Hematological and biochemical studies on filariasis of dogs

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Citation

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Abstract

The present work was done on eighty two stray dogs to be investigated for filariasis via hematological and serum chemistry profiles of naturally infested dogs. Out of the examined dogs, 14 (17.1%) were infested with Dipetalonema reconditum, 12 (85.7%) of them were males and 2 dogs (14.3%) were females. Microfilariae appeared as a snake like with a rapidly, forward movement across the microscopic field in wet smear while in Giemsa stained smears showed a coiled or twisted appearance. Hematological studies revealed hemolytic anemia associated with low erythrocyte counts, hemoglobin concentration and hematocrit value. A marked increase in erythrocyte sedimentation rate, reticulocyte, thrombocyte, total and differential leucocytic counts were encountered, in comparison with the control group.
Biochemical analysis of sera from infested dogs showed a significant changes in the determined parameters used for evaluation of liver and kidney functions.
It could be concluded that infestation of dogs with filariasis induced a hemolytic anemia, with disturbance in the liver and kidney functions.

INTRODUCTION

Filariasis is one of the most important parasitic diseases caused by the filaroid nematodes with a world wide distribution and affects both man, animals and birds. In Egypt, while many previous studies on herbivorous animal filariasis were conducted both in Sharkia and other provinces throughout the country ([1,2,3,4,5,6]) few studies dealt with filariasis of dogs ([6,7,8,9]) and no records of filariasis in this animal species in Sharkia province were documented. On the other hand, local studies on hemoparasites in dogs with particular relation to hematological and biochemical dimensions are limited ([10]).

From this point of view and since filariasis of dogs (dirofilariasis) represent a public health hazards to man ([11,12]), this study was conducted to investigate the dogs for filariasis and to study the blood cellular and biochemical changes in naturally filariasis infested dogs.

MATERIALS AND METHODS

DOGS AND BLOOD SAMPLES

Blood samples were collected from 82 middle aged stray dogs and examined directly for filariasis. For haematological and biochemical analysis, blood samples were collected from ten microfilaraemic dogs proved free from internal and external parasites through naked eye, blood and faecal examinations. As a control group, five dogs of a comparable age were treated with Praziquantel (5 mg/kg body weight, orally) and Ivermectin (1 ml/50 kg body weight, subcutaneously) and proved to be free from internal and external parasites through repeatedly naked eye, faecal and blood examinations over a period of three months post treatment were used. Blood samples for haematological and biochemical analysis were divided into two portions as following: The 1st portion (5ml) put in clean dry test tubes containing anticoagulants as sodium citrate 3.8% for determination of erythrocyte sedimentation rate, dipotassium salt of EDTA for studies of erythrogram and leucogram, and ammonium oxalate 1% for platelet counts. The 2nd portion (6ml) put in plain centrifuge tubes, left undisturbed for clotting of the blood and the clear straw-coloured serum was carefully separated after centrifugation at 3000 r.p.m. for 15 minutes and kept in the deep freezing at -200C until subsequent biochemical analysis.

PARASITOLOGICAL STUDIES

Wet smears, modified Knott technique ([13]) as well as Giemsa stained blood films were used to investigate dogs for microfilariae. The microfilariae were measured using a calibrated eye micrometer and photographed using Leitz

microscope (Germany) and Canon digital photo camera (Japan). To study the microfilarial periodicity, blood samples were collected every three hours from three microfilaraemic dogs and used to investigate the day periodicity of microfilariae using the technique of Ezzat and Tadros ([14]). In brief, 0.5 ml of freshly collected blood was added to 1.5 ml of 2% glacial acetic acid in distilled water tinged with gentian violet. After thorough mixing, the tubes were left for 5 min. then the number of microfilariae was counted in 0.1 ml of the mixture and multiplied by 40 to give the number of microfilariae in one ml blood.

HEMATOLOGICAL ANALYSIS

The hematological parameters included erythrocyte sedimentation rate (ESR), red blood cell count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), reticulocyte count (using Brillient cresyl blue stained film), platelet count as well as total and differential leucocytic counts were performed using standard techniques as described by Feldman et al. ([15]). The blood indices included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

BIOCHEMICAL ANALYSIS

Serum samples were colorimetrically analyzed for the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (total, direct & indirect), glucose, total proteins, albumin, globulins (calculated as the difference between total proteins and albumin) as a biochemical indicators for liver function. Serum levels of urea nitrogen, creatinine, inorganic phosphorus, calcium, sodium, potassium and magnesium were used for evaluation of kidney function. All the biochemical analyses were measured using the determination methods according to manufacturer instructions (kits from Bio-merieux, France).

STATISTICAL ANALYSIS

The obtained data in this study were computed and statistically analyzed using student's “t” test according to Tamhane and Dunlop ([16]).

RESULTS

PREVALENCE OF FILARIASIS OF DOGS

Out of 82 examined dogs, 14 dogs (17.1%) were proved to be infested with Dipetalonema reconditum according to the microfilarial identification. Out of 14 infested dogs, 12 dogs (85.7%) were males and 2 dogs (14.3%) were females.

MORPHOLOGY OF THE MICROFILARIA

In wet blood smears, the microfilariae appeared as a snake like with a rapidly, forward movement across the microscopic field. Stained microfilariae appeared coiled or twisted to various degrees (plate 1, A). The microfilarial length varied from about 250 – 260 µm (aver. 255± 2.4 µm), while the diameter varied from about 3.5 – 4.5 µm (aver. 4± 0.24 µm). The anterior end of the microfilariae devoid from nuclei to a distance about 7 – 8 µm (aver. 7 ± 0.45 µm), (plate 1, B). The nerve ring and excretory pore located at about 28 – 32 µm (aver. 30± 0.68 µm) and 40 – 44 µm (aver. 42± 0.84 µm) from the anterior end, respectively. The anal pore located at about 60 – 70 µm (aver. 65±0.98 µm) from the tail end which showed mostly a hooked appearance (plate 1, C).

Figure 1

Plate 1: The microfilaria of , Giemsa stained. (A): The whole microfilaria (Bar = 30 Âµm), (B): The anterior end of the microfilaria with no nuclei (Bar = 7 Âµm), (C): The posterior end of the microfilaria showing a characteristic hooked tail (Bar = 10 Âµm).

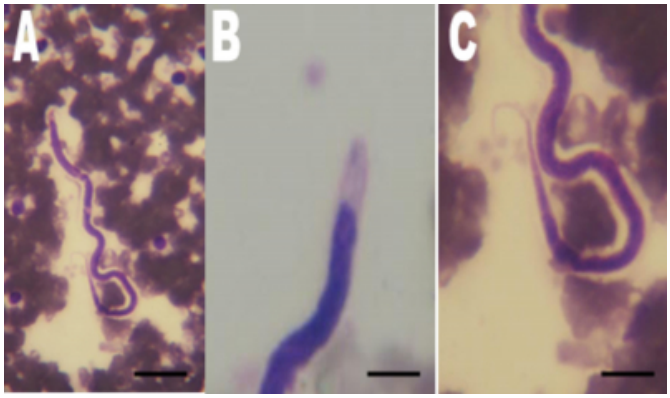
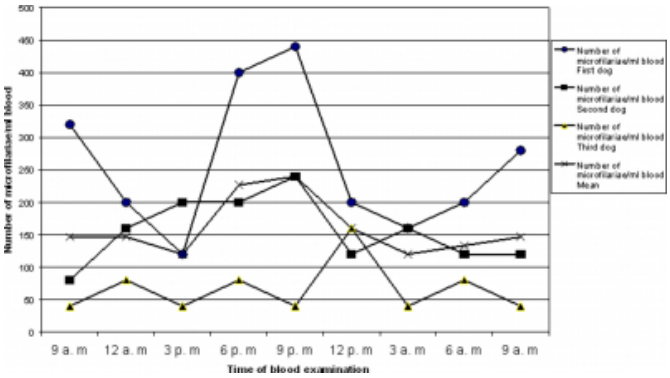


Figure 2

Figure 1: , a summer day microfilarial periodicity.



MICROFILARIAL PERIODICITY

As shown in Fig. 1, the number of microfilariae increased

significantly in the peripheral blood toward the evening (nocturnal periodicity) and peaked between 6 – 9 p. m.

BLOOD CELLULAR FINDINGS

Blood cellular analysis of Dipetalonema reconditum infested dogs revealed a significant reduction in RBCs counts, Hb content, PCV value and increase in reticulocyte count, MCV, MCH with a decrease in MCHC, indicating the presence of regenerative anemia of macrocytic hypochromic type. The values of ESR, reticulocyte, thrombocyte, total and differential leucocytic counts were significantly increased, in comparison with the control group (table 1).

BIOCHEMICAL FINDINGS

Liver function tests of sera from infested dogs showed a significant (P≤0.01) increase in the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serum bilirubin (total & indirect), total proteins, globulins and a decrease (P≤0.05) in the serum values of glucose and albumin, with insignificant change in the serum direct bilirubin, when compared with control (table 2).

Kidney function tests in infested dogs revealed a significant increase in serum urea nitrogen (P≤0.01), creatinine, inorganic phosphorus, potassium and a decrease in the serum calcium and sodium levels (P≤0.05) while, the serum magnesium level showed insignificant change, comparatively with control (table 3).

Figure 3

Table 1: Blood cellular parameters in the control and infested dogs with (Mean values ± SD).

Parameters	Normal dogs	Infested dogs
Erythrocyte sedimentation rate (mm/h)	2.45 ± 0.12	3.73 ± 0.16*
Red blood cell count (x10^6/µl)	6.5 ± 0.45	3.4 ± 1.12**
Hemoglobin concentration (g/dl)	16.0 ± 1.12	9.5 ± 1.12**
Packed cell volume (%)	40.0 ± 2.42	28.0 ± 1.12**
Mean corpuscular volume (Fl)	61.53 ± 5.6	82.35 ± 1.12*
Mean corpuscular hemoglobin (Pg)	24.61 ± 3.15	27.94 ± 1.12*
Mean corpuscular hemoglobin concentration (%)	40.0 ± 4.37	33.92 ± 1.12*
Reticulocytes (%)	1.2 ± 0.02	2.5 ± 1.12**
Platelets (x10^3/µl)	450± 17.61	760± 17.61**
Total leukocytic count (x10^3/µl)	8.5 ± 1.12	16.6 ± 1.12**
Segmented Neutrophils (x10^3/µl)	4.0 ± 0.2	6.5 ± 0.2*
Band neutrophils (x10^3/µl)	0.1 ± 0.001	0.4 ± 0.01**
Lymphocytes (x10^3/µl)	3.4 ± 0.15	5.4 ± 0.15*
Monocytes (x10^3/µl)	0. 7 ± 0.05	2. 5 ± 1.35**
Eosinophils (x10^3/µl)	0.3 ± 0.01	1.8 ± 1.25**

*Significant at probability ≤0.05 **Significant at probability ≤0.01

Figure 4

Table 2: Liver function tests in the control and infested dogs with (Mean values ± SD).

Parameters	Normal dog	Infested dog
Alanine aminotransferase (U/l)	55.24±2.05	120.66 ± 4.30**
Aspartateaminotransferase (U/l)	80.0 ± 1.4	150.0 ± 5.33**
Total bilirubin (mg/dl)	0.4 ± 0.06	1.05 ± 0.2**
Direct bilirubin (mg/dl)	0.15 ± 0.01	0.15 ± 1.12
Indirect bilirubin (mg/dl)	0.25 ± 0.03	0.90 ± 0.02**
Glucose (mg/dl)	86.0 ± 1.76	40.8 ± 1.12*
Total protein (g/dl)	7.00 ± 0.75	8.95 ± 0.16*
Albumin (g/dl)	3.70 ± 0.55	3.0 ± 0.04*
Globulins (g/dl)	3.30 ± 0.61	5.95 ± 0.32*

*Significant at probability ≤0.05 **Significant at probability ≤0.01

Figure 5

Table 3: Kidney function tests in the control and infested dogs with (Mean values ± SD).

Parameters	Normal dog	Infested dog
Urea nitrogen (mg/dl)	25.0 ± 1.77	73.92 ± 2.04**
Creatinine (mg/dl)	1.2 ± 0.02	1.5± 0.02*
Inorganic phosphorus (mg/dl)	2.8 ± 0.02	3.5 ± 1.12*
Calcium (mg/dl)	10.5 ± 1.1	8.4 ± 1.12*
Sodium (mEq/l)	141.4 ± 12.5	125.8 ± 17.61*
Potassium (mEq/l)	4.5 ± 1.12	6.6 ± 0.72*
Magnesium (mg/dl)	1.9 ± 0.2	2.0 ± 0.2

*Significant at probability ≤0.05 **Significant at probability ≤0.01

DISCUSSION

In the present study, a survey was conducted to investigate the dogs for filariasis, as well as the blood cellular and biochemical changes in naturally infested dogs. Out of 82 examined dogs, 14 (17.1%) were infested with Dipetalonema reconditum. A nearly similar infestation rates of dogs with Dipetalonema reconditum were also reported, in which 22.6% of dogs were infested in Brazil([17])and 15.9% infestation rate was reported in dogs from South Italy ([18]).While, lower infestation rates with Dipetalonema reconditum were also recorded in dogs in Egypt and other countries, where 0.063% of dogs from Abu Rawach, Giza, Egypt proved to be infested (9), 1.0% of dogs in Spain were infested ([19]), less than 0.5% infestation rate in the State of Washington ([20]) and 6% in Western Sicily, Italy ([21]). Reasons for these differences in infestation rates in these studies may be attributed to the locality, distribution and prevalence of the arthropod vectors of this parasite such as fleas, lice and ticks, which in great part affected by the different climatic conditions in these regions as well as the methods of examination of dogs for filariasis. High infestation rate was recorded in male dogs (85.7%) than in females (14.3%). Similar results were stated by Falls and Platt ([22]) and Amer ([9]). This is might be returned to hormonal effect on

susceptibility of dogs to infestation.

Regarding the observed characteristic morphological features of Dipetalonema reconditum microfilariae in this study, there was no contradiction with the previous descriptions ([23,24,25,26]). Concerning with the microfilarial periodicity of Dipetalonema reconditum, this study showed a nocturnal periodicity of the microfilariae and peaked between 6 – 9 p. m. These results were to some extent in agreement with the results of Newton and Wright ([13]) who reported a nocturnal periodicity of microfilariae of Dipetalonema reconditum with two peaks at 6.0 p. m and 12 p. m. and Korkejian and Edeson ([27]) who noticed nocturnal periodicity of microfilariae of Dipetalonema reconditum. Also, Amer ([9]) observed increase the number microfilariae of this parasite in peripheral blood of infested dogs between 6.30 p. m – 10.30 p. m during the different seasons of the year.

Concerning the hematological results in the present work, a regenerative anemia of macrocytic hypochromic type associated with a reduction in the RBCs count, Hb concentration and PCV value were recorded, with an increase of ESR, reticulocyte, thrombocyte, total and differential leucocytic counts. The macrocytosis and hypochromasia were due to reticulocytosis that seen in the infested dogs with microfilariae. The present anemia may be attributed to the hemolysis of RBCs as a result of destructive motility of microfilaria as reported by Ishihara et al. ([28]) and ([29]) and Kitagawa et al. ([30]) who showed a severe intravascular hemolysis with a significant reduction of RBCs count and Hb concentration in dogs with dirofilarial hemoglobinuria. Ziegler et al. ([31]) found intravascular hemolytic anemia (macrocytic up to 80 days after infection, subsequently normocytic and hypochromic), accompanied by reticulocytosis in the rodent, Mastomys natalensis, infested with Litomosoides carinii. Similar findings were obtained by previous authors ([10,32,33,34]).Moreover, Sharma and Joshi ([35]) showed a decrease in the erythrogram of microfilariae infested cattle. Reifur et al. ([17]) reported a significant macrocytic anemia in dogs infested with three different microfilariae: Dirofilaria immitis, D. reconditum, and the third (mf3) were not identified. The latter authors mentioned that D. reconditum was the species with the highest prevalence (22.6%), while Dirofilaria immitis was 5.47%. Our results disagree with Anuchai et al. ([36]) who found moderate microcytic anemia and severe thrombocytopenia in 7 dogs infested with dirofilariasis, ehrlichiosis, and babesiosis. The difference may be attributed to the complicated infestations in these dogs, while in our study; we found only D. reconditum microfilariae.

The higher ESR value in infested animals may be due to the anemia. It may also be due to auto-agglutination that is observed in this disease during infection. The increase in ESR has been observed in many other diseases where autoagglutination of red blood cells takes place as in malaria and tuberculosis ([37]). Similar results were obtained in canine with dirofilariasis ([10]), in microfilariae-infested cattle ([35]), in haemoparasitized camels with Trypanosoma evansi and Dipetalonema evansi,([38]) and in an owl with microfilaraemia ([39]).

Thrombocytosis observed in hemoparasitised dogs could be related to the hemolytic anemia ([10, 40]). On contrary, thrombocytopenia was obtained by Rawlings ([41]) and Anuchai et al. ([34]) in dogs infested with Dirofilaria immitis.

The leukogram revealed a marked leucocytosis with neutrophilia, eosinophilia, lymphocytosis and monocytosis. The higher blood neutrophil and monocyte counts were for the phagocytic removal of tissue breakdown products or microfilariae. Similarly, Paltrinieri et al. ([42]) showed neutrophilic leucocytosis in dogs with dirofilariasis. The observed eosinophilia was due to sensitivity to the foreign protein of a parasite which may be a part of an immune phenomenon ([15]). The lymphocytosis which develops in dogs infested with blood parasite is presumably due to intense antigenic stimulations which increase the demands for lymphocytes to be transformed into plasma cells for antibodies production.Yamagata et al. ([43]) found lymphocytosis with increases in IgE values in dogs experimentally co-infested with Dirofilaria immitis and Ancylostoma caninum. The authors mentioned that parasitic nematodes that undergo blood and tissue migrations increased IgE and IgG values. The results of the leukogram were in agreement with the findings of others ([10, 34, 35, 41, 44]).

Concerning the biochemical results, an increase in the serum enzyme activities (ALT & AST), serum bilirubin (total & indirect) and a decrease in the serum glucose level were observed in the dogs infested with D. reconditum, when compared with the non infested one. The increased serum enzymes and hypoglycemia demonstrated in microfilariaemic dogs suggested liver dysfunction secondary to circulatory disturbance. In addition, the hypoglycemia was attributed to glucose consumption by the Dipetalonema viteae and B. pahangi parasites ([45]). The hyperbilirubinemia

(total & indirect) may be attributed to hemolytic anemia with resultant hemolytic jaundice. The obtained results were in harmony with earlier findings ([10, 31, 34, 36, 38,46]).

Protein profile of serum samples showed an increase in the total protein and globulins concentration with a decrease in the albumin values in the infested dogs with microfilariae comparatively with non-infested one. The observed hyperproteinemia can be attributed on the one hand to an increase in the ?-globulin concentration in response to the parasitic antigens and on the other had to a release of hemoglobin from destructed erythrocytes ([47]).The obtained hypoalbuminemia probably corresponds to the degenerative changes in the haemoparasitized organs (mainly liver). Similar results have been reported previously ([32, 35, 38, 48]).

The significantly higher serum urea nitrogen, creatinine, inorganic phosphorus, potassium and lower serum calcium and sodium levels in infested dogs than in non infested one might result from more severe kidney dysfunction, metabolic acidosis, as well as intravascular hemolysis ([30, 32, 36]).

In conclusion, a hemolytic anemia with disturbance in the liver and kidney functions were the main results in canine filariasis, caused by Dipetalonema reconditum.

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