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ORIGINAL PAPER

ELISA and some biochemical tests of heterophyidae infection in laboratory animals

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Abstract Heterophyiasis is an important food-borne parasitic zoonosis in Egypt, among the inhabitants living around brackish-water lakes especially fishermen, and it is a common human parasite in the Nile Delta. The experiment was done on two laboratory animals (rats and dogs), and the time of sample collection was done periodically at 6, 9, 15, 21, and 28 days post-infection to evaluate different tests required. Whole blood was collected with heparin or ethylenediamine tetra-acetic acid as anticoagulant to help in the hematological studies such as red blood cells count (RBCs), white blood cells count, packed cell volume (PCV), and hemoglobin (Hb). Only marked increase in the total leuckocytic count was recorded while RBCs, PCV, and Hb were decreased in most of the results obtained. Total protein and globulin decreased while albumin and A/G ratio increased. Liver enzymes showing marked increase in aspartate aminotransferase and increase in alanine aminotransferase in dogs and rats denoting that liver has a role in the response to that infection. Kidney-function tests, urea, and creatinine showed slight increase at 6 days post-infection (d.p.i.). After preparation of different Ag (antigen) from different collected helminthes, the protein content of each was determined. The sera of infected animals were collected to find antibodies in their blood against the parasite using enzyme-linked immunosorbent assay and using crude heterophyid antigen collected from their intestines after scarification. The worms washed, homogenized, and then centrifuged to collect supernatant fluid as antigens. The results indicated that antibody starts to appear at 9 d.p.i. and increases till 21 and 28 d.p.i. and detection depends on antigen concentration.

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Introduction

Tilapia species and cat fish (*Clarias lazera*) are common fish species which are consumed in large quantities due to their relatively cheaper price than other fish species. The environment of Egypt and abundance of water canals are helping the intensive production of these fish kinds.

Metacercariae of the family Heterophyidae are liberated from their cysts in all laboratory animals. Full development may occur or the trematodes may live only for a short time and then be expelled (Kuntz and Chandler 1956). Out of 1,593 examined *Tilapia* fish specimens, 566 were found to be infected (34.9%), and the infection rate in the case of *C. lazera* was 39.02%.

Among the final hosts in which the worms reach maturity, some are more suitable than others. Previously, Raef (1994) found that albino rats allowed full development of the heterophyid flukes in their intestines. The viability of *Heterophyid* metacercariae in the muscle of fish hosts is an important factor in acquiring this infection.

Heterophyiasis is an important food-borne parasitic zoonosis in Egypt, among inhabitants living around brackish-water lakes especially fishermen, and it is a common human parasite in the Nile Delta (Rifaat et al. 1980).

The current method of diagnosing heterophyiasis is based on the demonstration of fluke eggs in stool samples (coprological diagnosis). The technique is reliable only when the intensity of infection is high.

Methods of detection is based on immunological principles have resulted in some interest. An enzyme-linked immunosorbent assay (ELISA) method for antibodies detection has been developed.

Serological assays may have potential for the diagnosis of infection when eggs cannot be detected at all times in the fecal samples while antibodies release within a few days of parasitic infections.

Materials and methods

Experimental infection of laboratory animals used for hematology, biochemical, immunoassay, and histopathological studies:

A total number of 100 albino rats (outbreed albino Swiss) with weight of 100–120 g/each were used. These rats were divided into five groups with each group subdivided into two subgroups (ten each) of one as infected and the second left as a negative control group. All infected and control groups were scarified at 6, 9, 15, 21, and 28 days post-infection (d.p.i.). These groups were infected orally with 100–150 encysted metacercariae (EMC) through stomach tube. This experiment was repeated twice.

Rearing and infection of puppies

A total number of six newly born mongrel stray puppies (less than 2 months old) were separated from their mothers and kept individually in sufficient clean screened-bottom wire cages and in hygienic conditions.

The puppies were divided into two groups with one group (of five puppies) infected and the other (of a single puppy) left as a control; each puppy was infected with 4,000–5,000 EMC through the stomach tube.

Prophylactic dose of praziquantal (50 mg/10 kg body weight) as anthelmintic drug was given once a week before experimental infection. The animals were supplied with balanced ration and clean water. The feces of the used animals were carefully examined daily (looking for the eggs of parasites according to the method described by Faust et al. 1976) for 2 weeks before the beginning of the experiment to ensure that they were parasite free.

Collection of adult worms from experimentally infected animals:

A number of experimentally infected rats (five rats each time) were killed at 6, 9, 15, 21, and 28 days post-infection after starvation of 1 day.

The puppies were examined daily for eggs detection. Blood samples were collected at 6, 9, 15, 21, and 28 d.p.i. from the infected dogs for hematological studies and separation of serum for biochemical analysis. The puppies were killed at the end of the experiment.

The small intestines of the infected laboratory animals were removed and immediately examined after dissection in a Petri dish with warm saline under a stereo-microscope, and the mucous membrane was scraped, then incubated at 37°C for 2 h, during which the worms got detached. The grossly recognized worms were collected, counted, and worm burden was estimated. The recovered worms were divided into two sections; one was permanently mounted for identification and the other was used for antigen preparation. Identification of adult worms occurred according to the key of Yamaguti (1958).

Blood samples

Blood samples were taken directly from the medial canthus of the eye by disposable sterile capillary tube from rats while dog's blood samples were collected from the jugular vein.

Whole blood About 1–2-ml whole blood was collected in a clean small plastic tube containing a suitable amount of di-potassium salt of ethylenediamine tetra-acetic acid (EDTA) as anticoagulant. These samples were used for hematological studies.

Blood serum About 3–4-ml whole blood was collected in plain test tubes kept in an inclined position for 20 min at room temperature, then placed in a refrigerator to avoid glycolysis and shrinkage of the clot. The samples were centrifuged at 14,000 rpm for 14 min, and the clear serum was separated, carefully collected, and stored in eppendorf tubes at −20°C until used.

Determination of hemoglobin (Hb) was done according to Van Kampen and Zijlstra (1961).

The packed cell volume (PCV) was estimated according to Coles (1989) using the microhaematocrit method.

Total leuckocytic count was performed using the improved hemocytometer with Tukey's solution as a diluting fluid according to Jain (1986).

Leuckocytic counts

Two glass slides were prepared from each animal. The air-dried film, fixed by methyl alcohol for 2 min then transferred to a staining jar containing freshly prepared Giemsa stain (diluted by adding 1 ml of concentrated stock Giemsa solution to 10-ml neutral distilled water) for 30 min. The stained films washed with water. Differentiation and counting was done according to Coles (1989).

Serum biochemical studies

Determination of serum total protein

Serum total protein was determined using a kit provided by Biogamma according to Doumas (1975).

Determination of albumin

Albumin was determined using a kit provided by Spectrum Diagnostics according to Doumas et al. (1971).

Determination of total globulin

Total globulin was determined mathematically by subtraction of albumin value from total protein value (total globulin = total protein − albumin).

Serum biochemical studies included determination of the activities of serum total protein, albumin, urea, and creatinine.

Determination of ALT and AST

Alanine aminotransferase (ALT = GPT) and aspartate aminotransferase (AST = GOT) were determined using a kit provided by Randox according to Reitman and Frankel (1957).

Determination of urea

Urea was determined using a kit provided by Spectrum Diagnostics according to Shephard and Mezzachi (1983).

Determination of serum creatinine

Creatinine was determined using a kit provided by Spectrum Diagnostics according to Doolan et al. (1962).

Antigen preparation The worms were washed in 0.01 M PBS at pH 7.2 and then individually homogenized for 15 min to disrupt the remaining intact worms. The sonicated materials were centrifuged at 20,000 rpm for 45 min at 4°C. The protein content of the antigen was determined according to Lowry et al. (1951).

Table 1 Haematological picture of infected rats at different days post-infection (mean values±SE)

d.p.i.	PCV (%)	RBCs (10^6/UL)	Hb (gm/dl)
6 d.p.i.	59.06±2.6*	6.5±0.2*	17.68±0.08*
Control	64.9±6	7.2±0.6	19.7±2.09
9 d.p.i.	50.7±5.4*	5.6±0.6*	14.9±1.8*
Control	53.4±2.5	5.9±0.2	15.9±0.75
15 d.p.i.	65.17±6.3**	7.2±0.69**	19.61±2**
Control	62.9±1.5	6.99±0.16	17.9±0.95
21 d.p.i.	56.8±2.5**	6.4±0.3**	16.9±0.85**
Control	53.4±2.5	5.9±0.2	15.9±0.75
28 d.p.i.	58.3±7.7**	6.48±0.8**	17.7±2.3**
Control	62.9±1.5	6.9±0.16	17.9±0.95

d.p.i. days post-infection, *PCV* packed cell volume, *RBCs* red blood cells count, *Hb* hemoglobin

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

Enzyme-linked immunosorbent assay

ELISA was performed according to Iacona et al. (1980); the optimal reaction conditions regarding sensitizing antigen concentration, antibody, and conjugate dilutions were chosen for use with micro-ELISA after preliminary checker board titration. In the present study, the

Table 2 Haematological picture of infected dogs at different days post-infection (mean values±SE)

d.p.i.	PCV (%)	RBCs (10^6/UL)	Hb (gm/dl)
6 d.p.i.	46.06±2.6*	4.7±0.5*	14.2±2.1*
Control	46.6	4.8	14.8
9 d.p.i.	41.7±5.4*	4.1±0.9*	11.9±1.2*
Control	43.4	4.6	15.3
15 d.p.i.	45.17±6.3**	4.4±0.2**	13.41±1.2**
Control	48.9	4.9	14.9
21 d.p.i.	40.8±2.5**	4.9±0.6**	14.9±0.85**
Control	43.4±2.5	5.1	15.2
28 d.p.i.	41.8±3.7**	4.6±0.3**	14.7±2.1**
Control	46.6	4.9	14.9

d.p.i. days post-infection, *PCV* packed cell volume, *RBCs* red blood cells count, *Hb* hemoglobin

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

Table 3 Leuckocytic values of infected rats at different days post-infection (mean values±SE)

d.p.i.	WBCs(10^3/UL)	Neutrophil (%)	Esinophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)
6 d.p.i.	20.16±3*	25±2.7*	2.16±1.2*	1.6±0.95*	55.16±3.3*	16±3.5*
Control	18.4±1.1	40±1.2	5±0	0	47.5±2.5	7.5±2.5
9 d.p.i.	27±3.9*	33±6.6*	4.6±10.0*	0	53.3±2.4*	9±2.64*
Control	15±1.5	38±3.7	2.8±0.81	0.4±0.3	48.4±4.6	10.4±0.7
15 d.p.i.	15±2.1**	31.5±6.5**	1.5±0.9**	0	60±6.6**	7±2.8**
Control	11.4±0.8	34.4±2.3	2±0	0.8±0.4	53.8±1.8	9±1.5
21 d.p.i.	23.1±2.6**	38±1.9**	5±1**	1±1**	47±5**	9±3**
Control	15±1.5	38±3.7	2.8±0.81	0.4±0.3	48.4±4.6	10.4±0.7
28 d.p.i.	24±3.3**	25.5±4.9**	4.5±2.2**	0	61.7±3.6**	8.25±2.71**
Control	11.4±0.8	34.4±2.3	2±0	0.8±0.4	53.8±1.8	9±1.5

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

optimum conditions were 5 μg/ml coating buffer antigen concentration, 1:40 serum dilution, 1:4,000 antimouse polyvalent immunoglobulins (IgG, IgA, and IgM), alkaline phosphatase (Sigma) as conjugate and ABTS solution, and one component (Sigma) as substrate. All incubation steps were carried out at 37°C in a moist chamber. The positivity threshold value was determined as a double fold of the mean cut-off value of negative sera. The application of ELISA is according to Iacona et al. (1980) and El-Dakhly (2003).

Statistical analysis

The obtained results were statistically analyzed using analysis of variance test, mean, standard deviation, and standard error according to Tamhans and Dunlop (2000) to compare between different readings of laboratory animals and control using MINITAB statistical software (copyright 1992. Release 8).

Results

Hematological picture

Concerning hematological picture (Table 1) RBCs, PCV, and Hb increased only at 21 d.p.i. and decrease in all d.p.i.

In dogs (Table 2) at 6 d.p.i., no change was observed in these parameters, but a decreased was noticed in other infected dogs.

Differential leuckocytic (Table 3) white blood cells count (WBCs) was increased in all infected rats, neutrophils, and basophils did not increased than the control while esinophils increased at 9, 21, and 28 d.p.i. Lymphocytes increased in all d.p.i. and decreased at 21 d.p.i. for all groups and—that is the main response—monocyte were increased at 6 d.p.i.

In the dogs' (Table 4) WBCs, esinophils, and lymphocytes were increased than control and neutrophils increased at 6, 9, and 21 d.p.i. Basophils increased at 15,

Table 4 Leuckocytic values of infected dogs at different days post-infection (mean values±SE)

d.p.i.	WBCs (10^3/UL)	Neutrophil (%)	Esinophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)
6 d.p.i.	33.5±1.3*	46±2.9*	8±2.2*	2±0.6*	44±3.6*	4±1.71*
Control	30.2	40	5	2	37.5	7.5
9 d.p.i.	37±2.4*	40±6.6*	9.6±10.0*	2±0.1*	43.3±2.4*	5±2.14*
Control	35.3	38	4	2	38.4	6
15 d.p.i.	35±1.2**	41.5±6.5**	7.5±0.9**	3±0.3**	50±2.4**	5±2.8**
Control	31.4	44.4	3	2	33.8	5
21 d.p.i.	36.1±2.6**	40±1.9**	9±1**	2±0.1**	47±5**	4±3**
Control	35.5	38	4	1	36.8	6
28 d.p.i.	34.5±0.8**	42±2.1**	9.5±2.2**	2±0.4**	41.7±3.1**	4.25±0.71**
Control	32.8	43.4	6	2	33.8	4

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

Table 5 Values of total serum proteins, albumin, and globulin in infected rats at different days post-infection (mean values±SE)

d.p.i.	Serum total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Alb. Glob. ratio
6 d.p.i.	16.95±2.48*	5.85±0.26*	11.1±2.22*	0.52±0.17*
Control	21.82±1.6	5.31±0.2	16.51±1.6	0.3±0.2
9 d.p.i.	16.07±4.9*	5.44±0.07*	10.63±5.01*	0.51±0.05*
Control	19.79±3.98	4.67±0.1	15.12±3.99	0.3±0.02
15 d.p.i.	17±1.67**	5.53±0.37**	11.66±1.32**	0.47±0.28**
Control	20.59±2.27	4.85±0.266	15.74±2.28	0.3±0.11
21 d.p.i.	16.36±2.24**	6.05±0.34**	10.3±2.54**	0.58±0.13**
Control	19.79±3.98	4.67±0.1	15.12±3.99	0.3±0.02
28 d.p.i.	18.03±2.9**	6.21±0.177**	11.82±2.95**	0.52±.06**
Control	20.59±2.27	4.85±0.266	15.74±2.28	0.3±0.11

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

21, and 28 d.p.i. while monocytes showed no change than controls.

Biochemical analysis

Some biochemical tests were performed on the blood and serum of the experimentally infected laboratory animals. These results were used as a guide to clarify the effect of infection on the final host. These results also give an indication in the diagnosis efforts, especially for the early one or developed a utility of detecting the parasite.

Total protein (Tables 5 and 6) in all rats and dogs were lower than the controls, while albumin and A/G ratio in infected rats were higher and globulin was lower than control.

Liver enzymes GPT (ALT) and GOT (AST) in Table 7 of rats GPT (ALT) were elevated in all infected animals than control; also, GOT (AST) elevated except at 9 d.p.i. decreased than control. In Table 8, both enzymes were elevated in the infected dogs.

In kidney-function tests (Table 9), urea was elevated in the infected rats at 6 d.p.i. then returns to normal at 15, 21, and 28 d.p.i. in comparison with the control. Also, creatinine increase in 6 and 21 d.p.i.

In Table 10, urea was elevated in dogs at 6 d.p.i. then returns to normal level while creatinine increased a little at 6 d.p.i. and elevated at 21 and 28 d.p.i. in comparison with the control.

ELISA test

The crude heterophyids extract and cestode crude antigen were used as antigens at different concentration for detecting specific antibody to heterophyid in the serum of infected animals at different days post-infection

Table 11 showed the results of experimentally infected rat serum samples after ELISA test by using different antigen concentration.

When crude heterophyids extract was used at 4 μg/ml in the ELISA test and the mean (0.457) was used as a cut-off limit between positive and negative heterophyiasis, it was found that in experimentally infected animals at 6 d.p.i. 20% were presumptive and 80% were negative while at 9 d.p.i., 10% were positive, 10% were presumptive, and 80% were negative. The sera of infected animals at 15 d.p.i. showed 40% positive, 30% presumptive, and 30% negative while at 21 d.p.i., 50% were positive, 40% presumptive, and 10% negative. At 28 d.p.i., all samples were positive (100%).

Table 6 Values of total serum proteins, albumin, and globulin in infected dogs at different days post-infection (mean values±SE)

d.p.i.	Serum total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Alb. Glob. ratio
6 d.p.i.	20.52±1.3*	5±0.177*	15.52±1.1*	0.32±0.15*
Control	23.82	4.03	19.79	0.2
9 d.p.i.	19.07±4.9*	5.24±0.21*	14.46±4.6*	0.36±0.045*
Control	21.79	4.2	17.59	0.238
15 d.p.i.	19±1.67**	5.03±0.12**	13.97±1.55**	0.36±0.077**
Control	20.59	4.05	16.54	0.24
21 d.p.i.	18.36±2.24**	5.12±0.3**	11.24±1.9**	0.45±0.15**
Control	19.79±3.98	4.1	15.69	0.26
28 d.p.i.	21.03±2.9**	5.21±0.2**	15.82±2.7**	0.329±0.07**
Control	22.88	4.08	18.8	0.22

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

Table 7 Liver function activities of infected rats at different times of infection (mean values±SE)

d.p.i.	ALT (IU/ml)	AST(IU/ml)
6 d.p.i.	15.37±3.1*	119.62±37*
Control	14±10	82.5±6.5
9 d.p.i.	22.5±2.75*	40.25±9.25*
Control	19.5±3.6	62±5
15 d.p.i.	23.5±3.5**	83.25±15.34**
Control	21.25±4.25	64±9.9
21 d.p.i.	25.75±1.9**	84.75±32.17**
Control	19.5±3.6	62±5
28 d.p.i.	28.5±9**	122.5±36**
Control	21.25±4	64±9.9

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

Table 9 Kidney-function activities of infected rats at different days post-infection (mean values±SE)

d.p.i.	Urea (mg/dl)	Creatinine (mg/dl)
6 d.p.i.	35.7±5*	9.27±1.3*
Control	25.4±8.5	8.3±0
9 d.p.i.	35.3±5*	7.88±0.64*
Control	47.65±5.6	9.78±0.67
15 d.p.i.	32.8±5.9**	4.6±1.9**
Control	46.82±2.9	7.9±0.94
21 d.p.i.	38.7±10**	10.73±0.18**
Control	47.65±5.6	9.78±0.67
28 d.p.i.	30.29±4.4**	7.2±1.09**
Control	46.82±2.9	7.9±0.94

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

When crude heterophyids extract was used at 8 μg/ml in the ELISA test and the mean (0.540) was used as a cut-off limit between positive and negative heterophyiasis, it was found that 30% of the experimentally infected animals at 6 and 9 d.p.i. were positive, 20% were presumptive, and 50% were negative. While at 15 d.p.i., 60% were positive, 30% were presumptive, and 10% were negative. The infected animals at 21 and 28 d.p.i. were positive (100%).

When crude cestodes (*Hymenolepis diminuta*) extract was used at 10 μg/ml in the ELISA test, and the mean (0.469) was used as a cut-off limit between positive and negative heterophyiasis, it was found that all experimentally infected animals at 6, 9, and 15 d.p.i. were negative while at 21 and 28 d.p.i., 20% were positive, 10% were presumptive, and 70% were negative.

This revealed that at high concentration of cestode antigens, there was a cross reactivity between cestodes and heterophyid infection especially after 3 weeks of infection.

Discussion

The present study was performed to investigate the immunological aspects and some clinical responses to the infection of laboratory animals with heterophyid parasites. The present work revealed that out of 1,593 examined *Tilapia* fish specimens, 566 were found to be infected (34.9%) and the infection rate in case of *C. lazera* was 39.02%.

Study of hemogram showed unconstant variation of values in PCV, RBCs count, and Hb in rats that increase at 21 d.p.i. and dogs. This coincided with Sung et al. (1990) and Molina et al. (2006) who showed that increase in Hb

Table 8 Liver function activities of infected dogs at different days post-infection (mean values±SE)

d.p.i.	ALT (IU/ml)	AST(IU/ml)
6 d.p.i.	16±9*	76±16*
Control	12	34
9 d.p.i.	23.5±1.05*	38.25±1.2*
Control	18.5	29
15 d.p.i.	23.2±2.5**	43.25±2.1**
Control	16.3	32
21 d.p.i.	28.72±2.8**	44.75±2.3**
Control	16.34	33
28 d.p.i.	30.5±3.2**	72.5±1.3**
Control	18.6	31

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

Table 10 Kidney-function activities of infected dogs at different days post-infection (mean values±SE)

d.p.i.	Urea (mg/dl)	Creatinine (mg/dl)
6 d.p.i.	51.69*	7.29±1.4*
Control	42.37	7.3
9 d.p.i.	51.32*	5.80±0.60*
Control	47.65±5.6	6.78
15 d.p.i.	49.4±2.3**	6.2±1.1**
Control	66.82	6.9
21 d.p.i.	55.7±1.6**	8.71±0.12**
Control	67.65	7.78
28 d.p.i.	48.29±2.3**	7.2±1.09**
Control	56.82	6.9

*$P \leq 0.05$, significant to control group; **$P \leq 0.01$, significant to control group

Table 11 Status of experimentally infected rats serum after ELISA test (optical densities at 405 nm, serum dilution at a dilution of 1:40)

Antigen	Heterophyids extract (4 μg/ml)			Heterophyids extract (8 μg/ml)			Cestode extract (10 μg/ml)		
	+ve %	±ve %	−ve %	+ve %	±ve %	−ve %	+ve %	±ve %	−ve %
6 d.p.i.	0	20	80	30	20	50	0	0	0
9 d.p.i.	10	10	80	30	20	50	0	0	0
15 d.p.i.	40	30	30	60	30	10	0	0	0
21 d.p.i.	50	40	10	100	0	0	20	10	70
28 d.p.i.	100	0	0	100	0	0	20	10	70

+ve confirmed heterophyiasis, *±ve* presumptive heterophyiasis, *−ve* negative

content and decrease in PCV and RBCs count in *Fasciola gigantica* infection. While Ahmed (1996) showed decreases in these results, those of Kumar et al. (1991) and Matanovi et al. (2007) in Fascioliasis of sheep and goat agreed with the obtained results showing low RBCs, PCV, and Hb levels in rat and dogs while Jane et al. (1986) showed increase in these volumes. The occurrence of anemia could be a result of blood loss or due to impaired erythropoiesis produced by a variety of causes including mineral and specific amino acids deficiency from disturbed protein metabolism and toxic marrow suppression, all resulting from chronic parasitism (Jain 1986). It was noticed that anemia in dogs was more pronounced than in rats. The susceptibility to fluke's infestation is known to differ among the laboratory animals, and dogs are considered as an optimum final host in which the development of worms is good and not in rats.

The leukogram in the present study revealed leukopenia due to neutropenia in rats. The number of nutrophils in circulation is dependent on the balance between production and release from the bone marrow, size of the marrow reserve pool and utilization by the tissues. Neutropenia resulted when overwhelming tissue demand and utilization of neutrophils to combat infection was too high than the production and release from the bone marrow, a result agreed with that mentioned by Bush (1991). Also, the cause of lymphopenia in rats observed at 21 d.p.i. can be explained on the basis that lymphocytes which were carried in lymph were lost and never returned to the circulating blood as seen in the case of enteritis. This was proved by histopathological changes in the intestine and mesenteric lymph nodes. On the contrary, leuckocytosis observed in rats and dogs was mostly due to neutrophilia and/or lymphocytosis. The causes of neutrophilia may be attributed to its greater production and release from the bone marrow in response to infestation (Robert and Keith 1986).

Esinophilia was observed in both dogs and rats in the early stages. It is most evident that the parasite invades the tissues denoting an allergic response to parasitic antigen. Responsible antigens react with specific IgE that has been bound to the mast cells and, thus, initiates the histamine release. Esinophiles arrive latter to the affected tissue to neutralize histamine and when localized in the tissue their number begin to be slightly decreased in blood (Bush 1991). The results of esinophilia in this work agreed with Demirci et al. (2006) in fascioliasis and Oliveira et al. (2007) in schistosomiasis.

In the present study, total protein and globulin decreased while albumin and A/G ratio increased (Vercruysse et al. 1988) in goat infected with *Schistosoma* and (Ayaz et al. 2007) in sheep infected with fascioliasis, the albumin decreased and globulin and total protein increased. While (Fahim et al. 2000) in patients infected with *Schistosoma*, the albumin, total protein, and A/G ratio decreased and globulin increased.

The present study showed that the ALT and AST were elevated in the infected animals than the control ones. This result was coordinated with Ahmed (1996) and Shalaby et al. (2003a, b) and similar to Fahim et al. (2000) in the case of *Schistosoma* patient (Manga-Gonz Lez et al. 2004; Gonz Lanz et al. 2006) in *Dicroceolium dendiriticum* but Matanovi et al. (2007) recorded a decrease in AST (GOT) in sheep fascioliasis. Degenerative changes and necrosis in hepatic parenchyma were observed during histopathological examination which might be the contributory factors in their elevation.

Analysis of urea and serum creatinine revealed a slight elevation than normal in some infected animals within different d.p.i. Such increase may indicate reduction in glomerular filtration rate as well as impairment of renal blood flow, and this is supported by the histopathological changes seen in the kidneys. Also, excessive protein catabolism secondary to small bowel hemorrhage and necrosis could be a second cause of increase in blood urea nitrogen in infested animals. This coordinated with Ahmed (1996) and Shalaby et al. (2003a, b) in heterophyid infection and Hassounah et al. (1995) in *Schistosoma* infection while Matanovi et al. (2007) recorded a decrease in these levels with fascioliasis. On the other hand, Lopes Filho and Haddad (1998) recorded no significant alteration in *Schistosoma* patient.

ELISA results by using heterophyids crude somatic antigen showed that specific antibodies start at 9 d.p.i. and increase gradually till 21 and 28 d.p.i., and the detection of specific antibodies in ELISA depends on concentration of antigen. Cross reactivity between cestode and trematode antigen appear only later after 28 d.p.i.; these results agreed with Mahdy et al. (2000) who stated that the antibody levels increased gradually till 21 d.p.i. and nearly remain in constant levels till 30 d.p.i. cross reaction between cestodes and trematode was recorded by several authors: Klimenko and Belozerov (1986) who stated that there was a cross reaction between *F. hepatic* and Hydatiosis and *Cysticercus tenicollis*; Abdel-Rahman and Mageed (2000) and Abdel-Rahman et al. (2003) recorded cross reactions between *F. gigantica*, *Toxocara vitulorum*, and *Moniezia expansa* and cross reaction between *F. gigantica* and *Echinococcus granulosus*, respectively. On the other hand, Wang et al. (1985) stated that there was no cross reaction between *Fasciola hepatica* and *M. expansa*, *C. tenicollis*, and *Coenurus cerebralis*. Elshazly et al. (2008) recorded that the sensitivity percentage of both counter-current immunoelectrophoresis and indirect fluorescent immunoassay were 20 and 40, respectively, for antibody detection at early infection that coincided with our results in using ELISA test.

References

Abdel-Rahman EH, Mageed KN (2000) Molecular identity of major cross reactive adult antigens in *F. gigantica, Toxocara vitulorum* and *Moniezia expansa*. JEgypt Parasit 30(2):561–571

Abdel-Rahman EH, Abdel-Mageed KN, Abu El-Ezz NM (2003) Cross-reaction: a common trail among helminthes. J Egypt Soc Parasitol 33(2):457–471

Ahmed AR (1996) Clinicopathological and histopathological changes associated with experimental infestation of dogs and rats with some fish borne trematodes. Benha Vet Med J 7(2):184–202

Ayaz E, Ertekin A, Ozdal N, Ta Z (2007) Some biochemical parameters in sheep infected with endoparasites (*Fasciola* spp., *Dicrocoelium dendriticum*, hydatid cysts, Trichostrongylidae and Protostrongylidae). Turkiye Parazitol Derg 31(1):57–61

Bush BM (1991) Interpretation of laboratory results for small animal clinicians, 1st edn. Wiley, New York

Coles EH (1989) Veterinary clinical pathology, 4th edn. W.B. Sounders, Philadelphia

Demirci M, Kaya S, Cetin ES, Aridogan BC, Korkmaz M (2006) Eosinophil cationic protein in patients with fascioliasis: its probable effects on symptoms and signs. Scand J Infect Dis 38 (5):346–349

Doolan PD, Aplen EL, Theil GB (1962) A clinical apprasial of the plasma concentration and endogenous clearance of creatinine. Am J Med 32:65

Doumas BT (1975) Standarad for total protein assays—a collaborative study. Clinc Chemist 21:1159–1166

Doumas BT, Watson WA, Biggas HG (1971) Albumin standarad and the measurement of serum albumin with bromocresol green. Clinc Chem Acta 31:87–96

El-Dakhly KM (2003) Characterization and evaluation of specific epitope derived from excretory/secretory antigen against sheep fascioliasis. Ph.D. thesis, Fac. Vet. Med., Cairo University

Elshazly AM, Elsheikha HM, Rahbar MH, Awad SI (2008) Comparison of three immunodiagnostic tests for experimental Heterophyes heterophyes infection in dogs. Vet Parasitol 151(2–4):196–202, Epub 2007 Nov 12

Fahim FA, Esmat AY, Hassan GK, Abdel-Bary A (2000) Biochemical changes in patients with combined chronic schistosomiasis and viral hepatitis C infections. Dis Markers 16(3–4):111–118

Faust EC, Russell PF, June RC (1976) Criag and Faust's Clinical Parasitology, 8th edn. Lea & Febiger, Philadelphia, p 787

Gonz Lanz C, Magna A, Lez MY, Revilla NB (2006) preliminary protective capacity in study of a *D. dedriticum* antigenic protein in hamster. Parasit Res 99(6):749–752

Hassounah OA, Bayoumy AS, Abdel-Wahab MM (1995) Studies on the effect of acute doses of indomethacin (prostaglandins inhibitor) during induced liver failure. J Egypt Soc Parasitol 25 (2):443–452

Iacona A, Pini C, Vicari G (1980) Enzyme linked immunosorbent assay (ELISA) in the serodiagnosis of hydtid cyst. Am J Trop Med Hyg 29(1):95–102

Jain NC (1986) Schalm's veterinary hematology, 4th edn. Lea & Febiger Publisher, Philadelphia

Jane EH, Michos C, Fried B (1986) Clinical and pathological effects of *Echinostoma revolutum* in the golden hamster, *Mesocricetus auratus*. Parasitology 93:505–515

Klimenko VV, Belozerov SN (1986) Diagnostic efficacy of purified antigen-proteinase of *Fasciola hepatica* and the ELISA in sheep fascioliasis. Byulleten Vsesoyuzongo instituta Skryabian 44 (16):31–38

Kumar P, Srivastava AK, Pandey BB, Rai P (1991) Studies on the erythrocytic indexes in experimental opithorchiasis in dogs. Indian Veterinary Medical Journal 15(2):142

Kuntz RE, Chandler AC (1956) Studies on Egyptian trematodes with special reference to the heterophyids of mammals. I. Adult flukes, with descriptions of *Phagicola longicollis* n. sp., *Cynodiplostomum namrui* n. sp., and a *Stephanoprora* from cats. J Parasitol 42:445–459

Lopes Filho GJ, Haddad CM (1998) Late clinical, biochemical, endoscopic and electroencephalographic evaluation of patients with schistosomal portal hypertension treated with distal splenorenal shunt. Int Surg 83(1):42–47

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin-phenol reagent. J Biol Chem 193:265–275

Mahdy OA, Mousa WM, Kandil Omnia M (2000) Some serological and biological control studies on *Heterophyes heterophyes* and *Prohemistomum vivax* in experimentally infected rats. Assuit Vet Med J 43(85):186–198

Manga-Gonz Lez MY, Ferreras MC, Campo R, Gonz Lez-Lanza C, Prez V, Garca Marn JF (2004) Hepatic marker enzymes, biochemical parameters and pathological effects in lambs experimentally infected with *Dicrocoelium dendriticum* (Digenea). Parasitol Res 93(5):344–355, Epub 2004 Jun 10

Matanovi K, Severin K, Martinkovi F, Simpraga M, Janicki Z, Barisi J (2007) Hematological and biochemical changes in organically farmed sheep naturally infected with *Fasciola hepatica*. Parasitol Res 10(6):1657–1661, Epub 2007 Aug 13

Molina EC, Lozano SP, Barraca AP (2006) The relationship between haematological indices, serum gamma-glutamyl transferase and glutamate dehydrogenase, visual hepatic damage and worm burden in cattle infected with *Fasciola gigantica*. J Helminthol 80(3):277–279

Oliveira FL, Frazo P, Chammas R, Hsu DK, Liu FT, Borojevic R, Takiya CM, El-Cheikh MC (2007) Kinetics of mobilization and

differentiation of lymphohematopoietic cells during experimental murine schistosomiasis in galectin-3 mice. J Leukoc Biol 82 (2):300–310, Epub 2007 Apr 24

Raef Azza M (1994) Role of marine fish in transmission of some parasites to animals and birds. Ph. D. thesis (Parasitology), Fac. Vet. Med., Zagazig Univ

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamicpyruvic transaminases. Amer J Clic Path 28:56

Rifaat MA, Salam SA, El-Kholy SI, Hegazi MM, Youssef M (1980) Studies on the incidence of *Heterophyes heterophyes* in man and fish in Dakahlia Governorate. J Egypt Soci Parasitol 10(2):369–373

Robert JD, Keith WP (1986) Veterinary laboratory medicine, 2nd edn. Iowa State University Press, Ames

Shalaby SI, Hassan NR, El-Mahdy MM, Anter OM, Mohamed EB, Neelima G (2003a) Parasitological and pathological studies on extra-intestinal heterophyiasis. J of Parasitic Diseases 27(1):39–46

Shalaby SI, Hassan Nabiha R, Raafat A, Anter Olfat M, El-Mahdy MM, Mohamed IB, Neelima G (2003b) Effect of parasitization by certain heterophyid species in rats and dogs in Egypt. Biological- Memoris 29(1):21–29

Shephard MD, Mezzachi RD (1983) A colorimetric method for the determination of serum urea concentration. Clinc Biochem Revs 4:61–67

Sung TH, Woon GK Sun H, Jae RY, Jong YYC, Eui CK, Soon HL (1990) Changes in histopathological and serological findings of the liver after treatment in rabbit Clonorchiasis. Seoul J of Med 31(2):117–127

Tamhans AF, Dunlop D (2000) Statistics and data analysis from elementary to intermediate. Prentice-Hall, Upper Saddle River

Van Kampen E, Zijlstra WG (1961) Standardization of hemoglobinometry. II. The hemiglobincyanide method. Clinc Chim Acta (6):538–44

Vercruysse J, Fransen J, Southgate VR, Rollinson D, Majeleine W (1988) Clinical pathology of experimental *Schistosoma curassoni* infections in sheep and goats. Res Vet Sci 44(3):273–281

Wang PY, Jin JS, Liu JF, Tang LQ, Yuan WZ (1985) A study on ELISA for *Fasciola hepatica*. Chinese Journal of Veterinary Science and Technology. Zhongguo Shouyi Keji 8:3–7

Yamaguti S (1958) Systema helminthum Vol. 1. The digenetic trematodes of the vertebrates. Parts I and II. Interscience, New York