Tikrit Journal of Veterinary Sciences (2026) 5(1): 86-93
DOI : 10.25130/tjvs.5.1.10

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Science

Assessment of Serum Concentrations of IL-4, IL-6, and TNF-α in Cattle Infected with *Trypanosomiasis*

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ARTICLE INFO.

Article history:
-Received:17/7/2025
-Received In Revised Form:25/8/2025
-Accepted: 8/9/2025
-Available online: 30/6/206

Keywords:
bovine *Trypanosomiasis*; *Trypanosoma*; interleukin-6; tumor necrosis factor-α; interleukin-4; ELISA; cytokine biomarkers.

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ABSTRACT

Background and aim: Bovine Trypanosomiasis, caused by *Trypanosoma* spp., is an economically important parasitic disease that adversely affects cattle health and productivity in tropical and subtropical regions. The host cytokine response is a key determinant of disease progression and severity. This study aimed to evaluate the serum levels of IL-4, IL-6, and TNF-α in naturally infected cattle and determine their association with bovine Trypanosomiasis in Diyala Province, Iraq.

Materials and Methods: A total of 550 cattle were screened for Trypanosoma spp. infection by microscopic examination, and 43 animals were identified as infected. Serum samples from 40 infected cattle were analyzed for IL-4, IL-6, and TNF-α using enzyme-linked immunosorbent assay (ELISA). Cytokine prevalence and serum concentrations were statistically analyzed, with significance set at $p < 0.001$.

Results: The prevalence of *Trypanosoma* spp. infection was 7.8% (43/550). Positive cytokine expression was detected in 70.0% of cattle for IL-4, 87.5% for IL-6, and 82.5% for TNF-α. Serum concentrations of all three cytokines were significantly higher in infected cattle than in non-infected controls ($p < 0.001$), indicating a strong association between cytokine response and infection.

Conclusions: Serum levels of IL-4, IL-6, and TNF-α were significantly elevated in cattle with bovine Trypanosomiasis, indicating their important role in the host immune response. IL-6 showed the strongest diagnostic potential, while TNF-α and IL-4 reflected inflammatory and immune regulatory responses. These cytokines may serve as useful biomarkers for disease diagnosis and monitoring.

Introduction

Bovine *Trypanosomiasis*, a vector-borne parasitic disease caused by *Trypanosoma*, threatens cattle and human health in tropical/subtropical regions, especially Africa, Asia, and South America. *T. congolense, T. vivax, and T. evansi* mainly affect cattle, [1] spreading via tsetse flies and other means. It causes anemia, weight loss, reproductive problems, organ dysfunction in animals and death [2]. The immune response of the host is crucial in determining the infection's outcome [3]. Innate immunity, through macrophage activation and cytokine production, initially helps limit parasitemia. Adaptive immunity, particularly the production of antibodies against variable surface glycoproteins (VSGs), aids in parasite clearance [4]. However, the parasite's ability to undergo antigenic variation allows it to evade immune detection, [5] resulting in persistent infections. Chronic infection can provoke immunopathology characterized by cytokine imbalance, [6] systemic inflammation, and anemia. Moreover, *Trypanosoma* spp. manipulate host immunity by promoting regulatory cytokines and inducing T-cell exhaustion, which hampers effective clearance [7].

Cytokines such as IL-6, IL-4, and TNF-α play key roles in the disease's pathogenesis and immune regulation [8]. IL-6 acts as an inflammatory mediator, influencing fever, immune cell migration, and neuro inflammation; however, sustained elevation can lead to chronic inflammation [9]. IL-4 supports antibody-mediated and anti-inflammatory responses; maintaining proper levels helps clear parasites while preventing excessive inflammation [10). TNF-α helps control parasite growth via nitric oxide but can also cause tissue damage, leading to anemia and neurological issues [11].

Materials and methods

Samples and data collection

The current study was conducted from July 2024 to May 2025, involving the examination of 550 cattle of various ages and sexes in Diyala province. The cattle presented with clinical signs, including anemia (characterized by pallor of the mucous membranes and vaginal area in females), swelling of the pre-femoral and pre-scapular lymph nodes, severe emaciation, and general malaise. Blood samples (5 mL) were collected from each animal via jugular venipuncture. Of the total volume, 2 mL was transferred to EDTA tubes for Giemsa-stained thick and thin microscopic examination, wile 3 mL of blood was collected in gel tubes for serum separation through centrifugation, and the samples were then transported in refrigerated boxes to the RNA Laboratory, a private facility in Mosul. Following 5-10 minutes for clotting at room temperature, the tube were centrifuged for 5-10 minutes at 2000-3000 RPM, (12). The serum was pipetted off and stored frozen in a sterile tube for interleukin concentration measurements (ELISA for IL-4, IL-6, and TNF-alpha).

Laboratory examination

Microscopic examination

Diagnosing *Trypanosoma* involves analyzing Giemsa-stained thin and thick blood smears, [13]. The preparation process includes spreading blood onto a slide, fixing it with absolute methanol once it has dried, and then staining with Giemsa dye for 10 to 15 minutes. Following staining, the slides are rinsed with a buffer solution and left to air dry, then viewed under a microscope at 100X magnification with oil immersion lens, [14].

Serological examination

The study analyzed serum samples from infected cattle to determine interleukin-4, interleukin-6, and tumor necrosis factor-alpha levels using sandwich ELISA, [15].

In the current study conducting the examination, the levels of interleukin-4, interleukin-6, and tumor necrosis factor-alpha were measured using specific kits for each from the company (Hangzhou, China). The tests and procedure were performed according to the instructions of the ELISA kit manufacturer.

Results

Microscopic examination Giemsa staining of blood samples from 550 cattle revealed a 7.82% *Trypanosoma* infection rate. Detection rates were determined using thick and thin smear techniques, as show figure (1).

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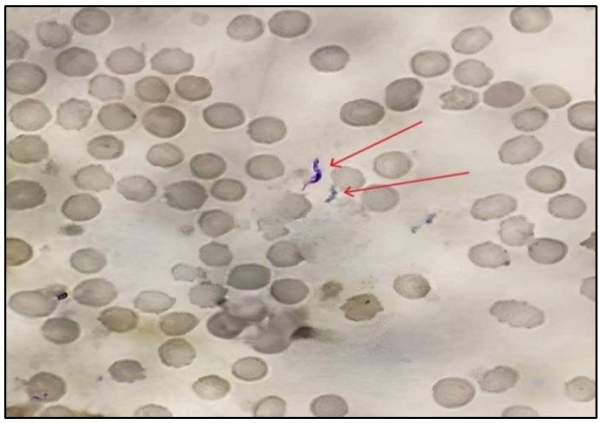

Figure (1) *T. congolense* by Giemsa stain under power 100 X Light Microscope.

Elisa result: **Measurement of IL-4, IL-6, and TNF-α cytokine concentrations by sandwich ELISA.**

IL-4, the analysis revealed that 70% of infected bovines tested positive for IL-4 (28 out of 40), while 30% of uninfected bovines tested negative (12 out of 40) as shown in Figure (2). A chi-square value of 6.40 with a significance level of P=0.010 indicates a significant relationship between IL-4 test results and the studied condition as shown in Table (1)

Table 1: Distribution of IL-4 in infected cattle with *Trypanosoma.*

IL-4 result	NO.	Percentage %
Positive	28	70.00
Negative	12	30.00
Total	40	100%
Chi-Square (P-value	…….	6.40 ** (0.010)
** (P≤0.01).		

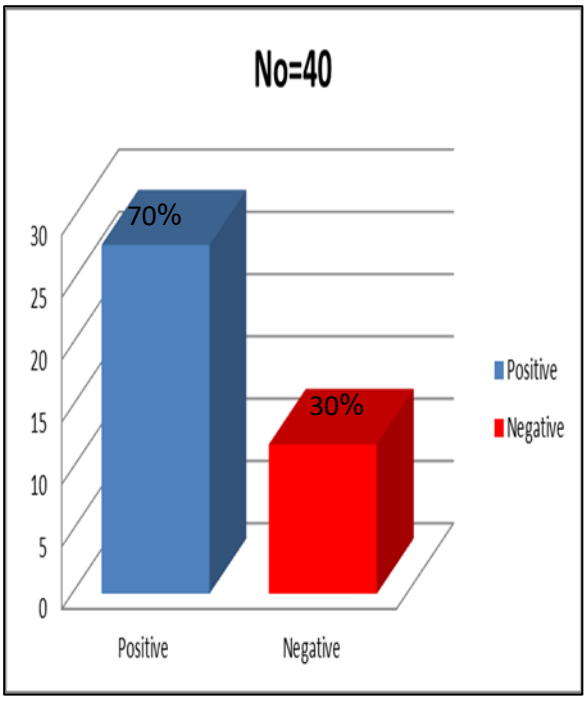

Figure (2) comparison between positive and negative IL4 in examined cattle.

IL-6, the study results indicated that 87.5% of infected bovines tested positive for IL-6 (35 out of 40), while 12.5% tested negative (5 out of 40) as shown in Figure (3). The chi-square test results showed a statistically significant difference, with a significance level of P = 0.0001 and a chi-square value of 22.50. This demonstrates a strong, significant relationship between the IL-6 test results and the condition being studied, as shown in Table (2).

Table 2: Distribution of IL-6 in infected cattle with *Trypanosoma*.

IL-6 result	No	Percentage (%)
Positive	35	87.50
Negative	5	12.50
Total	4o	100%
Chi-Square (P-value)	……	22.50 ** (0.0001)
** (P≤0.01).		

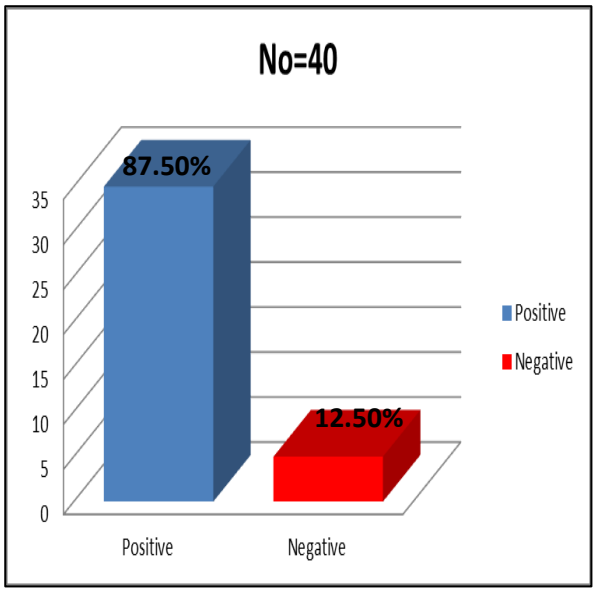

Figure (3) comparison between positive and negative IL6 in examined cattle.

TNF-α, according to the analysis's findings, 82.5% of the cows had positive TNF-α tests (33 out of 40), and 17.5% had negative tests (7 out of 40) as shown in Figure (4). With a chi-square score of 16.90 and a p-value of 0.0001, a chi-square test revealed a statistically significant difference. This data emphasizes the involvement of TNF-α in the immunological response associated with the illness and implies a strong and substantial link between the test results and the condition under study, as shown in Table (3).

Table 3: Distribution TNF-α in infected cattle with *Trypanosoma*.

TNF-α	No	% percentage
Positive	33	82.50
Negative	7	17.50
Total	40	100%
Chi-Square (P-value)	…….	16.90 ** (0.0001)
** (P≤0.01).		

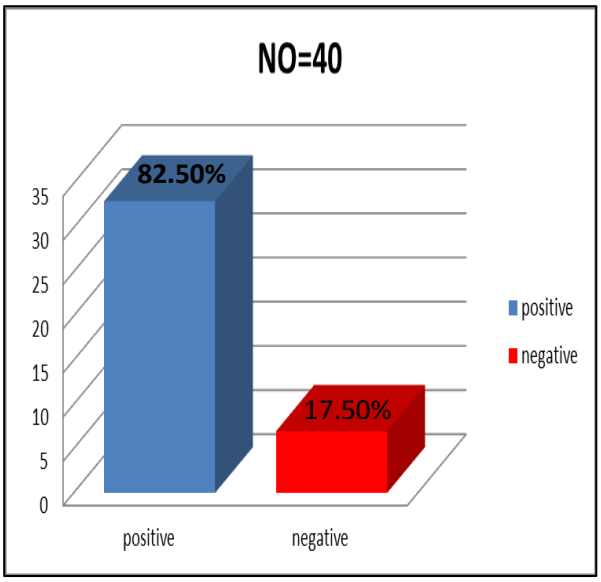

Figure (4) compration between positive and negative TNF-α examined cattle.

Discussion:

Bovine *Trypanosomiasis* leads to significant losses in livestock productivity and economic stability. Reliable diagnosis in endemic areas typically relies on laboratory methods such as microscopic blood smears and molecular techniques like PCR, [16], this study used ELISA to profile cytokines in infected cattle. Results showed high positivity rates for IL-4 (70%), IL-6 (87.5%), and TNF-α (82.5%) with significant χ^2 values ($p \leq 0.01$), consistent with previous findings.

Elevated levels of IL-4, which indicate a type of immune response that helps reduce inflammation, are often observed in chronic infections. Evidence from a previous study reported increased levels of IL-4 and IL-10 in cattle exposed to parasites, suggesting changes in immune activity, [17]. Similarly, another study found that IL-4 mRNA peaked around 32 days' post-infection in trypanotolerant cattle, indicating a potential protective role, [18]. Our study's finding that 70% of the infected animals tested positive for IL-4 ($p = 0.010$) supports its role as an immune response marker. In contrast, studies on naturally infected cattle have shown little to no variation in IL-4 levels, [19]. Additionally, another study reported no significant difference in IL-4 levels between infected and uninfected cattle. These discrepancies are likely due to IL-4's rapid degradation in the body, emphasizing the importance of timing when studying IL-4 in real-world scenarios, [20].

IL-6, a key pro-inflammatory cytokine, plays a significant role in the immune response to

trypanosome infections. A study reported a rapid rise in IL-6 mRNA expression in susceptible Boran cattle around 11 days' post-infection, which remained elevated through days 21 and 32. In contrast, levels of IL-6 peaked at a later stage in trypanotolerant N'Dama cattle, indicating a delayed immune response compared to susceptible Boran cattle. This suggests IL-6 may reflect susceptibility and disease progression, [21]. According to a study on experimental *T. vivax* infections, cattle that effectively controlled parasitemia maintained high levels of IL-6. In contrast, animals with poor control experienced declines in both IL-6 and TNF-α, [22]. Our study confirmed the presence of IL-6 in 87.5% of infected cattle, underscoring its role in disease progression and validating it as a reliable biomarker for infection severity.

in bovine *Trypanosomiasis*, effective immune responses during natural infections maintain high levels of the pro-inflammatory cytokine TNF-α, whereas cattle unable to control the infection show reduced levels. This reflects the activation of both Th1 (IL-6, TNF-α) and Th2 (IL-4) pathways, with IL-6 and TNF-α contributing to early parasite control and inflammation, [23].In our study, TNF-α was detected in 82.5% of infected cattle ($p < 0.0001$), consistent with previous reports linking elevated TNF-α to parasitemia and anemia, [24]In contrast, *T. congolense* infections typically induce minimal changes in TNF-α mRNA and weak cytokine production, indicating a milder immune response, [25].These differences likely result from variations in parasite species, host genetics, infection stage, sampling timing, and methodology (mRNA vs. serum protein). Consequently, TNF-α serves as a reliable marker of inflammation in *T. vivax* infections but is less informative in *T. congolense*, [26).

The statistically significant elevations in IL-4, IL-6, and TNF-α observed in this study align with existing research and suggest their potential as biomarkers for bovine *Trypanosomiasis*. Cytokine panels incorporating these markers could improve diagnostic precision and differentiate between acute and chronic infection phases.

Conclusion:

The findings of this study demonstrate a significant association between elevated levels of IL-4, IL-6, and TNF-α and the infection status of bovines. The high percentage of positive test results among infected animals, along with statistically significant chi-square values for all three cytokines ($P \leq 0.01$), indicates the crucial role of these inflammatory markers in the immune response to the studied condition. Specifically:

IL-4 demonstrated a significant association with infection, indicating its pivotal role in regulating immune responses.

IL-6 displayed the highest positive rate and the strongest statistical association, underscoring its potential as a critical and sensitive biomarker for determining infection status.

TNF-α also exhibited a robust and significant connection to the condition, further solidifying its crucial involvement in the inflammatory response linked to the disease.

Overall, these findings underscore the importance of analyzing cytokines in understanding disease progression and suggest that IL-4, IL-6, and TNF-α may serve as valuable markers for diagnosing and monitoring this infection in bovines.

Acknowledgement

I extend my sincere gratitude to the College of Veterinary Medicine, Tikrit University (Dean's office), the Microbiology Department, the Postgraduate Division, and my supervisors, Asst. Prof. Dr. Omaima Ibrahim Mahmoud, for their invaluable support throughout my study.

Declaration of interests

The authors declare no conflict of interest.

Funding Sources

This research received no financial support or specific grant from any funding agency in the commercial, public, or not-for-profit sectors.

Publication consent

All authors have critically reviewed and approved the final version of the manuscript and have provided their formal consent for its publication.

Data and material availability:

All data analyzed and generated in this study are included in this published research.

Author contribution:

All authors participated in the study design and conception, data analysis, data collection, performance of the results, and assent to the final version.

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Tikrit Journal of Veterinary Sciences (2026) 5(1): 86-93
DOI : 10.25130/tjvs.5.1.10

قياس مستويات الانترليوكين السادس ,الرابع وعامل النخر الورمي الفا في مصل الابقار المصابة بالمثقبيات.

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الملخص

الخلفية والهدف يعد داء المثقبيات البقري، الناجم عن طفيليات *Trypanosoma spp* من الأمراض الطفيلية ذات الأهمية الاقتصادية، إذ يؤثر سلبًا في صحة الأبقار وإنتاجيتها في المناطق المدارية وشبه المدارية. وتُعد استجابة السيتوكينات لدى العائل عاملًا رئيسيًا في تحديد تطور المرض وشدته. هدفت هذه الدراسة إلى تقييم المستويات المصلية لكل من IL-4 و IL-6وTNF-α في الأبقار المصابة طبيعيًا، وتحديد ارتباطها بداء المثقبيات البقري في محافظة ديالى، العراق.

المواد طرق العمل : شملت الدراسة فحص 550 رأسًا من الأبقار للكشف عن الإصابة بطفيليات *Trypanosoma spp*. باستخدام الفحص المجهري، حيث تم تشخيص إصابة 43 حيوانًا. وتم تحليل عينات المصل لـ 40 بقرة مصابة لقياس مستويات IL-4 و IL-6وTNF-α باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA). كما أُجريت التحليلات الإحصائية لتقييم معدلات ظهور السيتوكينات وتراكيزها في المصل، واعتمد مستوى الدلالة الإحصائية عند ($p < 0.001$).

النتائج: بلغت نسبة انتشار الإصابة بطفيليات *Trypanosoma* spp7.8% (43/550). وكُشف عن التعبير الإيجابي للسيتوكينات بنسبة 70.0% لـIL-4 ، و87.5% لـIL-6 ، و82.5% لـ TNF-α. كما كانت التراكيز المصلية لجميع السيتوكينات الثلاثة أعلى بصورة معنوية في الأبقار المصابة مقارنةً بالأبقار غير المصابة (المجموعة الضابطة $p < 0.001$) مما يشير إلى وجود ارتباط قوي بين استجابة السيتوكينات والإصابة.

الاستنتاجات: أظهرت الأبقار المصابة بداء المثقبيات البقري ارتفاعًا معنويًا في المستويات المصلية لكل من IL-4 وIL-6 وTNF-α، مما يدل على دورها المهم في الاستجابة المناعية للعائل. وأظهر IL-6 أعلى قدرة تشخيصية محتملة، في حين عكس كل من TNF-α وIL-4 الاستجابات الالتهابية والتنظيمية للمناعة. وتشير هذه النتائج إلى أن هذه السيتوكينات يمكن أن تُستخدم كمؤشرات حيوية واعدة لتشخيص المرض ومتابعة تطوره.

الكلمات المفتاحية: المثقبية البقرية؛ المثقبيات؛ إنترلوكين-6؛ عامل نخر الورم الفا؛ إنترلوكين-4؛ إليزا؛ مؤشرات السيتوكينات الحيوية.