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Clinical, seroepidemiological and hematological study of Buffaloes Babesiosis in Basrah, Iraq

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Abstract:

Objective: Babesiosis is one of the important diseases in cattle and buffaloes, the current study conducting to diagnoses and evaluate the infection by *B.pigeamina* in buffaloes during the period of November 2022 to September 2023.

Methods: The study included 210 animals of both sexes and varied ages. The blood sampling from 220 water buffaloes (*Bubalus Bubalis*) in the north of Basrah governorate during the period of November 2022 to September 2023; the samples collected from both sexes and different ages ranged between 1 to 5 years. At these ten months 20 blood samples was randomly collected each month to compared with the control 10 animals.

Results: The blood smear from infected buffaloes show the parasite clearly while the clinical signs reported as: babesiosis confirm 16% (32 affected animals) represented by fever, emaciation, anorexia, low milk production, jaundice of mucous membrane, corneal opacity and hemoglobinuria. The subclinical infected buffaloes which represented 41% (82 affected animals) appear healthy with no clinical signs. Also indicate that were no significant differences ($P < 0.05$) between the infected and control group. The hematological parameters show significant differences ($P < 0.05$) in RBCs count, total WBC count, deferential LC, Hb concentration and PCV in the infected buffaloes compare to the control animals while there were no significant differences ($P > 0.05$) between the subclinical and control buffaloes except the RBCs count. The serological examination revealed to significant differences ($P < 0.05$) in the infected group in posit to the control especially the liver enzymes and kidney function test enzymes which include: AST, ALT, ALP, BUN, creatinine and TP. Moreover, According the season and months the study detects the high infection rate in the summer while there were decrease in the infection rate in the mild or cold weather months. Moreover, the study indicates the high infection percentage in females compare to the male sex.

Conclusion: Babesiosis is very important disease in Buffaloes in north of Basrah and there are no significant differences in infection rates between male and female.

Keywords: Babesiosis; Buffaloes; *Bubalus bubalis*; *Babesia pigemina*.

1 Introduction

Babesia bigemina is an intraerythrocytic protozoan that belonging to Babesiidae family of Apicomplexa phylum. The disease classified as tick-borne and the *B. bigemina* is detected for the first time by Vector Babes in 1888, so described as the more prevalent causative species for babesiosis disease in cattle and buffaloes (Bock et al 2004, Maharana et al 2016 & Olaya et al 2017).

It is regarded as one of the important diseases as great losses because of decreasing the animal production, using the approaches of controlling and treatment, with the influencing on trading international bovine animals (Ghosh et al 2007 & Olaya et al 2017). With acute phase of infection, babesiosis is characterized by fever, anorexia, lethargy, haemoglobinuria and diarrhea, as well as to emaciation, hemolytic anemia and jaundice in more prolonged affected severe cases (Brites-Neto et al 2015). In acute infections, the animals frequently subclinical infections that act as a source of babesiosis infection and latent vector for natural transmission (Sharma et al 2016). In general, babesiosis disease may be diagnosed depending on the clinical signs of acute cases and can be demonstrated, microscopically, by blood smears with Giemsa dye. Although, this method remains as a most suitable "Gold Standard" way for detection of acute infections, it's low in sensitivity and specificity in carrier animals and required for effective diagnostic tests to detect of *B. bigemina* (Oliveira-Sequeira et al 2005). Serological techniques for babesiosis detection are applied in field studies (Terkawi et al 2012). However, *B. bigemina* can be detected by the classic serological examination and hematological changes. This assay is characterized by a high sensitivity for determination of clinical and subclinical infections (Costa et al 2015). On the other hand, the *B. bigemina* in carrier animals is easily detected by blood smear (Goff et al 2008 & Olaya et al 2017). Nonetheless and according to several studies, evaluation of babesiosis especially during the epidemiological investigations is easy to do (Adham et al 2009, Terkawi et al 2011 & Terkawi et al 2012). Hence, the

goal of current study was to detect the prevalence of *B. bigemina* parasite in carrier buffaloes by using of diagnostic blood smear's microscopy, serological examination and hematological alteration then explain the relationship between the and sex with the infection of buffaloes.

2 Materials and Methods

The vital signs recorded carefully (body temperatures, heart and respiratory rates); also, the clinical signs of each examined animals were done.

Sampling: The blood sampling from 220 water buffaloes (*Bubalus Bubalis*) in the north of Basrah governorate during the period of November 2022 to September 2023; the samples collected from both sexes and different ages ranged between 1 to 5 years. At these ten months 20 blood samples was randomly collected each month to compared with the control 10 animals.

Twelve milliliter of blood samples were taken from the Jugular vein after made the complete control to the animal; this quantity was divided into: 10 millimeters without EDTA tubes for serum collection and 2 milliliters with EDTA tubes for the hematological analysis and blood smears.

Giemsa Staining: Blood smears were prepared, and after air drying, the smears were fixed in methanol alcohols for three minutes, then stained with Giemsa stain for twenty minutes then examined with light microscope under an oil immersion lens to diagnose Babesia parasites based on parasite morphologically. (Mohammed et al 2020 & Sawitri et al 2021)

The hematological analysis included: red and white blood cells count (RBCs and WBCs count), packed cell volume (PCV), hemoglobin concentration (Hb %) and differential leukocytes count.

The serum was used in biochemical analyses that included: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine and total protein (TP). (Sawitri et al 2021) The statistical analysis was made by using one-way ANOVA.

3 Results

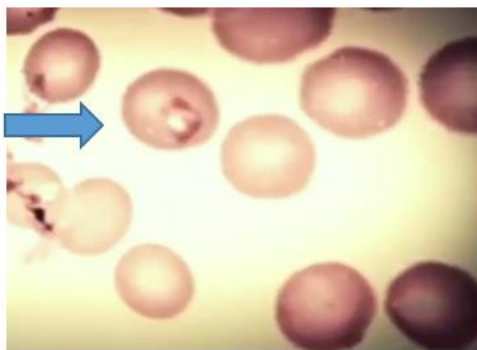


Figure 1: *B. bigemina* in buffaloes 1000X (the photo was deal by word explanation program)

The signs reveal in clinically infected buffaloes with *B. bigemina* were suffering from acute form of the babesiosis which confirm 16% (32 affected animals) represented by fever, emaciation, anorexia, low

milk production, jaundice of mucous membrane, corneal opacity and hemoglobinuria. The subclinical infected buffaloes which represented 41% (82 affected animals) appear healthy with no clinical signs.



Figure 2: severe emaciation in the infected buffaloes.



Figure 3: Corneal opacity of the infected eye

The vital signs illustrated in (Table 1) indicate that there were no significant differences ($P < 0.05$) between the control and subclinical infected buffaloes while

there were significant differences between the infected and control group.

Table 1: Show the vital signs parameters in the infected buffaloes with *B. bigemina*.

Vital signs	Control group Mean±S.E.	Infected animals Mean±S.E.	P Value	Subclinical infected Mean±S.E.	P Value
Body temperature	37.6±0.7	40.7± 0.5	$P < 0.05$	37.8± 0.19	$P > 0.05$
Heart rat/ mints	56.3±5.1	84.5±3.33	$P < 0.05$	59.2±3.0	$P > 0.05$
Respray rate/ mints	29.8±3.5	60.2±6.1	$P < 0.05$	31.7±4.1	$P > 0.05$

The hematological parameters show significant differences in RBCs count, total WBC count, deferential LC, Hb concentration and PCV in the

infected buffaloes compare to the control animals while there were no significant differences between the subclinical and control buffaloes except the RBCs count as shown in (Table 2).

Table 2: Illustrates the significant differences in the hematological values among the infected and control buffaloes.

Parameters	Control group Mean±S.E.	Infected animals Mean±S.E.	Subclinical infected Mean±S.E.
RBCs count X10 ⁶ µl	7.3 ± 0.71	4.9 ± 0.9*	5.8 ± 0.37*
Hb concentration mg/dl	12.1 ± 1.2	7.45 ± 1.1*	11.5 ± 1.6
PCV %	35.2 ± 2.2	26.3± 2.12*	33.8 ± 1.63
WBCs count X 10 ³ µl	10.62 ± 2.11	14.1± 0.8*	10.8± 1.44
Neutrophils X 10 ³ µl	44.43 ± 3.33	47.26 ±3.21*	46.52 ± 0.9
Basophils X 10 ³ µl	1.41± 0.2	2.0 ± 7.1*	2.1± 0.3
Eosinophils X 10 ³ µl	3.8 ± 1.9	3.66 ± 1.0	3.2 ± 0.7
Lymphocytes X 10 ³ µl	40.8± 4.14	45.8± 1.7*	43.6 ± 2.0
Monocytes X 10 ³ µl	3.2 ± 1.29	3.0 ± 63	3.1 ± 0.5

*($P < 0.05$) significantly differences among the infected and control buffaloes.

The serological examination revealed to significant differences in the infected group in posit to the control especially the liver enzymes and kidney function test enzymes which include: AST, ALT,

ALP, BUN, creatinine and TP. as explained in (Table 3)

Table (3): The serological values of the liver and kidney function enzymes.

Enzyme	Control group Mean±S.E.	Infected animals Mean±S.E.	Subclinical infected Mean±S.E.
AST U/L	50.12 ± 12.5	79.99 ± 8.44*	54.4 ± 7.2
ALT U/L	43.5 ± 10.9	76.26 ± 10.7*	45.9 ± 11.15
ALP U/L	61.8 ± 6.7	83.92 ± 14.33*	59.58 ± 17.31
BUN mg/dl	21.48 ± 6.62	32.8 ± 9.14*	23.52 ± 4.6
Creatinine mg/dl	1.76 ± 1.3	2.9 ± 1.6*	1.9 ± 1.12
TP g/dl	11.2 ± 8.2	16.1 ± 0.9*	9.82 ± 3.36

*(P<0.05) significantly differences among the infected and control buffaloes.

According the season and months the study detects the high infection rate in the summer (April, may, Jun, July and august) while there were decrease in the infection rate in the mild or cold weather months (November, December, January, February and March). Moreover; the study indicates the high infection percentage in females compare to the male sex.

4 Discussion

The Babesiosis is one of the most important local diseases of the blood parasitic and tick born disease in Iraq and especially Basrah governorate as explained in the previous studies such as (Sawitri et al 2021 ., Moayad and Hasso , 2019) this may be due to the tropical or semi tropical in Basrah as mentioned by (Vikrant Sudan et al 2013 & Olaa et al 2017) due to the high temperature and wet weather moreover this weather will help the tick infestation which regard as the main source of babesiosis., this agree with (Alsaad et al 2016 & Sobhy et al 2022)

The clinical signs which include the fever is due to the immunity reaction as we know and explained by (Sawitri et al 2021& Jasim et al 2023), in the other hand the anorexia and inappetance which lead to the loss of the body weight then emaciation and low milk production while the jaundice mucous membrane in fact result in due to the anemia which caused by the *B. bigemina* that cause severe decrease in the red blood corpuscles due to the damage of it then leading to hemoglobinuria that affect the kidney function this results agree with (Olaa et al 2017 , Sawitri et al 2021& Mohammed et al 2020).

Corneal opacity may be caused by the physiological alteration in the body protein and circulation disturbances. The same finding reported by (Sawitri et al 2021 &Waseem et al 2023).

The subclinical infected buffaloes which represented 41% (82 affected animals) appear healthy with no clinical signs that mean the immune tolerance and alteration to the long period infection as continuous with (Sawitri et al 2021& Waseem et al 2023) results.

The vital signs which studied in this research revealed to increase in the body temperatures, heart and respiratory rate ...this may due to the severe anemia and the reduction of the RBCs number as compensatory mechanism to the reduction of the oxygenation and nutrient distribution because the anemia and the (Mohammed et al 2020) mention resample explanation for this point.

On the other hand there were significant (P<0.05) decreases in the RBCs, PCV, and Hb concentration that explained by the damage of the RBCs due to the *Babesia bigemina* infestation and this lead to decrease of the hemoglobin concentration percentage and low packed cell volume, while there were manifestation significant increase (P<0.05) in the total WBCs count and deferential leukocytes count as well as there were neutrophilia , basophilia and eosinophilia as immune reaction to the *Babesia bigemina* in the infected buffaloes and these results corresponding tom(Sawitri et al 2021) results.

The significant differences in the lever and kidney function test (P<0.05) came from the dysfunction and physiopathy changes in the hepatic and nephrotic tissues which normally response to these changes by increase in these enzymes values with variation that agree with (Olaa et al 2017 & Mohammed et al 2020)

The season and months the study detects the high infection rate in the summer because the disease is a tick born that mean the high incidence of the diseases increase with diffuse of the tick which become active in the summer such results reported by (Alsaad et al 2016) while the low incidence of the disease in the mild or cold weather months due to decrease of the tick activities in this season.

The study indicates the high infection percentage in females compare to the male sex because the high percentage of the females compare to the male in the fields as continues to (Arwa & Kawan 2022, Maharana et al 2016 &, Ateaa and Alkhaled 2019) results.

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