

Animal Health Research Institute
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**STUDIES ON SOME BLOOD PARASITES OF DOGS
IN ASSIUT GOVERNORATE WITH SPECIAL
REFERENCE TO MICROFILARIAE**
(With 5 Tables and 2 Plates)

By

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(Received at 15/3/2010)

دراسات على بعض طفيليات الدم في الكلاب بمحافظة أسيوط وخاصة
يرقات الفيلايريا

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تم عمل مسح طفيلي على عدد ١٤٥ كلب (١٢٥ من الكلاب الضالة من مناطق مختلفة في محافظة أسيوط، و ٢٠ من الكلاب المستوردة الشبرد التابعة لقسم الشرطة بمدينة أسيوط) وذلك لاستبيان معدل الإصابة بطفيليات الدم في هذه الحيوانات خلال الفترة من مارس ٢٠٠٩م حتى فبراير ٢٠١٠م. بلغت نسبة الإصابة الكلية بنوع واحد أو أكثر من طفيليات الدم ٦٥,٥١٪/موزعة كالتالي: يرقات الفيلايريا ١٤,٤٨٪ ، بابيزيا كانز ٢٣,٤٤٪/وهيباتوزون كانز ٢٧,٥٥٪. كان معدل العدوى في الكلاب الضالة بطفيليات الدم المختلفة ٧٢٪ في حين بلغ في الكلاب الأجنبية ٢٥٪ (دلاله احصائية عالية). وقد اوضحت الدراسة زيادة معدل الإصابة بطفيليات الدم المختلفة مع تقدم عمر تلك الحيوانات. وتم تصنيف الميكروفيلايريا إلى نوعين هما ديروفيلاريا إمييس وديروفيلاريا ريبنس وقد بلغت نسبة الإصابة بهما في الكلاب الضالة ٣,٢٪ و ١٣,٦٪ على التوالي بينما لم يتم العثور على اى من النوعين في الكلاب الأجنبية. وقد استخدمت أربعة طرق مختلفة لتشخيص والتعرف على أنواع الميكروفيلايريا المختلفة وهي: مسحات الدم الرطبة (الغير جافة) مسحات الدم السمكة، طريقة الترسيب الشعري وطريقة اختبار نوت واتضح أن الفحص الطفيلي بطريقة نوت هو أدق الطرق للتشخيص والتعرف على أنواع الميكروفيلايريا المختلفة حيث بلغت نسبة دقتها في تشخيص الميكروفيلايريا ٦٦,٦٦٪. كانت أعلى نسبة إصابة بطفيليات الدم المختلفة خلال فصل الصيف ٩٥,٥٪ والربيع ٧٨,٥٧٪ كانت نسبة الإصابة أقل في فصل الشتاء ٢٥٪ وقد وجدت دلاله احصائية عالية لنسبة الإصابة الموسمية وقد يفسر ذلك بالارتباط الوثيق بين هذه الطفيليات وانتشار وتكاثر الحشرات الناقلة للطفيليات في هذا الوقت من السنة.

SUMMARY

A parasitological survey was carried out on 145 dogs (125 stray dogs from different localities in Assiut Governorate and 20 foreign breed

Shepherd dogs in the security unit at Assiut city) to estimate the prevalence of different blood parasites during the period from March 2009 till February 2010. Out of 145 examined dogs 95 (65.51%) were infected with one or more parasitic species, 21 (14.48%) were positive for microfilariae, 34 (23.44%) with *Babesia canis* and 40 (27.58%) with *Hepatozoon canis*. The infection rate of stray dogs with different blood parasites was 72% while in foreign breed dogs was 25% (statistically highly significant). Mixed infection by more than one blood parasite was detected in 19 dogs. Two types of microfilariae were detected in examined animals: *Dirofilaria immitis* and *Dirofilaria repens* and their infection rate in stray dogs was 3.2% and 13.6% respectively. No microfilariae were detected in the blood films of foreign breed ones. Four methods were used in this study for the diagnosis of microfilariae: direct wet method, thick blood smears, capillary sedimentation technique and modified Knott's method. The modified Knott's test was the most reliable test, its sensitivity was 66.66%. The present work cleared that the incidence of infection of dogs with different blood parasites increased with age in both breeds. The highest rate of infection with different blood parasites was recorded during Summer (95.5%) and Spring (78.6%) while the lowest rate in Winter (25%). Seasonal variations were found statistically highly significant and this may be due to correlation between the infestation rate and the flourishing of the insect population and tick infestation during this period of the year.

Key words: Dog, blood parasites, microfilariae, prevalence.

INTRODUCTION

Dogs not only act as a dangerous reservoir host for most of the parasitic diseases but also they play a role in transmission of a number of important pathogens among domestic animals and human being that deteriorate their health and lead to enormous losses.

Dogs suffer from a variety of blood parasites as: *Babesia* (intra-erythrocytic protozoa), *Hepatozoon* (intra - leukocytic protozoa), *Trypanosoma* (inter-erythrocytic protozoa) and filarial worms (Gadahi *et al.*, 2008).

Heartworm disease in dogs and related canines is caused by a filarial nematode (a large thread-like round worm), *Dirofilaria immitis*. The adult worms live in the right side of the heart (right ventricle) and adjacent blood vessels (pulmonary arteries), and because of their location, are commonly called dog heartworms. It is particularly

common in the tropics and subtropics. It seems that all breeds of dogs are equally susceptible (Retnasapathy and San 1976). It is also of public health importance because it often produces nodules in the pulmonary parenchyma and subcutaneous tissue or even the eyes of humans (Seo Youngwoo *et al.*, 2001).

Dirofilaria repens is commonly found in the subcutaneous tissue of dogs in Southern Europe, Africa and Asia (Zahler *et al.*, 1997). Subcutaneous and subconjunctival migration of immature nematodes has been reported in human beings (Pampiglione *et al.*, 1995).

Both dirofilariasis due to *D. immitis* and *D. repens* are transmitted by the bite of anopheline and culicine mosquitoes harboring the infective third stage larvae.

In epizootiologic surveys of dirofilariasis in dogs, various methods for determining infection status have been used. These have included microscopic examination of blood smears, blood sample concentration techniques (e.g Knott's test) for detecting circulating microfilariae, examination for identifying adult worms at necropsy, radiographic and angiographic evaluations (Knight, 1977 and Thrall *et al.*, 1980).

Babesia spp. parasitize erythrocytes, causing anemia in dogs. Different species exist with varying host specificity. *B. canis* and *B. gibsoni* are two organisms commonly known to infect dogs. *B. canis* is commonly known to infect dogs and it is comparatively highly pathogenic for both young and old dogs. It is found throughout Asia, Africa, Europe, the Middle East, and North America (Levine, 1985).

Hepatozoon is a genus of protozoa belonging to phylum Apicomplexa. Dogs are known to be susceptible to infection by *Hepatozoon canis* and *Hepatozoon americanum*. The detection of capsule-like gamonts in the cytoplasm of neutrophils in blood smears is helpful for diagnosing hepatozoonosis (Baneth *et al.*, 2001).

Both *Babesia* and *Hepatozoon* parasites are transmitted by ticks in which sexual stages occur (Levine, 1961).

In Egypt dog's blood parasites were studied previously by several authors (Botros *et al.*, 1973, Sakla, 1975, Amer, 1986, Mahmoud, 1998 and Abd -el Rahim, 1998).

The aim of the present work is to throw further light on some of the blood parasitic diseases infecting dogs in Assiut Governorate with special reference to microfilariae which have public health hazards (zoonotic importance). In addition, to evaluate the sensitivity of different techniques used for diagnosis of microfilariae.

MATERIALS and METHODS

Animals

During the period from March 2009 to February 2010, one hundred and forty five dogs (125 stray dogs from different localities in Assiut Governorate and 20 foreign breed Shepherd dogs in the security unit at Assiut Governorate, upper Egypt) aged between 6 months and over 7 years old were used in this study.

Sampling:

5ml of blood was drained from each dog from the cephalic vein in vacum tubes for blood parasite examination.

Parasitological examination: Four parasitological techniques were done for detection of the most reliable one for diagnosis of microfilariae.

- Fresh blood was examined for detection of motile microfilariae with direct wet smear method (Levine, 1985).
- Haematocrit centrifugation technique and Knott's technique were done for detection of mild microfilaraemia (Lowernce & Thomas, 1987).
- Blood films (thin and thick) were prepared from fresh blood, fixed by absolute methyl alcohol, stained by Giemsa stain and examined by light microscope for detection of different blood parasites.
- The detected parasites were microphotographed and identified according to the description given by Levine (1985) and Soulsby (1986).
- The average dimensions of various anatomical regions were determined by eyepiece micrometer. Seasonal investigation of the detected blood parasites was done.

Postmortem examination: Infected dogs were thoroughly examined for adult *Dirofilaria immitis* and *Dirofilaria repens* during necropsy in the Veterinary Medical Hospital of Assiut Governorate.

Statistical analysis: Obtained data were subjected to a software program (SPSS) according to Borenstein *et al.* (1997).

RESULTS

Out of 145 examined dogs 95 (65.51%) were infected with one or more parasitic species, 21 (14.48%) were positive for microfilariae, 34 (23.44%) with *Babesia canis* and 40 (27.58%) with *Hepatozoon canis*. The infection rate of stray dogs with different blood parasites was 72% while in foreign breed dogs was 25%. Mixed infection by more than one blood parasite was detected in 19 dogs (18 stray dogs and 1 foreign breed dog).

Two types of microfilariae were detected in examined animals: *Dirofilaria immitis* and *Dirofilaria repens* and their infection rate were 2.75% and 11.72% respectively. Both types of microfilariae were detected in the blood films of stray dogs only (Table 1). Mixed infection with the two types of microfilariae was recorded in 3 stray dogs.

Data concerning the seasonal variation and the effect of age on the rate of infection with different blood parasites are shown in (Table 2 and 3).

Blood parasites detected in the peripheral blood of dogs are:

1- *Dirofilaria immitis* microfilariae:

Out of 125 examined stray dogs, 4 (3.2%) were positive for *D. immitis* microfilariae. The microfilaria was unsheathed. It is characterized by a tapered cranial end, straight caudal end and a straight body shape. The body cavity was filled with a large number of minute oval deeply stained nuclei appeared as one mass interrupted at certain anatomical landmarks (Plate 1, Table 4). Their total length ranged from 291.6 - 346.66 μm (325 μm) and their width ranged from 5.4 - 7.2 μm (6.75 μm).

2- *Dirofilaria repens* microfilariae:

It was detected in 17 (13.6%) of examined stray dogs. The microfilaria was non sheathed and lie in graceful curves in stained blood smears. It had a blunt cranial end, a button shaped caudal end and a crescentic shaped body. A single row of somatic nuclei is faint stained throughout the whole length of the body of the microfilariae which appeared as spiral shaped line. The somatic nuclei clump together but not mask the anatomical landmarks in most cases of the microfilariae. Their total length ranged from 289 - 330 μm (310 μm) and their width ranged from 3.75: 6.25 μm (5.75 μm).

The microfilarial landmarks were detected in the form of interruptions in the column of nuclei (Plate 1, Table 4).

The result of the comparative sensitivity of different techniques used for the detection of the microfilariae indicated that modified Knott's technique is the most sensitive technique (66.66%) followed by wet blood film technique (42.85%), thick blood film (33.33%) and haematocrit centrifugation (28.57%) (Table 5).

Routine P.M examinations of 100 dogs at the veterinary clinical hospital in Assiut University failed to detect any adult worms.

3- *Babesia canis*:

Out of 145 examined dogs, 34 (23.44%) were positive for *Babesia canis*. The incidence was 24% and 20% among stray and foreign breeds dogs respectively (Table 1)

Various forms of *Babesia canis* have been detected in the peripheral blood of dogs. Ovoid shaped mostly single or multiple. It had one marginally located chromatin mass in addition to central vacuole. It measured 3.5: 4.7µm in length by 2.1: 2.8 µm in width (average 3.8: 2.4 µm) (Plate 2, Fig. 2). Amoeboid forms measured 3.5: 4.8 µm in length by 2: 3.4 µm in width. Centrally located vacuoles are commonly seen (Plate 2 Fig. 1). Pyriform or pear shaped forms are commonly detected. They are found in pairs and less frequently in single infection. They measured from 3.5 µm in length by 2: 2.6 µm breadth (Plate 2 Fig. 3).

4- *Hepatozoon canis*

Out of 145 examined dogs 40 (27.58%) dogs were infected with *Hepatozoon canis*. The incidence was 31.2% and 5% among stray dogs and foreign breed ones respectively (Table 1).

Morphological observations of gametocytes:

A- Macrogametocytes

These are ovoidal bodies with blunt rounded ends measuring 9: 11 µm long and 5: 7.5 µm wide. The centrally located nucleus is large compact strippled and spherical. The cytoplasm is granular and stains dark blue with Giemsa stain. Macrogametocytes are surrounded by delicate fine capsule. (Plate 2 Fig. 4,5).

B- Microgametocytes

They are elongate, rectangular, sausage shaped bodies with blunt rounded ends measuring 8.5-11.2 µm long by 3.4- 4.8 µm wide, they stain pale blue with Giemsa stain. The nucleus is large, compact and strippled and is usually located at one pole of the parasite. The microgametocytes are enveloped by delicate fine capsules. On several occasions microgametocytes were found to lie within the nuclear substance of the polymorphnuclear leucocytes. (Plate 2, Fig. 6). Sometimes the parasite was found to lie within the cytoplasm of the host cell. Free gametocytes devoid of their capsules were usually encountered anywhere out side the host cell.

Table 1: Prevalence of different blood parasites in examined dogs.

Examined dogs	No. examined	No. infected	%	Infected dogs								
				Microfilariae			B.canis					
				Diro. immitis %	Diro. repens %	Total %	B.canis %	H.canis %	%			
Stray dogs (local breeds)	125	90	72	3.2	17	13.6	21	16.8	30	24	39	31.2
Foreign breeds	20	5	25	-	-	-	-	-	4	20	1	5
Total animals	145	95	65.5	2.75	17	11.72	21	14.48	34	23.4	40	27.55

Data were found statistically highly significant.

Chi square 16.85 P(probability) <0.0001

Table 2: Relation between the age and the prevalence rate of infection with dog's blood parasites

Age of examined dogs	No. examined dogs	No. infected	%	Infected dogs								
				Microfilariae			B.canis					
				Dirofilaria immitis %	Dirofil. repens %	Total %	B.canis %	H.canis %	%			
0.6: 3 years	30	15	50	-	2	6.66	2	6.66	5	16.66	8	26.66
4:6 years	55	35	63.63	1.8	6	10.9	7	12.72	14	25.45	14	25.45
Over 7 years	60	45	75	6.66	9	15	12	20	15	25	18	30

Table 3: Seasonal prevalence of dog's blood parasites.

Season	••Total examined	Infected dogs											
		Microfilariae					B. canis						
		Dirofilaria immitis	%	Dirofilaria repens	%	Total	%	Dirofilaria immitis	%	B. canis	%	Total infected	%
Autumn	30	1	3.33	2	6.66	3	10	4	13.33	5	16.66	12	40.0
Winter	28	-	-	-	-	-	-	3	10.71	4	14.28	7	25.0
Spring	42	2	4.76	6	14.28	8	19	12	28.57	13	30.95	33	78.6
Summer	45	1	2.22	9	20	10	22.22	15	33.33	18	40	43	95.6
Total	145	4	2.75	17	11.72	21	14.48	34	23.44	40	27.58	95	65.5

Highly significant statistical variations between seasons and prevalence of infection.
 Chi square 50.133
 P (probability) <0.00001

Table 4: Measurements of *Dirofilaria immitis* and *Dirofilaria repens* microfilariae landmarks in micron Giemsa stained.

	Dirofilaria immitis		Dirofilaria repens	
	Range	Mean	Range	Mean
Total length	291.6:346.66	325	289:330	310
Width	5.4: 7.2	6.75	3.75: 6.25	5.75
Cephalic space*	3.75: 5	4.25	2.5 : 5.25	3.50
Nerve ring*	45.5:65	62	55.25: 75	70
Excretory cell*	80: 100	98	55: 95	85
Anal pore*	199: 239	210	216.6: 266.66	247.5
Last tail nucleus*	229: 312	265	256.6: 309.16	288

* Microfilarial landmarks are determined from the anterior end.

Table 5: Comparative sensitivity of different techniques for the detection of microfilariae.

Technique	Tested	Positive	Sensitivity %
Wet Blood Film	21	9	42.85
Thick blood film	21	7	33.33
Haematocrit test	21	6	28.57
Modified Knott's	21	14	66.66

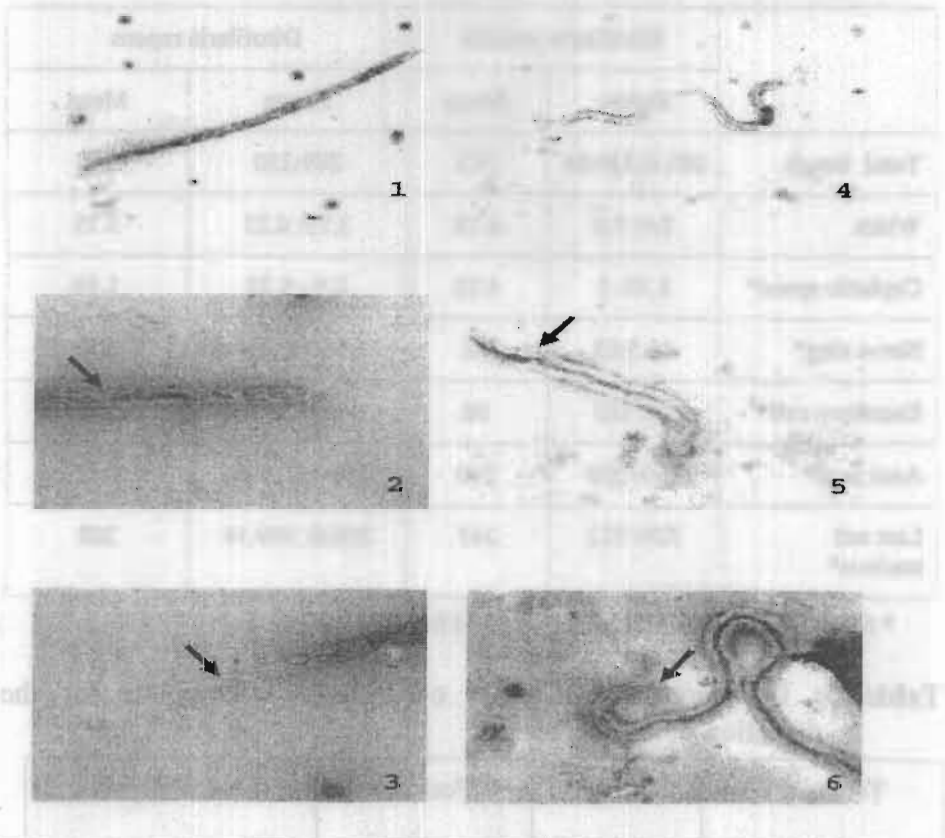


Plate 1: Microfilariae of dogs (Stained with Giemsa stain)

- 1- *Dirofilaria immitis* microfilaria x 100.
- 2- Anterior end of *D.immitis* showing nerve ring (arrow) x 1000
- 3- Posterior end of *D.immitis* microfilaria showing straight tail (arrow) x 1000
- 4- *Dirofilaria repens* microfilaria x 100.
- 5- Anterior end of *D.repens* microfilaria showing nerve ring (arrow) x 1000.
- 6- Posterior end of *D.repens* microfilaria showing button shaped tail (arrow) x 1000

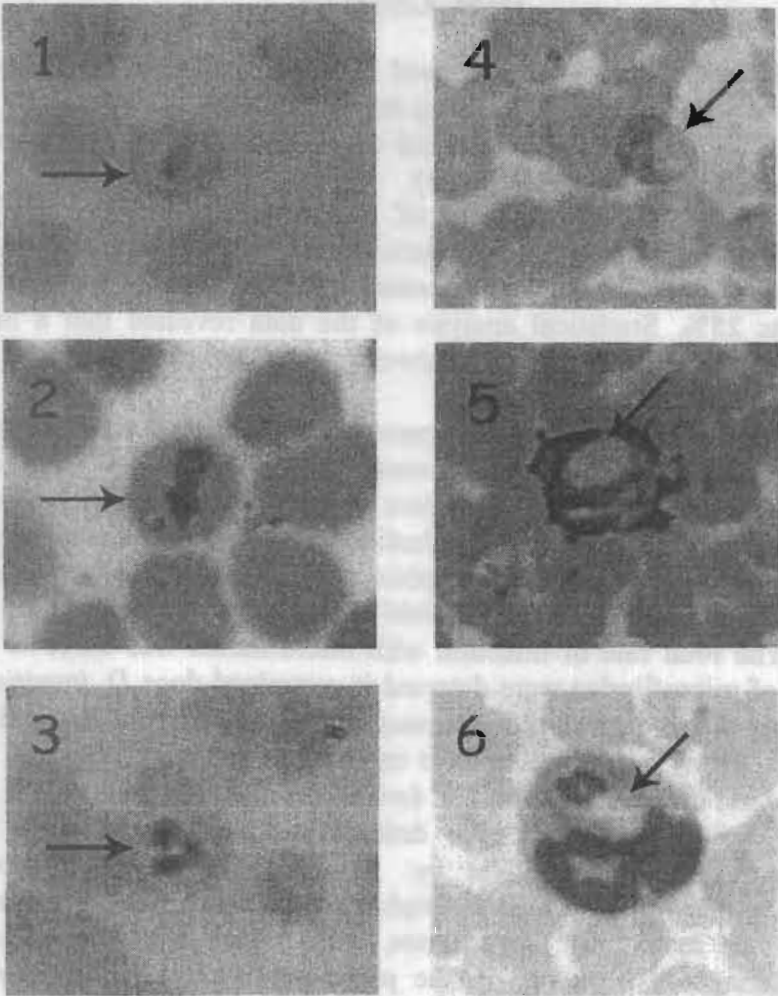


Plate 2: Different forms of protozoan blood parasites in blood films of naturally infected dogs (Giemsa stained).

Fig. 1-3: Different forms of *Babesia canis* X1000. (arrow)

Fig. 4-5: Macrogametocytes of *Hepatozoon canis* in blood films of dogs (arrow) X 1000

Fig. 6: Microgametocytes of *H. canis* in blood films of dogs X 1000 (arrow)

DISCUSSION

The parasitic diseases caused by blood parasites are found all over the world and may be causing severe infection in dogs. Each year thousands of dogs become disabled or die from problems caused by blood parasites (Gadahi *et al.*, 2008).

In the present work out of 145 examined dogs 95 (65.51%) were infected with one or more parasitic species. The infection rate of stray dogs with different blood parasites was 72% while in foreign breed dogs was 25%. Statistical analysis of the data revealed that a highly significant statistical variations between breeds of examined dogs and the prevalence rate of infection.

High prevalence of blood parasites in stray dogs detected in the present work may be due to the greater exposure of the stray dogs to the vector intermediate host, in addition to the better veterinary services beside the use of prophylactic medication as the best protective measures to the foreign breed dogs. This opinion coincides with Poglajen *et al.* (1996), Song *et al.* (2003), and Gadahi *et al.* (2008).

The total rate of infection with microfilariae was 14.48%. Two species of microfilariae were detected in examined dogs *D. immitis* and *D. repens*. Their prevalence rate was 2.75% and 11.72% respectively. Both species of microfilariae were detected in the blood films of stray dogs and not detected in examined foreign breed dogs.

Species of microfilariae detected in the present work were reported previously in Egypt by: Mahmoud (1998) who detected *D. repens* microfilariae in the peripheral blood of 13.3% of examined stray dogs in Assiut Governorate, Abd-el-Rahim (1998) detected microfilariae of *D. immitis* in the peripheral blood of 42.2% foreign breed dogs in Assiut city, Ahmed *et al.* (1986) who detected *D. repens* microfilariae in 1.27% of examined dogs in Abu. Roash in Giza province. In the present work detection of both types of microfilariae in Assiut Governorate may be attributed to the methods of diagnosis and the high number of the examined dogs. Also in other countries they were reported by Cancrini *et al.* (2000), Wu & Fan (2003) and Rossi *et al.* (2004).

Our finding fall within the trend of results obtained by various workers mentioned above.

Several factors play an important role in the prevalence of microfilariae in dogs such as: climatic and environmental conditions, humidity and mild fluctuation in day and night temperature. Also the

unhygienic disposal of manure of animals favors the flourishing of the insect population which plays an essential role in filarial transmission (Samano *et al.*, 1996).

Differentiation between different types of microfilariae by motility via direct wet smear method is difficult, where the microfilariae are unstained and large numbers of cells obscure their view. In the present work among the various techniques used, modified Knott's technique was found to be the most sensitive technique for the detection of microfilariae and its sensitivity was 66.66%. These results confirmed the results recorded by Lindsey (1965), Stein and Lawton (1973) and Todaro *et al.* (1977).

During the course of the present work no adult filarial worms were detected from 100 dogs at autopsy. The present findings are in agreement with that of Schillhorn (1974) and Mahmoud (1998) who failed to detect *D.immitis* adult worms in Nigerian dogs and stray ones in Assiut Governorate respectively. Detection of circulating microfilariae without adult *D. immitis* may be due to death of the adults (Newtown 1968), transplacental transmission of microfilariae (Mantovani and Jackson 1968), ectopic infections with adult *D.immitis* in unusual sites (Otto 1975) or recently terminated infections (Knight *et al.*, 1984). However, *Dirofilaria repens* adults inhabit subcutaneous tissues, hence the difficulty in getting them.

The prevalence rate of infection with both *Babesia canis* and *Hepatozoon canis* was 23.44% and 27.58% respectively. Their prevalence of infection among stray and foreign breed dogs was 24%, 31.2%, and 20%, 5% respectively. The same blood parasites (*B.canis* and *H.canis*) were detected in the same locality by Sakla (1975) who reported that the natural infection rate of dogs in Assiut city with *B. canis* and *H. canis* was 7.7% and 22.7% respectively.

The morphological characters of *B.canis*, microgametocytes and macrogametocytes of *H. canis* agreed with description reported by Sakla (1975), Levine (1985), Gossett *et al.* (1985) and Soulsby (1986).

During the present work it was found that infection of dogs with different blood parasites occur in young as well as in aged dogs but the incidence increase with age in both breeds. This may be attributed to the greatest exposure of the aged animals to the vector intermediate host than young ones. In addition to the low immunity to fight blood-parasite infections in old age, these results coincide with those of (Gadahi *et al.*, 2008).

Data concerning the seasonal variation on the rate of infection with different blood parasites of dogs showed that the highest rate of infection was during Summer and Spring and the lowest in Winter. Statistical analysis of seasonal variations was highly significant. This might be due to correlation between the infestation and increase in the density of mosquito population and the high peak of abundance of tick vector which plays an essential role in transmission of blood parasitic diseases during such months (Sakla 1973, Todaro *et al.*, 1977 and Samano *et al.*1996).

Conclusions: Based on the present observations it may be concluded that infection of dogs with different blood parasites is widespread at Assiut Governorate particularly in Stray dogs. The prevalence of infection with blood parasites depending on the age of dogs which increased gradually up to the age of 6 years. Stray dogs usually harbor various parasitic infections due to improper care and unhygienic conditions. Since they did not receive any medicine for treatment so these infections usually persisted for a long time. Such dogs act as a reservoir and carriers of infections for the pet dogs and human being. Prophylactic measures such as accurate diagnosis and proper antihelmintic treatment of imported dogs, in addition to destruction of stray dogs will help in decreasing the risk of infection to other foreign breed ones and humans in the vicinity of infected animals when suitable mosquito vectors are present.

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