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Investigation of Microfilaria in Cattle and Buffaloes by Using Conventional Techniques in Mosul City

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Abstract

This study included 200 cattle and buffaloes in Mosul city from August 2025 to January 2026 to detect microfilariae in cattle and buffalo blood using Knott's technique, Giemsa stain, fluorescent acridine orange stain, Hematoxylin and eosin stain, Leishman stain. Three types of infection were detected including mild, moderate, and severe, using the same method, with significant differences ($P < 0.05$). The percentage of Microfilaria infection in blood was 40% in cattle and 60% in buffaloes. Results indicated a low rate of infection below one year old (26.7%) in cattle and (40%) in buffalo, while a high percentage of infection in animals over three years old (55%) in cattle and (75%) in buffalo. significant differences were observed A high percentage of infection in females (45.5%) in cattle and (66.7%) in buffalo while the percentage of infection in males (33.3%) in cattle and (50%) in buffalo. The relationship between animal husbandry and infection rates indicated an increase level of infection in outdoor systems (46.2%,64.3%) in cattle and buffalo respectively than in indoor systems (28.6%, 50%) in cattle and buffalo respectively. The relationship between the months and infection rates diagnosed that presences an increase in the level of infection in summer seasons in cattle and buffaloes (61.1%,80%) respectively than in winter seasons in cattle and buffalo respectively (12.5% ,26.67%). Geographical distribution of the infection reviled that the higher infection rate observed in Badush region than anther regions in both cattle and buffalo (60%,72%) respectively.

Keywords: Microfilaria, cattle, buffalo, Blood, M.G. Giemsa stain, Fluorescent.

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Introduction

Bovine microfilariosis is a vector-borne, chronic wasting disease affecting cattle and buffaloes, caused by microfilariae of different types of Filarioid nematodes (1). Some blood-sucking insects, like black flies, midges, and mosquitoes, can spread infection by acting as intermediate hosts for many kinds of parasites (2). Intermediate hosts consume the blood of the definitive host, acquiring microfilariae along with the blood meals (3). The prevalence of infection with this parasite is contingent upon the presence of hot climatic conditions and the availability of intermediate hosts (4). Filarioid worms are in the class Nematoda and the order Spirurida. They are in the families Filariidae and Onchocercidae. Within these two families are many genera that infect human beings and animals (5). They reside in various regions of the inflammation (6). Many infectious and non-infectious aetiologies frequently manifest as corneal opacity (7). Anaemia, debility, loss of appetite, oedematous swelling (primarily in the abdominal region), elevated heart rate, and respiration rate are the primary clinical signs of microfilariosis (8). Microfilariosis can be diagnosed by preparing thin blood smears and staining them with different dyes, such as Giemsa stain and methylene blue stain, using the modified Knott's technique. Acridine orange stain is also used to stain blood smears (9).

Materials and methods

Sample collection

Two hundred blood samples, which include one hundred from cattle and one hundred from buffaloes. Were taken from both sexes of cattle and buffaloes. The animals' ages varied from under a year to eight years. Samples were collected from a variety of regional breeds, considering things like feeding habits, rearing techniques, and past medical care. The sampling was done in various parts of Nineveh governorate. The samples were tested for microfilariae infection at the University of Mosul's College of Veterinary Medicine between August 2025 and January 2026, following the collection of a case history from the owners' animals. collected 3 milliliters of blood from buffalo and cattle, and numerous clinical symptoms, such as pneumonia, signs of weakness, depression, irregular appetite, and locomotor dysfunction. Jugular veins were used to draw blood samples, which were then placed in EDTA tubes. To find microfilaria, we employed the Modified Knott technique, MGG quick stain, acridine orange stain, and hematoxylin and eosin stain. The diagnosis of microfilariae species based on morphometric research was established using Foreyt (10).

1-Modified Knott’s technique

Place one milliliter of blood sample and take nine milliliters of 2% formalin and gently stir it until it’s nicely mixed in a centrifuge tube and centrifuging it for five minutes at 1500 rpm, a drop of sediment and a drop of 1% con. Now collect a drop of this sediment, placing it carefully on a clean glass slide. Next, introduce a little drop of methylene blue stain, and gently place a cover slip. Finally, peer through your light microscope, starting with the 4X and then moving up to 10X to get a good look at your masterpiece. (11).

2- May Grunwald Giemsa stain (M G G quick stain)

After a careful drop of blood was placed on a glass slide, a light smear took shape, patiently left to dry for three minutes. It was then fixed with methyl alcohol for five minutes, much like one would fix a loose button, and stained with MGG quick stain for a brief two minutes. A rinse under running water followed, and once it was completely dry, the slide was scrutinized through an ocular micrometre, either under 40x or 100x magnification. This little effort was to measure and count the microfilariae, as noted by Sharkey and Heinrich (12).

3-Hematoxylin and Eosin Stain

A thick blood smear was prepared by placing a small drop of blood onto a clean glass slide and spreading it in a circular motion using another slide to form a small, thick smear. The smear was allowed to air dry completely for 30-45 minutes. Hemoglobin was removed by immersing the slide in distilled or tap water for 3-5 minutes until the smear became pale, indicating hemoglobin lysis. The slide was then air-dried completely. Fixation was carried out by immersing the slide in methanol for 1 minute, followed by air drying. The slide was subsequently stained with hematoxylin for 5-10 minutes, then gently rinsed with running water for 1 minute. Bluing was performed using running tap water or a bluing solution for 1-2 minutes until the nuclei appeared dark blue. The slide was then counterstained with eosin for 30-60 seconds, briefly rinsed with water, air dried, and examined under a light microscope using 4× and 10× objective lenses (13)

4-Acridine orange stain

After the hematological smears are completely air-dried and fixed, they are immersed in a 0.01% acridine orange solution for 3 minutes. The stained slides are then gently rinsed with tap water in the dark and allowed to air dry fully. Finally, the dried slides are examined under a fluorescent microscope using sequential filters (B, BF, G) to differentiate various cell types (14).

5-Leishman’s Staining Procedure

The prepared modified Leishman stain is gently applied to an air-dried blood smear and left for one minute to allow fixation. After that, the stain is diluted by adding twice its volume of buffered water directly onto the slide. The diluted stain is then allowed to remain on the smear for approximately one and a half minutes to achieve proper staining. The slide is carefully rinsed with clean water to remove excess stain. Finally, the back of the slide is wiped clean, and the slide is placed in a nearly horizontal position and left to air dry completely (15).

Statistical analysis

We took a good look at the results with the help of the IBM SPSS version 22 statistical program. To figure out the significant differences between the various factors and the infection rate, we employed the well-respected two-sided Chi-square test and the Fisher test (16).

Results

The present study showed that when we took a good look at 200 blood samples under the microscope, we found that half of them (50%) had positive microfilariae. To be more specific, 20% of the cattle and 30% of the buffalo were found to be infected, with the presences of significant differences between them, as indicated by a significant P value (P<0.05) in Table (1).

Table (1): Total infection rate of microfilariae in cattle and buffalo using modified Knott's technique

Numbers of examined blood sample	Numbers of positive blood sample	Infection rate%	P-Value
Buffalo 100	60	30a	
Cattle 100	40	20b	0.0072
Total 200	100	50	

Vertical different letters (a, b) mean a significant P value (P<0.05)

The severity of microfilariae infection in cattle and buffalo was mild, moderate and severe, with the percentages of 72%, 20% and 8%, respectively. It implies a huge difference in susceptibility to infection that is significant at the probability level (P < 0.05 we can infer).

Table (2) Intensity of infection with microfilariae in cattle and buffaloes.

Severity of infection	Total No. of Infection	No. of infected animals	Percentage%	P-Value
Mild infection (50-<25) *Mf.	100	72	72%c	0.0000
Moderate infection (25-<50) Mf.		20	20%b	
Severe infection (50-100) Mf.		8	8%a	0.0236 0.0000

Vertical different letters (a, b, c) mean significant P value (P<0.05)

The effect of risk factors on the severity of the infection, such as age, revealed significant differences. We demonstrated a low rate below one year old (26.7%) in cattle and (40%) in buffalo, while a high percentage of infection in animals over three years old (55%) in cattle and (75%) in buffalo (Table 3,4).

Table (3): The relationship between the infection rate of microfilariae and the age of the cattle.

Age of cattle	No. of examined cattle	No. of positive cattle	Infection rate%	P-Value
< 1 Year-1	30	8	26.7a	
				0.7781
2-3 years	30	10	33.3a, b	
				0.1191
> 3 Years	40	22	55b	0.0334

Vertical different letters (a, b) mean a significant P value (P<0.05)

Table (4) The relationship between the infection rate of microfilariae and the age of the buffalo.

Age of buffalo	No. of examined buffalo	No. of examined buffalo	Infection rate%	P-Value
< 1 Year-1	25	10	40a	
				0.2948
2-3 years	35	20	57.1a,b	
				0.1641
> 3 Years	40	30	75b	0.0104

Vertical different letters (a, b) mean a significant P value (P<0.05)

A high percentage of infection in females (45.5%) in cattle and (66.7%) in buffalo, while the percentage of infection in males (33.3%) in cattle and (50%) in buffalo does not significantly differ from one another. Table (5,6).

Table (5): The relationship between the infection rate of microfilariae and the sex of the cattle.

Sex of cattle	Number of examined cattle	Number of Positive cattle	Infection rate%	P-Value
male	45	15	33.3a	
female	55	25	45.5a	0.3050

Vertical different letters (a, b) mean a significant P- value (P<0.05)

Table (6): The relationship between infection rate and the sex of the buffalo.

Sex of buffalo	Number of examined buffaloes	Number of Positive buffaloes	Infection rate%	P-Value
male	40	20	50a	
female	60	40	66.7a	0.1447

Vertical different letters (a, b) mean a significant P- value (P<0.05)

The relationship between the months and infection rates diagnosed shows a significant increase in the level of infection in summer seasons in cattle and buffaloes (61.1%,80%), respectively, than in winter seasons in cattle and buffalo, respectively (12.5%,26.67%) Table (7,8).

Table (7): The relationship between the infection rate of microfilariae in cattle and the months of study.

Month	No of examined cattle	No. of Positive cattle	Infection rate%	P-Value
August 2025	18	11	61.1b	0.0052
September 2025	17	9	52.9b	0.0255
October 2025	17	7	41.2a	0.1175
November 2025	16	6	37.5a	0.2199
December 2025	16	5	31.3a	0.3924
January2026	16	2	12.5a	

Vertical different letters (a, b) mean a significant P- value (P<0.05)

Table (8): The relationship between the infection rate of microfilariae in buffalo and the months of study.

Month	No. of examined buffalo	No. of Positive buffalo	Infection rate%	P-Value
August 2025	20	16	80b	0.0023
September 2025	20	14	70b	0.0175
October 2025	15	12	80b	0.0092
November 2025	15	8	53a	0.2635
December 2025	15	6	40a	0.6998
January 2026	15	4	26.67a	

Vertical different letters (a, b) mean a significant P- value (P<0.05)

The relationship between animal husbandry and infection rates indicated an increased level of infection in outdoor systems (46.2%,64.3%) in cattle and buffalo, respectively, than in indoor systems (28.6%, 50%) in cattle and buffalo, respectively, but these percentages did not significantly differ from one another in Table (8,9).

Table (9): The relationship between the infection rate of microfilariae in cattle and the feeding system.

Feeding system	No. of examined cattle	No. of positive cattle	Infection rate%	P-Value
Open grazing system	65	30	46.2a	0.1341
Closed grazing system	35	10	28.6a	

Vertical different letters (a, b) mean a significant P- value (P<0.05)

Table (10): The relationship between the infection rate of microfilariae in buffalo and the feeding system.

Feeding system	No. of examined buffalo	No. of Positive buffalo	Infection rate%	P-Value
Open grazing system	70	45	64.3a	0.2654
Closed grazing system	30	15	50a	

Vertical different letters (a, b) mean a significant P- value (P<0.05)

Geographical distribution of the infection revealed that the higher infection rate observed in the Badush region than in other regions in both cattle and buffalo (64%,72%), respectively, in Table (11,12).

Table (11): Regions based on geographical distribution for the blood sample of microfilariae in cattle.

Regions	No. of examined cattle	No. of Positive cattle	Infection rate%	P-Value
Badush	25	16	64b	0.0274
Aski mosul	20	8	40a,b	0.4194
Hawi al-Kanisa	10	4	40a,b	0.6284
Tal asfur	10	3	30a,b	1.0000
AL-saadoun massacre	15	4	26.6a	1.0000
Zumar	10	3	30a	1.0000
Haleela	10	2	20a	

Different superscript letters (a–d) indicate significant differences among groups (P ≤ 0.05).

Table (12): Regions based on geographical distribution for the blood sample of microfilariae in buffalo.

Regions	No.of examined buffalo	No. of Positive buffalo	Infection rate%	P-Value
Badush	25	18	72a	0.1232
Tal asfur	20	12	60a	0.4421
AL-saadoun massacre	10	6	60a	0.6562
Tel kaif	10	5	50a	1.0000
AL-Samsiat	15	9	60a	0.4283
Hamidat	10	6	60a	0.6562
Haleela	10	4	40	

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

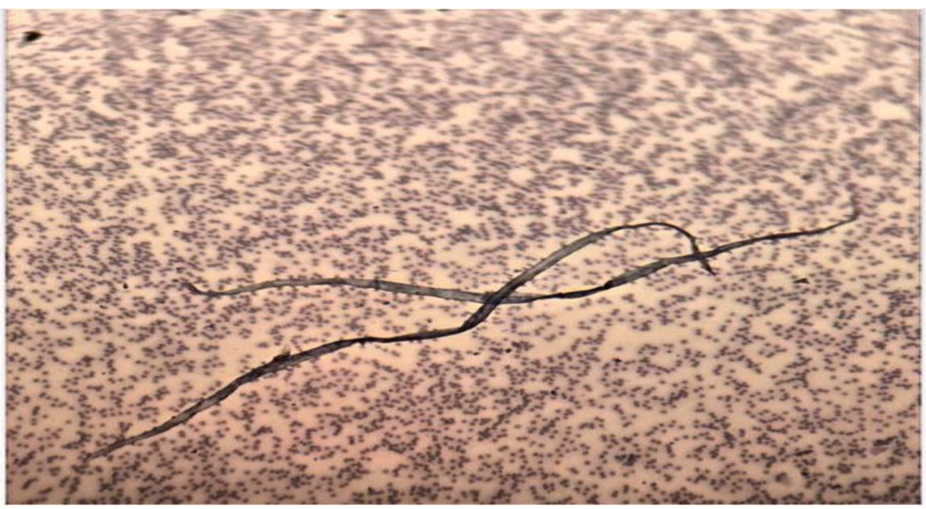
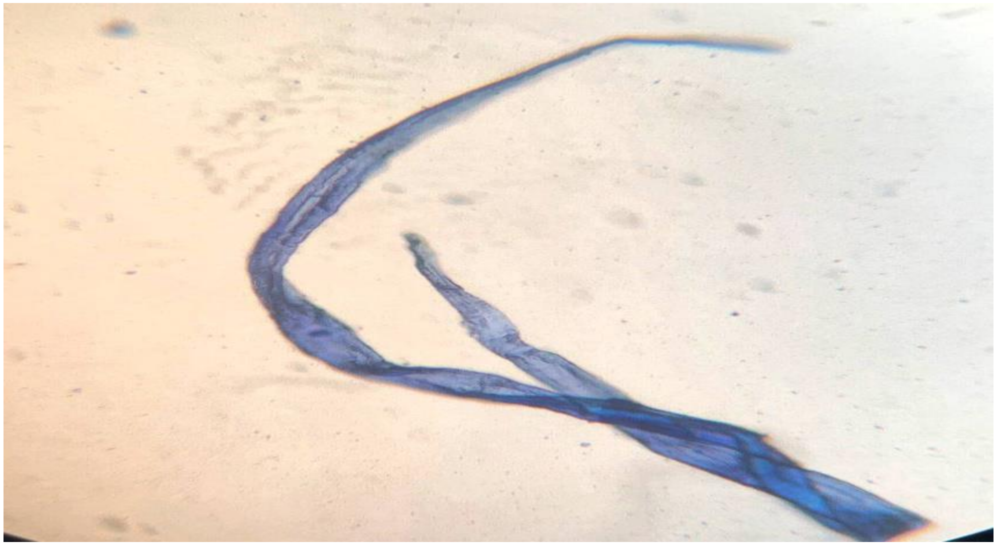


Fig (A): Blood smear stained with M.G. G.Quick stain (Giemsa Stain) showing microfilaria under power magnification(X4), by using a digital camera.



Fig(B)Microfilaria stained with methylene blue stain under power magnification(10X), using a digital camera.

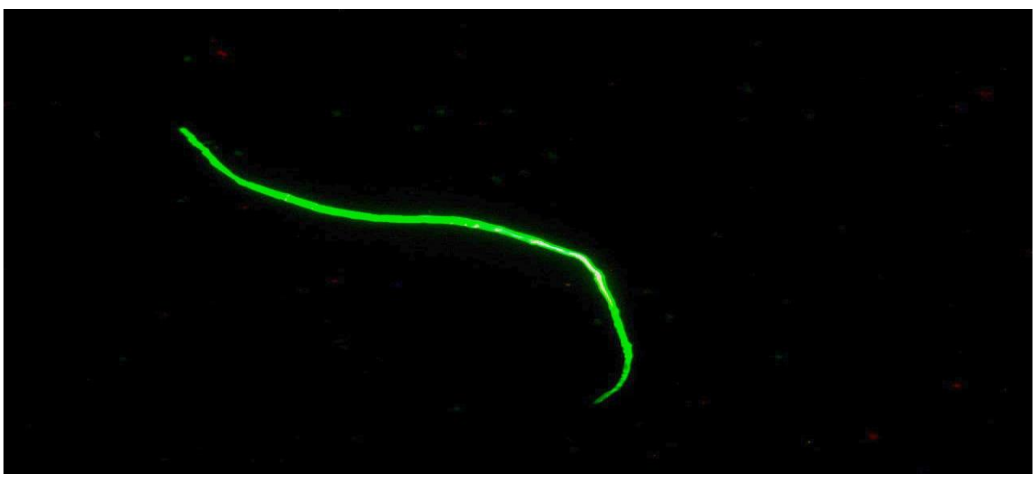
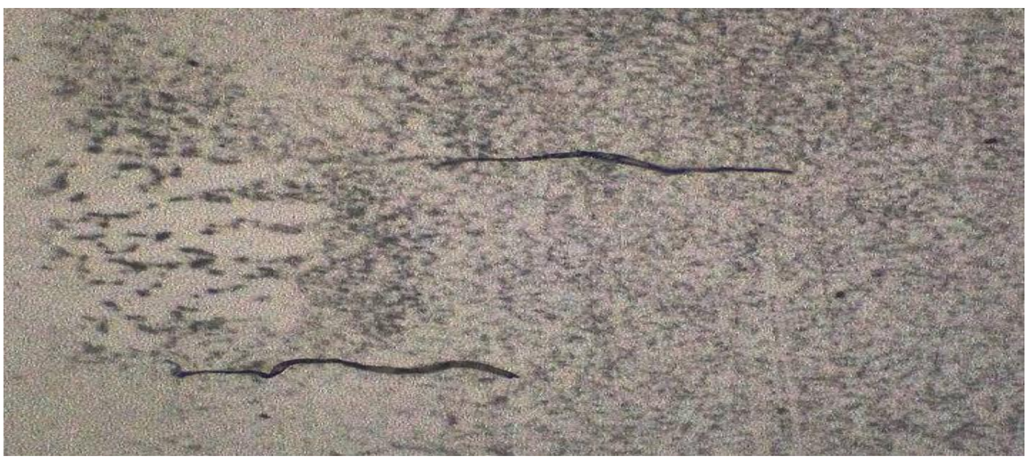


Fig (C): Blood smear stained with Acridine orange fluorochrome showing microfilaria under magnification (4X) power by using a digital camera.

Fig(D): Blood smear stained with Hematoxylin and eosin stain showing sheathed microfilaria magnification (10X) power by using a digital camera.



Fig(E): Blood smear stained with Leishman stain showing microfilaria under magnification (4X) power by using a digital camera.

Discussion

Microfilariosis in cattle and buffaloes reached 50% overall infection rate, which indicated an increase in the disease's incidence. This high infection rate may be attributed to the availability of intermediate hosts, like flies and mosquitoes, which are responsible for transmitting microfilaria (9). Our results agreed with Alabadi and Aghwan (17), Karki (18) and the study of Hussein and Aghwan (9), who recorded a total infection rate of microfilariae in sheep (56%, 60%,65%), respectively. The researchers (19,20,21) also recorded high percentage rates of microfilaria in farm animals (54.28% ,55%, 70%), respectively.

Many studies that recorded the occurrence of microfilaria in Mosul city have documented variation in infection rates in farm animals ranging from 0% to 100% (9,18,19,20,21,22,23). The parasitism in various parts of the world could be explained by differences 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 (22). We studied the relationship between some risk factors and intensity of the infection rates, like age of the animals, and we noticed that a low rate below one year old, while a high percentage of infection in both cattle and buffalo over three years old. The causes may also be due 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 (22). This is consistent with what was mentioned by (17,23-25), who indicated that the infection rate with microfilaria increases with the animal's age.

Severity of microfilariae infection showed three categories of infected (mild, moderate and severe) by the Modified Knott Technique. These differences in findings may be due to the difference in immunological status of animals. Our results were in agreement with the results of (17),

The relationship between age and infection rates showed a high percentage of infection in animals over three years old. 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. The relationship between sex of the animals and infection rates showed no significant differences in the percentage of infection in females and males. This result agreed with (19,24-27), and our study agreed with (17), who demonstrated that no significant difference was observed in the infection rate between males and females. Also, the result is in agreement with (28,29), who stated that the infection has occurred in both sexes equally. There were significant differences between animal husbandry and infection rates, with a higher rate in outdoor systems than in indoor systems. The reason may be attributed to the increase in their sensitivity to the microfilariae infection and inhibition of animal immune responses, as well as the effects of environmental conditions, herd infection, breeding conditions, field cleanliness, and the number of sucking insects present in the field. Our results were in agreement with the study of (17), who noticed that there were significant differences between animal husbandry and infection rates, a higher rate in outdoor systems (61.6%) than indoor systems (45.7%) in sheep. This study used different types of dyes for staining of microfilariae, such as methylene blue, M G G quick stain, Hematoxylin and eosin stain and finally acridine orange to identify microfilaria in cattle and buffalo blood samples.

These stains were characterized by high sensitivity for diagnosing this parasite at different microscopic magnifications of 4X, 10X, and 40X. Also, it was easy and quick to identify infection with the microfilaria because of the very short time during staining and examination, not more than 5 minutes (9,17,30). This suggests that M.G G quick stain and Acridine orange are reliable in detecting microfilariae infection in sheep.

Conclusion

The modified Knott's approach is a suitable procedure for detecting microfilariae, it is time-consuming and requires a centrifuge during the test. M.G rapid stain (Giemsa) is a reliable, quick procedure for detecting microfilariae and distinguishing their morphological properties. This stain is quick and easy to use, making it ideal for epidemiological research with a high number of samples. Acridine orange stain has a great sensitivity in recognizing microfilariae at microscopic magnifications of 4X and 10X.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical approval

The study and use committee of Veterinary Medicine College, University of Mosul, approved the study on 9/7/2025 (UM.VET.2025.048 decision number).

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التحري عن الخمج باليرقات الخيطية الدقيقة في الابقار والجاموس باستخدام الطرائق التقليدية في مدينه الموصل

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الخلاصة

أُجريت هذه الدراسة على 200 رأس من الأبقار والجاموس في مدينة الموصل خلال الفترة من آب 2025 إلى كانون الثاني 2026، بهدف الكشف عن وجود الميكروفيلاريا في دم الأبقار والجاموس باستخدام تقنية نوت (Knott's technique)، وصبغة جيمزا، وصبغة الأكريدين البرتقالي الفلورية، وصبغتي الهيماتوكسيلين والإيوزين، وصبغة ليشمان. أظهرت النتائج وجود ثلاث درجات من الخمج بالميكروفيلاريا هي: الخفيفة، والمتوسطة، والشديدة، مع وجود فروق معنوية ($P < 0.05$) . وبلغت نسبةالخمج 20% في الأبقار و30% في الجاموس خلال فترة الدراسة. كما لوحظت فروق معنوية بحسب عمر الحيوان؛ إذ سُجلت نسبة منخفضة في الحيوانات أقل من سنة واحدة بلغت 26.7% في الأبقار و40% في الجاموس، في حين ارتفعت نسبة الخمج في الحيوانات التي تزيد أعمارها عن ثلاث سنوات لتصل إلى 55% في الأبقار و75% في الجاموس. وأظهرت النتائج نسبة خمج أعلى في الإناث مقارنة بالذكور، حيث بلغت 45.5% في إناث الأبقار و66.7% في إناث الجاموس، بينما كانت نسبة الخمج في الذكور 33.3% في الأبقار و50% في الجاموس. كما بينت العلاقة بين نظم تربية الحيوانات ونسب الخمج زيادة الخمج في أنظمة التربية الخارجية، إذ بلغت 46.2% في الأبقار و64.3% في الجاموس، مقارنة بأنظمة التربية الداخلية التي سجلت 28.6% في الأبقار و50% في الجاموس. وأوضحت النتائج وجود زيادة في مستوى الخمج خلال فصل الصيف في الأبقار والجاموس بنسبة 61.1% و80% على التوالي، مقارنة بفصل الشتاء الذي بلغت فيه نسبة الخمج 12.5% في الأبقار و26.67% في الجاموس. كما كشفت الدراسة عن التوزيع الجغرافي للإصابة، حيث سُجلت أعلى نسبة خمج في منطقة بادوش مقارنة بالمناطق الأخرى، إذ بلغت 60% في الأبقار و72% في الجاموس.

الكلمات المفتاحية: اليرقات الخيطية الدقيقة، الابقار، الجاموس، الدم، گيمزا، التألق.