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List of abbreviations

μg:	Microgram
μl:	Microlitre
ADP:	Adenosine diphosphate
ALT:	Alanine aminotransferase
AST:	Aspartate aminotransferase
ATP:	Adenosine triphosphate
B. bigemina:	Babesia bigemina
B. bovis:	Babesia bovis
Chol:	Cholesterol
CIC:	Circulation immune complexes
EDTA:	Ethylene diamine tetracetic acid
GGT:	Gamma glutamyl transferase
GPO:	Glycerol 3-phosphate oxidase
HDL-C:	High density lipoprotein-cholesterol.
IFAT:	Immunofloresence antibodoy technique.
IFCC:	International Federation of Clinical Chemistry
LDL-C:	Low density lipoprotein-cholesterol.
PCR:	Polymerase chain reaction
POD:	Peroxidase
PPE:	The percentage of parasitizied erythrocytes
RBCs:	Erythrocytes
SGOT:	Serum glutamate oxaloacetate transaminase
SGPT:	Serum glutamate pyruvate transaminase
T.bil.:	Total bilirubin
TAG:	Triacylglycerol

SUMMARY

Babesiosis is important haemoprotozoal diseases affecting various kinds of domestic animals and result in a substantial economic losses in several important animal species. In Egypt, *Babesia bigemina* was widely spread. The main symptom in cattle infected with blood parasite is anemia and haemoglobinurea which may lead to death in severe cases.

The present work was planned to study some biochemical alterations in the blood of cattle infected with *Babesia bigemina* and treated with antiparasitic drug.

Fifty four cattle were used for performing the present study their ages ranged from 4 to 6 years for females, from 2 to 3 years for males and from 6 months to 1 year for calves.

After clinical and parasitological examination the cattle under study were classified into:

Group I: Comprised nine young cattles (calves) their ages ranged from 6 months to 1 year. These animals were microscopically positive for babesiosis and treated with Berenil at a dose of 3.5 mg/kg body weight intramuscularly for one shot. Nine young cattles (calves) were used as control (apparently healthy and free from internal& external parasites).

Group II: Comprised nine adult male cattles, their ages ranged from 2 to 3 years old. These animals were microscopically positive for

babesiosis and treated with Berenil at a dose of 3.5 mg/kg body weight intramuscularly for 1 shot. Nine adult male cattles were used as control (apparently healthy and free from internal & external parasites).

Group III: Comprised nine adult female cattles their ages ranged from 4 to 6 years old. These animals were microscopically positive for babesiosis and treated with Berenil at a dose of 3.5 mg/kg body weight intramuscularly for 1 shot. Nine adult female cattles were used as control (apparently healthy and free from internal& external parasites).

The blood samples were collected from calves, adult males and adult females before and one week after treatment with berenil at a dose of 3.5 mg/kg body weight.

The blood samples were collected from control and infected animals before and one week after treatment.

The results of the present study were presented as follows:

1. Serum aspartate aminotransferase activity (AS-T) in sera of calves, adult males and adult females infected with *Babesia bigemina* and treated with Berenil showed the following:

   For calves, there were non-significant increase in the mean values in after treatment group compared to control group and significant increase in before treatment group compared to control and after treatment groups.

For adult males, there were significant increase in the mean values in before treatment group compared to control and after treatment groups.

For adult females, there were significant increase in the mean values in before treatment group compared to control and after treatment groups.

2. Serum alanine aminotransferase activity (AL-T) in sera of calves, adult males and adult females infected with *Babesia bigemina* and treated with berenil showed the following:

   For calves, there were non-significant increase in after treatment group compared to control group and a significant increase in before treatment group compared to control group.

   For adult males, there were non-significant increase in after treatment group compared to control group and a significant increase in before treatment group compared to control and after treatment groups.

   For adult females, there were non-significant increase in after treatment group compared to control group and a significant increase in before treatment group compared to control and after treatment group.

3. Serum Gamma Glutamyl transferase activity (GGT) in sera of calves, adult males, and adult females infected with *Babesia bigemina* and treated with berenil showed the following:

   For calves, there were significant increase in before and after treatment groups compared to control group and a significant decrease in after treatment group compared to before treatment group.

   For adult males, there were significant increase in before and after treatment groups compared to control group. Also, there were significant decrease in after treatment group compared to before treatment group.

For adult females, there were significant increase in before and after treatment groups compared to control group. Also, there were significant increase in before treatment group compared to after treatment group.

4. Serum total bilirubin in sera of calves, adult males, and adult females infected with *Babesia bigemina* and treated with berenil showed the following:

   For calves, there were non-significant decrease in before treatment group compared to control group and a significant decrease in after treatment group compared to control and before treatment groups.

   For adult males, there were significant increase in before treatment group compared to control group and a non-significant increase in after treatment group compared to control group and a significant increase in before treatment group compared to control and after treatment group.

   For adult females, there were significant increase in before treatment group and after treatment groups compared to control group also there were significant decrease in after treatment group compared to before treatment group.

5. Serum triacylglycerol in sera of calves, adult males, and adult females infected with *Babesia bigemine* and treated with berenil showed the following:

   For calves, there were significant decrease in before treatment group compared to control group and after treatment groups. Also, there were significant increase in after treatment group compared to before treatment group.

   For adult males, there were significant decrease in before and after treatment groups compared to control group.

For adult females, there were significant decrease in before and after treatment groups compared to control group. Also, there were non-significant changes between males and females before treatment but there were significant decrease in adult males and adult females compared to calves group before treatment but also there were non-significant changes in adult males, adult females and calves after treatment.

6. Serum total cholesterol in sera of calves adult males and adult females infected with *Babesia bigemina* and treated with berenil showed the following:

   For calves, there were significant decrease in before treatment group compared to control group and a non-significant decrease in after treatment group compared to control group also there were significant decrease in after treatment group compared to before treatment group.

   For adult males, there were significant decrease in before treatment group compared to control and after treatment groups. Also, there were significant decrease in before treatment group compared to after treatment group.

   For adult females, there were significant decrease in before treatment group compared to control and after treatment groups while between after and before treatment, there were significant decrease in before treatment group compared to after treatment group. Also, there were non significant changes between adult males and adult females and calves also adult males were more susceptible to infection than adult females than calves.

7. High density lipoprotein cholesterol (HDL-C) in sera of calves, adult males, and adult females infected with *Babesia bigenia* and treated with berenil showed the following:

For calves, there were significant decrease in before treatment group compared to control and after treatment groups. Also, there were significant decrease in after treatment group compared to control group. For adult males, there were significant decrease in before treatment group compared to after treatment group than control group. Also, there were significant decrease in after treatment group compared to control group.

For adult females, there were significant decrease in before treatment group compared to control group and significant decrease in after treatment group compared to control group but there were significant decrease in before treatment group compared to after treatment group. Also, the mean value for adult males showed non-significant changes with adult females in before treatment group and a significant decrease in calves in the same group and in after treatment groups.

8. Low density lipoproteins cholesterol (LDL-C) in sera of calves, adult males, and adult females infected with *Babesia bigemina* and treated with berenil showed the following:

   For calves, there were significant increase in before and after treatment groups compared to control group and non-significant increase in before treatment group compared to after treatment group.

   For adult males, there were non-significant changes among before, after treatment and control groups.

   For adult females, there were non-significant decrease in before treatment group compared to after treatment and control groups and non-significant decrease in control group compared to before treatment group and non-significant increase in after treatment group compared to control group.

There were non-significant changes in calves compared to adult females in before and after treatment and significant decrease in adult males compared to calves and adult females in before and after treatment groups.

9. Serum calcium level in sera of calves adult males and adult females infected with *Babesia bigemina* and treated with berenil showed the followings:

   For calves, there were non-significant changes among before, after treatment and control groups.

   For adult males, there were non-significant increase in before treatment group compared to control group and there were significant decrease in after treatment group compared to control group and before treatment group.

   For adult females, there were non-significant decrease in before and after treatment groups compared to control group. Also, there were significant decrease in adult females before treatment group compared to adult males, and non-significant decrease in calves and adult males in before treatment groups. Also, there were non-significant decrease in adult males compared to adult females and significant increase in calves compared to adult males and females in after treatment groups.

10. Serum phosphorus level in sera of calves adult males and adult females infected with *Babesia bigemina* and treated with berenil showed the following:

    For calves, there were significant decrease in before treatment group compared to after treatment group but there were non-significant increase in control group compared to before treatment group.

For adult males, there were non-significant increase in after treatment group compared to control group and significant decrease in before treatment group compared to control and after treatment groups.

For adult females, there were non-significant decrease among before, after treatment and control groups. Also, there were significant changes among calves, adult males and adult females before and after treatment groups.

11. Serum total protein level in sera of calves adult males and adult females infected with *Babesia bigemina* exhibited the following:

    For calves, there were non-significant decrease in infected group compared to control group.

    For adult males, there were non-significant decrease in infected group compared to control group.

    For adult females, there were non-significant decrease in infected group compared to control group.

    For infected group, there were non-significant decrease in calves compared to adult females and a significant decrease in calves compared to adult males.

12. Serum albumin level in sera of calves adult males and adult females infected with *Babesia bigemina* showed the following:

    For calves, there were significant decrease in infected animals compared to control groups.

    For adult males, there were significant decrease in infected group compared to control group.

    For adult females, there were significant decrease in infected animals compared to control group.

For infected groups, there were non-significant increase in calves, adult females and adult males compared to control groups.

13. Serum total globulins in sera of calves, adult males and adult females infected with *B. bigemina* showed the following:

    For calves, there were non-significant increase in infected group compared to control group.

    For adult males, there were non-significant increase in infected group compared to control group.

    For adult females, there were significant increase in infected group compared to control group.

    For infected group, there were significant increase in the following manner adult males > adult females > calves.

14. Serum alpha-globulin in sera of calves adult males and adult females infected with *B. bigemina*s showed the following:

    For calves, adult males and adult females there were non-significant increase in infected groups compared to control groups. Also, there were non-significant increase in adult males compared to adult females and significant increase in adult males compared to calves.

15. Serum beta globulin in sera of calves, adult males and adult females infected with *B. bigemina* showed the following:

    For calves, and adult males, there were non-significant increase in infected groups compared to control group.

    For adult females, there were significant increase in infected group compared to control group.

16. Serum gamma globulin in sera of calves, adult males and adult females infected with *B. bigemina* exhibited the following:

For calves, adult males and adult females, there were non-significant increase in infected groups compared to control groups.

Also, there were non-significant increase in calves compared to adult males and significant decrease in adult females compared to calves and adult males in infected group.

17. Serum albumin-globulin ratio in sera of calves adult males, and adult females infected with *B. bigemina* showed the following:

For calves, adult males and adult females, there were significant decrease in infected groups compared to control groups and significant decrease in adult males and adult females compared to calves.

Also, for infected group, there were significant increase in claves and adult females compared to adult males, but there were significant variation between calves and adult males.

Generally there were significant decrease in infected group of calves, adult males and adult females compared to the corresponding control levels.

Diagnosis of Babesiosis using Polymerase Chain Reaction (PCR)

The present work was planned for using the polymerase chain reaction (PCR) in diagnosis of *Babesiosis* by modern and accurate technique for detection of very low number of parasites in the blood of infected animals even this number reach to one parasite in blood in apparently healthy cattle (negative blood smear examination) PCR can detect it by accurate steps:

1. DNA isolation:

For DNA extractions, the erythrocytes pellets were suspended in lysis buffer at 4^{o}C and were digested from 1 h to over nigh with proteinase K and 1% sod. dodecyl sulfate at temperature 37^{o}C lysates were djusted to 1 M NaCl and extracted with an equal volume of phenal-chloroform-isoamyl alcohol (25: 24: 1). The DNA in the aqueous phase as precipitated with 2 volumes of cold 95% ethanol and pelleted by centrifugation at 10000 x g. The precipitated DNA was washed once with 70% ethanol and resuspended in 50 µl of tris-EDTA [TE] before being used as template for PCR.

2. Primer:

The common PCR primer used in this study was obtained from the sequence of a portion of a pocytochrome b gene of *B. bigemina*. This primer pair set was designed from the regions conserved in *Babesia bigemina* to amplify a fragment of 278 (bp) from *B. bigemina* and designed on the basis of a G+ C content that ranged from 40 to 60%.

Forward primer:

5'-CATCTAATTTCTCTCCATACCCCTCC-3'

Reverse primer:

5’-CCTCGGCTTCAACTCTGATGCCAAAG-3’

3. PCR amplification:

The PCR amplification was done in 50 microliters of reaction mixture using 10 µg DNA as a template 1 x PCR buffer, 50 mM KCl, 0.01% gelatin, 2.5 Mg Cl_2, primers, deoxynucleotide triphosphates 2.5 µ of Taq polymerase. The PCR were carried out in an automated DNA thermal cycles for 34 cycles each cycle consisted of 1 min. 94^{o}C for denaturation (5 min. for the first cycle), at

70^{o}C for 1 min for annealing and 72^{o}C for 1 min. for polymerization with an additional 7 mi. at 72^{o}C after last cycle.

The amplified fragments were visualized by electrophoresis in a 1.2% agarsose gel containing ethidium bromide run at 90 V for 1 h and photographed in UV lamp using Digital V. camera.

Carrier cattle infected with *Babesia bigemina* are difficult to detect because of the low numbers of parasites in peripheral blood. Diagnosis of these carrier status is important for evaluating the efficacies of vaccines and in the epidemiological studies. The present study used the polymerase chain reaction (PCR) to amplify a portion of the apocytochrome b gene from *Babesia bigemina* in one PCR reaction using a common PCR primer pair set conserved in *Babesia bigemina* and tested the ability of this method to detect the Egyptian strains. The same amplified band was generated and identified by southern blot hybridization with non-radioactive species specific probes on the Egyptian control samples. The sensitivity of the extra chromosomal DNA based PCR test was 50 femtogram of DNA/100 ML extracted genomic DNA of each parasite independently form one ml blood.

Microscopic examination of blood smears of the carrier cattle showed negative (no parasite existed) results.

This PCR method provides a useful diagnostic tool for detecting carrier cattle infected with *B. bigemina* and the sensitivity is significantly improved over that of current methods. The present investigation also suggest that characteristics of the apocytochrome b gene may make this available target DNA for PCR-based detection of other hemopearasites in Egypt.

The appearance of DNA bands in the blood Berenil-treated animals although disappearance of clinical symptoms could be attributed to pre-immunization (carrier cases), so it's advisable to use Berenil for several doses.

CONCLUSION

In Egypt the blood parasites *Babesia bigemina* causes great economic losses in cattle. The drug Berenil which is the most extensively used for treatment of animals suffering from these parasites in our veterinary practice.

The obtained data revealed a significant changes in the levels of most enzymes and some biochemical parameters studied besides, calcium, phosphorus and total protein fractions. These enzymes and biochemical parameters effected by invasion of the parasites into the body of the animals and affect on health, production and substantial economic losses in several important animals. Berenil can overcome the problem in our field but no spend on all the parasites on the animal.

Microscopic examination using peripheral blood films for detection of babesiosis fail to detect carrier cattle, subclinical babesiosis. PCR is the effective technique for diagnosis of such cases which appeared negative microscopically, but the parasite still in the blood of animals.

We developed a PCR-based method for the direct detection of *B. bigemina* carrier cattle that is superior to existing methods. The method is highly sensitive, is broadly applicable to strains of the parasite from diverse geographic regions, and is specific for *B. bigemina* rather than the other hemoparasites tests. The sensitivity of this method will facilitate analysis of vaccines and their ability to induce or prevent the carrier stage. Additionally, the method may have use for testing the efficacies of drugs against the parasites and in studies on the transmission and epidemiology of the disease.