated tissue cysts at 0.8 KGy.

Group 8: 30 mice were inoculated with irradiated tissue cysts at 0.8 KGy, and after 15 days infected with non-irradiated tissue cysts as a challenge dose.

Group 9: 30 mice were infected only once with non-irradiated tissue cysts (positive control group 1).

Group 10: 30 mice were infected twice with non-irradiated tissue cysts at 15 days intervals (positive control group 2).

Immune Response in Mice: All animals surviving were bled 3, 15 and 30 days after the single injection and 3, 15 and 30 days after challenge injection (Omata *et al.*, 1996) and IgM antibody was determined by ELISA (Acebes *et al.*, 1994).

Statistical Analysis: The survival rate was evaluated by the Complete Randomized Design (CRD). The means of absorbance of ELISA were examined using the Duncan's Multiple Range Test (Duncan, 1955), at 0.05% level of probability.

Results and Discussion

Animal Surviving:

All non-immunized (non-inoculated with irradiated tissue cysts), 30 mice (100%) infected with non-irradiated *Toxoplasma* tissue cysts single dose (positive control group 1), and 30 mice (100%) infected with non-irradiated *Toxoplasma* tissue cysts double dose (positive control group 2) become ill starting 2-4 days. In this study, 14 (46.7%) out of 30 mice of positive control group 1 died starting 20-28 days post infection. Whereas, 21 mice (70%) of positive control group 2 died starting 8-16 days post challenge. All immunized 120 mice (100%), inoculated with X-ray irradiated *Toxoplasma* tissue cysts at 0.4 and 0.8 KGy single or double dose were survived. Experimentally group which infected with non-irradiated *Toxoplasma* tissue cysts first dose, and with irradiated tissue cysts at 0.4 KGy second dose only 20 out of 30 mice (66.7%) were survived. Whereas, 21 mice (70%) injected with non-irradiated tissue cysts first dose, and with irradiated tissue cysts at 0.8 KGy second doses were survived.

Finally, all 60 mice (100%) immunized with irradiated *Toxoplasma* tissue cysts at 0.4 or 0.8 KGy, and after 15 days challenged with non-irradiated tissue cysts were survived.

It has been explained that X-ray causes many changes in the parasite which is ranged between decreasing its ability to cause infection and retaining its antigen effect to killing this parasite by using high doses of irradiation (Dubey & Thayer, 1994; Dubey *et al.*, 1996; Omata *et al.*, 1996; Dubey *et al.*, 1998 and Lin *et al.*, 1999).

Antibody Response:

When ELISA test was used, a significant increase was noticed in IgM level for the injected mice with one dose (figure 1 D1 - D2, figure 2), two doses (figure 1 E2 - E3, figure 2) of non-irradiated *Toxoplasma* tissue cysts (positive control groups 1 & 2). It is shown in figure 1 (G1 - A2), that the IgM level was higher at day 15 post injection and then started to decline. On the other hand, injection with two doses of non-irradiated *Toxoplasma* tissue cysts led to a significant increase in IgM level since the third day after the second injection (figure 1 E2 - B3) and this increase continued till day 15 and then declined (figure 1 C3 - E3, figure 2).

It has been shown that giving a second dose of non-irradiated *Toxoplasma* tissue cysts caused an increase in the IgM level, as well as, its availability in the body (Buxton & Innes, 1995; Lunden, 1995 and Wastling *et al.*, 1995). Its appearance as a high level at day 3 post challenged and remained in the peak at day 15 post challenged. On the other words, it

remained in the high level till day 30 post challenged which indicated to the activation of humoral immune response for the mice injected with two doses of non-irradiated *Toxoplasma* tissue cysts (Barriga, 1981; Remington & McLeod, 1981; Dubey, 1986; Buxton *et al.*, 1989; Denkers & Gazzinelli, 1998 and Dunn *et al.*, 1999).

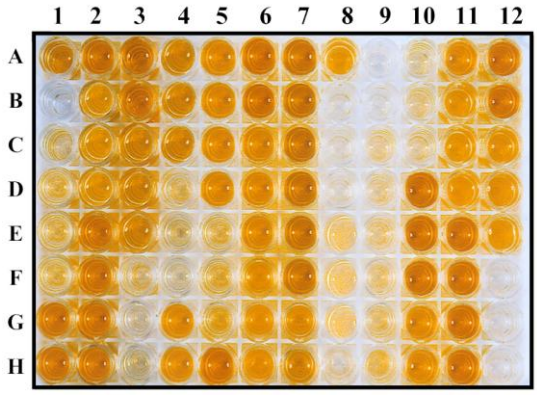

Fig.1: Photo of standardization measurement plate which used in ELISA to determine IgM antibody titters in positive control groups (1&2) and in mice inoculated with X-ray irradiated *Toxoplasma* tissue cysts at 0.4 KGy .

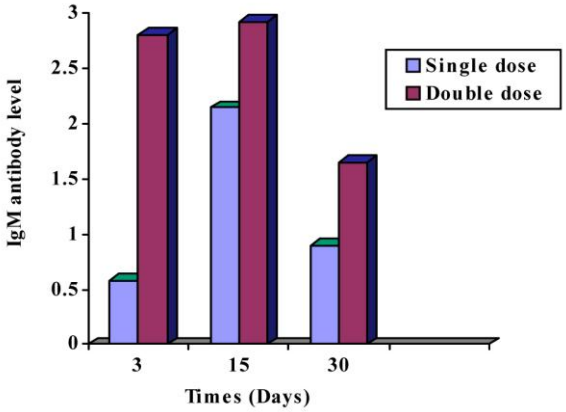

Fig. 2: IgM antibody level after different times (days) in mice inoculated with single and double dose of non-irradiated *Toxoplasma* tissue cysts (positive control 1&2) by using ELISA

Mice injection with X-ray irradiated *Toxoplasma* tissue cysts at 0.4 and 0.8 KGy revealed a significant reduction in IgM level for all animals under investigation at the days 3, 15 and 30 after injection compared with positive control groups 1 and 2 (figure 3, figure 4). This reduction was inversely proportional with the increase of irradiation dose. The above

results were confirmed by other investigators (Casaratt, 1968; Assmer *et al.*, 1999 and Agwan, 2005).

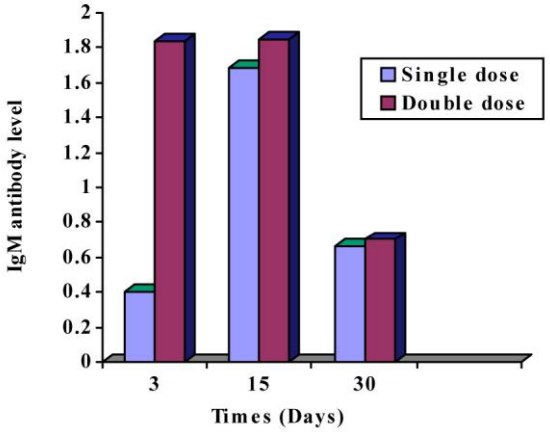

Fig. 3: IgM antibody level after different times (days) in mice inoculated with single and double dose of X- ray irradiated *Toxoplasma* tissue cysts at 0.4 KGy by using ELISA

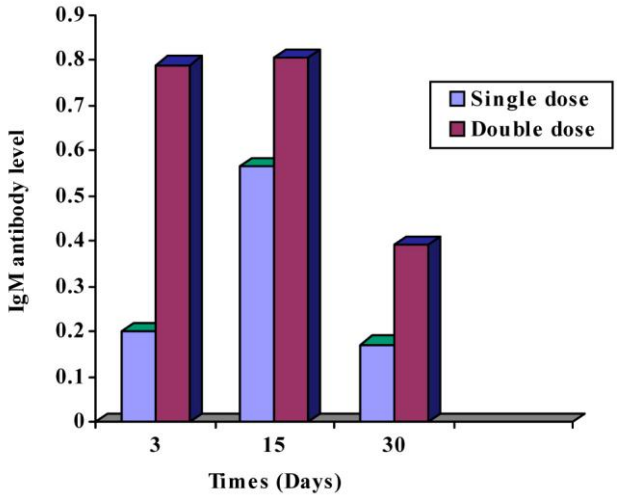

Fig. 4: IgM antibody level after different times (days) in mice inoculated with single and double dose of X- ray irradiated *Toxoplasma* tissue cysts at 0.8 KGy by using ELISA

Mice injection with one dose of X-ray irradiated *Toxoplasma* tissue cysts at 0.4 KGy, led to a significant decrease in IgM level (figure 1 F3 - F4) compared with positive control group 1 (which was received one dose of non-irradiated tissue cysts) (figure 1 D1 - D2). Also, it noticed in figure 1 (A4 - C4) that the IgM level was higher at day 15 after injection then started to decline. Injection of mice by two doses of X-ray irradiated

Toxoplasma tissue cysts at 0.4 KGy (figure 1 G4 - G5, figure 3) enhanced a little increase of IgM level since day 3 after second injection (figure 1 G4 - A5) compared with its analogous for the one dose (figure 1 F3 - F4). IgM level remained low compared with positive control group 2 (which was received two doses of non-irradiated tissue cysts). Generally, the IgM level was higher at day 15 after second injection (figure1 B5 - D5) and then started to decline at day 30 (figure 1 E5 - G5). The above results were confirmed by other investigators (Remington *et al.*, 1995 and Agwan, 2005).

The use of X-ray irradiated tissue cysts at 0.8 KGy one dose and two doses revealed a significant decrease in IgM level compared with their analogous at 0.4 KGy till day 15 after injection. This decrease stayed to be significantly at day 30 after injection (figure 3, figure 4). This can be confirmed by comparing figure 5 (F3 - F4) with its analogous in figure 1 (F3 - F4), for the injection with one dose of X-ray irradiated tissue cysts. However, the injection with two doses showed no differences compared to one dose, where the same decrease in IgM level was noticed. This can be detected when comparing the figure 5 (G4 - G5) with the figure 1 (G4 - G5). The above result was confirmed by Dubey *et al.* (1998).

The immune response was lower for the mice injected with X-ray irradiated tissue cysts at 0.4 and 0.8 KGy (one or two doses). It looks as, there is a reversible relationship between the intensity of immune response and the irradiation dose. It can be explained that irradiation effect on parasite antigen on one way or another for inducing humoral immune response which led to a decrease in its ability. This result was previously noticed by Agwan (2005) which mentioned that different physical treatments (ultrasonic waves, irradiation with laser or microwaves, ect) which affected in parasite antigens and weakened its ability to induce humeral immune response.

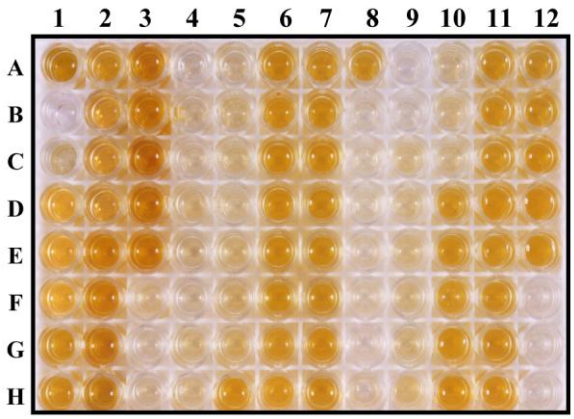

Fig. 5: Photo of standardization measurement plate which used in ELISA to determine IgM antibody titters in positive control groups (1&2) and in mice inoculated with X-ray irradiated *Toxoplasma* tissue cysts at 0.8 KGy

Mice injection with two doses of *Toxoplasma* tissue cysts (the first dose non-irradiated and the second one was irradiated with X-ray at 0.4 KGy) revealed a significant decrease in IgM level at day 3 after the second injection (figure 1 H5 - H6, figure 6) compared with the positive control group 2 (which was received two doses of non-irradiated tissue cysts). This decrease was continued till day 30 after second injection. When comparing the IgM level with that of its analogous at previous experiment (mice inoculated with two doses of X-ray irradiated tissue cysts at 0.4 KGy), it can be noticed that IgM level was increased at the second case compared with its analogous at the first case. IgM level reached its maximum peak at the first 3 days after second injection, then started to be declined. Figure 1, demonstrates the above results, where the IgM level appeared to be higher at the first 3 days (figure 1 H - B6) compared with it's analogous at day 15 after second injection (figure 1 C6-E6) which was higher than it's analogous at day 30 after second injection (figure 1 F6- H6).

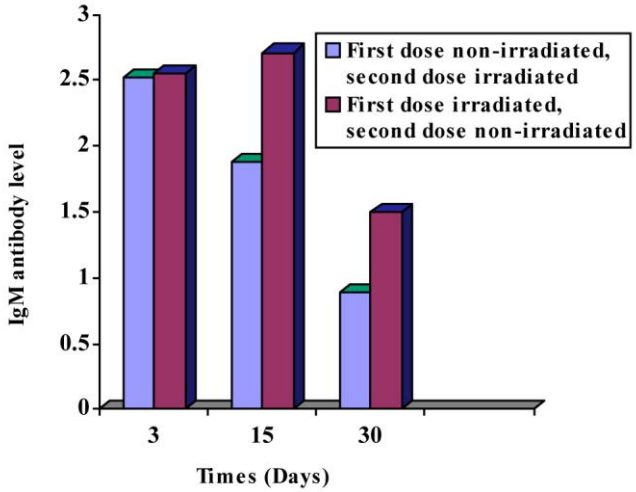

Fig. 6: IgM antibody level after different times (days) in mice inoculated with different doses of *Toxoplasma* tissue cysts (first dose non-irradiated, second dose irradiated and first dose irradiated, second dose non-irradiated) irradiated with X- ray at 0.4 KGy by using ELISA

Mice injection with two doses of *Toxoplasma* tissue cysts (the first dose was non-irradiated and the second dose was irradiated with X-ray at 0.8 KGy) led to decrease in IgM level which was higher than that of 0.4 KGy, since the third day of the second injection. This decrease was significant since day 15 after the second injection. It looks as, inversely relationship between IgM level and irradiated dose (figure 7). The above observation can be confirmed when comparing figure 5 (H5 - H6) with it's analogous in figure 1 (H5 - H6). This result was previously confirmed by Agwan (2005).

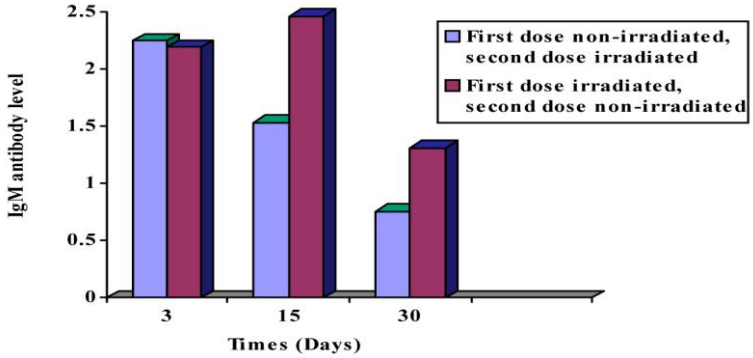

Fig. 7: IgM antibody level after different times (days) in mice inoculated with different doses of *Toxoplasma* tissue cysts (first dose non-irradiated, second dose irradiated and first dose irradiated, second dose non-irradiated) irradiated with X- ray at 0.8 KGy by using ELISA

Similar results were obtained when mice received two doses of *Toxoplasma* tissue cysts (the first was irradiated with X-ray at 0.4 KGy and the second one was non-irradiated) (figure 6). A significant reduction was noticed in IgM level compared with its analogous positive control group 2 which was received two doses of non-irradiated tissue cysts. The IgM level reached its peak at day 15 after second injection. Generally, the IgM level was higher than its analogous when using two doses of X-ray irradiated tissue cysts at 0.4 KGy, and two doses of *Toxoplasma* tissue cysts (the first was non –irradiated and the second one was irradiated with X-ray at 0.4 KGy). This was obvious on figure 1 (A7–A8), since this appeared at day 15 after the second injection (D7–F7) which was approximately similar to its analogous at day 3 after the second injection (A7–C7) except a little difference which could not be seen in the figure.

A significant reduction was observed when mice were injected with two doses of tissue cysts (the first one was irradiated with X-ray at 0.8 KGy and the second one was non-irradiated) compared with a positive control group 2 which was received two doses of non-irradiated tissue cysts, since day 3 after the second injection. On the other hand, the reduction in IgM level at 0.8 KGy was significantly higher than that of 0.4 KGy since day 3 after the second injection, but it was not significant at day 30 after the second injection. When comparing the foregoing results with these analogous for mice injected with two doses of X-ray irradiated tissue cysts at 0.8 KGy, one can noticed that the IgM level was higher for the three periods (3, 15 and 30 days after the second injection).

A significant reduction on IgM level was noticed only at day 3 after the second injection compared with its analogous which was received two doses of tissue cysts; the first one was non-irradiated and the second was irradiated with X-ray at 0.8 KGy. However, the IgM level was higher than that of mice received two doses of tissue cysts; the first one was irradiated at 0.8 KGy and the second non-irradiated compared with its analogous for the injected mice with two doses; first one was non-irradiated and the second one was irradiated with 0.8 KGy, since the day 15 after the second injection.

It has been known that the challenging dose usually increase the immune response for the host, this result was previously noticed by (Omata *et al.* 1996), but the present results indicated that it is preferably that the challenging dose in non-irradiated parasites to preserve parasite antigen in a healthy state and non-effected by any physical treatment.

A positive results were obtained when ELISA-IgM used were applied through the injection of mice by X-ray irradiated *Toxoplasma* tissue cysts at 0.4 and 0.8 KGy although the mice stayed a life. This can be reasoned

that bradyzoites inside the tissue cysts lost their abilities for reproduction at the same time, they retain their metabolic functions. These results are in accordance with that reported by (Hiramoto *et al.*, 2002) when they observed that *Toxoplasma gondii* RH strain failed to reproduce when exposed to γ-ray at 200 Gy invitro and invivo.

Conclusions

1. IgM level was lower in mice infected with irradiated tissue cysts, with no consideration to irradiation dose, compared with those infected with non-irradiated tissue cysts.
2. IgM level was higher in mice infected with tissue cysts irradiated with X-ray at 0.4 KGy compared with those infected with tissue cysts irradiated with X-ray at 0.8 KGy.
3. Irradiation with 0.4 and 0.8 KGy of X-ray resulted in attenuating tissue cysts as to evoke immunological response without causing infection.
4. IgM level was higher in mice infected first with irradiated and then with non-irradiated tissue cysts than those infected first with non-irradiated and then with irradiated tissue cysts.

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الحماية ضد داء المقوسات في الفئران البيض الممنعة بطفيلي *Toxoplasma Gondii* المضعفة

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الخلاصة

يهدف البحث الحالي الى دراسة تطور الاستجابة المناعية ضد داء المقوسات الكونيدية Toxoplasmosis في الفئران البيض السويسرية، نوع *Mus musculus*، وسلالة BALB/c، حيث اصيبت تجريبيا بطفيلي المقوسات الكونيدية *Toxoplasma gondii* لغرض تحفيز العائل لمقاومة اي اصابة بالمرض مستقبلا. وللوصول الى هذا الهدف فقد تم عزل الاكياس النسجية لطفيلي المقوسات الكونيدية من مشائم نساء مجهضات وضعفت الاكياس باستخدام جرعتين من الاشعة السينية بمقدار ٠,٤ و٠,٨ كيلوكري.

حقنت الفئران تحت غشاء الجنب (الخلب) مرة واحدة او مرتين بالاكياس النسجية المضعفة بما يعادل ١٠٠ كيس/فارة. كما حقنت مجموعة من الفئران باكياس غير مشعة لاستخدامها كمجموعة ضابطة موجبة. استخدم معيار دراسة الاستجابة المناعية الخلطية المتمثلة بالضد IgM باستخدام اختبار ارتباط انزيم الادمصاص المناعي (ELISA IgM) لاظهار الاختلافات في مستوى الضد IgM بين الفئران المحقونة بالاكياس النسجية للمقوسات الكونيدية المشعة وتلك المحقونة بالاكياس النسجية غير المشعة.

اظهرت النتائج امكانية استحداث المناعة عن طريق استخدام الاكياس النسجية المشعة للمقوسات الكونيدية والتي تحمي الفئران جزئيا من الاصابة المستقبلية عن طريق انتاج اجسام مضادة نوع IgM متخصصة لهذا الطفيلي.