# CATTLE BABESIOSIS AND ASSOCIATED BIOCHEMICAL ALTERATION IN KALUBYIA GOVERNORATE.

**Ola, F. A. Talkhan**; Mervat E. I. Rdwan.*; Ali. M. A****

* Veterinary Hospital –Benha University **Animal Health Research Institute, Shebin El-kom.

drofscience@ymail.com

**Abstract :** Members of genus babesia are tick transmitted intra erythrocytes proto zoon parasites, many species are of considerable economic importance in live stock industry, additionally some species are zoonotic and affected on human health, so this investigation performed to differentiated between traditional and some modern methods for diagnosis of bovine babesiosis, a total of 100 animals from private farms located in different places in Kalubia aged from 1-3 years the samples were collected from clinically infected animals that suffered from fever (41 C°) , Anorexia, depression, weakness, pale mucos membrane, emaciation, weight loss hemoglobin urea with accelerate heart and respiratory rates and animals appearan healthy in contact with this animals, laboratory examination two blood samples were collected from each animals from juglar vein samples with anticoagulant for blood film stain and PCR while second without anticoagulant for biochemical the result of our study revealed a great significant Increase in urea , creatinine, AST, Alt and globulin in clinical cases of babesia bigemina but non significant changes in sub clinical cases Also the result revealed significant increase in serum iron ,Total iron binding capacity transfferin total protein, However There are non significant increase in albumin and A/G ration. 2010;8(3):29-36]. (ISSN: 1545-0740).

***Keywords:*** Babesiosis, Cattle, Early diagnosis, Pathogenic Alteration.

## Introduction

Cattle and buffaloes play an important role in our life. They comprise the main source of milk and meat. The general health condition of them was impaired by the parasitic diseases generally and blood parasites specially. Babesia is considered one of the most important blood parasites affecting cattle and buffaloes.

Negative microscopic examination doesn't exclude the possibility of infection as in very early and chronic stage of disease and in subclinical infection the detection of babesia in Gimsa- stained blood smear was chllicult 50 it was necessary to develop xeno dignosis and molecular detection, these techniques are more reliable in this respect because they directly determine the presence of viable parasites and parasite DNA respectively. Particularly PCR have revolutionized this approach **(Annetta Zinil *et al.* 2003).**

## Material and Methods

**Animals:**

Total 100 animals bovine aged from 1-3 years were examined for the existence of the bovine babesiosis. Samples were collected from animals that showed clinical signs suspected to be bovine babesiosis and from apparently healthy in contact animals. The animals from private farms located in Kaliobia Governorate.

**Samples**

The blood samples were collected from the juglar vein by using steril sharp needle with wide pore two samples were collected from each animal the samples that used for blood smear and PCR analysis were collected in clean and dry test tube containing Di-sodium EDTA as anticoagulant. For biochemical examination for separation of serum for determination of serum Asparate amino transferase (AST) and alanine animo transferase (ALT) **Reitman and Frankel , (1957)**, urea **Patton and Crouch , (1997)** creatinine **Henry, (1974)** total protein **peters, (1968)** albumin **Rodkey, (1965)**, iron

**Bauer, (1984)** total Iron binding capacity **Fairbanks and Klee , (1987)**

**Blood smear:**

Some precautions should be taken into account like using very clean, dry glass slides, clean, dry regular edges speeder slides, sharp sterile needle, and absolute methyle alcohol.

**Giemsa stain stock solution:**

Giemsa powder (0.5g) dissolved in glycerin absolute(33ml),the powder was dissolved in glycerin with vigorous shaking. Then the mixture was added to one liter methyle alcohol. The stain was transferred to a tightly stopper brown bottle and stored in dark place for two months the stain was filtered through filter paper prior to use. PCR procedure: polymerase chain reaction.

**Blood film:**

- Three thin blood film were prepared and left in air to dry and fixed in absolute methyle alcohol for 1-2min.
- Staining with freshly filtered and diluted Giemsa stain for 30-45 minutes then washed with distal water to remove excess of stain.
- The Slides were left to dry, then put one drop of cider oil examined under oil immersion lens according to **(Coles, 1986).**

**Examination of blood film for babesia**

1/4 – 1/2 inch from the end of the film and transferred from one side of film to other (cross – sectional method) to give constant and representative examination according to **Barrent (1965)** animal can be considered negative if the three slides were negative.

**Serum samples for biochemical examination:**

Serum were separated by centrifugation at 300 rpm for 10 minutes then clear supernatant serum aspirated carefully into dry sterile labeled wails and used for serum analysis to differentiated between liver, kidney function and iron in clinically infected animals and animals apparently health in contact .

A total of 15 bovine blood samples included in this study collected from ----- with clinical suspicion of babesiosis. Blood samples submitted in EDTA containing tubes and stored at -20°C until subsequent DNA purification

**1- DNA extraction from blood**

The DNA was extracted from each sample by chloroform- isoamyl extraction method (All buffers used according to *Sambrook et al. (1989).*

Blood samples typically were obtained as 1 ml of whole blood stored in EDTA vacutainer tubes . To each 1 ml sample, add 0.8 ml 1X SSC buffer, and mix. Centrifuge for 1 minute at 12,000 rpm in a microcentrifuge. Remove 1 ml of the supernatant and discard into disinfectant. Add 1 ml of 1X SSC buffer, vortex, centrifuge as above for 1 minute, and remove all of the supernatant. Add 375 ul of 0.2M NaOAc to each pellet and vortex briefly. Then add 25 ul of 10% SDS and 5 ul of proteinase K (20 mg/ml H2O) (Sigma P-0390), vortex briefly and incubate for 1 hour at 55°C. Add 120 ul phenol/chloroform/isoamyl alcohol and vortex for 30 seconds. Centrifuge the sample for 2 minutes at 12,000 rpm in a microcentrifuge tube. Carefully remove the aqueous layer to a new 1.5 ml microcentrifuge tube, add 1 ml of cold 100% ethanol, mix, and incubate for 15 minutes at -20° C. Centrifuge for 2 minutes at 12,000 rpm in a microcentrifuge. Decant the supernatant and drain. Add 180 ul 10:1 TE buffer, vortex, and incubate at 55°C for 10 minutes. Add 20 ul 2 M sodium acetate and mix. Add 500 ul of cold 100% ethanol, mix, and centrifuge for 1 minute at 12,000 rpm in a microcentrifuge. Decant the supernatant and rinse the pellet with 1 ml of 80% ethanol. Centrifuge for 1 minute at 12,000 rpm in a microcentrifuge. Decant the supernatant, and dry the pellet in a Speedy-Vac

for 10 minutes (or until dry). Resuspend the pellet by adding 200 ul of 10:1 TE buffer. Incubate overnight at 37 C, vortexing periodically to dissolve the genomic DNA. Store the samples at -20°C

**2 - PCR**

Specific PCR has been used to detect the gene encoding for *B. bigemina* 18S ribosomal RNA (18SrRNA) within the DNA extracts of the suspected animals for infection.

Forward and reverse primers were designed using primer premier 5 software with contribution of genebank data for *Babesia bigemina* isolate BRC02 18S ribosomal RNA gene, partial sequence( accession no. FJ426361.1). The primers sequences were:

Forward: GAGAAACGGCTACCACAT;

Reverse CATTACCAAGGCTCAAAA

The PCR master mix was comprised of PCR buffer (300 mM Tris, 75 mM ammonium sulfate, pH 9.0), 2.5 mM MgCl, 400 µM dNTPs, 20 pmol of each primer, and 2 U µl–1 taq DNA polymerase. The PCR cycling parameters were one cycle of 94 ° C for 5 min, 35 cycles of 94° C for 30 s, 49° C for 30 s and 72 ° C for 1 min, with a final extension step of 72° C for 10 min. PCR products were analyzed by electrophoresis on 1.5% agarose gel documented with documentation system.

**Statistical analysis:**

Data were analyzed using T-Test as described by **Petrie and Waston 1999**

**Results**

The results obtained in the PCR assay showed 11 out of 15 samples positive for *B. bigemina of* expected molecular weight 409 base pair.

Number of examined animals were 100 aged from 1-3 years were examine for bovine babesiosis about 38 calves suffered from fever (41°C) anaroxia, depression, weakness, pale mucous membrane. Emaciation, weight loss haemoglobin urea and accelerated heart and respiratory rates. The rest of examine animals were apparently healthy.

**Blood film:**

Examination of Giemsa stained blood smears with oil immersion lens revealed intra-erythrocytic double (pear shaped) of B. bigemina in 38 (out of 100) blood samples of examined animals while the other 62 examined animals appear free from developmental stages of B. bigemina in blood smears.

**PCR- Based Molecular Diagnosis of *Babesia bigemina***

The occurrence of bovine *Babesia* sp. in Egypt has been reported. An infection with *Babesia spp.* was suggested based on diagnosis by light microscopy of blood smears of a cow **(Brossard and Aeschlimann, 1975).** The aim of this study was the molecular – based early diagnosis of *B. bigemina* in Egypt.

Laboratorial diagnosis of clinical infection by babesia in cattle is usually based on the detection of the parasite in Giemsa-stained blood smears. Early detection of babesiosis in animals is very important to control the infection. Although serological tests can be used to detect circulating antibodies, cross-reactivity with antibodies directed against other species of piroplasms has been reported **(Papadopoulos *et al.*, 1996).** Moreover, antibodies tend to disappear in long-term carriers, whereas babesia persist. Therefore, animals with a negative serological test can still be the source of the infection. Several PCR-based diagnosis procedures

for the identification of these parasites have been developed **(Figueroa *et al.*, 1993; Birkenheuer *et al.*, 2003; Criado-Fornelio *et al.*, 2003; and Rampersad *et al.*, 2003)**

Regarding to the biochemical changes in serum of both clinical and subclinical cases table (1) revealed that there were highly significant increase in serum iron , globulin and AST, also significant increase in urea, albumin, A/G ratio and total iron binding capacity in case of subclinical cases also there were a highly significant increase in serum urea, creatinine, AST, ALT, iron, Total iron binding capacity , Total protein and Globulin and significant increase in transferase in case of clinically affected cases .

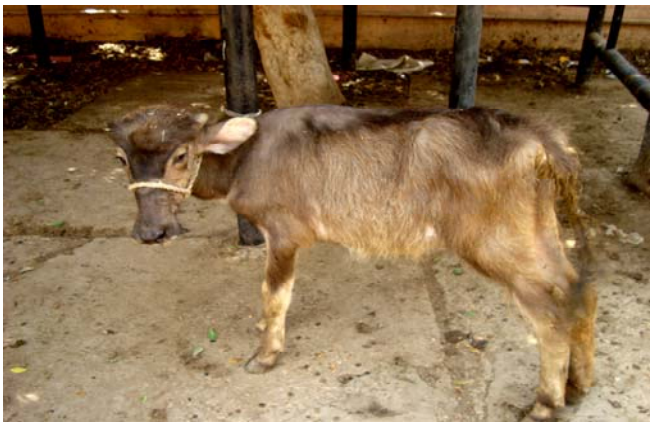
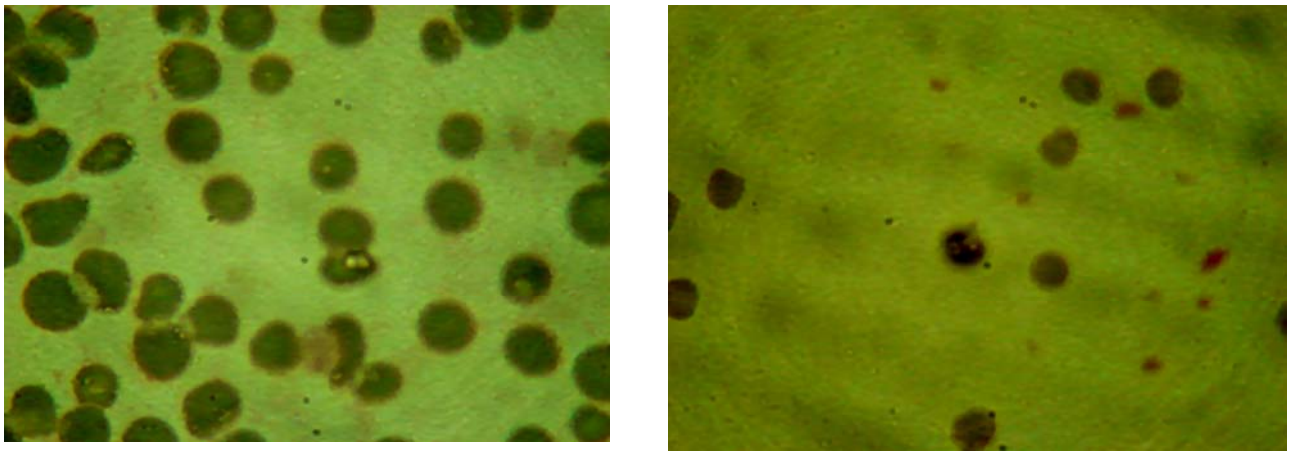


**Figure (1): Emaciated, Anoroxia and off food**

**Figure (2); Animal infected with ticks**

**Figure (3): tick born disease and mange**

**Clinical Signs of Babesiosis in Animals**



(A) (B)

**Figure (4):Giemsa stained blood film showing intraerythrocytic double pyriform (pear shape) of B. bigemina inside RBCs**

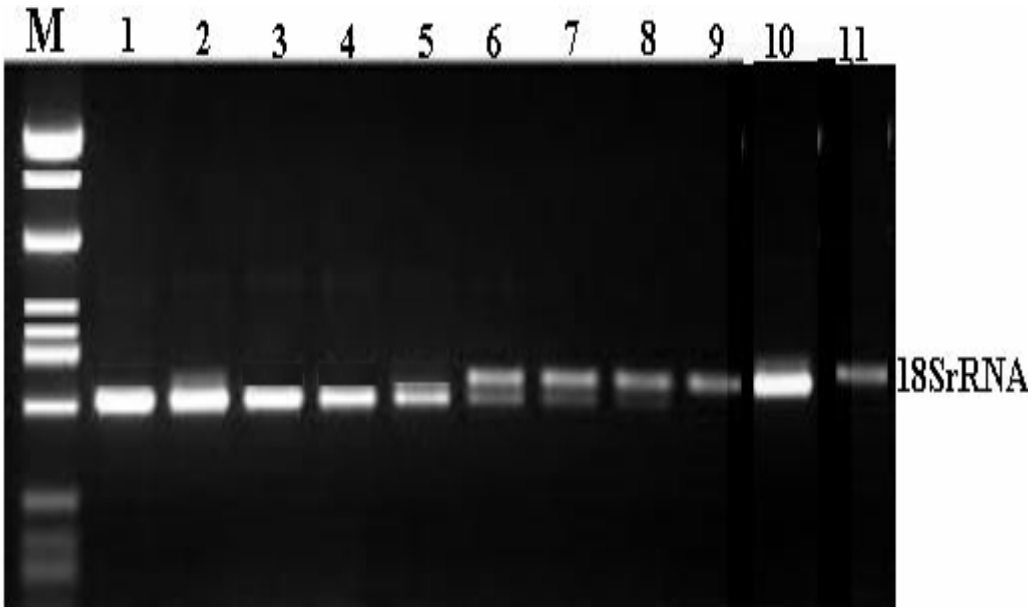



**Figure (5): The PCR product of amplified *Babesia bigemina* 18SrRNA gene separated on 1.5% agarose gel electrophoesis .**

**Table (1). Serum biochemical parameter in clinical, subclinical Babesiosis and Healthy control cattle.**

| P / Group | urea | creatinine | AST | ALT | Iron | TIBC | Transferin | T.prot | Albumin | globulin | A/G ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control Group | 15.45 ± 0.9 | 0.9 ± 0.03 | 40.3 ± 1.35 | 36.4 ± 1.14 | 130.8 ± 1.91 | 80.5 ± 1.24 | 0.58 ± 0.03 | 6.3 ± 0.07 | 3.5 ± 0.05 | 2.8 ± 0.06 | 1.2 ± 0.06 |
| Subclinical group | 20.14* ± 1.18 | 1.01 ± 0.05 | 71.6** ± 3.3 | 40.6 ± 1.41 | 161.4** ± 1.68 | 88.7* ± 2.06 | 0.62 ± 0.015 | 6.4 ± 0.05 | 3.11* ± 0.09 | 3.3** ± 0.09 | 0.9* ± 0.07 |
| clinical group | 34.0** ± 1.33 | 1.5** ± 0.04 | 132.1** ± 1.68 | 74.2** ± 1.87 | 168.2** ± 1.5 | 101.2** ± 2.45 | 0.7* ± 0.015 | 6* ± 0.06 | 2.6** ± 0.03 | 3.4** ± 0.07 | 0.8** ± 0.02 |

* Significant variation at ($P \leq 0.01$) ** highly significant variation at ($P \leq 0.001$)

**DISCUSSION**

Babesiosis is one of the most important diseases in our countries because it occurs sometimes in acute forms with serious recognized clinical manifestations yet lowering the productive performance of the affected animals.

The clinical picture in animals suffering from babesiosis Fig. (1, 2 and 3) include high temperature (90-91°C), loss appetite, cessation of rumination, anemia, laboured breathing and haemoglobin urea, such finding could be due to destruction of large number of erythrocytes by blood parasite resulting in hemoglobinaemia and consequently hemoglobinurea **Bron *et al.* (1992)**. On other hand, **Egell (1996) and Radostits *et al.* (2002)** attributed the sudden onest of high fever (40-41°C) as response to affect of un specific toxic substances produced during the metabolism of babesia on thermoregulatory.

Then the heart rate was increased, marked dyspnea was then developed and visible mucous membranes were first conjested but very soon became pale and in the terminal stages became icteric.

The method of chocice to detect babesia in blood of infected animals especially in acute cases was blood film examination **(Bose, *et al.* 1995)** in the present work, examination of Giemsa stained blood smear revealed intra-erythrocytic double pyriform (pean shaped) of B. bigemina inside RBCs of infected animals this is agreement with **Ahmed (1980), Smith (1990), Homer (2000) and Ali (2005)** added that round, oval and irregular froms may be observed depending on the developmental stage of B. bigemina inside erythrosytes,

Animals detected by blood film examination it was 38 out of 100 blood film represent 38% our result was in agreement with **El-Bahi (1989)** demonstrated babesia species in blood smears of 38.5% of tested cattle in Fayoum Fig. (4)

Exploitation of both the highly conserved and hypervariable sequences within the 18S rRNA gene permits design of a platform primers capable of early detection and specific pathogen identification in a single rapid detection platform. PCR analyses, permitting identification of definitive pathogen characterization of the species. Diagnostic accuracy of our assay was evaluated against conventional light microscopy-based methods. This assay may be a useful early diagnostic for *B. bigemina*, Fig (5).

Our present study indicate that the serum protein and globulin pattern was significantly altered by babesia bigemina infection. There was a highly significant increase in total protein and globulin in serum of infected clinical cases and these in accordance with data recorded in cattle **Ashmawy, et al (1994),** in calves by **Dwivedi and Gaytan, (1980)** and In buffaloes **Abd El-maksoud, et al (2000)** .

The increased value of globulin was attributed to the stimulation of immune system by the antigens of invaded parasites **El-Sayed and Rady, (1999)** and **Norimine, et al (2004).** Also the decrease in serum Albumin value is associated with the acute phase of many infectious diseases **Allen** and **Kuttler, (1981).** In addition, Albumin may be decreased due to decreased protein synthesis capacity of the affected Liver or prolonged insufficient caloric intake **Barbara, et al., (2008)**. It could be also attributed to loss of protein from distrusted RBCS and Its excretion in urine as albuminuria in addition to the malnutrition status occur during the disease **Henley and Judith, (1985).**

Concerning the effect of babesia Bigemina infection on activity of liver enzymes, the obtained results revealed a highly significant increase in serum AST and ALT. These results were in agreement with other previous studies reported by **Allen and Kuttler, (1981) Camacho, et al (2005)** and **Barbara, et al (2008).** The increase in enzymes activity may attributed to sever anemia that lead to hypoxic and toxic liver damages. Also massive hemolysis may occur which in conjunction with hypoxia may lead to hepatic cell degeneration and glomerular dysfunction leading to increase in AST, ALT and Bun, **Allen and Kuttler, (1981).**

Also there was a highly significant increase in both serum urea and creatinine level which in agreement with the result obtained by **Camacho et al, (2005)** and **Barbara, et al, (2008).**

Initially, increased urea and creatinine levels may be attributed to the rapid distraction of RBCs via phagocytosis in the reticulo-endothelial system **Ajayi, et al. (1979), Allen and Kuttler (1981)** and so the massive haemalysis occur during the period of infection with babesia bigemina and hypoxia lead to hepatic cell degeneration and glomerular dysfunction resulting in increased level of serum urea and creatinine.

Concerning the serum trace elements changes in babesia infected animals, the present data revealed a significant increase in iron, total iron binding capacity and iron saturation which was similar to that recorded by **El-Sayed and Rady, (1999)** and **Abd El-Maksoud**, **et al (2005)** such changes may be attributed to the hemolytic anemia induced by blood parasites and possibility of free radicals invading erythrocytes leading to distraction of their membrane **Academic and Itoh, (1992).** It could be concluded that babesiosis is a life threatened diseases accompanied by disturbance in serum protein Fractions, hepatic and renal dysfunction. The clinical infection were easily detected early by microscopic examination of giemesa stained blood smear but not subclinical cases in which there was an extremely low

parasitemia so PCR assays capable of detecting extremely low parasitemia as occur in carrier animals or subclinical infection and differentiating isolates, Table (1).

**Conclusion**

Clinical infection of cattle Babesiosis can be easily detected early by microscopic examination of giemesa stained blood smear but not in subclinical cases in which there was an extremely low parasitemia. PCR assays capable of detecting extremely low parasitemia as occurind in carrier animals or subclinical infection and differentiating isolates.

***Corresponding author***: Mervat Rdwan

Email: drofscience@ymail.com

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