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IMMUNODIAGNOSTIC STUDIES ON *TOXOCARA VITULORUM* IN RABBITS

(With 3 Tables and 4 Plates)

By

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دراسات مناعية تشخيصية علي التوكسوكارا فيتلورم في الأرانب

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في هذه الدراسة تم تشخيص التوكسوكارا فيتلورم في الأرانب وكذلك محاولة تحصينها، ولهذا تم تقسيم عدد ١٢ من ذكور الأرانب النيوزيلاندي الي ثلاث مجموعات متساوية، حيث كانت المجموعة الأولي (أ) مجموعة محصنة بمستخرج البويضات المعدية وتم عدوي المجموعة الثانية (ب) ببويضات النوكسوكارا فيتلورم بدون تحصين في حين تم إعطاء المجموعة الأخيرة (ج) الحاوي **adjuvant** فقط واعتبرت كمجموعة ضابطة سلبية في. وقد أجريت عدة اختبارات مناعية علي المجموعات الثلاث، شملت اختبار الأليزا واختبار اللطخ المناعي واختبار التكسير البروتيني لجزيئات مولد الضد المستخدم عن طريق الفصل الكهربي ، حيث اوضحت نتائج الأليزا للمجموعة الأولي أن ظهور أول مستوي ايجابي للأجسام المناعية المضادة (٠,٦٢٣) بدأ عند اليوم الـ ١٤ من التحصين ووصل الي أعلي مستوي له (٠,٩٣٦) عند اليوم الـ ٢١ من العدوي. وفي المجموعة الثانية كان أول ظهور للأجسام المضادة (٠,٤٩٦) عند اليوم الـ ٢١ من العدوي في حين كان أعلي مستوي لها (٠,٦٥٧) عند اليوم الـ ٣٠ من العدوي. وأخيرا في المجموعة الثالثة بدا ظهور اول مستوي ايجابي (٠,٤١٨) عند اليوم الـ ٢١ من التحصين وصولا الي أعلي تركيز (٠,٧٧٨) عند اليوم الـ ٣٠ من العدوي. وبالنسب لنتائج الفصل الكهربي الجزيئي لبروتينات مولد الضد المستخدم وجدت ٨ حزم بروتينية علي مدار التجربة وهي علي الترتيب ٢٤٠ و ١٣٥ و ٤٠ و ٣٣ و ٢٨ و ٢٤ و ٢٢ و ١٨ كيلو دالتون. وبالنسبة لأختبار اللطخ المناعي وجدت أيضا ٦ حزم بروتينية مختلفة وهي ٤٣ و ٤٠ و ٣٣ و ٢٤ و ٢٢ و ١٨ كيلو دالتون. هذا وقد وجدنا ان الحزم ٣٣ و ٢٤ و ١٨ كيلو دالتون قد ظهرت طوال فترة التحصين والعدوي أيضا مما يجعلنا نعتبرها حزم حامية يمكن استخدامها في ايجاد التحصين المناسب. ويجب أن نشير الي أن الحزم البروتينية ٤٣ و ٤٠ و ٣٣ و ٢٤ و ٢٢ و ١٨ قد ظهرت مبكرا بينما ظهرت الحزم البروتينية ٥٠ و ٦٠ كيلو دالتون متأخرة في نهاية التجربة. وبالنسبة للتغيرات الباثولوجية فقد أكدت ما وجدناه حيث تركزت في أكباد ورئات الحيوانات المعدية فقط حيث ظهرت البقع البيضاء بكثرة في الكبد وامتلأت الرئة بالبقع والعقد النزفية في حين أنه ظهرت هذه الأعضاء أقرب الي الشكل الطبيعي في المجموعة المحصنة.

SUMMARY

In the present study, serodiagnosis as well as trial for vaccination of *Toxocara vitulorum* were done. Twelve male New Zealand rabbits were divided into 3 equal groups; *Toxocara vitulorum* immunized group (A), control positive *Toxocara vitulorum* infected group (B) and control Freund's adjuvant group (C). Enzyme-linked immunosorbent assay (ELISA), Western Blot and protein fractionation by using Sodium Dodecyl Sulphate Gel Electrophoresis (SDS-PAGE) were done. Concerning ELISA results, *Toxocara vitulorum*-immunized group showed the first positive mean antibody titre (0.623) at the day 14 post immunization and reached the peak (0.936) at the day 21 p.i. In control infected group, the first positive mean antibody titre (0.496) appeared at the day 21 p.i and reached the peak (0.657) at the day 30 p.i. In control adjuvant group, the first positive mean antibody titre (0.418) appeared at the day 21 post immunization and reached the highest level (0.778) at the day 30 p.i Concerning results of SDS-PAGE, electrophoretic analysis with silver stain revealed 8 protein bands with molecular weights 240, 135, 40, 33, 28, 24, 22 and 18 kDa, respectively. The analysis of immunoblotting cleared polypeptide bands of molecular weights 43, 40, 33, 24, 22 and 18 kDa in different sequences along the experiment. It is concluded that, protein bands of molecular weights 33, 24 and 18 kDa appeared along the course of immunization and still also post infection. Therefore, they are considered as protective bands that can be used for preparation of a purified vaccine. It is worthy to mention that protein bands of molecular weights 43, 40, 33, 24, 22 and 18 kDa were detected early (early diagnostic bands); while those of molecular weights 60 kDa and 50 kDa were detected at the day 45 p.i (late diagnostic bands). Concerning to the histopathological results, lesions were prominent in liver and lungs of control infected group showing several hepatic milky spots as well as diffuse eosinophilic infiltration in addition to haemorrhagic patches and nodules in lung tissues. The picture in immunized group was greatly reduced appearing more or less normal.

Key words: *Toxocara vitulorum*, immunodiagnosis, rabbits

INTRODUCTION

Toxocara vitulorum is a pathogenic parasite of the small intestine of buffaloes. It is common to find buffalo calves highly infected between 15 and 90 days of age (Starke-Buzetti *et al.* 1983) and the main way of infection of the calf is via the transmammary route in neonatal calves (Starke-Buzetti *et al.* 1992). The main route for migration of

T. vitulorum larvae in the buffalo host is from the gut to the liver by passive blood transport through the portal vein and in a minor way, through mesenteric lymph nodes. In the resistant host, cows for instance, *T. vitulorum* larvae may reach the muscles where they survive, but do not molt, possibly as hypobiotic larvae (Omar and Barriga, 1991). Migration of larvae of *T. vitulorum* in white mice, manifested a behavior similar to other ascarids (Chauhan and Pande, 1972).

The adult parasites are relatively easy to remove from the intestines by chemotherapy, while the larvae are difficult to kill mainly when they are in the musculature and in the brain (Abo-Shehada and Herbert, 1984). Therefore, an alternative possibility of control is to immunize the dams to kill their tissue larvae before they are transferred to the calves. An immunological approach to the control of *T. vitulorum* has always been attractive, and it would be most advantageous if the larvae could be killed in less vital tissues, preferably the intestine (Barriga and Omar, 1992). However, little is known about the immunological responses of water buffalo against *T. vitulorum*

Amerasinghe *et al.* (1984) found anti-*T. vitulorum* antibodies in the serum of swamp buffalo cows before and after parturition and also in their colostrum after parturition. Their calves showed the same serum antibodies after birth, but they developed patent *T. vitulorum* infection. Later, Rajapakse *et al.* (1994 a) demonstrated antibodies against the excretory/secretory antigens of *T. vitulorum* larvae (E/S) in serum and colostrum of buffalo cows by ELISA (enzyme-linked immunosorbent assay). High titers of anti-larval E/S antibodies in buffalo cow colostrum and in the serum of their suckling calves, correlated with low *T. vitulorum* fecal egg counts in the calves and suggested that antibodies might have a protective role against activated or migrating larvae. Furthermore, Rajapakse *et al.* (1994 b) confirmed antibody-mediated protection against *T. vitulorum* larvae by the ability of buffalo serum or colostrum to inhibit migration of *T. vitulorum* larvae in mice. Besides, Amerasinghe *et al.* (1992) reported that, immunization of mice with *T. vitulorum*-perienteric antigen induced 100 % and 79 %, respectively, of inhibition of larval migration to their tissues, suggesting a potential protection against the infection.

The present study aims to immunize rabbits against embryonated egg antigen of *Toxocara vitulorum* together with using the possible immunodiagnostic techniques (SDS-PAGE, ELISA and Western Blot) for detection of time of peak antibody release and specific diagnostic polypeptide bands.

MATERIALS and METHODS

1. Collection and embryonation of eggs:

Adult *Toxocara vitulorum* worms were obtained from the slaughtered buffalo calves at Beni-Suef abattoirs, transported to Beni-Suef laboratory of Animal Health Research Institute, washed several times with saline and examined under microscope. Fertile eggs were obtained from uteri of the gravid females using blunt scissor, then, sieved, washed and precipitated several times using 1 % formol-saline followed by keeping in refrigerated adequate solution till use (Sabry, 1999).

The collected eggs were divided into several amounts put in clean petri-dishes and incubated at 28 °C, underwent several rewashing and good aeration followed by microscopical examination to observe the developmental changes and left to embryonate for 30 days (Omar and Barriga, 1991 and Mousa *et al.*, 2001). Some of the embryonated eggs were used for antigen production, and others were kept at 4°C for challenge.

2. Preparation of *Toxocara vitulorum* antigen:

The embryonated infective *T. vitulorum* eggs were washed several times with 0.01 M PBS (pH 7.4) by centrifugation at 1500 rpm. for 10 minutes to remove remnants of the formol-saline. Eggs were mixed with an equal volume of the solution, then homogenized at 6000 rpm for 5 minutes in ice bath. The homogenized samples were sonicated for 5 minutes at 5 pulse rate and 60-80 amplitude value using cole parner ultra sonic sonicator. Then the suspension was subjected to high speed centrifugation 14,000 rpm at 4 C° for 30 minutes, then the supernatant containing soluble antigenic materials was separated and stored at -20 C° in plastic vials till used (Sabry, 1999 and Arafa, 2008). Protein content of the antigenic components was determined according to Lowry *et al.* (1951).

3. Rearing and housing of rabbits:

Twelve male New Zealand rabbits of 1-1.5 kg body weight free from parasitic, bacterial or viral diseases were obtained, reared in clean and disinfected battery, then feed on maintenance ration provided with coccidicidal drugs. Temperature and ventilation were adjusted.

4. Experimental design:

4.1. Grouping of animals:

Rabbits were divided into 3 equal groups:

- *Group A* (Immunized group): Rabbits immunized against *T. vitulorum* antigen.

- *Group B* (Control positive group): Rabbits were only infected with embryonated eggs of *T.vitulorum* used without immunization.
- *Group C* (Control adjuvant group): Rabbits administered Freund's adjuvant only.

4.2. Protocol of immunization:

In groups (A and C), animals were administered complete Freund's adjuvant (600 µg protein) followed by incomplete one (400 µg protein) as the method described by Mousa *et al.* (2001).

4.3. Procedure of infection:

Each rabbit (of groups A and B) was challenged after the day 30 from the beginning of the experiment with 10000 embryonated *T. vitulorum* eggs orally using stomach tube.

4.4. Blood collection:

Blood was collected from the ear veins at the days zero, 14 and 21 post immunization and at the days 14, 21, 30 and 45 p.i., then serum was obtained by centrifugation and preserved at -20 C° till use.

5. Diagnostic tools:

5.1. Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis: (SDS-PAGE):

It is used for determination of molecular weights of polypeptide bands obtained by fractionation of *T. vitulorum* embryonated egg antigen according to Laemmeli (1970).

5.2. Enzyme-linked immunosorbent assay (ELISA):

ELISA was applied to detect antibodies against *T. vitulorum* antigen using 96-well flat microtiter plate according to Starke-Buzetti *et al.* (2001) and Arafa (2008).

5.3. Western Blot technique:

Polypeptide bands obtained from SDS-PAGE were electrophoretically transferred to nitrocellulose according Towbin *et al.* (1979) and the results were analyzed using Gel pro-analyzer 3.1.

6. Histopathological techniques:

Pieces from liver and lungs of the sacrificed rabbits were fixed in 10 % formalin, embedded in paraffin sections of 5 µm thickness, stained with haematoxylin and eosin and examined microscopically according to Bancroft and Stevens (1996).

RESULTS

1. Evaluating the egg antigen of *Toxocara vitulorum* using ELISA:

ELISA was performed on sera of different rabbit groups with *T. vitulorum* egg larval antigen at zero day, at days 14 and 21 post

immunization, then at days 14, 21, 30 and 45 post infection (p.i.). It has been found that, in Group (A); the first positive antibody titer (0.623) was found at the day 14 post immunization, increased to (0.925), (0.933), (0.936), (0.926) and (0.872) at the day 21 post immunization, 14, 21, 30 and 45 days p.i. respectively. In group (B); the first positive mean antibody titer (0.496) was at the day 21 p.i., increased to (0.657) at the day 30 and became (0.566) at the day 45 p.i. Moreover, in Group (C), the first positive mean antibody titer (0.418) at 21 days post immunization, followed by (0.480), (0.598), (0.778) and (0.743) at the days 14, 21, 30 and 45 p.i. respectively (Table 1).

It's worthy to clarify that, ELISA with *T. vitulorum* egg antigen showed sensitivity reached to 100% since all infected and immunized rabbits were positively reacted.

2. Characterization of *T. vitulorum* egg antigen by using SDS-PAGE:

Electrophoretic analysis of infective *T. vitulorum* egg antigen with SDS – PAGE after staining with silver stain revealed eight bands with molecular weights 240, 135, 40, 33, 28, 24, 22 and 18 kDa (Table 2 and Plate 1).

3. Detection of *T. vitulorum* specific epitopes by using Western Blot:

Reaction of *T .vitulorum* antigen with sera of rabbits in group (A) at day zero of the experiment did not reveal any bands due to absence of the specific antibodies against *T. vitulorum*, while the same reaction revealed three polypeptide bands of molecular weights 33, 24, and 18 kDa at the day 14 post immunization. The same bands appeared at the day 21 in addition to bands of 43, 40 and 22 kDa. Furthermore, six bands of molecular weights 43, 40, 33, 24, 22 and 18 kDa appeared at the day 15 p.i. (post infection). The same bands appeared at the days 30 and 45 p.i. in addition to bands of molecular weights 60 kDa and 50 kDa. It's worthy to mention that bands of 33, 24 and 18 kDa appeared along the course of the immunization and still post infection, so it may be the protective bands from which the purified vaccine may be prepared. Bands of molecular weights 43, 40, 33, 24, 22, and 18 kDa appeared early so, they may be considered as early diagnostic bands, while bands of 60 kDa and 50 kDa appeared at the day 45 p.i., so may used as specific diagnostic bands for the late infection (Table 3 and Plate 2).

4. Histopathological findings:

The pathological findings were prominently in liver and lungs due to the pattern of the migratory behaviour. In the immunized group (group A), gross lesions revealed relative lung resistance and more or

less normal hepatic configuration, while microscopic findings of these organs showed mild eosinophilic granulomas in the liver associated with normal lung outline.

On the other hand, in *Toxocara vitulorum* infected group (group B) milky spots were seen on liver surface as well as haemorrhagic patches in the lungs, while the microscopic picture revealed eosinophilic infiltration in both hepatic parenchyma and lung tissues (Plates 3 & 4).

Table 1: Evaluation the *T. vitulorum* eggs antigen for capture of their antibodies in different groups using ELISA.

Time \ Group		A	B	C
Zero day		0.204	0.206	0.208
Day 14 post immunization		0.618	0.195	0.347
		0.638	0.199	0.390
		0.612	0.210	0.351
	Mean	0.623	0.201	0.363
Day 21 post immunization		0.939	0.197	0.451
		0.886	0.199	0.401
		0.949	0.209	0.403
	Mean	0.925	0.201	0.418
Day 14 p.i		0.986	0.320	0.484
		0.894	0.399	0.475
		0.919	0.309	0.481
	Mean	0.933	0.343	0.480
Day 21 p.i		0.953	0.428	0.637
		0.919	0.563	0.624
		0.935	0.497	0.532
	Mean	0.936	0.496	0.598
Day 30 p.i		0.921	0.750	0.753
		0.915	0.629	0.763
		0.942	0.592	0.818
	Mean	0.926	0.657	0.778
Day 45 p.i		0.879	0.615	0.757
		0.848	0.551	0.731
		0.889	0.531	0.742
	Mean	0.872	0.566	0.743

(Cut off value = 0.410)

Table 2: Characterization of infective *T. vitulorum* egg antigen with SDS-PAGE.

*Marker	*T. vitulorum antigen*
250	
	240
160	
	135
105	
75	
50	
	40
35	
	33
30	
	28
25	
	24
	22
	18
15	
10	

* Amersham prestained broad range molecular weight protein marker (Bio-lab Company).

Table 3: Determination of specific epitopes of *T. vitulorum* antigen with sera of group (A) by using Western Blot.

Marker	Day zero	Day 14 p. imm	Day 21 p.imm	Day 15 p.i.	Day 30 p. i.	Day 45 p.i.
250						
160						
105						
75						
					60	60
50					50	50
			43	43	43	43
			40	40	40	40
35						
		33	33	33	33	33
30						
25						
		24	24	24	24	24
			22	22	22	22
		18	18	18	18	18
15						
10						

p.imm (meaning post immunization)

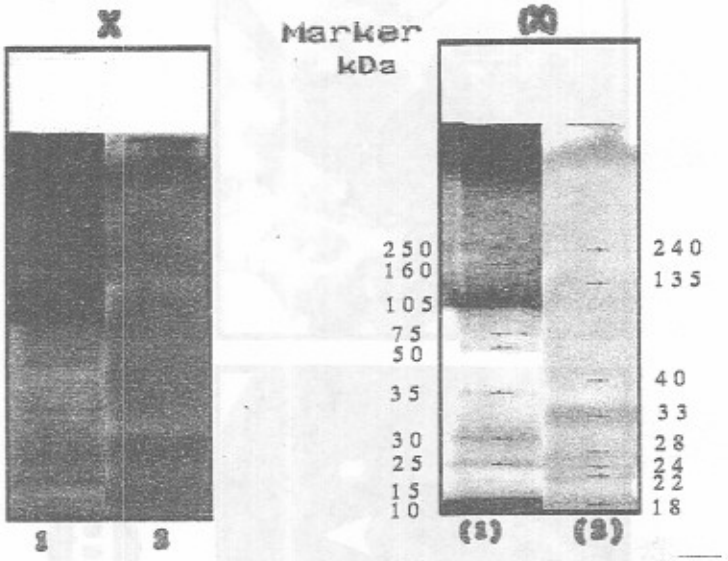

Plate 1: A, (A) Showing the polypeptide bands revealed from the electrophoresis of *T. vitulorum* eggs using SDS –PAGE

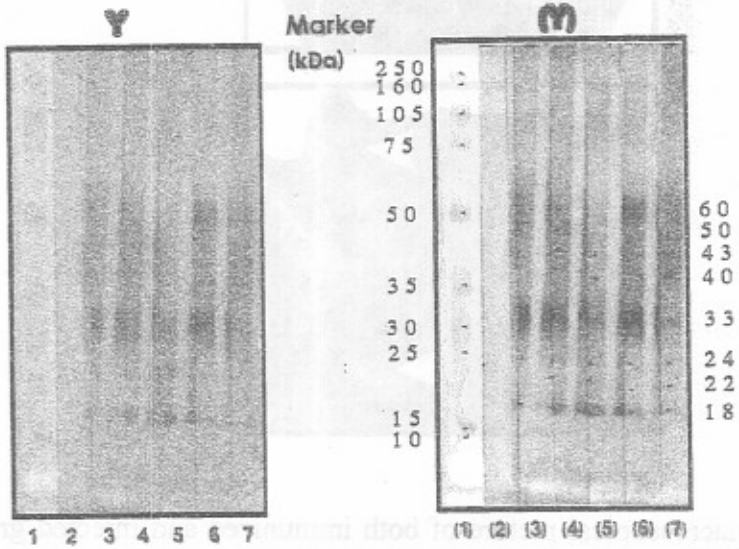

Plate 2: B, (B) showing polypeptide bands revealed from the reaction of *T. vitulorum* with sera of group (A)

(3), (4), (5), (6) and (7) revealed days 14 post. Imm., 21 post. Imm., 14 p.i, 30 p.i and 45 p.i

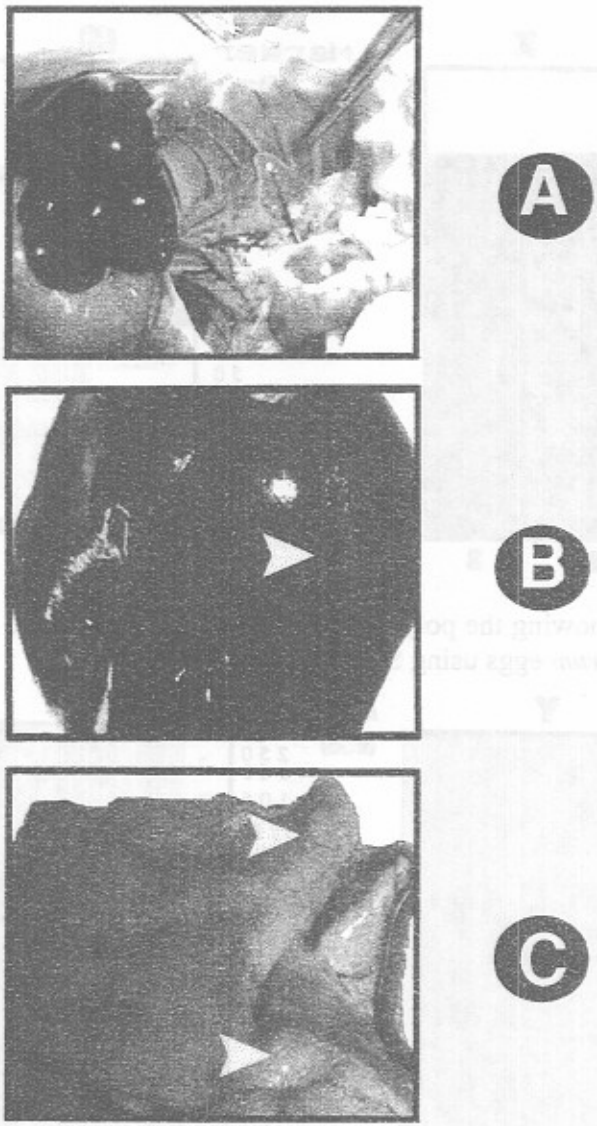

Plate 3: Macroscopical picture of both immunized and infected groups
A) Viscera of a rabbit in group (A) showing more or less normal organs
B) Liver of a rabbit in group (B) showing milky spots
C) Lung of a rabbit in group (B) showing nodules

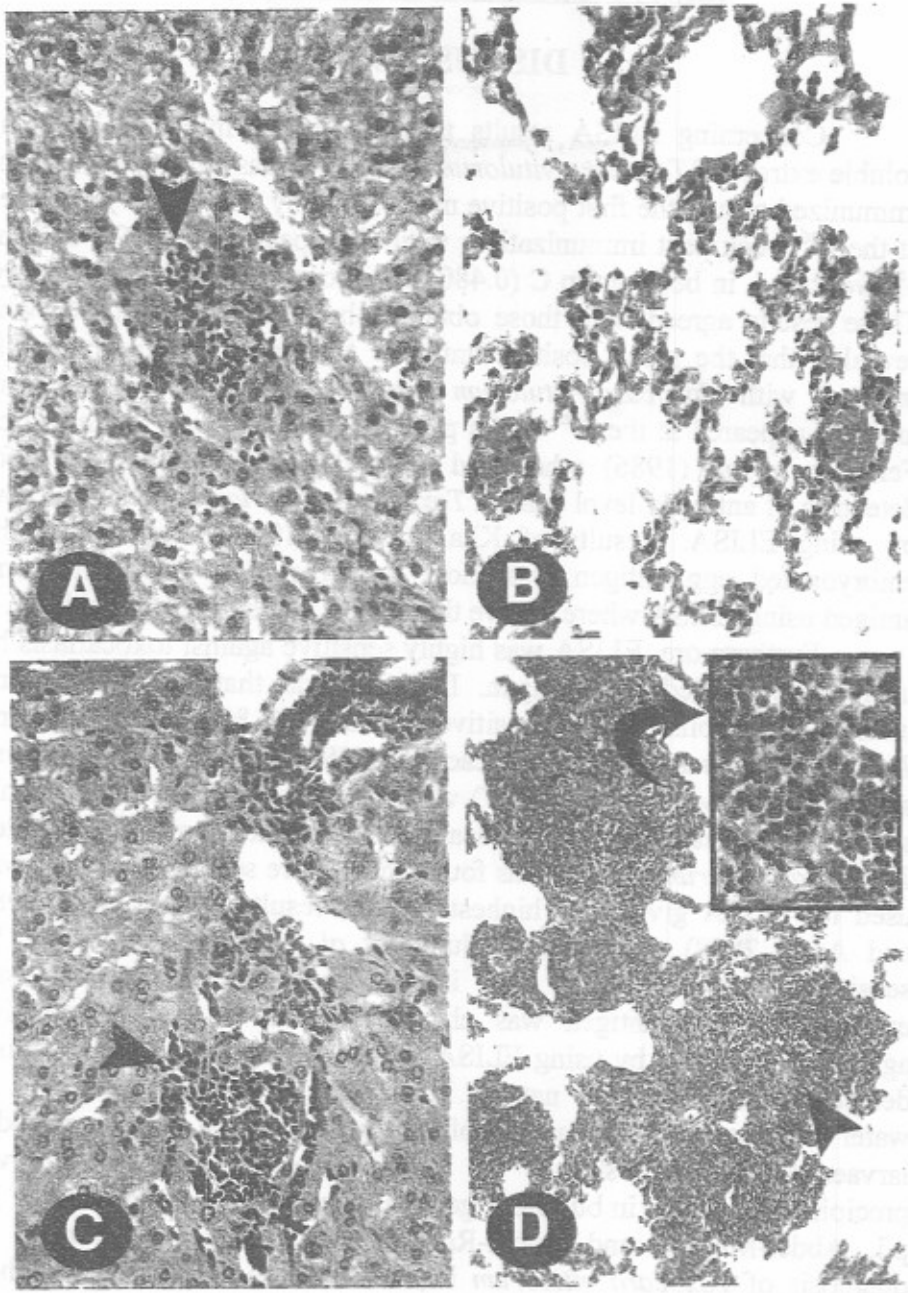

Plate 4: Histopthological picture of both immunized and infected groups
A) Liver in group (A) showing mild eosinophilic infiltration (H & E x 400)
B) Lung in group (A) appeared more or less normal (H & E x 400)
C) Liver in group (B) showing severe eosinophilic infiltration (H & E x 400)
D) Lung in group (B) showing diffused eosinophilic granulomatous reactions (H & E x 200, small square x 1000)

DISCUSSION

Concerning ELISA results to evaluate the infection by using soluble extract of *Toxocara vitulorum* antigen, it was evident that, in the immunized group, the first positive mean antibody titre (0.623) appeared at the 2nd week post immunization; while it appeared in the 2nd and the 3rd weeks p.i. in both group C (0.480) and group B (0.496) respectively. These results agreed with those obtained by Selim *et al.* (1986) who revealed that the mean positive antibody titre in mice experimentally infected with *Toxocara vitulorum* eggs by using the whole worm antigen, appeared at the 2nd weeks p.i. Similar results were obtained by Fernado *et al.* (1986) who used an embryonated egg antigen for detection of antibody level against *Toxocara vitulorum* infection in cows by using ELISA. Results of Khalil *et al.* (1989) showed that the embryonated egg antigen was more sensitive than the adult worm antigen using ELISA where it gave the highest percentage of positivity.

Furthermore, ELISA was highly sensitive against toxocariasis by using embryonated egg antigen. It was found that all infected and immunized rabbits showed positive results (100 % sensitivity) using *Toxocara vitulorum* antigen. Efficacy of ELISA as a diagnostic tool was supported by Cypess *et al.* (1977) who reported that ELISA was highly sensitive in diagnosis of toxocariasis. Extraction of embryonated *Toxocara vitulorum* antigen was found to be more sensitive, since it was used for ELISA giving the highest positive results (Khalil *et al.* 1989 and Arafa 2008). In addition, Nunes *et al.* (1999) revealed 100 % sensitivity after 20 days post inoculation in mice, so the used embryonated egg antigen was characterized to be highly sensitive against toxocariasis by using ELISA. Starke-Buzetti *et al.* (2001) could detect antibodies against natural infection of *Toxocara vitulorum* in water buffaloes by ELISA using soluble extract antigen of the nematodal larvae. Moreover, Usha *et al.* (1997) detected that circumlarval precipitin (CLP) test in buffaloes gave maximum antibody titre at day 14 p.i. Abdel-Megeed and Abdel-Rahman (2003) used 5 antigens in diagnosis of *Toxocara vitulorum* in buffalo calves using ELISA; they reported that egg antigen was the second potent antigen.

Concerning data of characterization of embryonated egg antigen of *Toxocara vitulorum* by using SDS-PAGE, it was evident that bands of molecular weights 240, 135, 40, 33, 28, 24, 22 and 18 KDa were found respectively. More or less similar results were obtained by Starke-Buzetti and Ferriera (2004) who revealed bands of molecular weights

190, 150, 110, 90, 64, 56, 48 and 19 kDa when they used excretory/secretory (E/S) antigen of *Toxocara vitulorum* larvae; the slight variation in the revealed bands might be referred to the type of the antigen used. Results obtained by Abdel-Megeed and Abdel-Rahman (2003) coincided with the present findings, where they detected polypeptide bands of molecular weights 137.7, 81, 75, 48 and 21 kDa. On the contrary, Abdel-Rahman (2000) revealed only 2 polypeptide bands of 92 kDa and 87 kDa when antigen of adult worm is analyzed by SDS-PAGE. This variation might be related to the difference of antigen used. He added that ELISA was more potent with using this antigen and gave 100 % sensitivity. Moreover, Ferreira and Starke-Buzetti (2005) used SDS-PAGE on perienteric fluid antigen obtained from buffalo calves and revealed nine bands of 11, 14, 38, 58, 76, 88, 112 and 165 kDa. They added that the last 3 bands remained during the peak of egg output.

Concerning results of western blot, five bands were recorded at the day 15 p.i. and these bands were 43, 40, 33, 24, 22 and 18 KDa and were identified as early diagnostic bands, and those of 60 and 50 KDa appeared at both 30 and 45 days p.i and they were identified as late diagnostic bands. Similar results were revealed by Barriga and Omar (1992) who stated that specific bands of soluble extract of embryonated eggs antigen against sera of rabbits experimentally infected with *Toxocara vitulorum* by using western blot appeared at day 15 p.i.

Concerning histopathological findings, rabbits of group (B) showed milky spots in the hepatic parenchyma and the pulmonary tissues showed haemorrhagic patches and some nodules. Unlikely, rabbits of group (A) revealed both normal liver architecture and more or less normal lung tissue indicating pulmonary resistance. Similar results were obtained by Gorp *et al.* (1987) who detected that mice experimentally infected with *Toxocara vitulorum* showed no or a low degree of cellular reactions but macroscopic hemorrhages might be observed. Also, Hussein *et al.* (1980) recorded no lesions in rats inoculated with *Toxocara vitulorum* eggs, and only few larvae were detected in their livers and lungs. Moreover, Abeydeera and Roberts (1991) evaluated the response of liver tissue in buffaloes experimentally infected with *Toxocara vitulorum* larvae, by which the lesions produced varied from mild cellular infiltrations in early stages to eosinophilic granulomas with degenerating larvae in later stage of the infection. On studying the course of experimental infection of rabbits with *Toxocara vitulorum* embryonated eggs Omar and Barriga (1991) revealed that size

of muscle larvae did not develop beyond the infective stage suggesting that they were hypobiotic larvae. Furthermore, Barriga and Omar (1992) concluded that, 3 times infections of rabbits with *Toxocara vitulorum* revealed that larvae declined steadily in liver and lungs from day 5 to 30 of the 1st infection, was absent in liver at the day 30 of the 2nd infection, and in both organs in day 30 of the 3rd one. In addition, Pramanik *et al.* (1994) reported that infection of rabbits with *Toxocara vitulorum* showed the highest recovery in liver then in lungs; and larval recovery was highest at day 3 p.i and declined to the lowest one at day 63 p.i.

It is worthy to mention that Srivastava *et al.* (1988) studied the histopathological findings in mice experimentally infected with *Toxocara vitulorum* eggs and found that migration of the larvae lead to degeneration of the hepatic cells together with leucocytic infiltration along the migratory tracts. The results confirmed that the migratory pattern of *Toxocara vitulorum* is closely related to that of *Toxocara canis*.

Finally, vaccine efficacy was evaluated by both positive antibody titre and the hepatic eosinophilic granulomas formation. These findings coincided with those obtained by Amerasinghe *et al.* (1992) showed that mice exposed to three or more infections with *Toxocara vitulorum* eggs lead to protection against the challenge. Following parentral immunization of mice with variety of *Toxocara vitulorum* soluble antigens (extract, E/S or perienteric) from adults, larvae induced statistically significant protection against the infection. They added that, the more immunogenic component was adult perienteric fluid (100 % protection) followed by larval E/S (92 % protection). They proved that oral infection of mice on 3 or more occasions with *Toxocara vitulorum* eggs induced protection against a challenge infection of the parasite. Besides, Rajapaske *et al.* (1994 a) demonstrated antibodies against E/S antigen of *Toxocara vitulorum* in serum and colostrum of buffalo calves by using ELISA and confirmed antibody-mediated protection against *Toxocara vitulorum* larvae by the ability of buffalo serum or colostrum to inhibit migration of *Toxocara vitulorum* larvae in mice.

Furthermore, Rajapaske *et al.* (1994 b) suggested that antibodies detected in mice immunized against *Toxocara vitulorum* might adversely affect the metabolism of larvae through antibody-mediated cell mediated cytotoxicity, where antibodies might bind to the surface of the migrating larvae.

Further studies must be done to find out more specific epitopes derived from *Toxocara vitulorum* embryonated eggs and/or larvae in

order to obtain more specific diagnostic bands that facilitate the arrival to appropriate diagnosis from which the proper vaccine can be obtained.

REFERENCES

Abdel-Rahman, E.H. (2000): Isolation and structural characterization of *Toxocara vitulorum* specific antigen and its potency in diagnosis of toxocariasis among buffalo calves. J. Egypt Soc. Parasitol.; 30 (2):387-400.

Abdel-Megeed, K.N. and Abdel-Rahman, E.H. (2003): Comparative immunodiagnostic approach of toxocariasis in buffalo calves. J. Egypt. Soc. Parasitol., 33 (2): 473 – 484.

Abeydeera, L.R. and Roberts, J.A. (1991): Histopathological study of the buffalo liver in experimental infection with *Toxocara vitulorum* infective larvae. J. Buffaloes, 7 (2): 165-170.

Abo-Shehada, M.N. and Herbert, I.V. (1984): Anthelmintic effect of levamizole, ivermectin, albendazole and fenbendazole on larval *Toxocara canis* infection in mice. Res. Vet. Sci., 36: 87 – 91.

Amerasinghe, P.H.; Massodi, M.A.; Samarasinghe, B.; Sivanathan, S.; Gunawardana, V.K. and Fernando, S.T. (1984): Immunological responses of pregnant swam and Murrah buffalo cows and calves to *Toxocara (Neoascaris) vitulorum* infection. In: The use of nuclear techniques to improve domestic buffalo production in Asia. Int. Atomic Energy Agency, Vienna, Austria., 161 – 170.

Amerasinghe, P.H.; Rajapakse, R.P.; Lloyd, S. and Fernando, S.T. (1992): Antigen-induced protection against infection with *Toxocara vitulorum* larvae in mice. Parasitol. Res., 78 (8): 643 – 647.

Arafa, W.M. (2008): Some studies on *Toxocara* species in model experimental animal. M.V.Sc Thesis, Fac. Vet. Med. Beni-Suef Univ.

Bancroft, J.D. and Stevens, A. (1996): Theory and Practice of Histological Techniques. 4th edition. chukhll livingstone new york.

Barriga, O.O. and Omar, H.M. (1992): Immunity to *Toxocara vitulorum* repeated infections in a rabbit model. Vet. Immunol Immunopathol., 33: 249 – 260.

Chauhan, P.P. and Pande, B.P. (1972): Migratory behaviour and histopathology of *Neoascaris vitulorum* larvae in albino mice. Ind. J. Exp. Biol., 10: 193 – 200.

Cypess, R.H.; Karol, M.H.; Zidian, J.L.; Glickman, L.T. and Gitlin, D. (1977): Larva-specific antibodies in patients with visceral larva migrans. J Infect Dis., 135 (4): 633-640.

Fernando, S.T.; Gunawardana, V.K.; Sivanathan, S. and Samarasinghe, B.T. (1986): Immunological response of pregnant buffalo cows and calves to *T. vitulorum* infection. Livestock production and diseases in the tropics. Proceedings of the 5th Inter. Conference, Kuala Lumpur, Malaysia.

Ferreira, F.P. and Starke-Buzetti, W.A. (2005): Detection of antibody to *Toxocara vitulorum* perieneteric fluid antigens (Pe) in the colostrum and serum of buffalo calves and cows by Western blotting. Vet. Parasitol., 129 (1-2): 119-124.

Gorp, K.V.; Mangelschots, M.; Brandt, J. and Van Gorp, K. (1987): *Toxocara vitulorum* a possible agent of larva migrans in humans. Helminth Zoonoses. Dordecht, Netherlands; Martinus Nijhoff publisher, 159 – 166.

Hussein, M.I.; Dewan, M.L. and Baki, M.A. (1980): Preliminary studies on the efficacy of tetramisole hydrochloride (ICI) against transmammary migration of *Toxocara* (*Neoascaris*) *vitulorum* larvae in buffalo cows. Bangladesh J. Agric. Sci., 7 (1): 25-28.

Khalil, H.M.; Azab, M.E.; Safar, E.H.; Bebars, M. A.; El-Hady, H.M. and Khattab, H. M. (1989): Immunodiagnostics of visceral toxocariasis. J. Egypt Soc Parasitol., 19 (2): 381-393.

Laemmli, U.K. (1970): Cleavage of structural protein during the assembly of the head bacteriophage T4. Nature. 227, 680-685.

Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L. and Randali, R.J. (1951): protein measurement with Folin- phenol reagent. J. Biol. Chem., 193: 265-275.

Mousa, W.M.; Ezz El-Dien, N.M. and Ibrahim, S.F. (2001): Evaluation of three *Toxocara canis* antigens for serodiagnosis of experimentally infected rabbits with visceral larva migrans. J. Egypt. Vet. Med. Ass., 61 (1): 173 – 180.

Nunes, C.M.; Tundisi, R.N.; Heinemann, M.B.; Ogassawara, S. and Richtzenhain, L.J. (1999): Toxocariasis: serological diagnosis by indirect antibody competition ELISA. Rev. Inst. Med. Trop., 41 (2):95-100.

Omar, H.M. and Barriga, O.O. (1991): Biology and pathophysiology of *Toxocara vitulorum* infections in a rabbit model. Vet. Parasitol., 40: 257 – 266.

Pramanik, S.; Basak, D.K. and Mukherjee, G.S. (1994): Pathology of induced *Toxocara vitulorum* infection in rabbits and guinea pigs. Indian J. of Vet. Pathol., 18 (2): 142 – 146.

Rajapakse, R.P.; Lloyd, S. and Fernando, S.T. (1994 a): Toxocara vitulorum: Maternal transfer of antibodies from buffalo cows (*Buablus bubalis*) to calves and levels of infection with *Toxocara vitulorum* in the calves. Res. Vet. Sci., 57: 81 – 87.

Rajapakse, R.P.; Lloyd, S. and Fernando, S.T. (1994 b): The effect of serum and colostrum immunoglobulines from buffaloes infected with *Toxocara vitulorum* on *Toxocara vitulorum* larvae in vitro and in vivo in mice. Parasitol. Res., 80 (5): 426 – 430.

Sabry, M.A. (1999): Epidemiological studies on Toxocariasis in animals and man. Ph.D. Vet. degree (zoonoses) Fac. Vet. Med. Cairo Univ.

Selim, D.A.; Tawfik, M.K.; Ali, L.M. and Ramadan, E.I. (1986): Some immunological studies on visceral larva migrans due to *Toxocara vitulorum* in experimental animals. Assiut Vet. Med. J., 16 (31): 105 – 115.

Srivastava, P.S.; Singh, S.P.; Sinha, H.K.; Sinha, S.R.P.; Sinha, B.K. and Prasad, L.N. (1988): Tissue changes during post intestinal migration of histotrophic larvae of *Toxocara vitulorum* in experimentally infected albino mice. Rivista di Parassitologia, 49 (1): 37-44.

Starke-Buzetti, W.A. and Ferreira, F.P. (2004): Characterization of excretory/secretory antigen from *Toxocara vitulorum* larvae. Ann. N. Y. Acad. Sci., 1026: 210-218.

Starke-Buzetti, W.A.; Machado, R.Z. and Zocoller-Seno, M.C. (1983): Natural course of gastrointestinal helminthic infections in buffaloes in Andradina County, Brazil. Arq. Bras. Med. Vet. Zoot., 40: 758 – 762.

Starke-Buzetti, W.A.; Machado, R.Z. and Zocoller-Seno, M.C. (1992): Transmammary passage of gastrointestinal nematode larvae to buffalo calves. II. *Toxocara vitulorum* larvae. Arq. Bras. Med. Vet. Zoot., 44: 97 – 103.

Starke-Buzetti, W.A.; Machado, R.Z. and Zocoller-Seno, M.C. (2001): An enzyme-linked immunisorbent assay (ELISA) for detection of antibodies against *Toxocara vitulorum* in water buffaloes. Vet. Parasitol., 97 (1): 55 – 64.

Towbin, H.; Stachelin, T. and Gordon, J. (1979): Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets, procedures and some application. Proc. Nati. Acd. Sci. USA., 76: 4350-4354

Usha, S.; Banerjee, D.P. and Ghosh, J.D. (1997): Immunodiagnosis of *Toxocara vitulorum* tissue-phase infection in buffaloes. Indian J. Anim. Sci., 67 (9): 95 – 104.