

SOME STUDIES ON MICROFILARIAE IN EQUINE'S BLOOD IN ASSIUT GOVERNORATE

(With 3 Tables and 4 Plates)

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بعض الدراسات علي يرقات الفيلاريا في دم الفصيلة الخيلية في محافظة أسيوط

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تم عمل مسح طفيلي علي ١٨٢ حيوان من الفصيلة الخيلية (١١٠ حمار و ٥٠ حصان و ٢٢ بغل) من مناطق مختلفة من محافظة اسيوط لاستبيان معدل الاصابة بيرقات الفيلاريا في دم هذه الحيوانات في الفترة من يوليو ٢٠٠٧ حتي مارس ٢٠٠٨ وبلغت نسبة الاصابة ٢٥,٨٢% موزعة كالاتي (١٨,٢٨% و ٢٦% و ١٣,٦٣%) بين الحمير والخيول والبغال علي التوالي (بدون دلالة إحصائية). وتم تصنيف الميكروفيلاريا الي نوعين الانكوسيركا ريتكيولاتا (١٧,٥٨%) والستاريا أكوينا (١٠,٩%) ولوحظ اثناء فحص مسحات الدم السمكية للحيوانات المصابة ان يرقات الانكوسيركا ريتكيولاتا تتجمع مع بعضها البعض في شكل يشبه خصلة من الشعر، ربما بسبب استعمال بعض الأدوية أو تغيرات مناعية وقد لوحظ عند اجراء الصفة التشريحية لبعض الحمير المصابة ان نسبة الاصابة بالطور البالغ من الستاريا اكوينا (٥٢%) وبفحص مسحات الدم السمكية لتلك الحيوانات المصابة كانت نسبة الاصابة بميكروفيلاريا هذا النوع (١٤,٢٨%) لذلك ينصح باستخدام طرق سيرولوجية مستقبلية للحصول علي نسب إصابة دقيقة. كانت أعلى نسبة أصابة خلال فصلي الصيف (٥٢%) والربيع (٣١,٨%) بينما كانت نسبة الاصابة اقل في فصل الشتاء (٤,٧٦%) وقد وجدت دلالة إحصائية عالية لنسبة الإصابة الفصلية وقد يفسر ذلك بتغير الظروف الملائمة لنمو وتوالد الحشرات الناقلة للطفيل والتي تتأثر بالتغيرات المناخية.

SUMMARY

A parasitological survey was carried out on 182 animals from the family Equidae, (110 donkeys, 50 horses and 22 mules) from different localities in Assiut Governorate, to clear up the prevalence of microfilariae in March 2008. Out of 182 examined animals the blood of these animals in the period from July 2007 till 25.82 % were harboring microfilariae. The incidence was (28.18 %, 26% and 13.63 %) in donkeys, horses and

mules respectively (with non significant statistical value). Two types of microfilariae were detected *Onchocerca reticulata* (17.58 %) and *Setaria equina* (10.9%). Examination of thick blood films of some cases revealed that the microfilariae of *Onchocerca reticulata* were aggregated together in the form of a bundle of hair, this may be due to the use of drugs or immunological reactions. Adults of *Setaria equina* were detected in the peritoneal cavities of 52.5% of necropsied donkeys. Examination of thick blood films of the same animals revealed that only 14.28 % harbor microfilariae of *Setaria equina* in the peripheral blood. It was concluded that future studies on filarial parasites in equines should be aided with serological techniques. The highest rate of infection was noticed in Summer (52 %) and Spring (31.8 %), while the lowest rate was in Winter (4.76%). Seasonal variations were found statistically highly significant and this may be correlated with the density of the arthropod vector which is affected by climatic variations.

Key words: *Microfilariae, Onchocerca reticulata, Stearia equina, equines*

INTRODUCTION

Members of the family Equidae serve as animals of work and transportation, especially in developing countries. In Egypt equines are considered to be indispensable animals to the farmers where they help in various field works and transportation. In addition, its manure is used as natural fertilizer. However, these animals have not been given sufficient care although they are always subjected to many parasitic diseases, which may lead to lowering their vitality and rendering them unable to perform their laborious work. Filariasis is one of the most important parasitic diseases affecting equines in Egypt which is caused by different species of filarial worms as *Onchocerca spp.*, *Setaria equina* and *Parafilaria multipapillosa*. Chronic septicaemia in equines arises as the result of metabolic products of filarial worms in the blood (Davidov, 1949). *Setaria equina*, a common parasite in all parts of the world, produces functional disorders which might lead to death (Martins *et al.*, 2002). *Setaria equina* and *Onchocerca reticulata* were recorded in various countries of the world (Francalanci and Manfredini, 1972, Coleman *et al.*, 1985 and Siddiqui *et al.*, 1996). In Egypt *Setaria equina* worms were found in the peritoneal cavity and produced many affections of the eyes and scrotum (Ahmed, 1984, El Seify *et al.*, 1985 and Abu El Magd and Ahmed, 1994). *Onchocerca reticulata* was found in the skin, subcutaneous tissues and induced ulcerative wounds on the back beside fistulous withers and nodular ulcerative dermatitis (Fahmy, 1972)

The Filarial infection rate among equines in Egypt was studied by various workers, In Giza Province (Selim and Fouad, 1964 and Fahmy, 1972), in Upper Egypt (Khailfia *et al.*, 1988, Mahmoud, 1998 and Arafa, 1998), Therefore the present investigation was conducted to assess a parasitological survey on blood microfilariae of equines in Assiut Governorate by examination of blood films and necropsy of available animals for detection of adult filarial parasites, in addition to study effect of seasonal variation on the rate of infection.

MATERIALS and METHODS

Animals: During the period from July 2007 to March 2008, one hundred and eighty two equines (110 donkeys, 50 horses and 22 mules) from different localities in Assiut Governorate were used in this study.

Sampling: Blood samples were drained from each animal from Jugular vein in vacum tubes. Parasitological examination: blood films (wet, and thick) were prepared from fresh blood, Buffy coats were obtained through the microhaematocrit centrifugation technique. Knott's technique was done for detection of mild microfilaraemia (Coles, 1986). The wet and stained blood films by Gimesa stain were examined by light microscope for detection of microfilariae (Lowernce and Thomas, 1987). 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 equine microfilariae in peripheral blood was done.

Postmortem examination: Forty adult donkeys were thoroughly examined for adult *Setaria equina* and *Onchocerca reticulata* during necropsy, peripheral blood examination of these animals was done for detection of microfilariae

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

RESULTS and DISCUSSION

Out of 182 examined animals, 47(25.82%) were positive for microfilariae. The incidence was 28.18% in donkeys, 26% in horses and 13.63% in mules (Table 1). Two types of microfilariae were detected in the blood films of examined animals: *Onchocerca reticulata* and *Setaria equina*. The rates of infection were 17.27% and 8.18% in donkeys, 12% and 10% in horses and 9% and 4.5% in mules respectively. Mixed infection with the two types was recorded in percentage of 2.72% in donkeys, 4% in horses and not detected in mules Table (1). Statistical analysis of the data revealed that there is no significant statistical variations between different species of the family Equidae and the prevalence rate of infection From these studies it was cleared that the filarial parasites were detected among different species of equines in Assiut Governorate. These results are lower than the results obtained by Khalifa *et al.* (1988) who found that the rate of infection with microfilariae of *O. reticulata* and *S. equina* ranged between 51.2% and 18.8% in donkeys, 41.2 % and 23.5 % in horses and 25 % and 16.7 % in mules respectively in Egypt. In other countries (Coleman *et al.*, 1985, Siddiqui *et al.*, 1996 and Hornok *et al.*, 2007) reported that the incidence of infection with microfilariae in Equidae ranged between 14.6%, 55.35% and 22.2% respectively. Meanwhile our results were higher than those reported by (Ahmed, 1984, El. Seify *et al.*, 1985, Mahmoud, 1998 and Arafa 1998). This may be due to many different factors 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.

Data concerning the seasonal variation on the rate of infection with microfilariae are shown in Table (3). These results 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 fly population during different seasons as reported by (Khamis *et al.*, 1973, Mohamed 1979, Khalifa *et al.*, 1988, Mahmoud 1998 and Arafa 1998).

Morphological characters of *Setaria equina* microfilariae and adults: Plate (1-2 and 4): The microfilariae were characterized by possessing a delicate transparent sheath, which protruded at both extremities for short distance. It took faint pink colour with Gimesa stain. The anterior extremity was rounded but its posterior end was tapering and ending in a fine tail. The total length of the body proper without the sheath ranged between 176- 228 μ (mean 199 μ) while its breadth ranged between 5- 6 μ (mean 5.4 μ) at the level of nerve ring. The body cavity was filled with a large number of minute oval deeply stained nuclei appeared as one mass interrupted at certain anatomical landmarks. Anteriorly, there was a cephalic space free of nuclei as well as the tail region. Below the cephalic region there were three small nuclei. The cuticle showed transverse striations especially at the posterior end. The internal body appeared as one or two unequal separated homogenous red masses that differed from the surrounding nuclei. These characters agree with the description of (Ahmed, 1984; Lix, 1990; Giannetto, 1996; Arafa, 1998 and Mahmoud 1998). On the other hand the morphological characters and the measurements of the adult worms agree with the description of Yamaguti (1961), Levine (1985) and Soulsby (1986). Milky white worms of *Setaria equina* in this study were detected in the peritoneal cavities of 21 out of 40 donkeys (52.5 %) these results agree with the finding of Buchwalder (1989) and Oge *et al.* (2003). The total length of the male ranged from 50-72mm \times 0.4- 0.6 mm in breadth while that of the female varies from 95- 125 mm in length and 0.7-1 mm in breadth. Morphological features of adults are shown in Plate (4). Thick blood films as well as concentrated blood examination of 21 donkeys which proved to harbor *S. equina* adults during P.M. examination revealed that only 3 animals (14.28%) had sheathed microfilariae in their peripheral blood, these results agree with the results obtained by (Mohamed, 1979; Ahmed 1984; Khalifa *et al.*, 1988 and Arafa 1998). The low incidence of microfilariae in the present work may be related to the chronic infection of *S. equina* which is characterized by absence of circulating microfilariae in the peripheral circulation. This may also indicate that accurate future estimation of incidence of equines filarial infection should be aided with serological techniques.

Morphological characters of *O. reticulata* microfilariae (Diesing, 1841), Plate (1, 2 and 3): The microfilariae were slender, unshathed and elongated tended to be coiled or slightly straight. The anterior extremity was rounded and of the same diameter as the rest of the body except posteriorly where it tapers gradually into a fine tail. The total length ranged between 150: 192 micron (mean 163.2 micron) while its breadth ranged from 4:6 micron (mean 4.8 micron) at the level of the nerve ring. The somatic nuclei were arranged in two to four oval nuclei well defined deeply stained compact nuclei, which did not reach the tail tip. They mostly clump together, more or less masked the anatomical landmarks of the microfilariae. Marked transverse striations obscured the tail nuclei. These results agree with the finding of Khalifa *et al.* (1988) and Mahmoud (1998). Adult specimens of *Onchocerca reticulata* were difficult to be detected during the present work. Examination of thick blood films revealed that the microfilariae of *Onchocerca reticulata* were aggregated together in the form of a bundle of hair as shown in Plate (3). No satisfactory explanation can be given to clarify this phenomenon whether the drugs or immunoresponse of the host plays a role for aggregation of the microfilariae is not yet clear. This phenomenon needs further future studies to be cleared.

In conclusion this study revealed that the rate of infection with adult worms of *S. equina* was commonly higher than their circulating microfilariae. Therefore proper diagnosis of *S. equina* could not be detected by blood film technique alone, but serological tests seemed to be important diagnostic techniques.

Table 1: Incidence of infection with microfilariae in equines in Assiut Governorate.

Table 1: Incidence of infection with microfilariae in equines in Assiut Governorate.

Animals	No. of Ex.	<i>Onchocerca reticulata</i>				<i>Setaria equina</i>				Mixed		Total *	
		No. of inf.	%	Single inf.	%	No. of inf	%	Single inf	%	No.	%	No.	%
Donkeys	110	22	20	19	17.27	12	10.9	9	8.18	3	2.72	31	28.18
Horses	50	8	16	6	12	7	14	5	10	2	4	13	26
Mules	22	2	9	2	9	1	4.54	1	4.54	-	-	3	13.63
Total	182	32	17.58	27	14.83	20	10.9	15	8.24	5	2.74	47	25.8

* Data were found statistically non-significant X^2 (Chi square) = 2.026 P (probability) =0.363

Table 2: Measurements in micron of *Setaria equina* and *Onchocerca reticulata* microfilariae.

	<i>Setaria equina</i>		<i>Onchocerca reticulata</i>	
	Range	Mean	Range	Mean
Total length	176:-228	199	150:192	163.2
Width	5:6	5.4	4:6	4.8
Cephalic space*	4.8:8.4	5.52	4.8:7.2	5.28
Nerve ring*	30:44.4	40.8	29:38.4	30
Excretory pore*	51.6:68.4	61.2	42: 44.4	42
Excretory cell*	64.8:82.8	69.6	51: 64.8	50.4
First genital cell *	75.6:120	97.2	78:96	85.2
Anal pore*	132:181.2	168	115:132	117.6
Last tail cell*	155:205	182.4	139:168	135
Tail	8.4:13	12.96	9:20	12

*: Microfilarial landmarks are determined from the anterior end

Table 3: Effect of seasons on the rate of infection with microfilariae among equines.

** Highly significant statistical variations between seasons and prevalence rate of infection.

χ^2 (Chi square) =33.807

P (probability) < 0 .001

Plate1: Wet smears of microfilariae of equines.

a: *Onchocerca reticulata* microfilaria X200 b : Anterior end of *O. reticulata* X400

c: Posterior end of *O.reticulata* X400 (d &e): Anterior end of *S. equina* showing sheath (arrow) X200

Seasons	Donkeys No: 110			Horses No.50			Mules No.22			Total No. 182		
	No** Exam	No. Of inf.	%	No.* * Exam	No. Of inf.	%	No. Exam	No. Of inf.	%	No** Exam	No. Of inf.	%
Winter	25	1	4	14	1	7.14	3	-	-	42	2	4.76
Spring	27	10	37	10	3	30	7	1	14.28	44	14	31.8
Summer	30	16	53.33	11	8	72.72	9	2	22.22	50	26	52
Autumn	28	4	14.28	15	1	6.66	3	-	-	46	5	10.86
Total	110	31	28.18	50	13	26	22	3	13.63	182	47	25.8

Plate 2: microfilariae of equines (stained with Gimesa stain)

a: *Setaria equina* microfilaria showing sheath (arrow) X400

b- anterior end of *S.equina* microfilaria X1000

c- extending of the sheath at the posterior end of *S. equina* X1000

d-*O.reticulata* microfilaria X400

e- anterior end of *O. reticulata* X1000

f- posterior end of *O. reticulata* X1000

Plate 3: *O. reticulata* microfilaria (stained with Giemsa stain)

A-severe infection with *O. reticulata* microfilaria X40

b- Aggregation of *O. reticulata* microfilariae together in the form of a hair like bundle X400

Plate 4: *S. equina* adult male and female

a- Anterior end of female X100 b- Posterior end of female X100

c- Anterior end of male X100 d- Posterior end of male showing spicules X100

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