Immunological Variables In Cattle Infected With Blood Protozoa Transmitted By Ticks And Horseflies In Samarra City

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KEYWORDS

Ticks, Immunoglobulins, Major Histocompatibility Complex (MHC), Horseflies

ABSTRACT

The results of the immunological tests on cattle infested with ticks and horseflies showed significant differences between the groups of infested cattle. There was a highly significant difference in the immunoglobulin IgM, which reached 54, compared to the healthy cattle group. Additionally, there were highly significant differences in the surface markers CD8, CD4, and the MHCII complex in the groups of cattle infested with ticks and horseflies compared to the healthy cattle groups. However, no significant differences were observed between the infested and healthy cattle groups in terms of immunoglobulin IgG and complement G3.

1. Introduction

Ectoparasites (such as ticks, mites, lice, blood-sucking flies, and fleas) are among the most significant factors affecting the economy of many countries that rely on livestock. These parasites cause physiological and pathological damage, negatively impacting the host's life, growth, and reproductio (Zaheer, et al., 2022). The phylum Arthropoda includes several subphyla, among them the subphylum Mandibulata, which includes various classes such as Insecta, comprising lice, fleas, mosquitoes, and horseflies. Another subphylum is Chelicerata, which includes the class Arachnida, comprising ticks and mites (Sebastin & Gautam, 2021) Hard ticks are arthropods of medical and veterinary importance, known for transmitting numerous pathogenic microorganisms, including protozoa, viruses, and bacteria. They feed on the blood of vertebrate animals, thriving in hot and humid environments, while cold and dry environments hinder their growth and development (Hurtado & Giraldo, 2018; Tenzin & Rinzin, 2023).

Among the protozoal diseases transmitted by ticks is Texas fever, which is prevalent in Europe, America, the Philippines, and Africa. The primary vector of Texas fever is B. annulatus, which transmits the parasite Babesia bigemina. Babesia is passed from one tick generation to the next through the eggs (Tawana, et al., 2021). Ticks can also cause tick paralysis in sheep and cattle, which is severe in domestic animals and presents as progressive motor weakness due to the inhibition of acetylcholine release at nerve junctions, resulting from the blockage of motor nerve fibers by toxins secreted by the tick's salivary glands (Diakou, 2024; Al-Lahaibi & Al-Taee,2019). Horseflies are among the most important arthropods belonging to the class Insecta and order Diptera, within the family Tabanidae. They cause significant economic losses and veterinary diseases in livestock due to their blood-sucking behavior. Their painful bite, attributed to their large and strong mouthparts, causes bleeding at the wound site. The mechanical transmission of diseases like anthrax, anaplasmosis, and jaundice, which can lead to abortion in infected animals and may even result in their death, is also of concern (Alam et al., 2022). Immunoglobulins (Ig) are glycoprotein molecules produced by plasma cells in response to an immunogen and function as antibodies (Nagres, 2017). These immunoglobulins are produced by B-lymphocytes and bind tightly to the antigen present within the pathogen, aiding in its recognition and subsequent attack, leading to its direct elimination. The body produces thousands of different antibodies, each specific to a particular antigen Delves, 2020).

Aim of the Study

To investigate the immunological changes (CD4, CD8, C3, IgG, IgM, MHC) that occur as a result of

infection with each type of blood-feeding parasite in the host, specifically due to infestation with ticks and horseflies.

2. Methodology

Collect Blood Samples:-

Blood samples were drawn from the jugular vein of 227 cattle using a 15 ml medical syringe. A volume of 15 ml was collected and divided into several portions. 2.5 ml was placed into tubes containing the anticoagulant EDTA, while the remaining amount was placed into Gel tubes, which do not contain an anticoagulant. The blood in the Gel tubes was then centrifuged for ten minutes at 3000 RPM to separate the serum, which was subsequently placed into small Eppendorf tubes and stored in a freezer at -20°C until further immunological and biochemical analysis (Hussein, 2017).

Immunological Examinations

Estimation of IgM Bovine Immunoglobulin M:- The concentration of IgM was estimated using a kit produced by the Chinese company Sunlong, following the steps provided with the kit.

Principle of the Method:- A sandwich ELISA kit was used, where a microplate was pre-coated with specific antibodies against the IgM enzyme, added by the manufacturer. Samples and standards were added to the small wells of the ELISA plate, where they bound to the specific antibodies. Subsequently, an avidin-HRP complex specific for IgM was added to each well in sequence, followed by incubation and washing. TMP solution was then added to each well. The wells containing IgM and the avidin-HRP complex coupled with antibodies against IgM turned blue, then changed to yellow after adding the stop solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm, with the OD value being proportional to the IgM concentration. The IgM concentration in the samples can be calculated by comparing the OD to the standard curve.

Procedure:-

1-Dilution of Standards: Five tubes were prepared for dilution and numbered from 1 to 5. Into tube 1, 300 µl of the standard solution was added along with 150 µl of standard diluent and mixed well. 300 µl was drawn from tube 1 and added to tube 2, along with 150 µl of standard diluent. In tube 3, 150 µl was drawn from tube 2 and 150 µl of standard diluent was added. In tube 4, 150 µl was drawn from tube 3 and 150 µl of standard diluent was added. In tube 5, 150 µl was drawn from tube 4 and 150 µl of standard diluent was added, yielding final concentrations of 36 ng/ml, 24 ng/ml, 16 ng/ml, 8 ng/ml, 4 ng/ml, and 2 ng/ml, respectively.

2-Sample Addition: In the ELISA plate, one well should be left blank. In the sample wells, 40 µl of the buffer solution and 10 µl of the sample were added (dilution factor 5). Samples should be added to the bottom of the well without touching the walls, and mixed well with gentle shaking.

3- Incubation: The plate was incubated for 30 minutes at 37°C.

4-Dilution: The buffer solution was diluted with distilled water (30 times for 96 samples and 20 times for 48 samples).

5-Washing: The plate was washed 3-5 times.

6-Addition of HRP Conjugate: 50 µl of HRP conjugate was added to each well except the blank well.

3- Incubation: As in step 3.

8- Washing: As in step 5.

9-Color Development: 50 µl of Chromogen A and 50 µl of Chromogen B were added to each well, mixed, and shaken well, then incubated at 37°C for 15 minutes.

10-Reaction Termination: 50 µl of stop solution was added to each well, terminating the reaction. The color in the wells should change from blue to yellow.

11-OD Reading: The OD was measured at a wavelength of 450 nm using a Microtiter plate reader, and the test should be conducted within 15 minutes after adding the stop solution.

Estimation of IgG (Bovine Immunoglobulin G): - The concentration of IgG was estimated using a kit produced by the Chinese company Sunlong, following the steps provided with the kit.

Principle of the Method: - The technique is similar to defined above for IgM, with precise antibodies and reagents for IgG.

Estimation of CD4 (Bovine Cluster of Differentiation4): - The concentration of CD4 become estimated the usage of a package produced with the aid of the Chinese employer Sunlong, following the steps provided with the package.

Principle of the Method: - The method is similar to described above for IgM, with precise antibodies and reagents for CD4.

Estimation of CD8 (Bovine Cluster of Differentiation 8): - - The awareness of CD8 changed into expected using a package produced by way of theChinese enterprise Sunlong, following the stairs supplied with the package..

Principle of the Method: - The approach is the same as defined above for IgM, with unique antibodies and reagents for CD8.

Estimation of C3 (Bovine Complement Component 3): - The awareness of C3 was envisioned the use of a kit produced by means of the Chinese corporation Sunlong, following the stairs supplied with the package.

Principle of the Method: - The method is the same as described above for IgM, with specific antibodies and reagents for C3.

Estimation of MHCII (Bovine Major Histocompatibility Complex II): - The concentration of MHCII was estimated using a kit produced by the Chinese company Sunlong, following the steps provided with the kit.

Principle of the Method: - The method is the same as described above for IgM, with specific antibodies and reagents for MHCII.

3. Result and Discussion

Immunological Variables of Cattle Infected with Ticks and Horseflies in Samarra City and Its Surrounding Areas.

The results presented in Table (1-3) show no significant differences in the average value of Immunoglobulin G (IgG) in cattle infected with ticks and horseflies, with a mean of 20.33 compared to 18.75 in the healthy group. However, there was a highly significant increase in Immunoglobulin M (IgM) in the infected group, with a value of 54.00 compared to 34.03 in the healthy group. The value of Cluster of Differentiation 8 (CD8) was significantly lower in the infected group, at 2979, compared to 3906 in the healthy group. As for Cluster of Differentiation 4 (CD4), there was a significant decrease in the infected group, with a value of 2429 compared to 3055 in the healthy group. No significant difference was observed in the Complement component C3, with the infected group having a value of 44.77 compared to 46.88 in the healthy group. However, a significant difference was noted in the Major Histocompatibility Complex Class II (MHCII), with the infected group having a significantly lower value of 1552 compared to 2025 in the healthy group.

The absence of great differences in the IgG degrees can be attributed to the fact that inflamed livestock have evolved a form of immune model in opposition to those parasites, making their immune response comparable regardless of infection. Research shows that diverse forms of flies, which includes horseflies and ticks, can purpose strain and reduce productivity in cattle, but their impact on precise immune additives along with IgG stages won't constantly be giant. For instance, horn flies and stable

flies are recognised to affect farm animals by way of decreasing their weight and milk production because of the strain and blood loss resulting from the flies (Campbell, 2008; Taylor, et al., 2012). This is constant with our have a look at, which located no great differences in IgG degrees among livestock infected with ticks and horseflies and healthful ones.

Recent and older studies have proven that the immune reaction, especially IgG degrees, may not range significantly among infected and non-inflamed cattle due to the fact IgG manufacturing is a complex system prompted via numerous factors, inclusive of the overall health of the animal, nutrients, and the presence of different sicknesses. Moreover, IgG degrees may be maintained inside everyday limits regardless of infections because of the cattle's adaptive immune responses, which goal to stability contamination response and homeostasis. While horsefly and tick infestations may have numerous poor effects on cattle, the particular impact on IgG degrees might not be extensive because of the complicated nature of immune responses and farm animals's capability to adapt to those stresses (Martins,et al., 2020; Taylor, et al., 2012).

Noted Qin, et al., (2017) of their study on unique cattle breeds and the impact of tick and horsefly infestations that resistant breeds just like the Nelore preserve solid IgG tiers at some point of infestation, while extra prone breeds like Holstein show a decline in IgG degrees particular to ticks. Additionally, IgG antibodies play an energetic function in opposition to tick saliva components, supporting to reduce the effect of infestations. Recent research at the impact of horsefly and tick infestations on IgG tiers in livestock in Brazil display that sure tick infestations can cause modifications in IgG degrees, in particular in greater inclined breeds like Holstein (Heylan, et al., 2023). Furthermore, studies carried out on the Kansas State Center of Excellence for Tick-borne Disease validated that IgG antibodies will be powerful towards tick saliva additives, helping in reducing the impact of infestations. This research included the development of recent vaccines and diagnostic equipment to manipulate tick-borne diseases in cattle (Bohn, 2021). The considerable increase in IgM tiers in livestock inflamed with ticks and horseflies in comparison to the wholesome institution may be attributed to the immune device's response to infections and parasites. When livestock are uncovered to ticks and horseflies, the immune device responds through producing IgM antibodies, one of the first antibodies produced in response to infection. IgM performs an crucial function within the on the spot immune response, operating to include the infection and fight parasitic infestations. Therefore, elevated IgM degrees are a demonstration of an active immune response in opposition to the infections due to ticks and horseflies. Additionally, those parasites can be chargeable for transmitting numerous infectious pathogens that require a strong immune response, leading to accelerated IgM production. Scientific sources have said on the immune reaction of farm animals to various tick antigens, where inflamed cattle show an increase in IgM ranges due to the immune system's interplay with proteins present in tick saliva, which are utilized in anti-tick vaccines (Tabor, 2018).

Noted Franzin, et al., (2017 that genetic differences and gene expression within the pores and skin of livestock can make them greater inclined or immune to ticks, highlighting that resistant livestock showcase more potent immune responses and improved IgM manufacturing in reaction to tick infestations.

A look at by using Scoles, et al., (2022) located that vaccinating cattle with synthetic peptides matching the expected extracellular domains of the Rh. Microplus tick aquaporin 2 protein led to an enhanced immune response, inclusive of accelerated IgM stages. This heightened immune response is likely due to continuous exposure to tick saliva, which includes diverse immune-stimulating components that promote the production of particular antibodies, including IgM. Scientific research has additionally targeted on figuring out particular IgM responses in farm animals uncovered to diverse pathogens, which include the ones transmitted through ticks and horseflies. These studies confirmed that the immune response in infected cattle entails a vast increase in IgM degrees, that's part of the primary immune reaction to new antigens introduced by means of the parasites. The outcomes of those studies advise that the cattle's immune gadget responds strongly to tick and horsefly infestations, with IgM antibodies playing a crucial role inside the early levels of this reaction (Poonsuk, et al., 2023).

The outcomes also indicated tremendous differences in the values of CD4 and CD8 clusters of differentiation between the infected institution and the healthful cattle. A big decrease in CD8 values was located in the inflamed organization, with a value of 2979 in comparison to 3926 within the healthy organization. Similarly, the CD4 price inside the inflamed institution become 2429 compared to 3055 inside the wholesome group. The decrease in these values in farm animals infected with ticks and horseflies is likely due to immune pressure. When inflamed with parasites like ticks and horseflies, livestock experience large immune stress, leading to the depletion of CD4 and CD8 cluster differentiation proteins. The parasites secrete substances that suppress immune gadget functions, decreasing the effectiveness of immune cellular responses (Okagawa, et al., 2018; Marima, et al., 2020).

These parasites reason chronic inflammatory responses within the frame, main to the long-term depletion of CD4 and CD8 cluster differentiation proteins. This continual inflammatory reaction extensively depletes the body's immune sources, further to the direct destruction of immune cells. Parasites goal and directly ruin immune cells through toxic secretions or by way of manipulating biochemical strategies inside immune cells. Moreover, they could transmit viral or bacterial illnesses that affect the immune system, leading to a discount within the range of CD4 and CD8 cells. These illnesses can cause direct harm to immune cells or lead to an excessive immune reaction that contributes to their depletion.

These combined factors reduce cattle's ability to fight parasitic and other Infections, resulting in lower degrees of immune cells, along with CD4 and CD8 differentiation proteins. This is steady with the outcomes of our take a look at Okagawa, 2018). Studies have proven that Rh. Microplus ticks modulate the immune response in livestock, and tick infestations can lead to an immune activation kingdom characterised through a decrease in CD4 and CD8 immune cells. This is possibly because of the suppression of tick salivary proteins, which own properties that modulate immunity and inhibit the activation and proliferation of CD4 and CD8 cells, main to their discount in instances of infection, as evidenced by the results of our examine (Frabetti, et al., 2023).

Similarly, current resources imply that horsefly bites can cause a widespread immune response, causing an inflammatory response on the chew sites and systemic immune modifications, together with a lower in CD4 and CD8 immune cells. This lower can be because of the strain and physiological modifications related to the inflammatory and immune reaction to the anticoagulant and immunosuppressive elements found in horsefly saliva (Kandel,et al., 2021).

Studies have additionally proven that during infections, there may be often an upregulation of immune inhibitory receptors along with LAG-3 and PD-1 on T cells, contributing to T mobile exhaustion. This was found in livestock infected with pathogens inclusive of bovine leukemia virus, main to a higher proportion of exhausted CD4 and CD8 cells, in addition deteriorating the immune reaction. These findings highlight the complicated interaction among external parasite infestations and the livestock immune gadget (Okagawa, et al., 2018).

The examine consequences additionally indicated no sizable differences in the cost of Complement (C3) in organizations infected with horseflies and ticks in comparison to wholesome agencies. This shows that the extent of this protein within the blood remains strong regardless of infection by using those parasites. It additionally indicates that the immune system isn't always extensively affected by these infections. The stages of the C3 component are a important a part of the immune device, especially within the complement device, which includes proteins that help cast off pathogens and broken cells from the frame. Furthermore, changes in C3 tiers can indicate diverse health situations. In the case of cattle inflamed with ticks and horseflies, research have shown that adjustments in C3 levels may occur because of the immune response brought about by these infections. For example, infections by way of parasites including ticks can spark off the supplement system, leading to the consumption of C3 proteins. This manner can assist the immune device of inflamed farm animals combat off the infections, but it also reflects the body’s reaction to the parasites (Merck, 2021; Birmingham, et al.,

2021).

The outcomes of the current look at align with research findings that show no tremendous differences in C3 degrees among livestock inflamed with horseflies and ticks and wholesome cattle. This suggests that at the same time as the immune system is activated, the baseline C3 tiers do not exchange significantly sufficient to be a reliable indicator of contamination severity (Talley, et al., 2023).

A take a look at by Marima, et al., (2020) at the genetic and immunological assessment of tick resistance in red meat farm animals after synthetic infestation with Rhipicephalus ticks involved thirty-six cows from Angus, Brahman, and Nguni breeds. The cows were divided into businesses: one organization was uncovered to ticks, and the other become now not. The breeds confirmed various resistance to ticks, indicating the presence of genetic factors that have an effect on tick resistance. One of the most vital genes identified was the Lum gene, that is a capacity marker for tick resistance in livestock, allowing them to fight parasites more successfully.

The outcomes presented in Table (32-4) show quite big differences inside the average cost of Major Histocompatibility Complex II (MHCII). A full-size lower turned into observed within the infected group, with a mean value of 1552±211.1 as compared to 2025±125.00 inside the wholesome group. According to studies, one of the main reasons for the lower in MHCII is that infections with horseflies and ticks suppress or inhibit the host's immune response to beautify their survival at the farm animals. External parasites secrete substances that modulate the immune system, leading to decreased expression of MHCII molecules on immune cells, which facilitates the parasites keep away from detection by the host's immune system (Machado, et al., 2010).

Moreover, tick and horsefly infestations lead to a robust inflammatory response and mobile damage, which may also reason mobile harm and regulate immune cell characteristic, doubtlessly major to a downregulation of MHCII expression because the body tries to control contamination and prevent immoderate tissue harm (Marima, et al., 2020).

The incredibly huge decrease in MHCII expression in cattle inflamed with horseflies and ticks in evaluation to healthful livestock has been confirmed by using way of severa studies. A have a study from Hokkaido University found that tick saliva consists of proteins that inhibit or suppress the immune response by using inhibiting the interest of Th1 helper T cells. Another take a look at confirmed that ticks take gain of anti-immune pathways in the host all through feeding, predominant to standard immune suppression, thereby decreasing MHCII expression in infected cattle. These studies highlight the tremendous impact of parasites on the cattle's immune device and verify the mechanisms these parasites use to avoid immune responses, which aligns with the findings of the present day take a look at (Sajiki, et al., 2021).

Many research have also highlighted the relationship among tick and fly infestations in farm animals and the discount in MHCII ranges. These studies have shown that ticks and flies can lessen the effectiveness of the cattle's immune system with the aid of suppressing immune cellular responses, which could cause reduced MHCII stages, making the farm animals greater liable to infections. Additionally, some research have indicated that ticks can suppress the immune machine in their hosts, leading to a reduction in immune responses, consisting of a decrease in MHCII tiers. This explains why inflamed farm animals are less able to face up to infections in comparison to healthy livestock. Furthermore, another take a look at showed that sure cattle breeds, which includes Bos indicus, exhibited extra resistance to ticks and flies in comparison to other breeds like Bos taurus. The extra resistant breeds proven a higher immune reaction, which includes better tiers of T-cells and basophils, even as the greater sensitive breeds confirmed a discount in those responses and an growth inside the expression of these receptors, including MHCII (Vlasova, et al., 2016; Tabor, et al., 2017).

Table (-13) Immunological Tests for Cattle Infested with Ticks and Horseflies in Samarra City and Its Surrounding Areas.

Groups	I g G	I g M	CD8	CD4	C3	MHC11

Patient	**20.33 ± 6.43**	**54.00 ± 19.40**	**2979 ± 89.00**	**2429 ± 43.40**	**44.77 ± 8.05**	**1552 ± 211.1**
Control	**18.75 ± 4.98**	**34.03 ± 5.15**	**3926 ± 34.20**	**3055 ± 47.30**	**46.88 ± 6.91**	**2025 ± 125.00**
P-Value	**0.511 ns**	**0.0002****	**0.0003****	**0.023 ***	**0.521 ns**	**0.0003 ****

(******) indicates the presence of highly significant differences between the treatments indicates (*) the presence of significant differences between the treatments.

(**ns**) indicates the absence of significant differences between the treatments

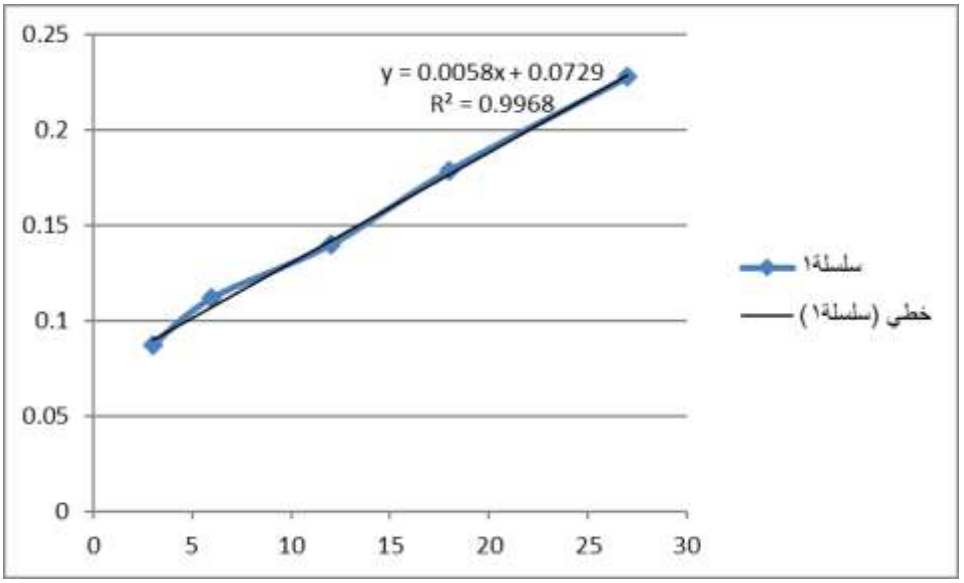
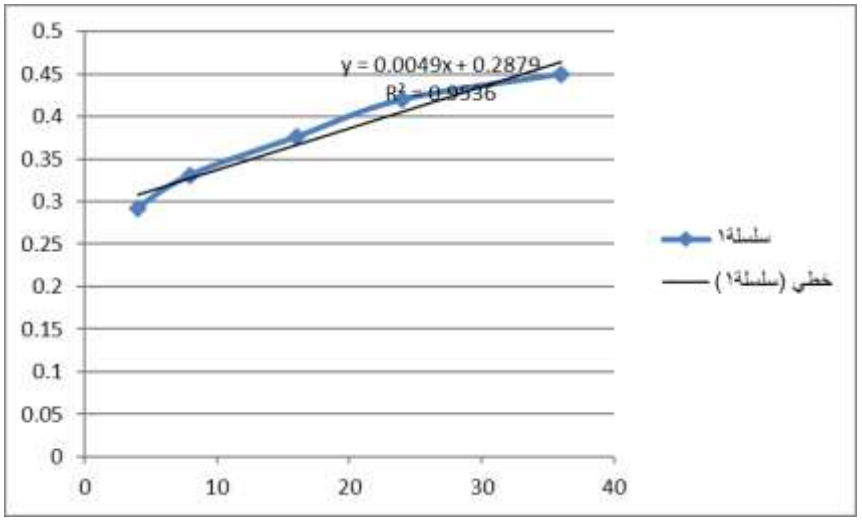


Figure (3-1) The standard curve for estimating the concentration of the enzyme (IgG) Bovine Immunoglobulin in cattle infested with ticks and horseflies.



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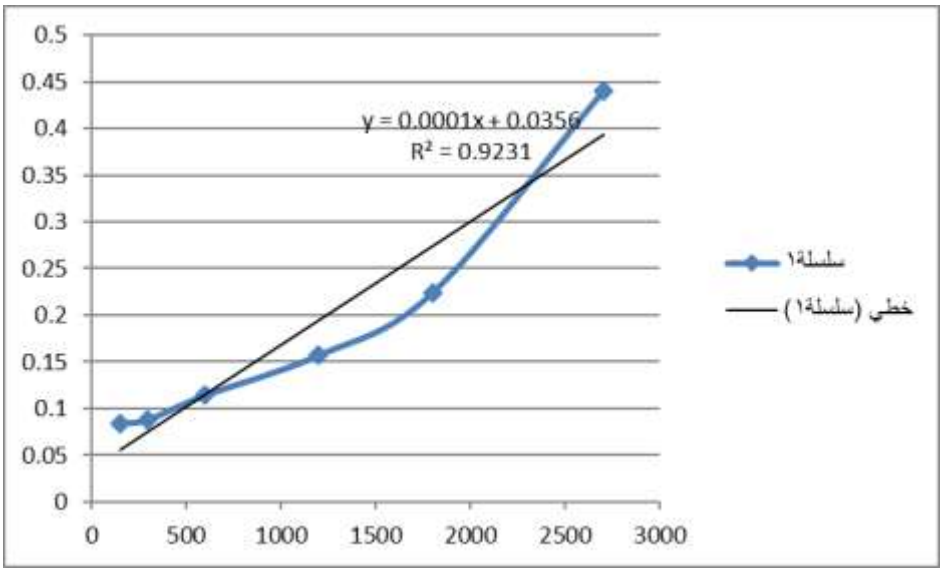

Figure (3-3) Estimation of the concentration of the enzyme Bovine Cluster of Differentiation 8 (CD8) in cattle infested with ticks and horseflies

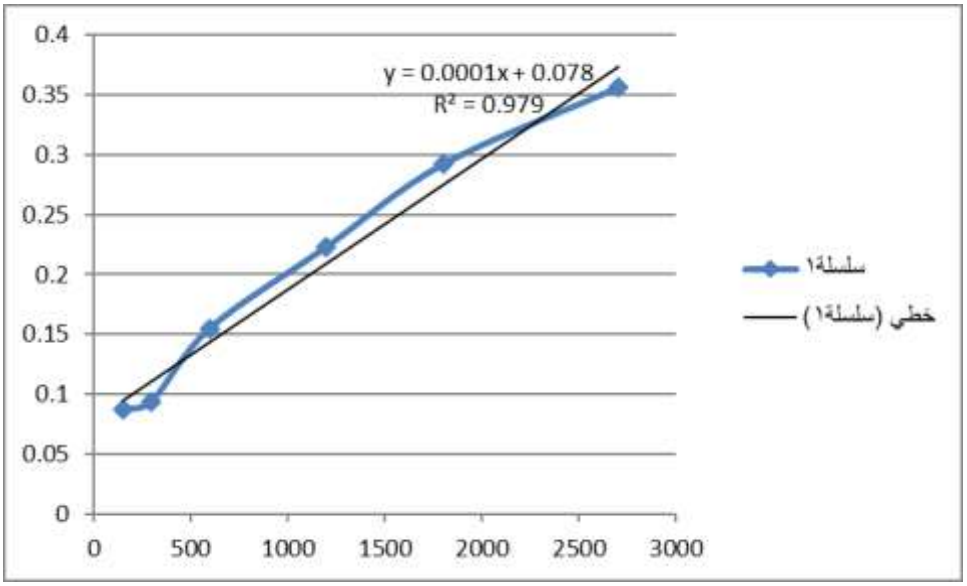

Figure (3-4) Estimation of the concentration of the enzyme Bovine Cluster of Differentiation 4 (CD4) in cattle infested with ticks and horseflies

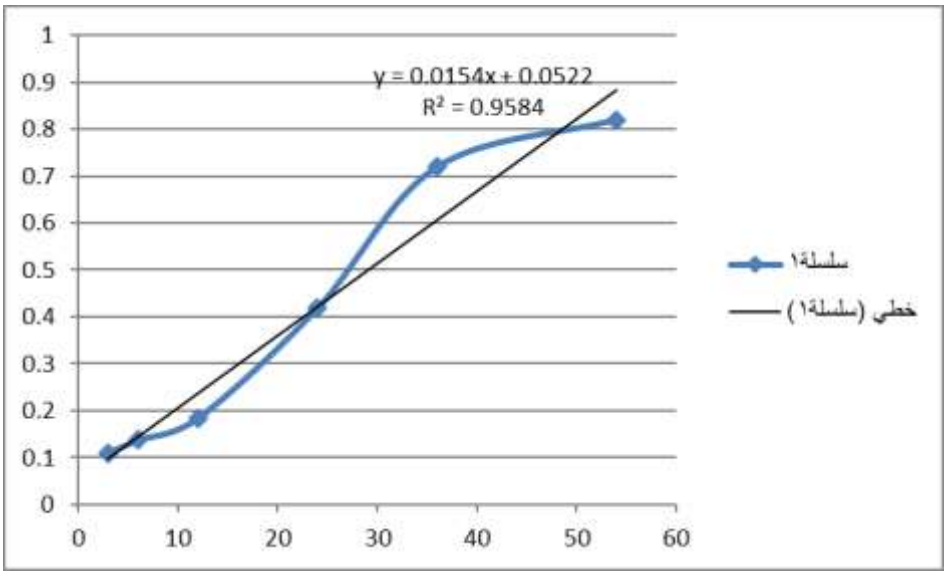

Figure (3-5) The standard curve for estimating the concentration of the enzyme Bovine Complement Component 3 (C3) in cattle infested with ticks and horseflies

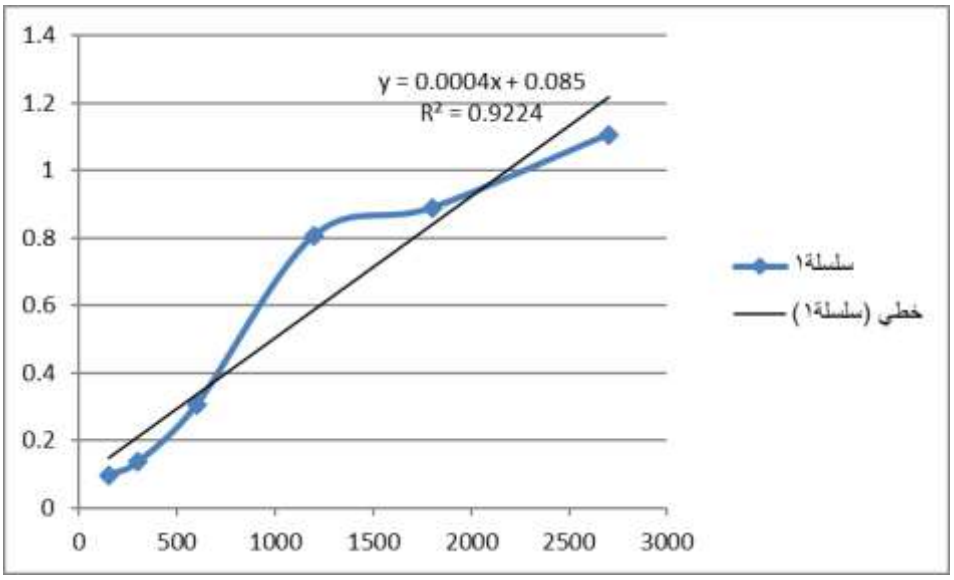

Figure (3-6): The standard curve for estimating the concentration of the enzyme Bovine Major Histocompatibility Complex II (MHCII) in cattle infected with ticks and horseflies.

4. Conclusion and future scope

-The results indicated significant differences in the value of immunoglobulin IgG, as the infected cattle developed a form of immune adaptation against these parasites, making their immune response similar regardless of the infection.

2-Immunoglobulin IgM showed a significant increase in the serum of cattle infected with ticks and horseflies compared to the healthy group, attributed to the immune system's response to infection and parasites.

3-The results demonstrated highly significant differences in the values of CD4 and CD8 cluster differentiation between the infected group and the healthy cattle. A significant decrease was observed in CD8 values, which reached 2979 in the infected group compared to 3926 in the healthy group. The CD4 value in the infected group was 2429 compared to 3055 in the healthy group.

4-The study results showed no significant differences in the value of Complement (C3) in groups infected with horseflies and ticks compared to the healthy groups, indicating that the level of this

protein in the blood remains constant regardless of infection with these parasites.

5-The results indicated highly significant differences in the average value of Major Histocompatibility Complex II (MHCII), with a significant decrease observed in the infected group, which recorded 1552 compared to 2025 in the healthy group.

Recommendations:-

1-Strengthening the role of regulatory bodies by conducting regular inspections of cattle infected with ticks and horseflies to treat and protect them from recurring infections by ticks and external parasites.

2-Implementing applied biological control to reduce infection severity by using alternatives like nano or plant-based extracts, which are considered safe for animals and their food products.

3-Studying the immune variables such as NK cells, Tumor Necrosis Factor (TNF), and Interleukins (IL) that occur in animals due to infection with ticks and horseflies..

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