



## Health importance of babesiosis infection in dairy cattle

Fadia Abd Al-Muhsin Al-Khayat\*

Dep. of Basic sciences, College of Dentistry, Baghdad university, Baghdad, Iraq.

\*Corresponding author: [fadia.khayat@codental.uobaghdad.edu.iq](mailto:fadia.khayat@codental.uobaghdad.edu.iq)

### Abstract

Babesiosis is haemoparasitic disease that transmitted by tick vector and have major importance for both human and animals. The study was carried out to determine the seroprevalence of Babesiosis infection in dairy cattle and to identify the most infected areas to reduce the possibility of disease outbreak with evaluate interleukin-6 expression (IL-6). Current study investigate babesiosis infection in 80 dairy cattle (20 control and 60 case) with different breeds and ages more than one year, distributed in surrounding regions of Baghdad Province which including Al-Tajy (North), Al-Duora (South), Abu-Graib(West) and Al-Nahraowan(East) for a period lasted from November/2022 to January 2023. Blood samples (3ml) were collected from the vein in the tail using gel tube, anti babesia IgG and IgM Antibodies were detected in order to determine the acute or chronic infection using enzyme-linked Immunosorbent assays (ELISA). On the other hand, expression of IL-6 was taken into account because of its diagnostic importance. The total incidence rate recorded 51.25% (41/80) with 51.2% for acute cases and 48.8% for chronic cases. Highly significant difference  $P < 0.001$  demonstrated in the levels of anti babesia antibodies between the study and control groups. According to the regions, the highest infection rate observed in Nahraowan (86.7%) followed by Al- Dora (73.3%) then Al-Tajy (60%) and finally Abu-Graib (53.3%). The acute cases reached the highest level in Al-Nahraowan (72.7%), followed by Al-Tajy (45.5%) and finally each of Al- Dora, Abu- Graib(36.4%), while chronic cases recorded the highest percentage in Al-Dora (63.6%), followed by Al-Nahraowan (45.5%) and finally each of Al-Tajy and Abu- Graib(36.4%). Highly significant differences was shown in the expression of IL-6 among studied group. In conclusions, babesiosis was largely present among dairy cattle in Nahraowan and Al-Duora, The infection has a clear effect on immunity through significantly elevated of inflammatory interleukin IL-6, it is necessary to highlight and raise awareness about the importance and risks of infections for limited and control.

**Keywords:** *Babesia bovis*, Babesiosis , ELISA, Antibodies, IL-6.

### Introduction

Babesiosis is a disease with public health importance to human and animals that caused by microscopic protozoan classified within phylum Apicomplexa, order Piroplasmida, genus Babesia, which infect red blood cells and is spread by certain ticks (Jaimes-Duenez., 2017). The most economically important species worldwide are *Babesia bovis* and *Babesia bigemina*. Many factors affecting on the wide variety of symptoms for this disease such as age, breed, species, and level of antibodies (Nejash et al., 2018). The incubation period usually ranges between 2 to 3 weeks, and symptoms of an animal infected with Babesia include high temperature of the infected animal, which is one of the most prominent symptoms , breakage of red blood cells which leads to the excretion of hemoglobin in the urine hence urine appears bloody Yellowing of all mucous membranes, Anorexia, Abortion of tenant cows, Sharp drop in milk production, Animal suffering from

acute anemia severe diarrhea, Loss of balance ,The animal may develop neurological symptoms (Chauvin et al., 2009; Abdel Aziz,et al., 2014). The infected animal may either become a chronic carrier or even die, the disease impacts the livestock industry and animal health (El-Ashker et al., 2015). Serious economic losses from the infection include abortion, mortality, reduction in meat and milk addition to the cost of control measures can cause impact on the livestock and dairy industry.( Kivaria, 2006; Onyiche et al., 2021).

Examination of light blood smears and staining them with the Chiemsa dye is the golden and routine examination, especially in acute infection, but in subclinical infection in which the parasitism rate is low, other techniques such as polymerase chain reaction PCR (Costa Júnior et al., 2006) and serologic methods (ELISA) can be used in seroepidemiologic surveys and as an adjunct in the diagnosis when infection is chronic or persistent (*Demessie, and*

*Derso*, 2015). Interleukins are proteins made in response to pathogens that regulate and mediate inflammatory and immune responses (Brown et al., 2006). Interleukin-6 (IL-6) is a cytokine with several functions and plays an important role in host defense due to its wide range of immunomodulatory and hematopoietic activities and the ability to trigger an acute phase response. (Goff et al., 2002; Talita et al., 2019)

### Materials and Methods

**Area of the study:** The population of dairy cattle is mainly distributed in surrounding regions of Baghdad Province which including Al-Tajy (North), Al-Duora (South) Abu-Graib (West) and Al-Nahraowan (East). These regions were identified to be infested with ticks which are the main carriers of the *B. bovis*. Different breed and age (more than one year) of cows showing some clinical signs with low milk production were selected for this study that lasted from November/2022 to January/2023.

**Blood samples:** Eighty blood sample were collected from dairy cattle (20 sample for each regions). Three ml of Blood from the vein in the tail was obtained from each cattle using Gel tube, centrifuge device at 3000 rpm for 5 minutes was used for separation of serum which was stored in Eppendorf tube at -20°C until use.

The enzyme-linked Immunosorbent assays (ELISA) are capable of detecting the infection and subsequence to determining the acute or chronic phase of *Babesia bovis*. Tow kits were intended for the qualitative detection of anti- *Babesia bovis* IgG, IgM antibody provided by Bio-sources (SL2832Hu). The microplate (96 well) has been pre-coated with antibodies specific to *babesia bovis*, as serum sample

was added followed by Horseradish Peroxidase (HRP)-conjugated antibody specific for babesiosis resulting in the antibody-antigen-Enzyme labeled antibody complex formation. Unbound reagent was removed by washing, then the TMB substrate solution was added to each well. For positive samples, blue color appeared in wells which turns after adding the stop solution to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. Competitive ELISA was used for the quantitative determination of bovine IL-6 (MBS733925). The microtiter plate has been pre-coated with goat-anti-rabbit antibody. Standards or samples were added to the appropriate microtiter plate wells with an antibody specific for IL-6 and Horseradish Peroxidase (HRP) conjugated IL-6. The competitive inhibition reaction was launched between with HRP labeled IL-6 and unlabeled IL-6 with the antibody. A substrate solution was added to the wells and the color develops in opposite to the amount of IL-6 in the sample. The color development was stopped and the intensity of the color was measured at a wavelength of 450 nm.

**statistical analysis:** All statistical analyzes were done using Spss.ver.24 including independent samples T test for comparison, descriptive statistics for mean and stander deviation, p value: if  $p < 0.05$  significant or If  $p > 0.05$  non-significant and if  $P < 0.001$  highly significant. (Thomas, 1967).

### Results and Discussion

Based on the results of current study, the incidence rate was recorded 51.25% (41/ 80) including acute infections with anti-*Babesia* antibodies IgM 51.2% (21/41) and chronic infections with anti-*Babesia* antibodies IgG 48.8% (20/41). Table 1.

**Table (1): Total infection rate among dairy cattle**

sample	Total infection		Acute stage		Chronic stage	
	No.	%	No.	%	No.	%
80	41	51.25	21	51.2	20	48.8

There is a general lack of studies performed on babesiosis in dairy cows, so it has been difficult to compare observational results with previous studies in my country. In Egypt, the results of a study found that the rate of infection with *Babesia* was 21.3% when 253 cattle examined (Mahmoud et al., 2015). In Qalubia, a study showed that the infection number of 89 examined cattle was 20 with infection rate 22.47%. (Hazem et al., 2014). Several factors play a role in the incidence of these parasites,

include vector numbers, vector transmissibility, and host susceptibility (Kocan et al., 2010).

For a comparison of Anti- *Babesia* antibodies, Table 2 revealed that there was a highly significant difference ( $P < 0.001$ ) between studies group and control group. The mean of Anti- *Babesia* IgM antibodies ( $2.283 \pm 0.179$ ) and the mean of Anti- *Babesia* IgG antibodies ( $13.305 \pm 0.474$ ) in infected cases was higher than control group ( $0.185 \pm 0.036$ ), ( $0.185 \pm 0.036$ ) respectively.

These results came in agreement with the findings of Bloch, et al., (2019) in that there was highly

significant differences between Anti-Babesia antibodies

**Table(2):Comparisons between the mean levels of anti babesia IgM and IgG antibodies among studied group**

Anti- Babesia antibodies	Study Groups	NO.	Mean	Std. Error Mean	T-test	P value
IgM	Case	21	2.283	0.179	11.223	0.000*
	Control	20	0.185	0.036		P<0.001
IgG	Case	20	13.305	0.474	27.55	0.000*
	Control	20	0.185	0.036		P<0.001

\*High significant

As shown in table 3, the distribution of infection according to regions showed the highest percentage in the Nahraowan region 86.7% followed by Al-Duora 73.35, Al- Tajy 60% and Abu-Graib 53.3% . The highest percentage of anti-babesia IgM antibodies was recorded in Nahraowan 72.7% while both of Al-Duora and Abu-Graib recorded the same percentage rate 36.4% and finally for Al-Tajy 45.5%. On the other hand ,anti-babesia IgG antibodies was highest in Al-Duora 63.6% and lowest in Nahraowan while Al-Tajy and Abu-Graib recorded the same percentage 36.4%. The differences in the level of infection are affected by several factors, including

the type of treatment used in the region, the vaccination status, the parasite strain, the breed and the age of the cattle. (Giglioti, et al., 2018) . Variation in the rate of infections due to differences in geographical location and climate. (Alkishe, et al., 2017). The results of a study conducted in Turkey revealed that babesiosis has been reported in all provinces but was more prevalent in central and high humidity areas .(Ozubek,et al.,2020). The high recorded percentage of diagnosed cases is due to the possibility of infecting cattle with the types of ticks spread in the study area.( Alvarez,et al., 2020).

**Table ( 3): Seroprevalence rates based on regions**

Region	Infected N=15		IgM		IgG	
	No.	%	No.	%	No.	%
Al-Duora	11	73.3	4	36.4	7	63.6
Nahraowan	13	86.7	8	72.7	5	45.5
Al-Tajy	9	60	5	45.5	4	36.4
Abu-Graib	8	53.3	4	36.4	4	36.4

Table 4 illustrated that the mean of IL-6 for study groups (13.924 ± 1.3533) compared to control groups (2.698 ± 0.5365) showed highly significant differences (P= 0.000). These results are consistent with what has been recorded by Feride and Fatma 2015, the level of IL-6 significantly higher in cattle infected with a low level of blood parasites when compared with healthy cattle. Also in Egypt, the results of a study conducted on four hundred cows

showed that there was a rise in the level of IL-6 in blood parasites infection.(Marwa and Marwa, 2023). Understanding the basics of immunity against diseases caused by blood parasites is necessary to produce an effective treatment and vaccine. There are no previous studies demonstrating the relationship between differential immune-related transcriptional profiles in cattle with acute or chronic conditions for babesiosis in Baghdad

**Table (4):Comparisons between the mean levels of IL-6, among studied group**

Test	Study Groups	NO.	Mean	Std.Error Mean	T-test	P value
IL6	Case	60	13.924	1.3533	4.729	0.000
	Control	20	2.698	0.5365		P<0.001 *

\*High significant

## Conclusions

The study showed that there was a significant increase in the incidence of babesiosis among dairy cattle in studied areas surround Baghdad , such as Nahraowan and Al-Duora areas. The study also showed that there was a rise in the concentrations of IL-6 in the serum of infected cattle, which was considered an immunological indicator that shows the effect of Babesia on the immune system.

## References

- Abdel Aziz, K. B., Khalil, W. K. B., Mahmoud, M. S., Hassan, N. H. A. and Mabrouk, D. M. (2014): Molecular characterization of babesiosis infected cattle: Improvement of diagnosis and profiling of the immune response genes expression. *Global Veterinaria*, 12(2): 197-206.
- Alkische, A.A., Peterson,A.T., Samy,A.M. (2017). Climate change influences on the potential geographic distribution of the disease vector tick *Ixodes ricinus*. *PLoS One*.;12(12):e0189092.
- Alvarez, J.A.,Rojas,C.,Figueroa, J.V. (2020). An overview of current knowledge on in vitro *Babesia* cultivation for production of live attenuated vaccines for bovine babesiosis in Mexico. *Front Vet Sci*.;7:364.
- Bloch, E. M., Mrango, Z. and Kasubi, M..(2019). The Babesia observational antibody (BAOBAB) study: A cross-sectional evaluation of Babesia in two communities in Kilosa district, Tanzania, *PLoS Negl Trop Dis*. 2019 Aug; 13(8): e0007632. doi: 10.1371/journal.pntd.0007632.
- Brownori, W., Norimine,J., Knowles,D. and Goff, W. (2006) Immune Control of Babesiabovis Infection. *Veterinary Parasitology*, 138, 75-87. <https://doi.org/10.1016/j.vetpar.2006.01.041>.
- Chauvin, A., Moreau,E., Bonnet,S., Plantard,O. and Malandrin ,L. (2009) Babesia and Its Hosts: Adaptation to Long-Lasting Interactions as a Way to Achieve Efficient Transmission. *Veterinary Research*, 40, 37. <https://doi.org/10.1051/vetres/2009020>.
- Chauvin, A., Moreau,E., Bonnet,S., Plantard,O. and Malandrin ,L. (2009) Babesia and Its Hosts: Adaptation to Long-Lasting Interactions as a Way to Achieve Efficient Transmission. *Veterinary Research*, 40, 37. <https://doi.org/10.1051/vetres/2009020>.
- Costa Junior , L.M., Rabelo ,E.M.L., Martin Filho O.A. & Ribeiro M.F.B. (2006). Comparison of different direct diagnostic methods to identify *Babesia bovis* and *Babesia bigemina* in animals vaccinated with live attenuated parasites. *Vet. Parasitol*. 139:231-236.
- Demessia, Y. and Derso, S. (2015): Ticks borne hemoparasitic diseases of ruminants: A Review. *Advances in Biological Research*, 94: 210-224.
- El-Ashker, M., Hotzel Otzel,H., Gwida,M., El-Beskawy,M., Silaghi,C. and Tomaso,H.( 2015). Molecular biological identification of Babesia, Theileria, and Anaplasma species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. *Vet. Parasitol*. 207: 329-334.
- Feride, K.S and Fatma, S.K. (2015). Acute-phase Proteins and Interleukin-6 (IL-6) in Cattle Infected with Subclinical Parasitic Infections. *Acta Scientiae Veterinariae*.43:1271.
- Giglioti, R., Oliveira, H.N., Bilhassi, T.B., Portilho, A.I., Okino, C.H., Marcondes, C.R. and OLIVERA, M.C.S. (2018) Estimates of Repeatability and Correlations of Hemoparasites Infection Levels for Cattle Reared in Endemic Areas for *Rhipicephalus microplus*. *Veterinary Parasitology*, 250, 78-84.
- Goff, W.L., Johnson , W.C., Tuo, W., Valdez , R.A., Parish, S.M., Barrington, G.M. and Davis, W.C. (2002) Age-Related Innate Immune Response in Calves to Babesiabovis Involves IL-12 Induction and IL-10 Modulation. *Annals of the New York Academy of Sciences*, 969, 164-168. <https://doi.org/10.1111/j.1749-6632.2002.tb04371.x>
- Hazem M. E. , Ebied , M.H. , Mohamed G. A. and Amr,A, E.(2014). Epidemiological Studies on Bovin Babesiosis and Theileriosis in Qalubia Governorate.Benha Veterinary Medical Journal,27(1):36-48.
- Jaimes –Duenez,J., Triana –Chavez,O. and Meifa – Jaramillo ,A.M. (2017). Parasitological and molecular surveys reveal high rates of infection with vector-borne pathogens and clinical anemia signs associated with infection in cattle from two important livestock areas in Colombia. *Ticks Tick-borne Dis*. 8: 290–299.
- Kivaria, F.M.(2006). Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania.*Trop. Anim. Heal Prod.*, 38 (2006), pp. 291-299, [10.1007/s11250-006-4181-2](https://doi.org/10.1007/s11250-006-4181-2) < <https://doi.org/10.1007/s11250-006-4181-2> > .
- Kocan, K.M., Dela La Fuente, J., Blouin , E.F., Coetzee J.F. & Ewing , S.A. (2010). The natural history of *Anaplasma marginale* *Vet. Parasitol*. 167:95-107.
- Mahmoud, M.S., Kandil, O.M., Nasr,S.M., Hendawy, S., Habeeb, S.M Marta,S. etal. (2015). Serological and molecular diagnostic surveys

combined with examining hematological profiles suggests increased levels of infection and hematological response of cattle to babesiosis infections compared to native buffaloes in Egypt. *Parasit Vectors.*;8(1):319.

Marwa, M.A and Marwa,M,K. (2023). Virulence of *Babesia bigemina* in infect infected cattle (*Bos taurus*): Molecular and immunological studies. *Research in Veterinary Science*. Volume 156:p.7-13.

<https://doi.org/10.1016/j.rvsc.2023.01.017>

Nejash, A., Nuraddis,I., Feyissa A,B. (2018) Prevalence, risk factors and vectors identification of bovine anaplasmosis and babesiosis in and around Jimma town, Southwestern Ethiopia *Acta Trop.*, 177 , pp. 9-18 , [10.1016/j.actatropica.2017.09.010](https://doi.org/10.1016/j.actatropica.2017.09.010)

Onyiche, T.E. , Raileanu,C., Fischer S., Silaghis I.S.(2021).Global distribution of *Babesia* species in questing ticks: a systematic review and meta-analysis based on published literature *Pathogens*, 2(10), pp. 1-26, [10.3390/PATHOGENS10020230](https://doi.org/10.3390/PATHOGENS10020230)

Ozubek, S., Bastos, R.G., Alzan, H.F., Inci,A., Aktas, M., Suarez, C.E.(2020). Bovine babesiosis in Turkey: impact, current gaps, and opportunities for intervention. *Pathogens*. 9:1041.

Thomas. R Taylor. (1967). *The principles of medical computing*, Blackwell. scientific publications, Oxford.