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Microscopic Diagnosis of Babesia (Piroplasm) Infection in Water Buffaloes (*Bubalus bubalis*) in Nineveh Governorate

Article Info.

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Abstract

The study results showed that the overall infection rate with Babesia spp. in water buffalo was 41.48%, based on microscopic examination of blood smears stained with Giemsa stain. The parasite was characterized by its appearance within red blood cells as a small, round, or oval shape, sometimes in pairs with a pear-shaped appearance, staining dark blue under the microscope. Examination using Acridine Orange stain also demonstrated high detection power for the parasite, showing the parasite shape as bright orange-yellowish within infected cells, while healthy cells appeared green. The study further demonstrated a statistically significant sex-related difference in infection prevalence ($P \leq 0.05$), with females showing a markedly higher infection rate of 52.32% compared with only 22.44% in males. The study detected infections which affected all age categories, but three-year-old animals and above showed the highest infection rate at 73.80%. The research established that different age groups showed substantial variations in their infection rates. The results also showed no significant differences ($P>0.05$) in infection rates between the regions included in the study.

Keyword: Babesia, Nineveh Governorate, buffalo, Acridine Orange stain.

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Introduction

Babesia spp. the three parasites represent major hemoprotozoan pathogens which affect ruminants to cause major health problems and economic losses throughout various hot and subtropical areas where tick vectors maintain their population (1). The parasites cause disease because they enter red blood cells to multiply, which results in various blood-related disorders that lead to anemia, jaundice, fever, weight loss and reduced animal output. The babesiosis develops when this condition affects specific animal species which act as natural hosts for the parasite. The disease acts as a major obstacle which prevents livestock development in numerous low- and middle-income countries (2,3).

The water buffalo (*Bubalus bubalis*) functions as a vital agricultural asset in Iraq because it generates substantial milk and meat output, which governorates like Nineveh depend on through their established buffalo breeding systems. Buffaloes show strong resistance to extreme environmental conditions, yet they face a high risk of blood-borne parasitic diseases, including *Babesia,* which damages their ability to produce and their general health condition (4,5,6).

Research on global epidemiology shows *Babesia bovis* and *Babesia bigemina* exist as the main buffalo-infecting species, which hard ticks from the Hyalomma and Rhipicephalus genera spread to their hosts. (7,8).

The clinical severity of babesiosis often escalates when co-infections occur with other hemoparasites such as *Theileria* spp. The immune system of animals becomes weaker, which makes them more prone to diseases, while insufficient food supply and environmental stressors increase their risk of getting sick (9). The combined effects of these infections result in major economic expenses because they cause decreased milk production, weight reduction, reproductive issues, death of animals, and higher costs for medical care and tick management programs (10).

Research on babesiosis in Iraqi cattle has expanded, but there is still a lack of studies which focus on *Babesia* infections that affect buffalo populations in the Nineveh governorate of northern Iraq. Epidemiological research needs to study current knowledge gaps because it will reveal disease transmission patterns, which will help develop specific control strategies for different geographic locations. This represents a clear knowledge gap, as buffalo herders in the region rely on traditional breeding and treatment methods, and cases are often diagnosed solely through clinical examination without the use of accurate laboratory diagnostic techniques. Furthermore, climate change, increased tick populations, and the expansion of buffalo farming in open, unprotected environments may lead to higher rates of babesiosis compared to previous years (11,12). Based on the limited number of studies in this field, this study comes to fill an important knowledge gap by determining the rate of Babesia infection in buffalo in Nineveh Governorate, with the Detection of the different species, and an accurate analysis of the influencing epidemiological factors, to support control programs and improve the health and productivity of buffalo in the region.

Material and Method

Collecting blood samples

One hundred and thirty-five blood samples were randomly collected from the water buffalo during field visits to various locations in Nineveh Governorate. The samples included both males and females of buffalo from different ages. Blood samples (3–5 mL) were collected from the jugular vein after thoroughly disinfecting the venipuncture site with 70% ethyl alcohol. Sterile, single-use, air-filled syringes were employed to maintain aseptic conditions and minimize the risk of contamination during collection. Immediately after collection, the blood samples were placed in special tubes containing the anticoagulant EDTA. After collection, all samples were transported in refrigerated boxes containing ice packs to maintain their quality and prevent spoilage during transport. The samples were subsequently transported to the Parasitology Laboratory at the College of Veterinary Medicine, University of Mosul, where they underwent comprehensive laboratory examinations and detailed analytical procedures (13,14)

Microscopic Examination of Blood Samples

Microscopic examination of blood samples was performed. Thin blood smears were prepared from all 135 samples and subsequently stained with Giemsa to facilitate microscopic identification of *Babesia* stages.

1- Preparation of 5% Giemsa Stain

A- Preparation of Giemsa Stock

Giemsa Stock was prepared by mixing 0.75 g of Giemsa powder with 25 mL of glycerin in a ceramic mortar until a homogeneous paste was formed. The mixture was then incubated for 30 minutes at 37°C, thoroughly mixed with 75 mL of absolute methyl alcohol, and incubated again for 24 hours at 37°C. (15)

B- Preparation of Giemsa Working Solution

The working Giemsa solution was prepared by diluting 5 mL of the stock reagent with 45 mL of distilled water, followed by filtration through standard filter paper. The prepared solution was then stored in dark glass bottles to prevent light-induced degradation before use.

C. Staining Method

All thin blood smears were prepared. The smears were fixed with absolute methyl alcohol for 3–5 minutes, and the smears were stained with 5% Giemsa for 30–60 minutes, rinsed gently with water, and allowed to air-dry. Once dried, they were examined under a light microscope at 100× magnification using immersion oil to facilitate accurate identification of *Babesia* parasites (15)

2-Preparation of Acridine Orange (AO) Stain:

Forty blood smears were randomly selected, fixed with absolute methyl alcohol, and stained with AO (16). Stock 0.5%: Dissolve 50 mg of AO powder in 10 mL of distilled water and store in an opaque bottle in the refrigerator for 4 weeks.

Working Solution (0.01%): Withdraw 1 mL of the stock solution, add 0.5 mL of glacial acetic acid, and bring the volume up to 50 mL of distilled water. The stain concentration is 0.01%, and the pH is 3.

Staining Procedure:

The smear was fixed with alcohol and left to dry, then immersed in working AO solution for two minutes, rinsed with water, and left to air dry. It was then examined using a fluorescence microscope (Optika-350B) at the University of Mosul.

Statistical analysis. The chi-square test was used to determine the significant differences in percentages between the different groups using SPSS version 25. The significance level was $P<0.05$.

Results

The results of the study showed that the overall percentage of infection with *Babesia* spp. Parasites in buffalo was 41.48%, based on microscopic examination of blood smears stained with Giemsa stain, where 56cases of infection were diagnosed out of a total of 135 samples in Nineveh Governorate (Table 1).

The *Babesia* parasites appeared inside the red blood cells as small, round or oval forms, and sometimes as paired pear-shaped structures. They stained a dark blue color under microscopic examination (Fig. 1). In this study, forty blood smears were examined with AO fluorescent stain. *Babesia* appeared as a bright orange-yellow within infected red blood cells, while uninfected cells had a green background.

The parasite was diagnosed in less than two minutes at 4X and 10X magnifications (Fig. 2). The study demonstrated that the infection rate with *Babesia* species in females was 52.32% (45/86), and it is more than in males 22.44% (11/49), with a significant difference ($P\leq0.05$). (Table 2).

The results indicated that Babesia infection occurred in all age groups, but with varying prevalence. A significant difference ($P\leq0.05$) was observed between younger and older animals. The highest infection rate was 73.80% (31/42) in animals older than three years. Animals aged more than one year showed an infection rate of 32.60 % (15/46), while the lowest rate, 21.27 % (10/47), was recorded in those younger than one year (Table3).

The results did not show any significant differences (P >0.05) in the infection rate of Babesia species according to the areas; the highest infection rate was in the Badoush area (50%) and the lowest in the Tel Kaif region (26.66%) (Table 4)

Table 1: Prevalence of Babesia spp. in Blood smears of water Buffaloes in Nineveh Governorate.

Number of examined	Number of infected buffalo	Percentage %
135	56	41.48

Table 2: Prevalence of *Babesia* spp. in water buffaloes according to sex.

sex	Number of samples examined	Number of positive samples	Percentage %
Male	49	11	22.44^{A}
Female	86	45	52.32^{B}

Distinct superscript letters within the same column indicate statistically significant differences at $P < 0.05$.

Table 3 :Prevalence of *Babesia* spp. in blood smears of buffaloes across different age.

Age/year	Number of samples examined	Number of positive sample	Percentage %
< 1	47	10	21.27^{A}
>1-3	46	15	32.60^{A}
> 3	42	31	73.80^{B}

Distinct superscript letters within the same column indicate statistically significant differences at $P < 0.05$.

Table 4 :Infection rates of *Babesia* spp. in blood smears of buffaloes based on the study regions

The areas of Mosul city	Number of samples examined	Number of positive samples	Percentage %
Badoush	20	10	50[A]
Hamidat	15	7	46.66[A]
Al-Saadoun slaughterhouse	10	4	40[A]
Hawi Al-Kanisa	13	6	46.15[A]
Tel Kaif	15	4	26.66[A]
Tal Asfour	14	6	42.85[A]
Zummar.	13	6	46.15[A]
sada bieuiza.	12	5	41.66[A]
Al-Shamsiyat	12	4	33.33[A]
Alslamia	11	4	36.36[A]

Distinct superscript letters within the same column indicate statistically significant differences at $P < 0.05$.

Discussion

Babesia infections in buffalo are a significant epidemiological problem in tropical and subtropical regions. Buffaloes are more susceptible to infection than some other ruminants due to traditional husbandry practices and high tick populations in the environment(17).

The results of the current study showed that 41.48% of the buffalo were infected with the *Babesia* parasite, a percentage close to that reported by researchers (18) and (19), whose infection rates were 45.74% and 41.66%, respectively. In contrast, our infection rate was higher than that reported by researchers (20,21) and (22), who recorded lower infection rates in buffalo of 31.1%, 16%, and 14.5%, respectively. Our study also showed a lower infection rate compared to that reported by the researcher (23), whose infection rate was 51.28%.

The variation in infection rates compared to other studies may appear to be related to weather changes, seasonal fluctuations, and age differences, factors that have also been shown to affect buffalo and many other animals, contributing significantly to reshaping the parasite distribution map.

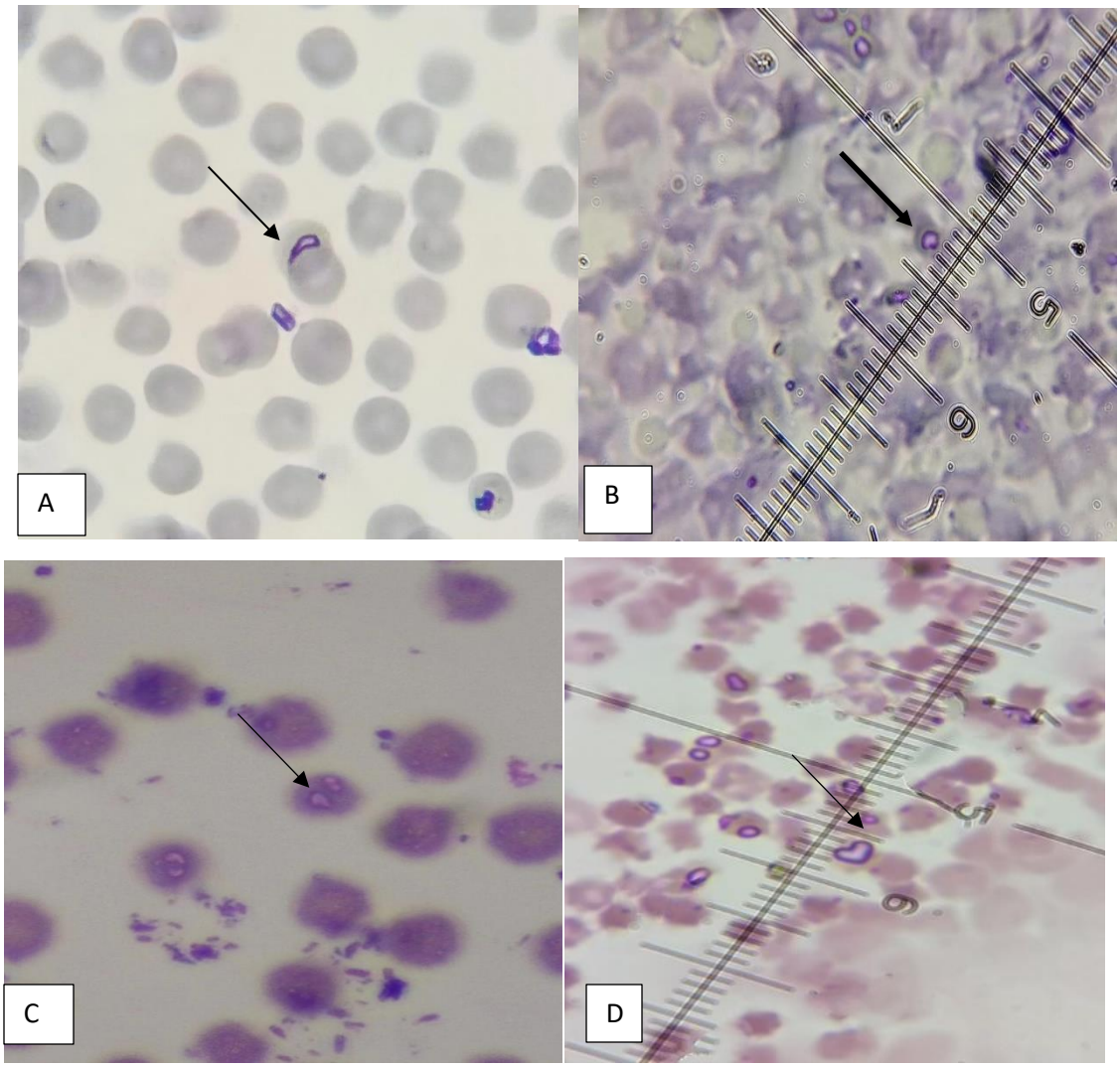

Fig 1*: Babesia* spp in different forms stained with Giemsa stain (A: amoeboid form; B: ring form, and C, D: pyriform in acute angle)X100.

The Giemsa-stained blood smears underwent microscopic analysis to show *Babesia* spp. intraerythrocytic stages, which were easily detectable. The parasites show their distinct shapes through pyriform and oval and sometimes double structures, which serve as the main features for parasite identification. The observed size range of approximately 1–5 µm matches the information found in standard parasitology references (24,25,26), which confirms that the studied morphological features match the diagnostic criteria for babesiosis

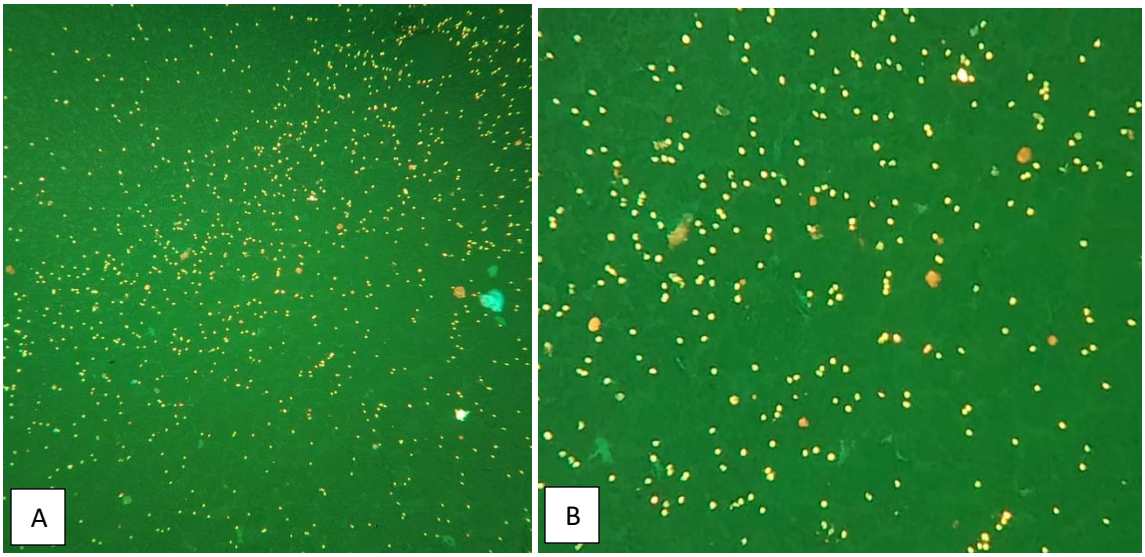

Fig2: Blood smear stained with AO showing *Babesia* parasite at 4X(A) and 10x(B) by using digital camera .

Acridine orange (AO) staining proved to be an essential diagnostic method which delivered advantages that surpassed conventional staining techniques. The fluorescence-based technique provided sensitive results at all magnification levels from 4× to 40×, and it processed big sample batches quickly because its staining method took under five minutes. The research findings demonstrate that AO staining provides useful results for large epidemiological studies because it enables fast diagnostic testing at large scales. The research results support previous studies, which demonstrated that this method works at the same speed as other methods (27).

The research results showed that infection prevalence between male and female participants differed significantly because female study participants developed infections at 52.32%, which exceeded the 22.44% infection rate among male participants. The different immune responses between pregnant and lactating women are due to the physical demands of pregnancy and lactation, which weaken their immune systems and make them more susceptible to tick-borne diseases. The duration of time that females spend with their herds leads to longer tick exposure periods. Research studies about ruminants under similar management systems have shown that sex-specific patterns exist according to previous investigations (28,29,30).

The study found that age served as a factor which determined infection risk because older animals developed the highest number of infections.

The pattern shows evidence of long-term tick vector contact, which resulted in increased chances of developing infections. Research conducted earlier has shown that the disease follows similar age-related patterns, which scientists believe result from multiple tick bites that affect the spread of babesiosis. The different study sites showed no substantial difference in infection rates, which indicates *Babesia* transmission patterns maintain similar patterns throughout the investigated areas. This finding corresponds with results from previous regional investigations, which similarly reported that intra-governorate geographic variation does not necessarily produce substantial differences in infection rates (29,32).

Conclusion

The study indicated that the overall infection rate of Babesia spp. in water buffalo was 41.48%. Giemsa and Acridine Orange stains proved highly effective in diagnosing the parasite. Significant differences in infection rates were observed based on sex, favoring females, and also based on age, with the highest rates observed in animals three years and older. No significant differences were found between the regions studied.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The work was completed in accordance with the ethical guidelines adopted by the College of Veterinary Medicine at the University of Mosul, and pursuant to official approval number UM.VET/2025/044

References

1. Abdullah, D. A., Ali, M. S., Omer, S. G., Ola-Fadunsin, S. D., Ali, F. F., & Gimba, F. I. (2019). Prevalence and climatic influence on hemoparasites of cattle and sheep in Mosul, Iraq. *Journal of Advanced Veterinary and Animal Research*, *6*(4), 492. doi: 10.5455/javar.2019.f373
2. Menshawy, S. M. (2020). A review on bovine babesiosis in Egypt. *Egyptian Veterinary Medical Society of Parasitology Journal (EVMSPJ)*, *16*(1), 8-19. DOI: 10.21608/evmspj.2020.81864

3. Herrera, P. C. T., Viloria, V. V., Balbin, M. M., & Mingala, C. N. (2017). Prevalence of babesiosis (Babesia bovis and Babesia bigemina) in cattle and water buffalo in Nueva Ecija, Philippines using nested polymerase chain reaction. *Annals of Parasitology*, *63*(4), 309-316.doi: 10.17420/ap6304.117.
4. Hildebrandt, A., Zintl, A., Montero, E., Hunfeld, K. P., & Gray, J. (2021). Human babesiosis in Europe. *Pathogens*, *10*(9), 1165.https:// doi.org/10.3390/pathogens10091165 .
5. Fesseha, H., Mathewos, M., Eshetu, E., & Tefera, B. (2022). Babesiosis in cattle and ixodid tick distribution in Dasenech and Salamago Districts, southern Ethiopia. *Scientific reports*, *12*(1), 6385.. doi: 10.1038/s41598-022-10416-4.
6. Rojas, C. E. S., Sivakumar, T., Mumbi, N. N. M., Ahedor, B., Valinotti, M. F. R., Acosta, T. J., & Yokoyama, N. (2025). Molecular epidemiological survey of Babesia species infecting cattle in Paraguay. *Veterinary Parasitology: Regional Studies and Reports*, *57*, 101162.. doi: 10.1016/j.vprsr.2024.101162.
7. Alvarez, J. A., Rojas, C., & Figueroa, J. V. (2019). Diagnostic tools for the identification of Babesia sp. in persistently infected cattle. *Pathogens*, *8*(3), 143.; https://doi.org/10.3390/pathogens8030143
8. Mirzayee, A. R., Fasehi, Z., Sapi, R. M., Himmat, A. M., & Karwand, B. (2024). Mechanism of Life Cycle and Transmission of Babesia. *British Journal of Biology Studies*, *4*(2), 19-28.. https://doi.org/10.32996/bjbs.2024.4.2.3
9. Vannier, E., & Krause, P. J. (2020). Babesiosis. *Hunter's tropical medicine and emerging infectious diseases*, 799-802.DOI: 10.1016/C2016-0-01879-X
10. Bock, R., Jackson, L., De Vos, A., & Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology*, *129*(S1), S247-S269.. doi: 10.1017/s0031182004005190.
11. Jalovecka, M., Sojka, D., Ascencio, M., & Schnittger, L. (2019). Babesia life cycle–when phylogeny meets biology. *Trends in parasitology*, *35*(5), 356-368.doi: 10.1016/j.pt.2019.01.007.
12. Ganguly, N., Parsani, H. R., Thakre, B. J., & Rathwa, S. D. (2024). Molecular Prevalence of Babesiosis in Buffaloes of North Gujarat. *Technical Advisors*, *20*(5), 61.. DOI: 10.48165/ijvsbt.20.5.11
13. Ali, K. N., & Marif, H. F. (2023). Babesiosis in cattle. *One Health Triad*, *3*, 114-121.. DOI: 10.47278/book.oht/2023.85
14. Yousef, S. G., Sobhy, N. M., Gouda, H., & Emam, M. H. (2024). Sero epidemiological study on bovine babesiosis in cattle and buffaloes in Sharkia Governorate, Egypt. *Open Veterinary Journal*, *14*(7), 1577.doi: 10.5455/OVJ.2024.v14.i7.7. Epub 2024 Jul 31.
15. Chaudhri, S. S., & Gupta, S. K. (2003). *Manual of general veterinary parasitology*. International Book Distributing Company..
16. Kronvall G. How to stain a clinical specimen with acridine orange of low pH. Available at: http://www.bioscand.se/kron/gk_bibliography.pdf. 2012.
17. Terkawi, M.A., Huyena ,N.X., Shinuoa, C., Inpankaew ,T., Maklon, K., Aboulaila ,M. (2011).Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in water

buffaloes in northeast Thailand. *Vet Parasitol*.;178:201–207. doi: 10.1016/j.vetpar.2011.01.041. Epub 2011 Jan 26.

18. Ateaa, R. S., & Alkhaled, M. J. A. (2019). Microscopic identification, molecular and phylogenetic analysis of Babesia species in buffalo from slaughter house in Al-Najaf city of Iraq.. DOI: 10.33899/ijvs.2019.162882
19. Tariq, M., Badshah, F., Khan, N. U., Zafar, M. I., De los Ríos-Escalante, P. R., Ibáñez-Arancibia, E., ... & Ben Said, M. (2024). Assessment of Babesia spp. prevalence in various domestic animals across Southern Punjab, Pakistan. *Brazilian Journal of Biology*, *84*, e277636.doi: 10.1590/1519-6984.277636. eCollection 2024.
20. Mahmmod, Y. (2013). Natural *Babesia bovis* infection in water buffaloes (Bubalus bubalis) and crossbred cattle under field conditions in Egypt: a preliminary study. *Journal of arthropod-borne diseases*, *8*(1), http://jad.tums.ac.ir
21. Alauttaish ,H.H., Abdulredha, N.W., Ali, D.H., Muhsen, R.K., AlSaad, I.A. (2023). Clinical, Sero epidemiological and hematological study of buffalo babesiosis in Basrah, Iraq. Methods. DOI: https://doi.org/10.31559/VMPH2024.5.2.8
22. Aziz, K. J., Waly, T. A., & Shekhmhamad, O. Q. (2025). Molecular Epidemiology of Babesiosis in Dairy Cattle in Erbil Province, Iraq. *Passer Journal of Basic and Applied Sciences*, *7*(1), 29-36.. 10.24271/psr.2025.214314
23. . Mohamed, G., & Ebied, M. (2014). Epidemiological studies on bovine babesiosis and theileriosis in Qalubia governorate. *BVMJ*, *27*, 36-48.
24. Soulsby EJL. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th ed. London: Baillière Tindall; 1982.
25. Suleiman, E., & Altaee, A. (2020). Microscopic And Molecular Study Of Babesia Spp. Babesia Bovis And Babesia Bigemina In Mosul City-Iraq. *Assiut Veterinary Medical Journal*, *66*(166), 1-13.DOI: 10.33899/ijvs.2017.145579
26. Garnham, P. C. C. (1980). Human babesiosis: European aspects.doi: 10.1016/0035-9203(80)90232-1.
27. 27. Ravindran, R., Lakshmanan, B., Sreekumar, C., John, L., Gomathinayagam, S., Mishra, A. K., & Rao, J. R. (2007). Acridine orange staining for quick detection of blood parasites. *journal of veterinary parasitology* 21(1):85-86.https://www.cabidigitallibrary.org › doi › pdf
28. Aktas, M., Altay, K., Dumanli, N. (2007).Estimation of prevalence and risk factors of babesiosis in cattle. *Vet Parasitol*.;148(2):187–193. doi: 10.1007/s00436-006-0345-2. Epub 2006 Nov 10.

29. Abed ,R.A., Hasso, S.A. (2019). Prevalence of theileriosis and babesiosis in cattle in Nineveh Province, Iraq. *Iraqi J Vet Sci*. DOI: https://doi.org/10.56286/bnznfk53

30.Anwar, K., & Din, A. (2017). Epidemiology of tick borne heamoprotozoan infection in ruminants in district Peshawar, and periphery, Khyber Pakhtunkhwa, (Pakistan). *Am. Sci. Res. J. Eng. Technol. Sci*, *35*, 191-200. http://asrjetsjournal.org/

31. Abbasi, F., Abbasi, I. H. R., Nissa, T. F., Bhutto, Z. A., Arain, M. A., Soomro, R. N., ... & Fazlani, S. A. (2017). Epidemiological study of tick infestation in buffalo of various regions of district Khairpur, Pakistan. *Veterinary world*, *10*(6), 688. doi: 10.14202/vetworld.2017.688-694.

32.Salim, B. (2015).Natural infection of Babesia and Theileria species in cattle and buffaloes in Sudan. *Vet Parasitol*.;214(1–2):39–45. https://doi.org/10.1016/j.parepi.2020.e00148

التشخيص المجهري لإصابة الجاموس بطفيلي الكمثريات في محافظة نينوى

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الخلاصة

أظهرت نتائج الدراسة أن معدل الإصابة الإجمالي بطفيلي الكمثريات في الجاموس 41.48%، وذلك بناءً على الفحص المجهري لمسحات الدم المصبوغة بصبغة كيمزا . وتميز الطفيلي بظهوره داخل خلايا الدم الحمراء على شكل أشكال صغيرة مستديرة أو بيضاوية، وأحيانًا على شكل أزواج ذات مظهر كمثري، ولون أزرق غامق تحت المجهر. كما أظهر الفحص باستخدام صبغة أكريدين البرتقالية قوة كشف عالية للطفيلي، حيث أظهرت أشكال الطفيلي أنها برتقالية-صفراء زاهية داخل الخلايا المصابة، بينما بدت الخلايا السليمة خضراء. وكشفت الدراسة أيضًا عن وجود فرق كبير في معدلات الإصابة بين الجنسين، حيث أظهرت الإناث إصابة بنسبة 52.32% مقارنة بنسبة 22.44% لدى الذكور. ولوحظت الإصابة في جميع الفئات العمرية، حيث سجلت الحيوانات التي يزيد عمرها عن ثلاث سنوات أعلى معدل إصابة بنسبة 73.80%، وأظهرت النتائج أيضًا عدم وجود فروق معنوية في معدلات الإصابة بين المناطق المشمولة بالدراسة.

الكلمات المفتاحية: الكمثريات، محافظة نينوى، الامراض المنقولة عبر القراد ، صبغة الأكريدين البرتقالية.