Attempts of immunization of mice against experimental infection by *Toxocara cati*

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Abstract:
studied the impact of muscular immunization to white mice (Balb / c) with serum and cell lymphoma of the spleen immunized mice with different antigens (mature eggs, live larvae, liquid hatch eggs, extract adult worm) of *Toxocara canis* to resist infection by the larvae of *Toxocara cati* in the case of hetero infection and against the larvae of *Toxocara canis* in similar infection case. The results showed that immunization with serum effect in reducing the rate of infection at the injection all different antigens (mature eggs, live larvae, liquid hatch eggs, extract adult worm) reached as prevention ratio against *T. cati* 65.64%, 41.60%, 60.68%, 73.28% respectively, and the proportion of total prevention against *T. canis* with same antigens 71.90%, 51.83 %, 53.84%, 71.23% respectively. But when immunization lymphoma cell of the spleen mice immunized proportion to protect against *T. cati* it has reached 64.96%, 51.36%, 48.63%, 67.68%, respectively against antigens (mature eggs, live larvae, liquid hatch eggs, extract adult worm), and amounted to prevention ratio against *T. canis* 59.24%, 53.75%, 39.01%, 69.65% respectively in same infection.

Key words: immunization, Toxocariasis.

Introduction:

The worms *Toxocara canis* and *Toxocara cati* are common worms between human, cats and dogs (1) and are widespread on a global level and it formed a dangerous on human humidity spicily where infect children between the ages of 4-10 years (2).

Human and other animals become infected when taking mature eggs, causing the Visceral Larva Migrants (VLM) where the larvae migrate to the eye, brain, liver and lung (3). Bouree and Guillot (3) said that the arrival to retina and the brain is one of the most serious complications in the case of human infection, and also demonstrated that increasing amounts of antibodies type IgG was after three weeks from experimentally cats infection. Gillert and Halliwell (4) showed that the increased production of IL 10 and lack of production in the IFNY in pregnant female dogs.

Received Torina *et al.* on the proportion of protection amounted to 90.15% at the muscles injection second stage larvae L2 of *T. leonine* as immunization dose against similar challenge doses and when peritoneal injected larvae L2 of *T. canis*, the prevention rate of 83.48% against similar challenge dose of immunization doses (5). received Al-Kubaisy (2004) on reduction ratio at 68.02% in the larvae of *T. cati* and 82.99% in the larvae of *T. leonine* when peritoneal immunization by excretory – secretary materials and give a similar challenge doses of the immunization doses (6). While Hosin (1992) received the highest percentage reduction in the preparation of larval *Toxocara cati* when giving dead larvae and excretory – secretary materials of *T. canis* larvae under the skin against the larvae of *T. cati*, which amounted to 75% (7).

Mahmoud (2014) got to 74.04% of the use of antigens extract worms *T. canis* when intramuscular immunization dose against variety challenge dose by the eggs of *T. cati* and 68.76% against similar challenge dose, and received the highest proportion of protection as a result of peritoneal immunization by excretory – secretary materials of the second phase larvae L2 of *T. canis* where the highest protection amounted to 74.74% against variety challenge dose of eggs *T. canis* and 69.71% against the same challenge dose (8).

The aim of this project is to study the possibility of immunity transmission indirectly through serum injection and lymphoid cells immunized and the possibility of providing protection against infection by the larvae of these worms.

Materials and methods:

A. Isolation of worm and preparation of antigens :

1-gathering worms: Bulk been hunting cats and dogs mediated by gunshot or poisoning substance (stryqnine) anatomy was on the ground, and isolated the small intestine and examined its contents, then collected worms from infected animals, diagnosed by (9).

2-lap & extract and eggs: eggs extracted by the way (10).

3-hatched eggs and larvae get: depending on the way Sprent (11) have been hatched eggs.

4-extracting larvae: method is used (12) for it.

5- larvae culturing: method is used by (7) and the media used as antigen of excretory – secretary materials.

6- preparation the antigens of extract worms *T. canis*: attended the antigens by the way (8).

7-serum and cells immunized: depend on the method used by Al-Gumaily (1990) (13).

B-design experience:

Group A: included 48 mouse was given intravenous immunization dose promissory note and in the order below:

(a) 8 mice were given a serum to immunizator mice by mature eggs of *T. canis* intravenous promissory note.

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(b) 8 mice were given a serum to immunizator mice by live larvae of *T. canis* intravenous promissory note.
(c) 8 mice were given a serum to immunizator mice by hatching liquid eggs of *T. canis* intravenous promissory note.
(d) 8 mice were given a serum to immunizator mice by extract worm antigens of *T. canis* intravenous promissory note.
The control group was included 8 mice and were given the serum for mice not immunizator then given to four of them challenge dose orally 1000 egg mature of *T. cati* and the other four were given a challenge dose in 1000 mature egg of *T. canis* after 24 hours of giving serum and after 10 days killed and explained mice and extracted larvae were counted.
Group B: 48 mouse were given immunization doses of *T. canis* in the order above in the first set, except that the doses were immunized mice was spleen cells. After 24-hour were given a half groups challenge dose 1000 mature egg of *T. cati* and the other half were given a challenge dose 1000 mature egg of *T. canis* and after 10 days giving the challenge dose and killed and explained mice larvae were counted.
The control group was included 8 mice given cells of lymphocytes of not immunizator mice and then given to half of them the challenge dose orally 1000 mature eggs of *T. cati* and the other half given 1000 egg mature eggs of *T. canis* .
C-statistical analysis: Use the T- test for the purpose of comparison between the transaction and its subsidiaries under the control of the level of probability ($p < 0.05$).

Results and Discussion:

Results indicate that the injection of serum resulting from immunization mature eggs gave the highest percentage of protection amounted to 71.90% at the serum injection resulting from injection eggs of *T. canis* against similar challenge dose and the proportion of protection amounted to 65.64% against variety challenge dose for *T. cati* and showed Results that the injection of serum significant effect on reducing the incidence (Table 1).
As Table (2) shows the effect of immunizatar serum by live larvae on the percentage of prevention against the same species and variant of the infection, it is noted that the highest percentage protection amounted to 51.83% against a similar challenge dose and the proportion of 41.60% against a variant challenge dose by eggs of *T. cati*.
When serum resulting from immunized mice injected with liquid hatching eggs of *T. canis* it was observed that the highest percentage protection amounted to 60.68% against variant challenge dose of the eggs of *T. cati* and the proportion of protection amounted to 53.84% against similar challenge dose by the eggs of *T. canis* (Table 3).
And Table (4) shows the effect of serum injection resulting from immunization extract body worms on the percentage of prevention against the similar and variant of the infection and is noted that the highest percentage protection amounted to 71.23% against similar challenge dose and the proportion of protection amounted to 73.28% against variant challenge dose the eggs of *T. cati* .
But when immunization by cell lymphoma of immunized mice spleen by mature eggs of *T. canis* against similar and variant infection was observed that the highest percentage protection amounted to 64.96% against variant challenge dose of *T. cati* and the proportion of protection amounted to 59.24% against similar challenge dose (Table 5).
But when the lymphocyte injection of spleen immunized mice by live larvae of *T. canis* we note that the highest percentage protection amounted to 53.75% against a similar challenge dose and ratio stood at 51.36 and protection against different dose challenge of the eggs of worms *T. cati*% (Table 6).
When immunization by lymphoma cell of the spleen immunized mice with liquid hatching eggs of *T. canis* ,we note that the highest percentage protection amounted to 48.63% against variant challenge dose of the eggs of *T. cati* and the proportion of protection amounted to 39.01% against similar challenge dose by the eggs of *T. canis* (Table 7).
But when immunization by lymphocyte cells of spleen immunized mice by extract worms *T. canis*, reaching the highest protection 69.65% against similar challenge dose while prevention was 67.68% percent against variant challenge dose the eggs of *T. cati* (Table 8).
The results above showed that injection of serum caused by the injection of mice of different antigens of *T. canis* for the prevention of similar and variant infection by *T. cati* have ability to reduce the rate of infection of these worms was the best effect is serum resulting injection for injection antigens extract worms *T. canis*, which amounted to 71.23 %% and 73.28 against similar challenge dose and adversity in a row, followed by the mature eggs of worms *T. canis*, which amounted to 71.90% and 65.64% against dose similar challenge respectively, and this is consistent with the findings of the (7,14), who asserted that the use of different antigens of *Toxocara canis* under the skin to resist infection by *Toxocara cati* and He stressed that these antigens stimulate an immune response in mice also agree with (15), which It found an increase in the amount of type IgG antibodies after three weeks injuring cats.
Torina *et al.* [5] (mature eggs, live larvae, liquid hatch eggs, extract adult worm) (mature eggs, live larvae, liquid hatch eggs, extract adult worm) (mature eggs, live larvae, liquid hatch eggs, extract adult worm) (5) showed that the increase in the production of interleukin-1L-10 and a decrease in the production of interferon IFNY in infected female dogs . And Hosin (9) said that may by the wall of the stomach and intestines and antibodies secreted IgG type role in

providing protection in immunized animals against subsequent challenge dose.

But when using lymphocytes of spleen immunized mice we note that the highest percentage protection amounted to 69.65%, 59.24%, 53.75%, 39.01% when immunization extract body antigens and mature eggs and live larvae and fluid hatching eggs respectively of *T. canis* against similar challenge dose, and reached prevention rate 67.68%, 64.96%, 51.36%, 48.63% respectively against variant challenge dose by the eggs of *T. cati* and this is consistent with the findings of the (7,10) when they use different antigens and by different ways injection as it got 68.18% when peritoneal immunization by liquid hatch eggs and 69.56% when immunization by excretory – secretory materials in peritoneal respectively.

These findings suggest the involvement of both cellular and humoral immunity in negative immune transfer of *T. canis* against infection by *T. canis* and *T. cati* and suggests that the combination of extract and extract - secretary of these worms are similar, which led to obtain the transfer of these immune from immunized mice to cells lymph and serum, which led to stimulate memory to produce immunity against similar and variant infection. This is consistent with (16,17). This refers to the necessarily to do extensive studies in this direction for the possibility of obtaining antigens with high efficiency for the prevention of infection by the larvae of these worms.

Table 1: injection effect of serum immunizater mice by mature eggs of *T. canis* in muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	33	1						56	90	65.64	9.03*
		T. canis	42	2	3					37	84	71.90	8.04*
control		*T. cati*	163	2	3					94	262		18.00
		T. canis	181	5	7			1		105	299		17.44

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05)

Table (2): effect injection of serum immunizater mice by live larvae of *T. canis* in the muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	86	2	1					64	153	41.60	3.5*
		T. canis	73	3	5					63	144	51.83	13.97*
control		*T. cati*	163	2	3					94	262		18.00
		T. canis	181	5	7			1		105	299		17.44

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05)

Table (3): injection effect of serum immunized mice with liquid hatching eggs of *T. canis* in muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	49		1					53	103	60.68	17.67*
		T. canis	72	1	2					63	138	53.84	18.28*
control		*T. cati*	163	2	3					94	262		18.00
		T. canis	181	5	7			1		105	299		17.44

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05).

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Table (4): injection effect of serum immunized mice with extract whole of *T. canis* in muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	17		1					52	70	73.28	3.3*
		T. canis	21		2					63	86	71.23	7.50*
control		*T. cati*	163	2	3					94	262		18.00
		T. canis	181	5	7			1		105	299		17.44

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05).

Table (5): injection effect of lymphoid cells of mice immunized by mature eggs of *T. canis* in muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	53	2	1					47	103	64.96	11.89*
		T. canis	79	2	6					54	141	59.24	12.52*
control		*T. cati*	189	5	3					97	294		22.64
		T. canis	213	7	18		2			106	346		35.78

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05)

Table (6): injection effect of lymphocytes immunized mice by live larvae of *T. canis* in the muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	76	2	2					63	143	51.36	8.97*
		T. canis	95	1	6					58	160	53.75	12.35*
control		*T. cati*	189	5	3					97	294		22.64
		T. canis	213	7	18		2			106	346		35.78

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05)

Table (7): injection effect of lymphocytes immunized mice by fluod hatching eggs of *T. canis* in the muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	97	2						52	151	48.63	20.33*
		T. canis	128	3	6					74	211	39.01	12.50*
control		*T. cati*	189	5	3					97	294		22.64
		T. canis	213	7	18		2			106	346		35.78

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05).

Table (8): injection effect of lymphoid cells immunized mice by whole extract of *T. canis* in the muscle

group	Primary immunized dose	Challenge dose	Stomach & intestine wall	liver	lung	kidney	spleen	brain	heart	muscle	total	Preservation comparative with control %	S.D.±
1	*T. canis*	*T. cati*	53	1	1		1			39	95	67.68	5.73*
		T. canis	51	3	6					45	105	69.65	6.44*
control		*T. cati*	189	5	3					97	294		22.64
		T. canis	213	7	18		2			106	346		35.78

(T-test) * refers to the presence of significant difference at the level of probability (P <0.05).

References:

1-Gortari, C.; Cazou, C. & Hours, R. (2007). Nematophaqous funqi of *Toxocara canis* eggs in aoublic place of la pleta, Arqentina, Rev. Iberoam Micolo., 24:24-28.

2-Machado, A. B. (2003). Visceral larva migrants : Case report. Anais Brasileros de Dematoloqia. 78: 215-219.

3-Guillot, J. & Bouree, P. (2007). Zoonotic worms from carnivorous pets: Risk Assessment & Prevention. Bull. Acad. Nat. Med., 101: 67-78.

4-Gilbert, S.; Halliwell, R. (2005). The effect 0f endoparasitism on the immune response to orally administereted antigen in cats. Veter. Immune. and immunopatho. 106: 113-120.

5- Torina, A.; Caracappa, S.; Barera, A.; Dieli, F.; Sireci, G.; Genchi, C.; Deplazes, P. and Salerno, A. (2005). *Toxocara canis* infection induces antigen specific IL 10 and IF ny production in pregnant dogs and their puppies. Veter. Immunol. Immunopatho., 108: 247-251.

6- Al-Kubaissi,A. B. (2004). Study of efficiency Vaccination with different antigen of *T.cati* and *T. leonine* immunizing white mice (Balb /c). Al-anbar J. of Agr. Sci., 2;(2).

7- Al-Kubaissi, A. B. (1992). Study of resistance of white mice Balb /c with *T.cati*, *T. leonine* infection. M. Sc., Thesis Univ. of Baghdad.

8-Mahmoud, A. J.(2014). The prevalence of Toxocariasis in Salah al-Din province with an immune attempt in laboratory mice Balb/c. Ph. D Thesis, College of education, Univ. of Tikrit.

9- Soulsby, E. J. (1982). Helminths, Arthropods And protozoa of domesticated animals. 7 th ed ., Bailliere Tindall , London . p : 150– 155 .

10- Fairbarin, D. (1957). Physiological hatching of *A. lumbricoids*. Experiment and technigues in parasitology free man and Co., Sanfrancisco . pp: 20-23.

11- Al-Tae, A. A.; Al- Bashir, N. M. & Mured, A. M. (1987). Artificial hatching of *T. canis* larvae using qut tissue and some chemicals. J. Biolo. Sci. Res., 18(3): 47-56.

12- Cleeland, R. and Laurence, K. A. (1961). In Vitro cultivation of *Ascaris lumbricoides* var suum. Larvae. J. of parasitol., 48 (1): 35-38.

13- Al-Gumaily,S.K.(1990). Study of the efficiency of vaccination with different antigens from *T.cati* , *T. leonine.* M. Sc., Thesis Univ. of Baghdad.

14- Al-Kubaissi,A. B.; Al-Ani, I. A. & Dawod, I. S. (2005). Efficiency white mice (Balb /c) immunization with somatic antigen of *T.canis* and *T. leonine* to resist infection with *T. cati, T. leonine* worm. Al-anbar J. of Agr. Sci., 3 (1): 251-256.

15- Hosin, A. B. (2004). Efficiency of immunization white mice Balb/c with *T. cati*, *T. leonine* (L_2) larvae. Al-Anbar J. of Agricultural sciences. 2(2): 407-414.

16- Al-Kubaissi, A.B. (2005). Efficiency of immunized white mice (Balb/c) immunization with somatic antigen of *T.cati* , *T. leonine* (L2) larvae. Al-anbar J. of Agr. Sci., 3 (1): 246-250.

17- Al- Azzawi, S. (1989). Biological studies on *T. cati* . M. Sc. Thesis, Univ. of Baghdad.

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محاولات تمنيع الفئران ضد الإصابة التجريبية بالسهمية القطية *Toxocara cati*

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الملخص

أجريت الدراسة الحالية لغرض معرفة تأثير التمنيع العضلي للفئران البيض (Balb/c) بمصل والخلايا اللمفاوية لطحال الفئران الممنعة بمستضدات مختلفة (البيوض الناضجة، اليرقات الحية، سائل فقس البيوض، مستخلص الدودة البالغة) لديدان السهمية الكلبية *Toxocara canis* لمقاومة الخمج بيرقات السهمية القطية *Toxocara cati* في حالة الخمج المغاير وضد يرقات السهمية الكلبية في حالة الخمج المماثل . أظهرت النتائج ان التمنيع بالمصل ذو تأثير في تقليل نسبة الخمج عند الحقن بكافة المستضدات اذ بلغت نسبة الوقاية ضد دودة *T. cati* 65.64% ،41.60% ، 60.68% ،73.28% على التوالي ضد المستضدات (البيوض الناضجة، اليرقات الحية، سائل فقس البيوض، مستخلص الدودة البالغة)، وبلغت نسبة الوقاية ضد ديدان *T. canis* 71.90% ،51.83% ،53.84% ،71.23% على التوالي عند التمنيع المماثل .أما عند التمنيع بالخلايا اللمفاوية لطحال الفئران الممنعة فقد بلغت نسبة الوقاية ضد دودة *T. cati* 64.96% ،51.36% ،48.63% ،67.68% على التوالي ضد المستضدات (البيوض الناضجة، اليرقات الحية، سائل فقس البيوض، مستخلص الدودة البالغة) ،وبلغت نسبة الوقاية ضد دودة *T. canis* 59.24% ،53.75% ،39.01% ،69.65% على التوالي عند التمنيع المماثل .