Efficiency of Immunization with the Hatching Fluid of *Toxocara canis* and *Toxascaris leonina* eggs against infection with *Toxascaris leonina* and *Toxocara cat*i larvae

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Abstract

The study was aimed to compare between three different routes of administration for immunization of white mice (Balb/c) with hatching fluid of *Toxocara canis* and *Toxascaris leonina* eggs against the experimental infection with *T.cati* and *T.leonina*.

Results showed a significant difference between mean values of larvae recovered from immunized mice compared to control. The immunization efficiencies that resulted from the different injection routes ranged from 56.25% in mice immunized subcutaneously with hatching fluid of *T.canis* eggs against experimental infection with *T.cati* eggs to 68.18% in mice immunized intraperitonially. Mice immunized with *T.canis* against *T.leonina* had an efficiency ranged from 61.22% when they immunized intraperitonially to 67.34 % when they immunized subcutaneously. The immunization efficiency in mice injected with *T.leonina* against *T.cati* was ranged from 42.61% when they immunized subcutaneously to 46.87% when they immunized intraperitonially.

Mice immunized with *T.leonina* against the experimental infection with the same worm had an immunization efficiency ranged from 62.75% when immunized intraperitonially to 65.05% when immunized subcutaneously.

It was concluded that the intraperitonial route is more efficient to stimulate immunity against *T.cati*, while subcutaneous route against *T.leonina*. In addition immunization by hatching fluid of *T.canis* is better than *T.leonina* against experimental infection with *T.cati*.

كفاءة التمنيع بسائل فقس بيوض *Toxocara canis* و *Toxascaris leonina* ضد الخمج بيرقات *Toxascaris leonina* و *Toxocara cati*

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الخلاصة

استهدفت الدراسة مقارنة ثلاث طرق حقن مختلفة لتمنيع الفئران البيضاء (Balb/c) بسائل فقس بيوض ديدان *Toxocara canis* و *Toxascaris leonina* ضد الخمج التجريبي بديدان *T.cati* و *T.leonina*.

أظهرت النتائج فرقا معنويا بين معدلات اليرقات المعزولة من الفئران الممنعة مقارنة بالسيطرة. تراوحت كفاءة التمنيع الناتجة عن طرق الحقن المختلفة بين 56.25% في الفئران الممنعة تحت الجلد بسائل فقس بيوض ديدان *T.canis* ضد الخمج التجريبي ببيوض ديدان *T.cati* الى 68.18 % في الفئران الممنعة بالبريتون.

تراوحت كفاءة التمنيع في الفئران الممنعة بديدان *T.canis* ضد *T.leonina* بين 61.22 % بالتمنيع في البريتون إلى 67.34 % بالتمنيع تحت الجلد، بينما تراوحت في الفئران الممنعة بديدان *T.leonina* ضد ديدان *T.cati* بين 42.61 % بالتمنيع تحت الجلد إلى 46.87 % بالتمنيع في البريتون.

في الفئران الممنعة بديدان *T.leonina* ضد نفس الديدان، تراوحت كفاءة التمنيع بين 62.75 % بالتمنيع في البريتون إلى 65.05 % بالتمنيع تحت الجلد.

يستنتج من ذلك، ان طريقة الحقن بالبريتون هي الطريقة الأكثر فاعلية في تحفيز المناعة ضد ديدان *T.cati*، بينما طريقة الحقن تحت الجلد هي الأكثر الفاعلية ضد ديدان *T.leonine*. إضافة إلى ان سائل فقس بيوض ديدان *T.canis* أفضل من سائل فقس بيوض ديدان *T.leonina* ضد الخمج التجريبي بديدان *T.cati*.

Introduction

Toxocara canis, *Toxocara cati* and *Toxascaris leonina* were considered important zoonotic worms and the environmental contamination with *Toxcara* spp. eggs is a public health problem (1) As, the human infections have a world – wide distribution, affecting mainly children under ten years of age, with a peak frequency between one and four years old (2).

When humans ingest infective *Toxcara* eggs, they hatch and release larvae that migrate anywhere in the body, causing a disease called visceral larva migrans (VLM). Common target organs are eyes, brain, liver, and lungs, with a risk of permanent visual, neurological and other tissue damages. Epidemiological studies have identified that pet dogs (particularly puppies) are the principal risk factor for human toxocarosis (3).

Carlos et al., (4) remarked that VLM granulomas are the most frequent granulomatous hepatitis in children in Brazil. Nicoletti et al., (5) found a significant association between toxocariasis and epilepsy, and suggest that toxocariasis may increase the risk of epilepsy in endemic areas.

In Spain, the infection rate with *T. canis* and *T.leonina* of 1161 dogs reached to 7.8 and 6.3% respectively (6), while in Turkey, *T.leonina* and *T. canis* were diagnosed in 21.8 and 13.3%, respectively in fecal samples from military working dogs (7), While *T. canis* was diagnosed in 21% of fecal samples from dogs in Ethiopia with a rate reached to 45% at the necropsy finding (8). Many researchers attempted to stimulate immunity against toxocariasis by administration of the *Ascaris* spp antigens; where (9) used extract of adult *Ascaris* larvae to stimulate immunity against the next infection with these worms.

The extract of *T.cati* worm was approved to improve the immunization efficiency against the experimental infection with the same worm (10), while (11) succeeded in immunizing of mice by *T.leonina* and *T.cati* against the experimental infections.

There was a cross reaction between antigens of *T. canis*, *T.leonina* and *T.cati* (12), and the recovered L_2 of *Ascaris suum* that stimulate the immunity against the next infection (13).

Materials and methods

- **Capture of cats and collection of worms:** Stray cats were captured by metal cages, then they weighted and injected intraperitoneally with 0.25 ml / kg B.W. of Amylobarbitone sodium as anesthetic.

The cats were killed immediately, then their digestive system was isolated, then checked, and worms were collected and washed by physiological saline solution. After that, female worms were isolated in small containers and put in a refrigerator. *T. canis* worms were obtained from the Biology laboratory of the College of Education.

1. **Recovery, incubation and hatching of eggs:** Eggs were recovered according to method of (14), and they Hatched according to the modified method of (15) as follows: The eggs were washed with distilled water several times by using a centrifuge to remove sulphuric acid, then washed with phosphate buffer solution pH= 7, and shake by a magnetic stirrer with magnetic bar for 24 hours at 33 °C.
2. **collection of hatching fluid:** After hatching of eggs, larvae were isolated by centrifuge at 1200 rpm, and the supernatant was collected and injected into mice at different routes at a dose of 1000 eggs.
3. **Injection of hatching fluid:** 40 mice were injected by hatching fluid of *T. canis* or *T. leonina* subcutaneously, intramuscularly or intraperitonially, with the following doses:

a) The first dose: 0.3 ml contain the hatching fluid of 1000 eggs.
b) The booster dose: 0.15 ml contain the hatching fluid of 500 eggs injected after 21 days.

Mice in each group were subdivided as fallows:

The first group: include 20 mice immunized with the hatching fluid of 1000 eggs subcutaneously as follows:

a) 5 mice were immunized with hatching fluid of *T. canis*, and administered orally with a challenge dose of 1000 mature egg of *T. cati*.
b) 5 mice were immunized with hatching fluid of *T. canis*, and administered orally with a challenge dose of 1000 mature egg of *T. leonine.*
c) 5 mice were immunized with hatching fluid of *T. leonina*, and administered orally with a challenge dose of 1000 mature egg of *T. cati*.
d) 5 mice were immunized with hatching fluid of *T. leonina*, and administered orally with a challenge dose of 1000 mature egg of *T. leonine.*

The second group: included 20 mice immunized with hatching fluid of 1000 eggs intramuscularly and divided by a similar pattern as in the first group.

The third group: included 20 mice immunized with the hatching fluid of 1000 eggs intraperitonially and divided by the similar manner as in the first group.

Control group: included 10 mice administered with the challenge dose as follows:

a) 5 mice were administered orally with a challenge dose of 1000 mature egg of *T. cati*.
b) 5 mice were administered orally with a challenge dose of 1000 mature egg of *T. leonine.*

Then mice were killed, subjected to the post mortem examination, and the larvae were recovered from certain organs 10 days after the administration of the challenge dose. The immunization efficiency was estimated according to the following equation:

$$\text{L. E.} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Results

1. **Subcutaneous injection**: Results in table (1) revealed that the highest immunization efficiency, in comparison with control, appeared in mice immunized with the hatching fluid of *T. canis* against a challenge dose of *T. leonina* was (67.34%). While the lowest immunization efficiency appeared in mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati* was (42.61%), Figure (1).

The mean value of larvae which recovered from wall of stomach and intestine of mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of the same worm was 48 larvae, while there were 37 larvae recovered from mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. leonina*. While, no larvae recovered from mice immunized with the hatching fluid of

T. canis eggs against a challenge dose of *T. cati*, and from mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati*.

The mean value of larvae which recovered from the liver ranged from 3 in mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of the same worm to 14 in mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. leonina.*

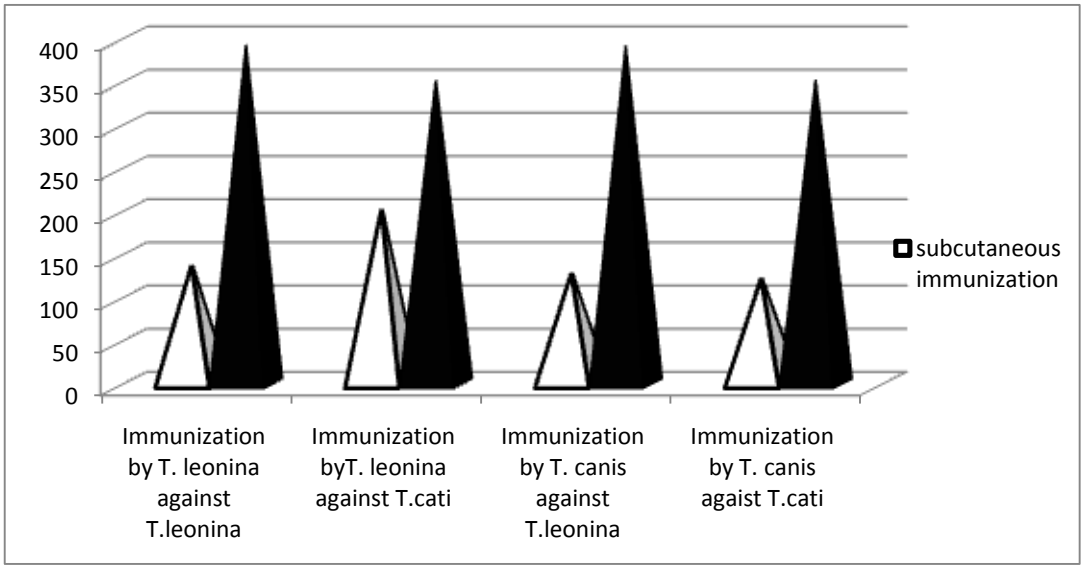
There were 10 larvae recovered from lungs of mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. leonine* and 8 larvae from mice immunized with *T. leonina* eggs against a challenge dose of the same worm, while there was no larvae in lungs of mice immunized with the *T. canis* eggs against a challenge dose of *T. cati* and that immunized with *T. leonina* eggs against a challenge dose of *T. cati* and from kidney, spleen, heart and brain in all mice immunized subcutaneously.

From the muscles, the mean value of recovered larvae ranged from 67 in mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. leonina* to 195 in mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati*, Table (1).

Table (1) The immunization efficiency and number of the larvae that recovered from the different organs of mice immunized with subcutaneous route

Group	Immunization dose	Challenge dose	Stomach wall & Intestine	Liver	L ung	Kidney	Spleen	Brain	Heart	Muscle	Sum of means	SD±	Immunization efficiency (%)
1st	*T. canis*	*T. cati*	-	4	-	-	-	-	-	118	122	20.69	65.34
	T. canis	*T. leonina*	37	14	10	-	-	-	-	67	128	23.55	67.34
	T. leonina	*T. cati*	-	7	-	-	-	-	-	195	202	34.52	42.61
	T. leonina	*T. leonina*	48	3	8	-	-	-	-	78	137	31.26	65.05
Control			-	-	-	3	-	-	-	349	352	49.88	
			192	18	28	-	-	-	-	154	392	23.55	

Figure (1) The mean values of the recovered larvae from the different organs of mice immunized subcutaneously compared to control



2. **Intramuscular injection**: Table (2) shows that the highest immunization efficiency was obtained in mice immunized with the hatching fluid of *T. canis* against a challenge dose of *T. leonina* (66.83%). While the lowest immunization efficiency was obtained in mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati*(43.75%),Figure (2).

The mean value of larvae recovered from the wall of stomach and intestine of mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of the same worm was 42 larvae, while there were 39 larvae recovered from mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. leonina*, while no larvae recovered from mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. cati*, and from mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati*.

The mean value of larvae that recovered from the liver was ranged from 4 in mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. leonina* to 14 in mice immunized with the hatching fluid of *T.canis* eggs against a challenge dose of *T. cati.*

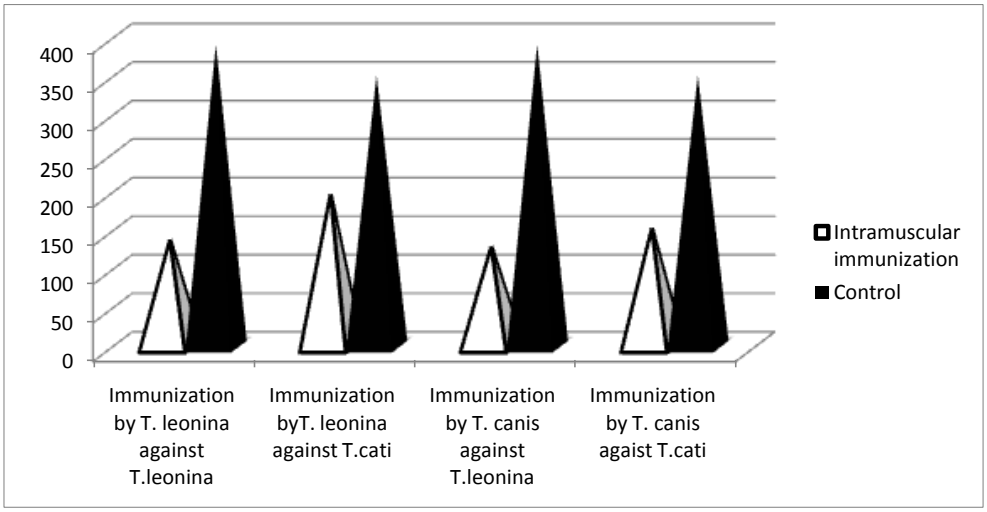
Nine larvae were extracted from the lungs of mice immunized with *T. leonina* against the same worm and 7 larvae were extracted from the lungs of mice immunized with *T. canis* against *T. leonina*, while there no larvae were recovered in mice immunized with *T. canis* against *T. cati* and mice immunized with *T. leonina* against *T. cati*.

There were no extracted larvae from the kidney, spleen, heart and brain. The mean value of larvae which recovered from the muscles ranged from 80 in mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T.leonina* to 193 in mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati.*

Table (2) The immunization efficiency and the number of the larvae that recovered from the different organs of mice immunized intramuscularly, in comparison with control group

Group	Immunization dose	Challenge dose	Stomach wall & Intestine	Liver	L ung	Kidney	Spleen	Brain	Heart	Muscle	Sum of means	SD±	Immunization efficiency (%)
2nd	*T. canis*	*T. cati*	-	12	-	-	-	-	-	142	154	29.85	56.25
	T. canis	*T. leonina*	39	4	7	-	-	-	-	80	130	33.66	66.83
	T. leonina	*T. cati*	-	5	-	-	-	-	-	193	198	54.68	43.75
	T. leonina	*T. leonina*	42	7	9	-	-	-	-	81	139	35.81	64.54
Control			-	-	-	-	-	3	-	349	352	49.88	
			192	18	28	-	-	-	-	154	392	23.55	

Figure (2) The mean values of recovered larvae from different organs of mice immunized intramuscularly, compared to control



3. **Intraperitonial injection**: Table (1) showed that the highest immunization efficiency was obtained in mice immunized with the hatching fluid of *T. canis* against a challenge dose of *T. cati* (68.18%). While the lowest immunization efficiency was appeared in mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati* (46.87%), Figures (1,4).

The mean value of larvae which recovered from wall of stomach and intestine of mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of the same worm was 54 larvae, while there were 46 recovered larvae from mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. leonina*, with no larvae recovered from mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. cati*, and from mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of *T. cati*.

The mean value of larvae which recovered from the liver was ranged from 2 in mice immunized with the hatching fluid of *T. canis* eggs against a challenge dose of *T. ca*ti to 8 in mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of the same worm.

There are 9 recovered larvae from the lung of mice immunized with the hatching fluid of *T. leonina* eggs against a challenge dose of the same worm,7 from mice immunized with *T. canis* eggs against a challenge dose of *T. leonina*, while there was no larvae in the lung of mice immunized with the *T. canis* eggs against a challenge dose of *T. cati* and that immunized with *T. leonina* eggs against a challenge dose of *T.cati* and from kidney,spleen, heart and brain in all mice immunized intraperitonially.

From the muscles,The mean value of recovered larvae ranged from 80 in mice immunized with the hatching fluid of *T.canis* eggs against a challenge dose of *T.leonina* to 193 in mice immunized with the hatching fluid of *T.leonina* eggs against a challenge dose of *T.cati*, Table(3).

Table (3) The immunization efficiency and the number of the larvae that recovered from the different organs of mice immunized intraperitonially, compared to control group

Group	Immunization dose	Challenge dose	Stomach wall & Intestine	Liver	Lung	Kidney	Spleen	Brain	Heart	Muscle	Sum of means	SD±	Immunization efficiency (%)
2nd	*T. canis*	*T. cati*	-	2	-	-	-	-	-	110	112	18.97	68.18
	T. canis	*T. leonina*	46	5	4	-	-	-	-	97	152	29.85	61.22
	T. leonina	*T. cati*	-	4	-	-	-	-	-	183	187	30.79	46.87
	T. leonina	*T. leonina*	54	8	11	-	-	-	-	73	146	31.4	62.75
Control			-	-	-	-	-	3	-	349	352	49.88	
			192	18	28	-	-	-	-	154	392	23.55	

Figure (3) The mean values of recovered larvae from different organs of mice immunized intraperitonially, compared to control group

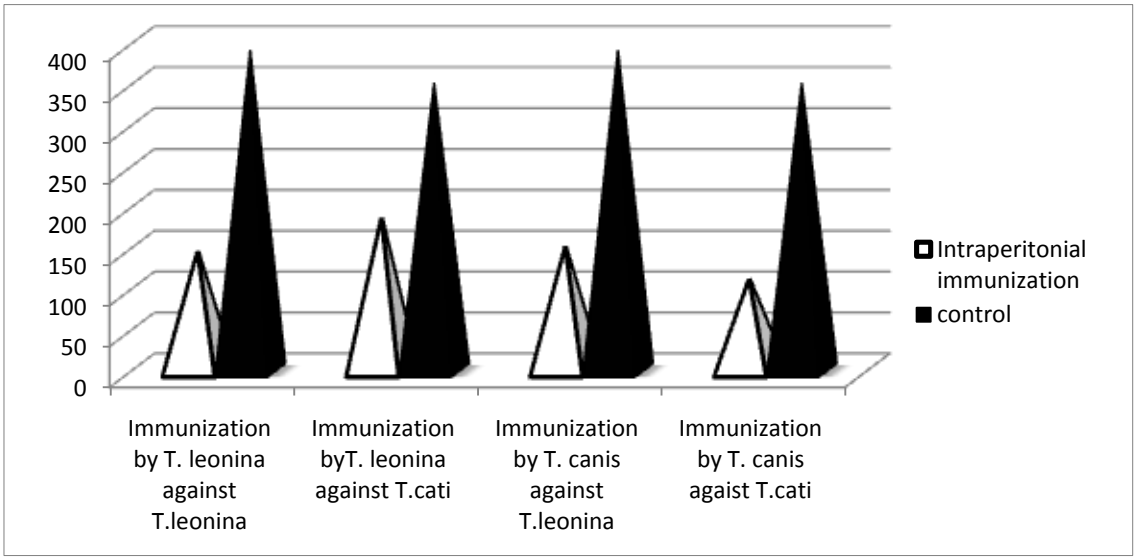
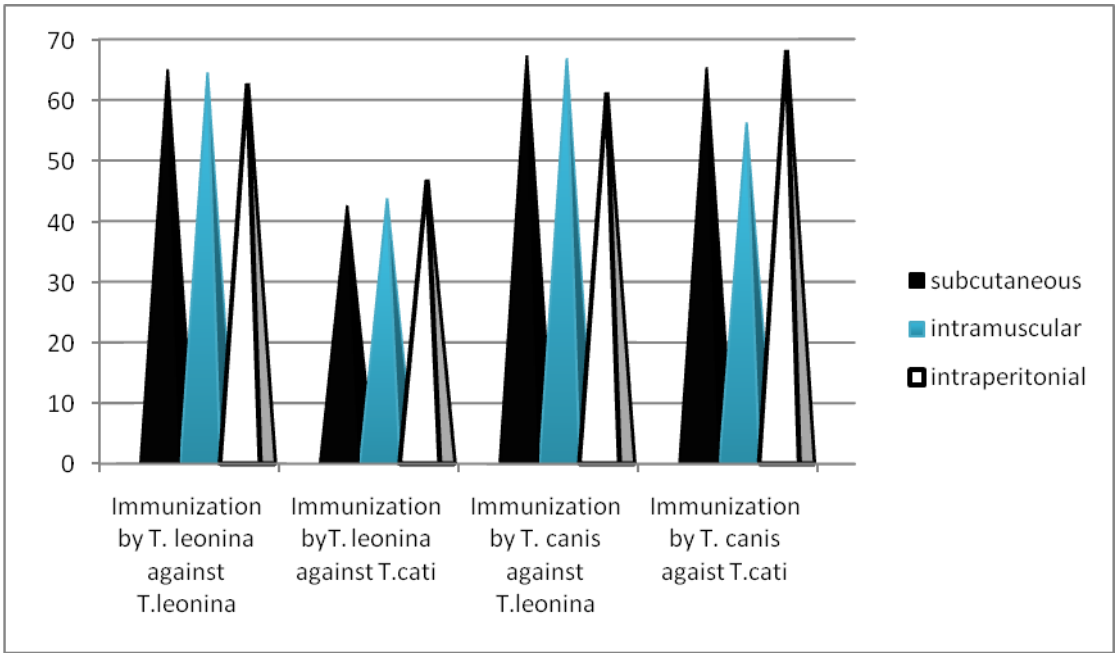


Figure (4) Comparison of the immunization efficiencies obtained by the three injection methods



Discussion

Gilbert & Halliwell (12) found that the amount of IgG antibodies increased 3 weeks post experimental infection of cats, and Hosin (16) concluded that the different antigens of *T.canis* had stimulated the immunological response against the VLM caused by *T.cati* in mice, and that these worms had a similar evolutionary composition, so that he obtained 75% immunization efficiency when he used the secretory / excretory materials of *T.canis* against the experimental infection with *T.cati*, and 72.53%, 69.43% and 69.1% when used the mature eggs, immature eggs and the live larvae of *T.canis* against *T.cati* ,respectively.

These results agree with our results, where the immunization efficiency which obtained in this study is 65.34% when using the hatching fluid of *T.canis* Subcutaneously to stimulate the immunity against the infection with *T.cati*, 65.05% when using *T. leonina* against the same worm. This result also agree with the result of Alkubaisi (17) who obtained 52.56% immunization efficiency.

Hosin (18) obtained 65.08% immunization efficiency by using the security excretory materials of *T.canis* against the experimental infection with *T.cati* and he concluded that the live larvae of *T.leonina* was the best antigens against the infection with the same worm with 52.68% immunization efficiency, then the live larvae of *T. canis* (51.15%).

Alkubaisi (19) was obtained 62.24% immunization efficiency when he used the hatching fluid of *T.cati* eggs for immunization with subcutaneous route against the infection with same worm, while the immunization efficiency resulted from subcutaneous immunization by hatching fluid of *T.leonina* against the same worm, *T.cati* against *T.leonina* and *T.leonina* against *T.cati* were 63.24% , 64.05% and 42.93 % respectively.

These results were in accordance with our recent results when using the hatching fluid of *T. canis* against experimental infection with *T.cati*, as well as *T.leonina* against the same worm, and the *T. canis* antigens was more efficient for stimulating the immunity against the experimental infection with both *T.cati* and *T.leonina.*

Hosin (18) suggested that the wall of stomach and the intestine may play an important role in resistance in treatment group for infection compared with the control, and that the subcutaneous route was an efficient route to enhance the resistance against the infection with these worms, and this agree with our results.

However, these results, in addition to our results, suggest that there is an antigenic interference between *T. canis*, *T.cati* and *T.leonina* antigens when different routes for immunization were used, and the antigens of *T. canis* lead to a higher level of immunity against the infection with *T.cati* than with that of *T.leonina* (19).

We suggests that the antibodies especially security immunoglobin A (SIgA) may have be an important role in preventing, or at least decreasing, the morbidity rate resulted from the infection with *Ascaris* spp where it is secreted in saliva and the intestine, in addition to the specific and non specific role of macrophages as the first defense line against these larvae in the intestine.

From these results, we can conclude that the intraperitonial injection is the more efficient route for stimulating immunity against the infection with *T.cati*, while the subcutaneous injection is the more efficient route for stimulating the immunity against the infection with *T.leonina*, and that the immunization by the hatchery fluid of *T. canis* eggs is more efficient than *T.leonina* for stimulating the immunization against the infection with *T.cati*, and the larvae of *T.cati* has the quickest migration to the muscles, therefore, they did not recovered from the organs of mice of treatment groups, while they are voluminously recovered from the muscles, as shown in tables (1,2 and 3).

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