

## Investigation of Microfilaria in Sheep by Using Conventional Techniques in Mosul City

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### Abstract

This study was performed on 300 sheep in Mosul city from August 2024 to October 2024 to demonstrate Microfilariae in sheep blood using various methods such as Knott's technique, M G G quick stain, and fluorescent acridine orange staining. When detecting microfilaria in the blood. The percentage of microfilaria infection is 56% during investigation by the modified Knott technique. There were three forms of infection: mild, moderate, and severe infection with microfilariae in sheep using the same method, showing significant differences in infection severity below the limit of level ( $P < 0.05$ ). Also, we studied risk factors such as age on the intensity of infection. There were significant differences. A high percentage of infection was noticed in animals over three years old (93.1%) while a low rate in sheep below one year old (27.1%). No significant differences were noticed between the females (58%) and males (54%). The animal husbandry indicated increased infection in door systems (61.6%) than indoor systems (45.7%). Also, there were significant differences between breeds of the animals.

**Keywords:** Microfilaria, Sheep, Blood, M.G. Giemsa stain , Fluorescent.

### Introduction

Filarioid nematodes are worldwide parasites transmitted by ' invertebrate's host causing filariasis in numerous vertebrate hosts except fish (1,2). Filarioid worms fall under the class of Nematoda, order Spirurida, above the family Filarioidea, which includes the

families *Filariidae* and *Onchocercidae*, and within these two families are many genera that infect humans and animals (3). Generally, *Parafilaria* and *Stephanofilaria* are small to medium-sized parasites, and the genus *Onchocerca* are medium-sized fibroids (4). The larvae of these worms are called microfilariae(mf), which are define as

serpentine organisms covered by sheath and contain many cell nuclei their length varies from 231-300  $\mu\text{m}$  and the width ranges from 5-7  $\mu\text{m}$  (5). They circulate in the peripheral blood and infect tissues and body cavities of vertebrate hosts (6) and larvae could be found in various body parts, including the peritoneal cavity, arteries, and subcutaneous tissue; they can cause skin filariasis, which is characterized by lesions that resemble scabies; they can also spread to the nervous system through blood circulation, resulting in neurological disorders; and when they get to the eye, they can cause blindness (7). Although adult nematodes are thought to be non-pathogenic in their natural hosts, hematophagous vectors can spread infectious stages to non-permissive hosts like sheep, goats, horses, cattle, and humans, leading to severe and frequently fatal neuropathological disorders known as cerebrospinal filariasis (8). (9) diagnosed for the first report of lumber paralysis due to infection of sheep with the larval stage of *Setaria* spp, in Iran. (10) showed in the brain cross-section of sheep the presence of nematode larvae with encephalomyelitis, lymphocytic, eosinophilic, and moderate leptomeningitis with many hemorrhagic tracts, degeneration, and necrosis brought on by larval migraines. The adult worm causes a syndrome called "Kumri", meaning weak back characterized by In coordination and a swinging back gait caused by paralysis of the lumbar region and one or both fore-hind limbs (11). This study aimed to use conventional methods to explore microfilariasis in sheep.

## Materials and methods

**Ethical approve:** The institutional animal care approved the study approved the study and use committee of Veterinary Medicine College, University of Mosul (UM.VET.2024.034 decision number in 1/9/2024)

Three hundred sheep samples, aged five months to seven years, were randomly selected from various Mosul city regions for blood collection. The samples were examined in the college of Veterinary Medicine University of Mosul during the period from August 2024 to October 2024 for the detection of microfilariae infection after taking a case history from the owners' animals that included information about (age, sex, treated or not, location of the animal, type of nutrition and rearing) took 3ml of blood samples from sheep appeared many clinical symptoms, including pneumonia, evidence of locomotor dysfunction, weakness, depression, and fluctuating appetite) Blood samples were collected from the jugular veins in EDTA tubes .We used the Modified Knott technique, M G G quick stain, and Acridine orange stain to detect microfilaria. The morphometric study-based diagnosis of microfilariae species was determined using (12 ,13).

**1-Modified Knott's technique:** After placing one milliliter of the sample blood and nine milliliters of 2% formalin in a tube and centrifuging the tube for five minutes at 1500 rpm, a drop of sediment was placed on a glass slide and a drop of 1% con. Methylene blue dye was added to the sediment drop using a pipette. The coverslip was then placed on the slide and examined with an ocular micrometer and an X 10 magnification power in 22 x 22

mm fields to determine the quantity and measurement of microfilariae (13).

**2-May Grunwald Giemsa stain (M G G quick stain):** After placing a single drop of blood on a glass slide, a light blood smear was created, allowed to dry for three minutes, fixed with methyl alcohol for five minutes, and then stained with M G G quick stain for two minutes. It was then rinsed under tap water, allowed to dry, and examined using an ocular micrometer and an (X40) or (X100) magnification power under fields to determine the quantity and measurement of microfilariae (14)

**3-Acridine orange stain:**After thoroughly dried in the staining solution, the blood smears were fixed with 100% methyl alcohol and left in the Acridine orange solution (0.01%) for two minutes. After carefully washing the dyed smears with regular water and letting them air dry completely, they were inspected using a fluorescence microscope with several filters (B BF G). The BF filter was used for the examination. (15)

**Statistical analysis:** The results were analyzed using the IBM SPSS version 22 statistical program, the two-sided Chi-square test, and

the Fischer test to determine the significant differences between the factors and the infection rate. The Kappa value was also used to establish the agreement between the different tests used in this study, namely, microscopic examination and modified nucleotide technology (16).

**Results**

The present study's findings demonstrated that, throughout the inquiry using the modified Knott approach, 168 sheep samples tested positive for microfilariae under a microscope, yielding a total percentage of 56%. in Table (1).

The intensity and prevalence of microfilariae infection in sheep was found to be mild, moderate, and severe, with percentage rates of 85.1%, 10.7%, and 4.1%, respectively. This indicates a significant difference in infection severity below the probability level ( $P < 0.05$ ) Table (2). The effect of risk factors on the intensity of the infection, like age, revealed significant differences. We noticed a high percentage of infection in animals over three years old (93.1%) while low rats in sheep below one year old (27.1%) Table (3).

**Table 1: Total rate of infection with microfilariae in sheep**

No. of examined blood samples	No. of positive samples	Percentage%
300	168	56

**Table (2) Intensity of infection with microfilariae in sheep by using the modified Knott method**

Severity of infection	Total No. of Infection	No. of infected animals	Percentage%
<b>Mild infection</b> (5-25) *Mf.		143	85.1a
<b>Moderate infection</b> (25-50) Mf.	168	18	10.7b
<b>Severe infection</b> (50-100 ) Mf.		7	4.1c

Different letters indicate a significant difference below the probability level ( $P < 0.05$ ).\*(MF): Microfilaria

**Table (3): Numbers and percentages of infection with microfilarial in sheep and their relationship to the age of the animal**

Age	No. of examined animals	No. of infected animals	Percentage %
<b>1 year &lt;</b>	55	15	27.2 a
<b>1-2 year</b>	96	36	37.5 a
<b>2-3 year</b>	61	35	57.3 b
<b>3 years &gt;</b>	88	82	93.1 c

Different letters vertically (A, B, C) indicate a significant difference below the probability level ( $P < 0.05$ )

The percentage of infection in sheep males (54%) and females (58%) did not significantly differ from one another Table (4). The relationship between animal husbandry and infection rates indicated an increase level of infection in door systems (61.6%) than in indoor systems (45.7%) in Table (5).

The present study used different stains for staining microfilariae, like Methylene blue stain, M G G quick stain, and Acridine orange stain. The uses of these stains revealed the presence of sheathed and non-sheathed microfilaria figures (A,B,C).

**Table (4): Numbers and percentages of infection with microfilariae larvae in sheep and their relationship with the ' 'animal's gender**

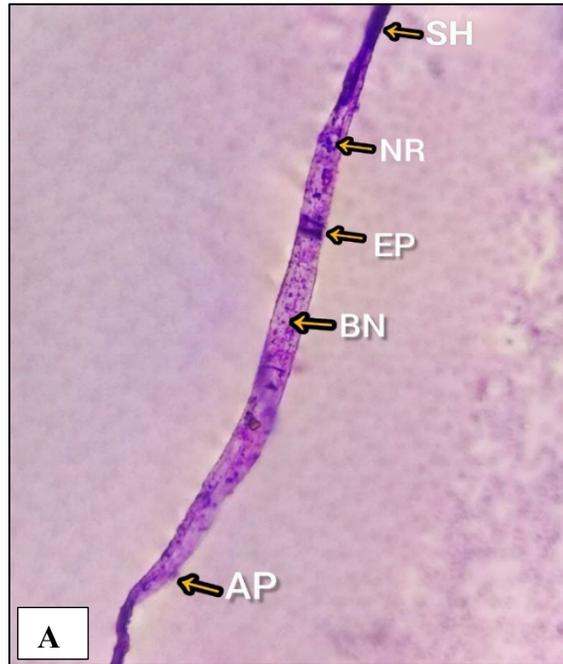
Gender of animals	No. of the examined animals	No. of the positive animals	Percentage%
<b>Males</b>	150	81	54a
<b>Females</b>	150	87	58a
<b>Total</b>	300	168	56

Similar letters indicate no significant difference below the probability level ( $P < 0.05$ ).

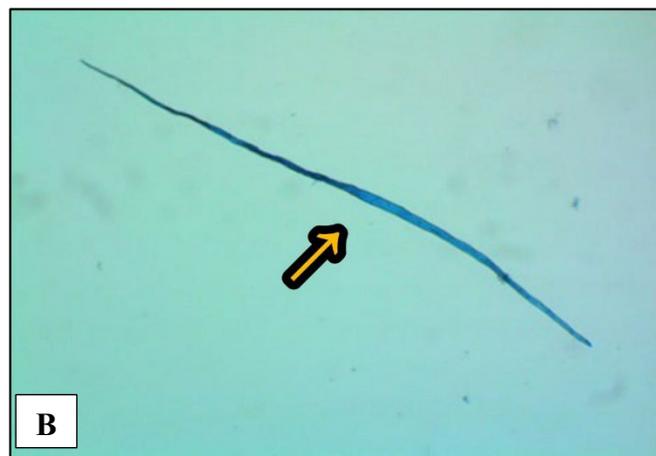
**Table (5): The relationship between animals' husbandry and infection rates**

Animals husbandry	No. of the examined animals	No. of the positive animals	Percentage%
Indoor	107	49	45.7 a
Outdoor	193	119	61.6 b

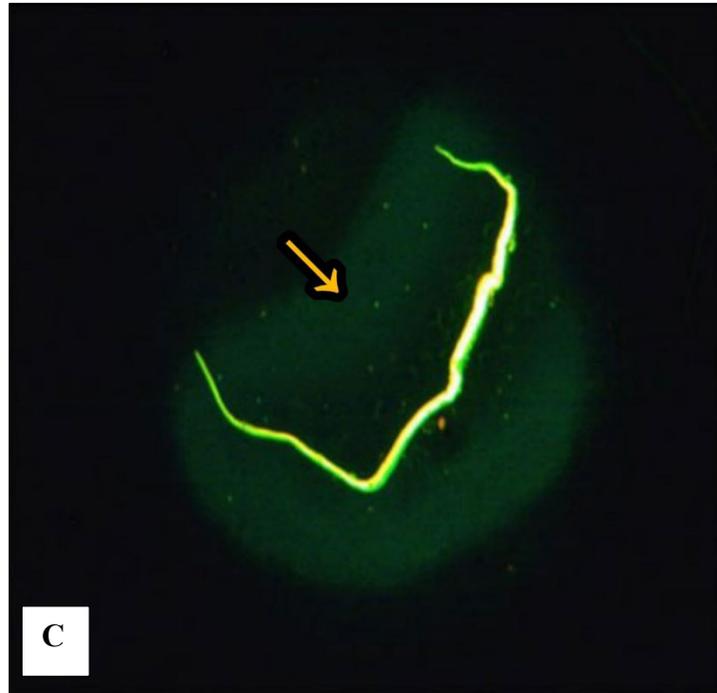
Different letters indicate a significant difference below the probability level ( $P < 0.05$ ).



**Fig (A): Blood smear stained with M.G.Quick stain (Giemsa Stain) showing microfilaria SH: Sheathing, NR: Nerve ring, EP: Excretory pore, BN: Body nuclei, AP: Anal pore, under power magnification (X100), by using a digital camera.**



**Fig (B): Microfilaria (*Setaria* spp.) stained with methylene blue stain under the power of magnification (X10) by using a digital camera.**



**Fig(C): Blood smear stained with Acridine orange fluorochrome showing microfilaria under magnification (X4 ) power by using digital camera.**

## Discussion

Sheep in Mosul city had a 56% overall infection rate with microfilaria, which indicated an increase in the disease's incidence. This high infection rate may be attributed to the availability of intermediate hosts, such as mosquitoes and flies, which are responsible for transmitting microfilaria (18). Our results were in agreement with (11) and the study of (18), who recorded a total infection rate of microfilariae in sheep (60% ,65%), respectively. Also, (19), (20), (21) recorded high percentage rates of microfilaria in farm animals (54.28% ,55%, 70%), respectively. Numerous researches about incidences of microfilaria in Mosul city have documented variation in infection rates in farm animals ranging from 0% to 100% (23-31). The parasitism

in various parts of the world could be explained by variations in the number of samples analyzed, the physiological state of the animal, and the time of sampling, as these larvae are characterized by a cyclic nocturnal-diurnal tendency (13). Studying the severity of the infection with microfilariae revealed three forms of infection, mild, moderate, and severe infection with microfilariae in sheep with percentages rates (85.1%,10.7%, and 4.1%) respectively by using Modified Knott Technique, these differences in the results may be attributed to the difference in immunological states of the animal. The study showed that people of all ages are susceptible to infection, with a significant difference in infection rates. We noticed a low rate in sheep below one year old (27.1%) while a high percentage rate of

infection in animals over three years old (93.1%). The reason may also be attributed to the long life span of these parasites inside their final host, like a period of larval development until they reach adult stages and shed microfilaria inside the host's body, consuming several months (13). This is consistent with what was mentioned by (24), (25), (31), (32), who indicated that the infection rate with microfilaria increases with the animal's age. As for the gender factor, no significant difference was observed in the infection rate between males (54%) and females (58%). This result agreed with (33,34), who stated that the infection has occurred in both sexes equally, while our study was in agreement with (24), (25), (19), (35), (36). There were significant differences between animal husbandry and infection rates, a higher rate in outdoor systems (61.6%) than indoor systems (45.7%). Also, there were significant differences between the breed of the animals and the infection rates. The reason may be attributed to the inhibition of animal immune responses and increase their sensitivity to the microfilariae infection, as well as the effects of environmental conditions, breeding conditions, herd infection, field cleanliness, and the number of sucking insects present in the field. This study used Acridine orange and M G G quick stain to identify microfilaria in sheep blood. Both stains were characterized by high sensitivity for diagnosing this parasite at different microscopic magnifications of 4X, 10X, and 40X. also, it was easy and speed to identify infection with the

microfilaria because of very short time during staining and examination not more than 5 minutes. (18, 37,44). This suggests that M.G G quick stain and Acridine orange are reliable in detecting microfilariae infection in sheep.

### **Conclusion**

Modified Knott's technique is a suitable procedure for the detection of microfilariae, but it is time-consuming and requires a centrifuge during the test. M.G quick stain (Giemsa) is a good standard quick technique for the detection of microfilariae and discrimination its morphological characteristics. This stain is fast and consumes less time, and it is beneficial in epidemiological studies in which the number of samples is large. The Acridine orange stain has high sensitivity in identifying microfilariae at microscopic magnifications of 4X and 10X.

### **Conflicts of interest**

The authors declare that there is no conflict of interest.

### **Ethical Clearance**

This work is approved by The Research Ethical Committee.

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## التحري عن يرقات الديدان الخيطية الدقيقة في الأغنام باستخدام التقنيات التقليدية في مدينة الموصل

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### الخلاصة

أجريت هذه الدراسة على 300 رأس من الأغنام في مدينة الموصل من شهر آب 2024 إلى شهر تشرين الأول 2024 لبيان وجود الميكروفالاريا في دم الأغنام باستخدام طرق مختلفة مثل تقنية تركيز نوت وصبغة جيمسا وبرتقالي أكرديدين الفلوري. تم الكشف عن الميكروفالاريا في مصل الدم. بلغت نسبة الإصابة باليرقات الخيطية الدقيقة (56%) أثناء الكشف باستخدام تقنية نوت المعدلة. كان هناك ثلاثة أشكال من الإصابة بالميكروفالاريا في الأغنام باستخدام نفس الطريقة وهي إصابة خفيفة ومتوسطة وشديدة بالميكروفالاريا في الأغنام باستخدام نفس الطريقة مما يشير إلى وجود فرق كبير في شدة الإصابة حيث تقل عن مستوى الاحتمالية ( $P < 0.05$ ). كما درسنا تأثير عوامل الخطر على شدة الإصابة مثل العمر وكانت هناك فروق كبيرة حيث لوحظت نسبة عالية من الإصابة في الحيوانات التي تزيد أعمارها عن ثلاث سنوات (93.1%) بينما كانت منخفضة في الأغنام التي تقل أعمارها عن سنة واحدة (27.1%). لم يلاحظ أي اختلافات معنوية بين الإناث (58%) والذكور (54%). أشارت نتائج تربية الحيوانات إلى زيادة مستوى الإصابة بالنظام الخارجي (61.6%) عن النظام الداخلي (45.7%). كما كانت هناك فروق معنوية بين سلالات الحيوانات، حيث كانت معدلات الإصابة أعلى في الأغنام المصدرة (90%) عن السلالة المحلية (49.5%).

**الكلمات المفتاحية:** اليرقات الخيطية الدقيقة ، الأغنام ، الدم، كيمزا، التآلق .