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PARASITOLOGICAL, BLOOD CELLULAR AND BIOCHEMICAL STUDIES ON FILARIASIS OF DOGS

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SUMMARY

The present work was done on eighty two stray dogs in three localities in Sharkia province to be investigated for filariasis, 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. The total length of the microfilariae ranged from 250 – 260 µm (aver. 255 µm) and the breadth was 3.5 – 4.5 µm (aver. 4 µm). The anterior end of the microfilariae devoid from nuclei to a distance of 7 – 8 µm (aver. 7 µm) while the posterior end showed a hooked tail. The micofilariae showed a nocturnal periodicity. This is the first record of filariasis in dogs in Sharkia province.

Hematological studies revealed hemolytic anemia (macrocytic hypochromic type) associated with low erythrocyte counts, hemoglobin concentration and hematocrit value. A marked increase in erythrocyte sedimentation rate (ESR), 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 increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, serum bilirubin (total & indirect), total proteins, globulins, urea nitrogen, creatinine, inorganic phosphorus, potassium and a decrease in serum glucose, albumin, calcium, and sodium values, with insignificant change in the magnesium level.

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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 (El-Seify et al., 1990; Sakla, 2000; El-Massry and Derbala, 2000; Arafa, 2002; Mahran, 2004 and Bahnass, 2005), few studies dealed with filariasis of dogs (Fahmy, 1972; Ahmed et al., 1986 and Amer, 1986) 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 (Sharma and Pachauri, 1982).

From this point of view and since filariasis of dogs (dirofilariasis) represent a public health hazards to man (Gorezis et al., 2006 and Sathyan et al., 2006), this study was conducted to investigate the dogs in Sharkia province for filariasis and to study the blood cellular and biochemical changes in naturally filariasis infested dogs.

MATERIALS AND METHODS

1- Dogs and blood samples:

82 middle aged stray dogs were collected from Zagazig, Burdain and El-Hosaniah regions. Blood samples were collected 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 (ESR), dipotassium salt of EDTA (Ethylene Diamine Tetra-Acetate) 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 -20°C until subsequent biochemical analysis.

2- Parasitological studies:

Wet smears, modified Knott technique (Newton and Wright, 1956) as well as Giemsa stained blood films were used to investigate dogs for mi-

crofilariae. 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 (1958). 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.

3- 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. (2000). The blood indices included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

4- 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).

5- Statistical analysis:

The obtained data in this study were computed and statistically analyzed using student's "t" test according to Tamhane and Dunlop (2000).

RESULTS

1- 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.

2- 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).

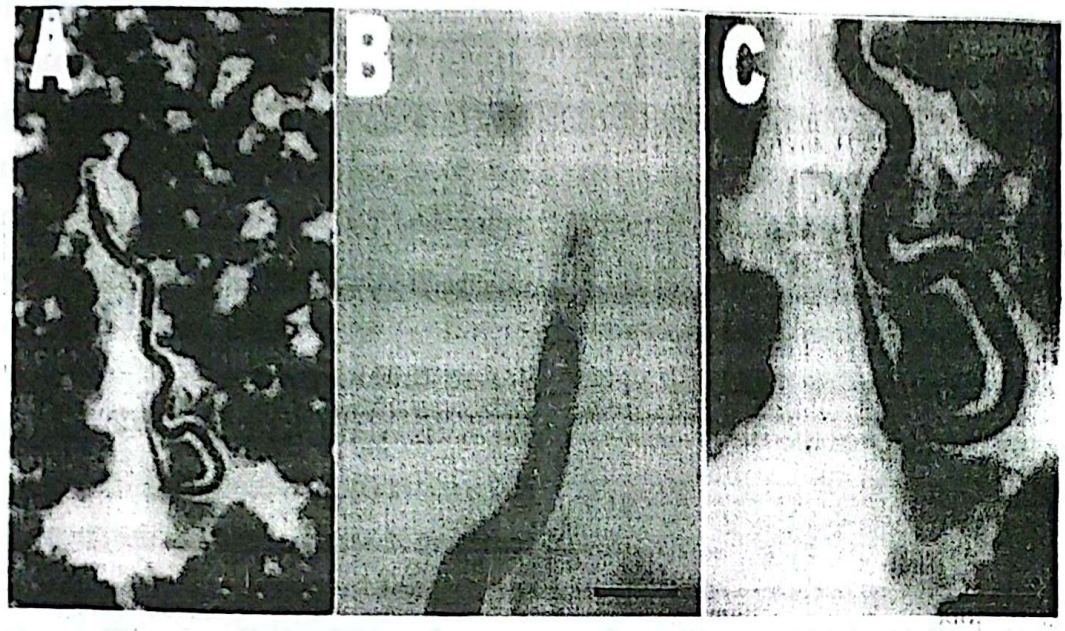

Plate 1: The microfilaria of *Dipetalonema reconditum*, Giemsa stained. (A): The whole microfilaria (Bar = 30 µm), (B): The anterior end of the microfilaria with no nuclei (Bar = 7 µm), (C): The posterior end of the microfilaria showing a characteristic hooked tail (Bar = 10 µm).

Fig. 1: ***Dipetalonema reconditum*, a summer day microfilarial periodicity.**

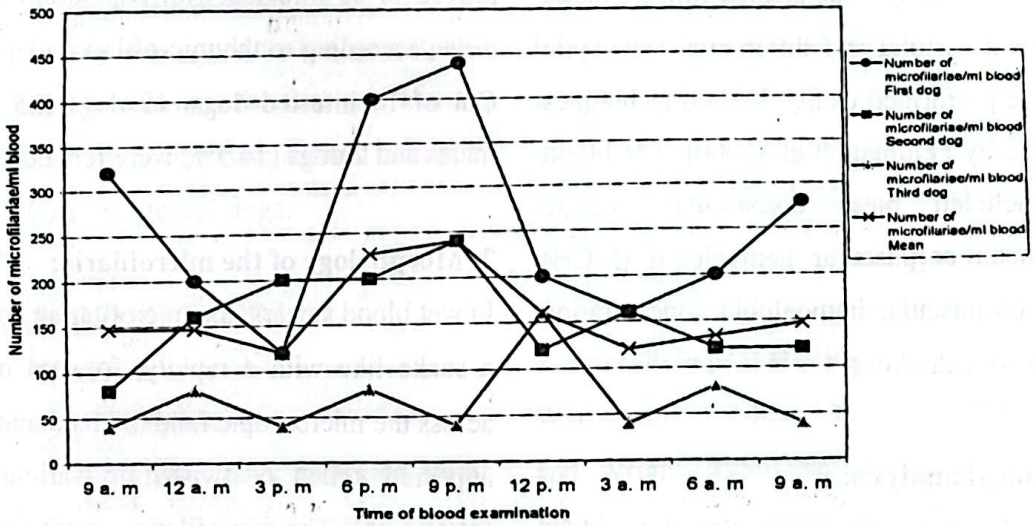

Fig. 1: *Dipetalonema reconditum*, a summer day microfilarial periodicity.

3- 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.

4- 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).

5- Biochemical findings:

Liver function tests of sera from infested dogs showed a significant ($p \leq 0.01$) increase in the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serum bilirubin (total & indirect), total proteins, globulins and a decrease ($p \leq 0.05$) 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 ($p \leq 0.01$), creatinine, inorganic phosphorus, potassium and a decrease in the serum calcium and sodium levels ($p \leq 0.05$) while, the serum magnesium level showed insignificant change, comparatively with control (table 3).

Table 1: Blood cellular parameters in the control and infested dogs with *Dipetalonema reconditum* (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 ($x10^6/\mu l$)	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 ($x10^3/\mu l$)	450± 17.61	760± 17.61**
Total leukocytic count ($x10^3/\mu l$)	8.5 ± 1.12	16.6 ± 1.12**
Segmented Neutrophils ($x10^3/\mu l$)	4.0 ± 0.2	6.5 ± 0.2*
Band neutrophils ($x10^3/\mu l$)	0.1 ± 0.001	0.4 ± 0.01**
Lymphocytes ($x10^3/\mu l$)	3.4 ± 0.15	5.4 ± 0.15*
Monocytes ($x10^3/\mu l$)	0. 7 ± 0.05	2. 5 ± 1.35**
Eosinophils ($x10^3/\mu l$)	0.3 ± 0.01	1.8 ± 1.25**

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

Table 2: Liver function tests in the control and infested dogs with *Dipetalonema reconditum* (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

Table 3: Kidney function tests in the control and infested dogs with *Dipetalonema reconditum* (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 in Sharkia province 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 (Reifur et al., 2004) and 15.9% infestation rate was reported in dogs from South Italy (Cringoli et al., 2001). 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 (Amer, 1986), 1.0% of dogs in Spain were infested (Ortega-Mora et al., 1991), less than 0.5% infestation rate in the State of Washington (Theis et al., 2001) and 6% in Western Sicily, Italy (Giannetto et al., 1997). 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 (1982) and Amer (1986). 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 (Nelson, 1962; Kelly, 1973; Watson et al., 1973 and Soulsby, 1982).

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 (1956) 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 (1978) who noticed nocturnal periodicity of microfilariae of *Dipetalonema reconditum*. Also, Amer (1986) 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 hypochro-

masia 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. (1978 and 1981) and Kitagawa et al. (1989) who showed a severe intravascular hemolysis with a significant reduction of RBCs count and Hb concentration in dogs with dirofilarial hemoglobinuria. Ziegler et al. (1991) 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 Sharma and Pachauri (1982), Kitagawa et al. (1998), Nielsen et al. (2006) and Anuchai et al. (2007). Moreover, Sharma and Joshi (2002) showed a decrease in the erythrogram of microfilariae infested cattle. Reifur et al. (2004) 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. (2006) 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 autoagglutination 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 (Hagan and Bruner, 1991). Similar results were obtained by Sharma and Pachauri (1982) in canine with dirofilariasis, Sharma and Joshi (2002) in microfilariae-infested cattle, Shafqaat et al. (2004) in haemoparasitized camels with *Trypanosoma evansi* and *Dipetalonema evansi*, and Bedin et al. (2007) in an owl with microfilaraemia.

Thrombocytosis observed in hemoparasitised dogs could be related to the hemolytic anemia (Makiya, 1997 and Sharma and Pachauri, 1982). On contrary, thrombocytopenia was obtained by Rawlings (1982) and Anuchai et al. (2007) 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. (1998) showed neutrophilic leucocytosis in dogs with dirofilariasis. The observed eosinophilia was due to sens

tivity to the foreign protein of a parasite which may be a part of an immune phenomenon (Feldman et al., 2000). 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. (1995) 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 Rawlings (1982), Sharma and Pachauri (1982), Gossett et al. (1987), Sharma and Joshi (2002) and Anuchai et al. (2007).

Concerning the biochemical results, increases 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 (table 2).

The increased serum enzymes and hypoglycemia demonstrated in microfilariaemic dogs suggested liver dysfunction secondary to circulatory disturbance. Moreover, Court et al. (1986) attributed the hypoglycemia to glucose consumption by the *Dipetalonema viteae* and *B. pahangi* parasites. The hyperbilirubinemia (total & indirect) may be attributed to hemolytic anemia with resultant hemolytic jaundice. The obtained findings and interpretations were in harmony with those of Sharma and Pachauri (1982), Ziegler et al. (1991), Kitagawa et al. (1997), Shafqaat et al. (2004), Anuchai et al. (2006) and Anuchai et al. (2007).

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 γ-globulin concentration in response to the parasitic antigens and on the other had to a release of hemoglobin from destructed erythrocytes (Moustafa et al., 1991). The obtained hypoalbuminemia probably corresponds to the degenerative changes in the haemoparasitized organs (mainly liver). Similar results have been reported by Safwat and El–Abdin (1982), Kitagawa et al. (1998), Sharma and Joshi (2002) and Shafqaat et al. (2004).

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 (Kitagawa et al., 1989 and 1998 and Anuchai et al., 2006).

For conclusion, this is the first clinico-pathological studies on canine filariasis in Egypt caused by *Dipetalonema reconditum* with a new record for this parasite in Sharkia province.

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