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# Molecular Characterization, Hematobiochemical Changes and Therapeutic Management of Tick Born Haemoparasites in Naturally Infected Cattle

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## Abstract

In this study, examination of infected cattle infested with tick, identification of collected tick samples were based on the12S rDNA PCR products as Rhipicephalus annulatus, the GenBank accession number is (OP650242). A total of 72 blood samples from crossbred cattle of both sexes were examined clinically and in the laboratory. Out of these, 43 cattle were healthy, while 19 (26.38%) had theileriosis and 10 (13.88%) had babesiosis. Hemogram analysis revealed distinct anemia patterns, with *Babesia*-infected cattle displaying macrocytic hypochromic anemia and *Theileria*-infected ones showing normocytic normochromic anemia, both with reduced platelet counts. *Babesia*-infected cattle had elevated total leukocyte counts, neutrophilia, eosinophilia, and lymphopenia, while *Theileria*-infected cattle had decreased total leukocyte counts, neutropenia, lymphocytosis, and eosinophilia. In infected cattle, serum biochemistry showed increased ALT, AST, creatinine, and urea levels in both *Babesia* and *Theileria* infections. There was decreased serum protein, and albumin, in both cases. Oxidative stress revealed elevated serum malonaldehyde (MDA), reduced glutathione peroxidase (GPx) and catalase (CAT) levels in infected animals compared to controls. After administering Imidocarb dipropionate (1mg/kg S/C) and Buparvaquone (1ml/20kg I/M) to animals with babesiosis and theileriosis, respectively, there was a positive change in the hematological and biochemical measures, bringing them closer to the normal values. There is a genuine danger to the cattle industry in Egypt due to the existence of babesiosis, theileriosis, and their vector. Modern techniques like PCR should be utilized for precise monitoring and to prevent spread of such diseases. Furthermore, adverse effect of *Babesia* and *Theileria* on hematological and biochemical parameters can be eliminated through the appropriate use of Imidocarb dipropionate and Buparvaquone for babesiosis and theileriosis respectively.

KEYWORDS
Blood parasite, Hematobiochemical changes, Molecular Characterization.

## INTRODUCTION

In Egypt, the climatic conditions, coupled with inadequate control measures and prophylactic, foster a conducive environment for various species of ticks (Casati *et al*., 2006; Al-Hosary *et al*., 2018). Ticks exert a detrimental influence on livestock, not only through their direct effects on infested animals but also by acting as vectors for life-threatening pathogens (Elsify *et al*., 2015). Ticks can also act as carriers for numerous pathogens affecting both animals and humans, such as *Theileria* spp. and *Babesia* spp. (Al-Hosary *et al*., 2021). Ticks commonly carry protozoan parasites known as piroplasms, which encompass *Babesia* spp. and *Theileria* spp. These parasites frequently lead to illnesses in both animals and humans. (Mohamed *et al*., 2021). Cattle theileriosis and babesiosis are notable hemoprotozoal diseases causative of substantial economic losses in cattle within tropical and subtropical regions (Noaman, 2013). These hemoparasites are widespread and exhibit significant prevalence in various geographical regions across Egypt (Abas *et al*., 2021).

Bovine babesiosis in Egypt, usually induces high economic losses in cattle due to hemolytic anemia, abortions, and even mortality, in addition to the negative effect on both milk and meat production (Bock *et al*., 2004; Adham *et al*., 2009; Fereig *et al*., 2017). Bovine babesiosis primarily affects cattle in tropical and subtropical areas and is caused by the hemoprotozoa *B. bigemina* and *B. bovis*, which reside within red blood cells (Bose *et al*.,1995). Millions of cattle perish yearly because of *Theileria* infection, and consequently, losses in production with cattle breeders worldwide are experiencing the burden of high expenses related to veterinary care and tick control measures, which have become increasingly challenging to manage (El-Dakhly *et al*., 2020).

Most cases, these protozoan diseases demonstrate themselves as a mixed and often latent invasion, which complicates its detection, treatment, and measures to eradicate it (Bursakov and Kovalchuk, 2019), so the demand for acaricide programs aimed at combating tick vectors and therapeutics programs targeting vertebrate hosts has seen a significant increase. (Bock *et al*., 2004). The primary clinical indicators of theileriosis, a tick-borne disease caused by *Theileria annulata*, include the inflammation of superficial lymph nodes, along with fever and reduced appetite (Radostits *et al*., 2006). *Theileria annulata*, a tick-borne pathogen, poses a significant threat to cattle in Egypt, resulting in consid-


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erable economic losses due to elevated morbidity and mortality rates, reduced productivity, increased treatment costs, and the necessity for preventive measures (Callow, 1984; Abdel-Hamied *et al*., 2020).

Clinical examination alone may not definitively identify the specific blood parasite in infected animals. The standard method for pinpointing tick-borne pathogens involves routine direct microscopy of Giemsa-stained blood smears (Nayel *et al*., 2012; Elsify *et al*., 2015). Nevertheless, in animals that serve as carriers or have a low level of parasitemia, this approach might produce constrained outcomes because of its insufficient sensitivity and specificity (Almería *et al*., 2001; Jacobson, 2006). Consequently, the utilization of molecular diagnosis through PCR enables the detection of subtle parasitemia that may go unnoticed by traditional methods employed for identifying blood protozoa, particularly in carrier states (Almería *et al*., 2001), and make the diagnosis of blood parasites more effective (Mosqueda *et al*., 2012).

Chemical treatment is the most usual method for treating animals for ticks, and also acaricides can aid in the management of tick-borne illnesses (Almazan *et al*., 2018). Numerous *Babesia*cidal compounds are effective in treating cattle with babesiosis, meanwhile practically , diminazene aceturate (3 to 5 mg/kg) intramuscular, amicarbalide (5 to 10 mg/kg) intramuscular, and imidocarb (1 to 3 mg/kg, IM) are mostly used (Kuttler, 1980; Mosqueda *et al*., 2012). Buparvaquone, a preferred medication among veterinarians, is commonly used to treat theileriosis in cattle (Masare *et al*., 2009). Buparvaquone belongs to the second generation of hydroxyl naphthoquinones and specifically targets the protozoa's electron transport chain system, without affecting the host's system (Hudson *et al*., 1985; McColm and McHardy, 1984).

Considering the substantial losses and the growing prevalence of babesiosis and theileriosis in cattle, the present study was conducted to diagnose cattle infected with Babesiosis and theileriosis. Additionally, the study aimed to evaluate the hematological and biochemical alterations in these animals during and after undergoing drug therapy.

## MATERIALS AND METHODS

*Ethical approval*

This work was accepted by the Animal Use Ethics Committee (ARC-IACUC) of agricultural research center, Egypt, (Approval no.: ARC-AH-22-15).

*Animal source and samples collection*

The current study, conducted between May and December 2022 in Ismailia governorate (30.6044 or 30 36′ 16″ north) (32.2771 or 32 16′ 38″ east), involved 72 Baladi-cross breed cattle aged 2-4 years. These cattle exhibited clinical symptoms of tick infestation, including fever and mucous membrane discoloration. Microscopic examination followed Benjamin's standard protocol (1978). Ticks were manually collected from infected cattle and preserved in 70% ethanol tubes.

A total of 72 blood samples were drawn from each suspected animal's jugular vein. Hematological examination and PCR were performed using EDTA-coated tubes. For serum biochemistry and assessment of oxidative status, blood was collected without anticoagulant. The collected blood samples were then centrifuged at 3000 rpm for 15 mi, and the resulting clear serum was stored at -20°C.

*Morphological identification of ticks*

Tick samples were prepared following Farid *et al*. (2021). Ticks were first morphologically identified following Hoogstraal (1956) and Walker (2003). by using an optical microscope (×400).

*DNA Extraction of ticks*

The QIA amp DNA Mini Kit (Qiagen) was used to extract the ticks' DNA after morphological identification conforming to manufacturer's instructions. The genomic DNA obtained was stored at a temperature of -20 °C until used. Tick samples molecular identification based on 12S rDNA Gene.

Amplification of the 12 S rDNA gene by PCR was carried out using specific primers (5′ GAGGAATTTGCTCTGTAATGG -3′ and 5′ - AAGAGTGACGGGCGATATGT-3′) and followed the cycling conditions described by (Norris *et al*., 1999). The products of amplification were electrophoretically separated on a 1.6% agarose gel with ethidium bromide.

*Sequence analysis*

After amplification, the PCR products were retrieved from the gel, purified, and subjected to sequencing. The sequencing process was carried out by Solgent Co. Ltd in South Korea using the Sanger sequencing technique. The obtained sequences were then assessed using BLAST® for analysis (Johnson *et al*., 2008).

*Molecular Piroplasma spp. (Babesia spp. and Theileria spp.) identification*

DNA Extraction

By following the guidelines provided by the manufacturer, the genomic DNA was purified from a 200 µl blood sample using the QIAamp DNA Blood Mini Kit from QIAGEN. The purified DNA was then stored at -20 °C until it was ready to be utilized.

PCR amplification

To evaluate which genetic marker, PCR amplifications of the same samples were done with general and species-specific primers, targeting different gene fragments.

PCR amplification of the 18S rRNA gene of *Babesia* spp. and *Theileria* spp. (general primers)

A 25µl PCR reaction tube was utilized, which contained 2 mM MgCl2, 0.2 mM of dnTPs, Taq polymerase (0.05 µl), 7µl of DNA, 4µl of nuclease-free water, and a set of primers (2 pmol). The amplification of the 18S rRNA gene was performed using the oligonucleotide primers Piro-18-F2 ACT GTC AGA GGT GAA ATT CTT AGand Piro-18-R all AAT AAT TCA CCG GAT CAC TCG. The PCR protocol involved an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds. A final extension step at 72°C for 5 minutes was conducted.

PCR amplification of *Babesia* spp. and *Theileria* spp. with species-specific primers

Polymerase chain reactions (PCR) targeting *T. annulata* (Tams1) {F 5′-GTT AAT GCT GCA AAT GAG GAT G3′andR5′-GGACTGATGAGAAGACGATGAG -3′} and *B. bigemina* SSrRNA {F5′TGTCCTCGTTTGCTTCTTAGAGGGACTCCT3′and R 5′GAG CAA ACA GCA AGG GCG CGT3′} were performed using species-specific

primers, as indicated. The PCR cycling conditions and gel electrophoresis were performed as described by Kirvar *et al*. (2000) and Adham *et al*. (2009).

*Haematological evaluation*

The red blood cell count (RBCs, $10^6$/µl), hemoglobin content (Hb g/dl), packed cell volume (PCV %), red blood cells indices (MCV, MCH, MCHC), white blood cells count (WBCs), differential leukocytic counts and platelets count (PLT x$10^3$/µl) were measured in blood samples obtained from healthy cows, as well as cows affected by babesiosis and theileriosis, both before and after treatment (Feldman *et al*., 2000).

*Biochemical evaluation*

Biochemical parameters were estimated spectrophotometrically as Aspartate amino transferase (AST), Alanine amino transferase (ALT), Urea, Creatinine, Glucose, total proteins, albumin, and globulin. Antioxidant enzymes in serum involves the determination of serum lipid peroxidation product Malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GPx) were estimated using test kits supplied by Bio-Diagnostic Company-Egypt. according to manufacture procedures.

*Chemotherapy study*

For control of tick: all animals infested with ticks were injected with ivermectin (Iveen®, ADWIA©, Egypt), (1 ml /50 kg by S/C) injection. Repeated dose after 14 days.

For blood parasite: All confirmed cases of *Babesia* spp. (*B. bigemina* infected group; 10) single dose of Imidocarb dipropionate was injected (1mg/kg by S/C route), (Imidocarb® Pharma Swede©, Egypt). Confirmed theileriosis cases (T. annulate infected group;10) treated with single dose of Buparvaquone 1ml/20kg by deep I/M route, Theil-Cure® Pharma Swede©, Egypt. Supportive treatment for all infected cattle comprised of meloxicam 0.5mg/kg I/M, Fercobsang® (iron, cyanocobalamin, Nicotinamide and Cobalt) 20ml daily I/M for 7 days. Blood samples were taken after 14 days of treatment, as described above, to show the effect of drug treatment.

*Statistical evaluation*

To determine the variance in the various hematological and biochemical parameters, one-way analysis of variance (ANOVA) was used. SPSS software (SPSS 20.0) was used to analyze animals from various groups.

## RESULTS

*Morphological identification of tick*

Rhipicephalus (Boophilus) annulatus

hexagonal basis capitulum, rounded or oval spiracular plate, and short, compressed, ridged palps. Males exhibited adanal shields and accessory shields, while females lacked a distinct anal groove. Festoons or ornamentation were not observed on these ticks (Fig. 1).

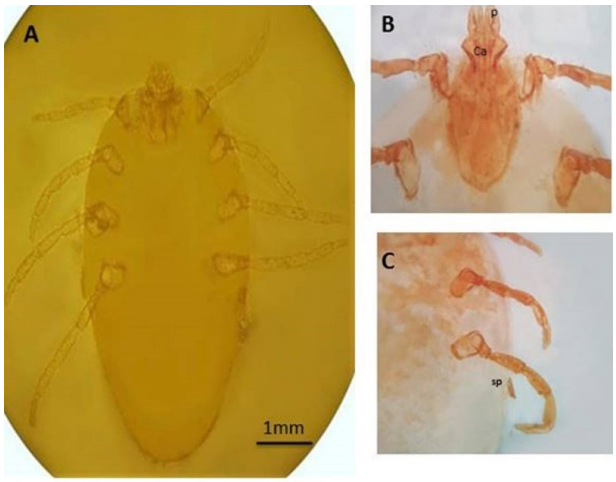


Fig. 1. Rhipicephalus (Boophilus) annulatus female A: ventral view B: anterior part and C: spicular plate. (Sp): Spicular plate, (ca): capituli (P): palpai.

*Molecular identification of tick species*

PCR amplification of the 12S rDNA gene was employed. The obtained PCR products confirmed the presence of Rhipicephalus

Table 1. Hematologic evaluation of erythrogram and leukogram of control, *Babesia*, and *Theileria* infected cattle before and after treatment.

| | | *Babesia* | | *Theileria* | |
|---|---|---|---|---|---|
| | Control | Infected | Treated | Infected | Treated |
| RBCs (x$10^6$/µl) | 8.06±0.25$^a$ | 5.66±0.10$^c$ | 7.07±0.15$^b$ | 5.30 ±0.11$^c$ | 7.15 ± 0.13$^b$ |
| HB (gm/dl) | 10.29±0.15$^a$ | 5.79±0.13$^d$ | 9.59±0.09$^b$ | 6.91± 0.13$^c$ | 9.76 ±0.14$^b$ |
| PCV (%) | 30.98±0.63$^a$ | 25.24±0.23$^c$ | 27.27±0.26$^b$ | 19.60±0.58$^d$ | 27.42 ±0.26$^b$ |
| MCV (fl) | 38.43±0.69$^b$ | 44.59±0.80$^a$ | 38.57±1.91$^b$ | 38.98±0.71$^b$ | 38.35±1.31$^b$ |
| MCH (pg) | 12.77±0.20$^a$ | 10.23±0.13$^b$ | 13.56±0.07$^a$ | 12.28±0.38$^a$ | 13.65±0.20$^a$ |
| MCHC (gm/dl) | 33.22±0.37$^b$ | 29.57±0.86$^c$ | 36.09±0.82$^a$ | 33.21±0.25$^b$ | 35.59±0.93$^a$ |
| PLT count (x$10^3$ /µl) | 316.0±13.9$^a$ | 218.40±5.49$^b$ | 307.2±13.4$^a$ | 241.00 ± 9.24$^b$ | 301.6± 14.5$^a$ |
| WBCs (x$10^3$ /µl) | 7.84±0.09$^b$ | 8.87±0.17$^a$ | 7.66±0.30$^b$ | 6.75±0.24$^c$ | 7.76±0.19$^b$ |
| Neutrophils (x$10^3$ /µl) | 3.10±0.11$^b$ | 5.27±0.13$^a$ | 3.09±0.08$^b$ | 1.16±0.06$^c$ | 3.00±0.1$^b$ |
| Lymphocytes (x$10^3$/µl) | 3.92±0.09$^b$ | 2.67±0.29$^d$ | 3.71±0.19$^c$ | 4.37±0.06$^a$ | 3.90±0.07$^b$ |
| Monocytes (x$10^3$ /µl) | 0.51±0.02$^b$ | 0.50±0.02$^b$ | 0.53±0.05$^b$ | 0.76±0.05$^a$ | 0.54±0.06$^b$ |
| Eosinophils (x$10^3$ /µl) | 0.30±0.04$^c$ | 0.42±0.10$^b$ | 0.32±0.03$^c$ | 0.45±0.06$^a$ | 0.30±0.04$^c$ |
| Basophiles (x$10^3$ /µl) | 0.01±0.002$^a$ | 0.01±0.002$^a$ | 0.01±0.002$^a$ | 0.01±0.003$^a$ | 0.02±0.02$^a$ |

Values (mean ± SE) in different columns with the different superscripts are significantly different at P<0.05.

annulatus, and the corresponding GenBank accession numbers is OP650242.

*Phylogenetic analysis*

Phylogenetic analysis was conducted using MEGA X10.1 software and the neighbor-joining (NJ) method. Analysis of the 12S rDNA gene sequences revealed minimal sequence variation among the Rhipicephalus annulatus species from Egypt, including the sequences obtained in this study.

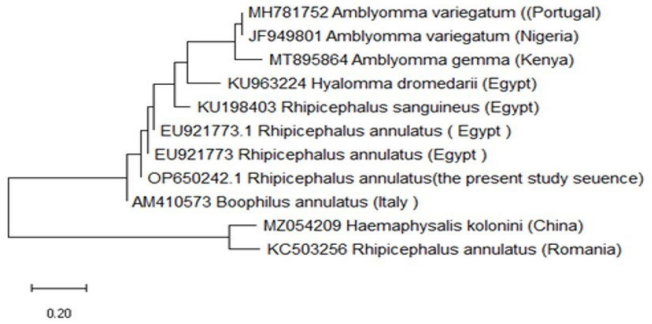


Fig. 2. Neighbor-joining (NJ) techniques were used in the phylogenetic study to build the tick phylogenetic tree from our sequencing data and some hard tick species sequences from the GenBank.

*Morphological identification of blood parasites*

The morphology of *Babesia* spp. and *Theileria annulata* in cows was observed as in Fig. 3 and Fig. 4, respectively.

Molecular identification of Piroplasm spp.

Out of the total, 24 blood samples yielded positive results during the morphological examination, the amplification process was carried out. The majority of these samples were successfully amplified using primers specifically designed for the target species, indicating the effectiveness of the species-specific primers in capturing the desired DNA fragments as for (*Tams1*) amplicon size was 768 bp and for (SSrRNA) amplicon size was 543 bp. Conversely, the general primers targeting Piroplasm failed to yield amplification for the majority of the samples, suggesting their limited suitability as diagnostic markers for the target tick-borne pathogens (Fig. 5).

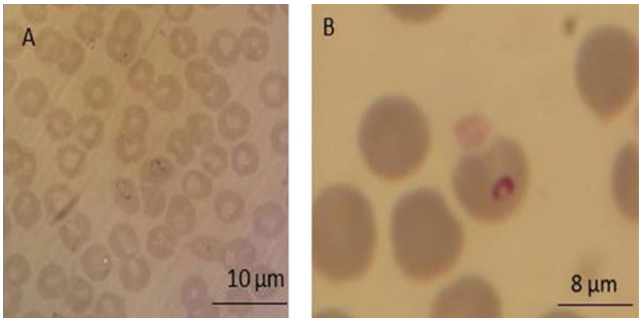


Fig 3. (A and B) *Babesia bigemina* with Pyriform body at acute angle inside the erythrocytes in Giemsa stained blood smear.

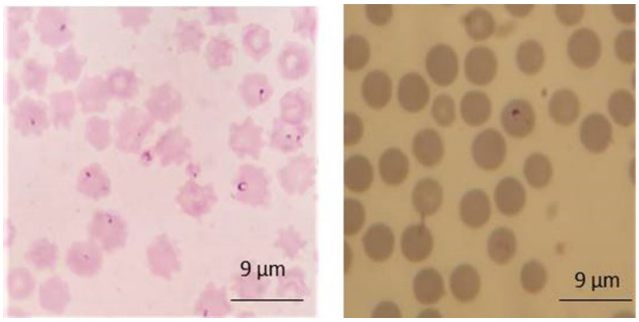


Fig. 4. *Theileria annulata* inside the erythrocytes in blood smear stained by Giemsa.

A total of 72 blood samples from crossbred cattle of both sexes were examined clinically, laboratory and by molecular identification. Out of these, 43 cattle were healthy, while 19 (26.38%) had theileriosis and 10 (13.88%) had babesiosis.

Diseased animals were divided into two groups: *B. bigemina*

Table 2. Serum biochemical evaluation of control, *Babesia*, and *Theileria* infected cattle before and after treatment.

| Parameters | | *Babesia* | | *Theileria* | |
|---|---|---|---|---|---|
| | Control | Infected | Treated | Infected | Treated |
| ALT (U/L) | 12.75± 0.61$^{c}$ | 22.65±0.73$^{a}$ | 15.68± 0.72$^{b}$ | 21.11± 1.4$^{a}$ | 14.27± 0.5$^{b}$ |
| AST (U/L) | 27.56± 3.8 $^{c}$ | 60.74±6.8 $^{a}$ | 34.38 ±1.7 $^{b}$ | 66.07± 11.9$^{a}$ | 38.72± 7.8$^{b}$ |
| Urea (mg/dL) | 31.04±0.92 $^{c}$ | 51.4±0.95 $^{a}$ | 43.00±1.9 $^{b}$ | 51.46± 0.63$^{a}$ | 40.07± 2.3$^{b}$ |
| Creatinine (mg/dL) | 0.88± 0.09 $^{c}$ | 3.35 ±0.21 $^{a}$ | 1.74±0.19 $^{b}$ | 3.84 ± 0.12$^{a}$ | 1.83± 0.34$^{b}$ |
| Total protein (g/dl) | 7.12± 0.13$^{a}$ | 5.92 ±0.21$^{c}$ | 6.12±0.31$^{b}$ | 5.20 ± 0.39$^{c}$ | 6.24± 0.13$^{b}$ |
| Albumin (g/dl) | 3.84± 0.007$^{a}$ | 1.97 ±0.14$^{c}$ | 2.32±0.24$^{b}$ | 1.35± 0.21$^{c}$ | 2.84± 0.12$^{b}$ |
| Globulin (g/dl) | 3.28± 0.08$^{c}$ | 3.95 ±0.4$^{a}$ | 3.80±0.41$^{b}$ | 3.85± 0.4$^{a}$ | 3.40 ± 0.52$^{b}$ |
| Glucose (mg/dL) | 78.50± 3.2$^{a}$ | 60.44 ±1.5$^{c}$ | 67.2±1.4 $^{b}$ | 58.5± 3.2$^{c}$ | 66.51± 2.6$^{b}$ |

Values (mean ± SE) in different columns with the different superscripts are significantly different at P<0.05.

Table 3. Serum Oxidant and antioxidant level of control, *Babesia*, and *Theileria* infected cattle before and after treatment.

| Parameters | | *Babesia* | | *Theileria* | |
|---|---|---|---|---|---|
| | Control | Infected | Treated | Infected | Treated |
| MDA (nmol/ml) | 2.88±0.07$^{c}$ | 7.46±0.21$^{a}$ | 4.94±0.24$^{b}$ | 7.65±0.17$^{a}$ | 5.76±0.17$^{b}$ |
| Catalase(U/ml) | 2.39±0.09$^{a}$ | 0.93±0.02$^{c}$ | 1.24±0.09$^{b}$ | 0.89±0.04$^{c}$ | 1.48±0.18$^{b}$ |
| GPx (nmol/l) | 5.72 ±0.12$^{a}$ | 2.37±0.20$^{c}$ | 3.77±0.16$^{b}$ | 2.07±0.25$^{c}$ | 3.45±0.18$^{b}$ |

Values (mean ± SE) in different columns with the different superscripts are significantly different at P<0.05.

(10 animals) and *T. annulata* (10 animals). Additionally, 10 clinically healthy cows, confirmed by laboratory tests and molecular identification of blood parasite, served as controls.

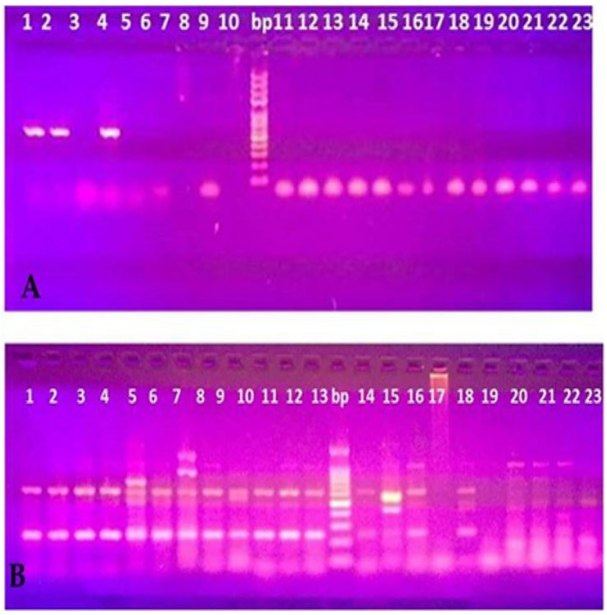


Fig. 5. PCR amplification from blood samples using: 18S rDNA Gene, (B) PCR amplification from the same blood samples using: Specific primers *T. annulata* (Tams1), and *B. bigemina* SSrRNA, Base pairs are used to represent fragment sizes (bp).

*Hematological findings*

The information for the hematological analysis is illustrated in Tables 1.

*Biochemical finding*

The information for biochemical and oxidant antioxidant profile illustrated in Tables 2 and 3.

## DISCUSSION

The current research aimed to validate tick identification using DNA sequences, addressing limitations in morphological identification, including the need for expertise and time (Dantas-Torres *et al*., 2013). Molecular tick recognition, utilizing DNA sequences, aims to enhance accuracy and efficiency. PCR amplification and DNA sequencing techniques offer a more objective and reliable approach, examining unique genetic markers for precise species identification.

For the molecular identification of *Babesia* spp., blood samples that tested positive in the morphological examination underwent PCR amplification. Species-specific primers successfully amplified most of the samples, whereas general primers targeting Piroplasma failed to amplify the majority. This suggests that the general primers may not serve as suitable diagnostic markers. Our results are similar with those of (El-Dakhly *et al*., 2020), who reported variations in the diagnostic marker for *Theileria* spp., even among isolates from the same geographic region. The existence of such molecular marker variations can impede the amplification of certain species.

Hematological findings in this study revealed significant drops in RBCs (x$10^6$/$mm^3$), Hb (g/dl), and PCV% in *Babesia*-infected cattle, along with rises in MCV and declines in MCHC, indicating macrocytic hypochromic anemia (Pandy and Misra, 1987; Ibrahim *et al*., 2009; Zulfiqar *et al*., 2012). This suggests severe intravascular red blood cell hemolysis in cattle with persistent babesiosis. Immune-mediated parasite damage may play a role in anemia development (Messick, 2004). Activated macrophages' increased erythrophagocytosis (Court *et al*., 2001) the generation of antibodies targeting red blood cells (Góes *et al*., 2007) could also play a role in the development of anemia, aligning with previous research findings (Mahmmod *et al*., 2011; Aziz *et al*., 2020).

Total leukocytic count in *Babesia*-diseased cows increased significantly, which may be because the host was mounted at the time of infection (Aulakh *et al*., 2005). Neutrophilia occurred in *Babesia* infected cows were noted by Saud *et al*. (2005); Mahmoud *et al*. (2015) and Ganguly *et al*. (2017). Lymphopenia observed in *Babesia* infected cattle, resembling findings were noted by Tufani *et al*. (2015) and Aziz *et al*. (2020).

Normocytic normochromic anemia of *Theileria* infected cattle affiliated with notable decrease in RBCs, Hb and PCV. Comparable results of decreasing in hemoglobin, packed cell volume and total erythrocyte count were reported by Sarma *et al*. (2016); Ayadi *et al*. (2017) and Kumar *et al*. (2018). MCV, MCH, and MCHC have already reported insignificant changes (Sandhu *et al*., 1998; Ibrahim *et al*., 2009). Thrombocytopenia was found in *Babesia* and *Theileria* infected cows. Thrombocytopenia is typically found with erythrocyte protozoal infections. Increased platelet consumption in severe diseases may be caused by intravascular disseminated coagulation or an immune-mediated mechanism (Taboada, 2006; Riond *et al*., 2008). However, Thrombocytopenia is commonly associated with the connection between enhanced platelet phagocytosis in response to antibodies binding to their surfaces and the activation of macrophages through inflammatory cytokines like macrophage colony-stimulating factor (M-CSF) and interferon gamma (IFN-) (Musaji *et al*., 2004). Regularly, enhanced destruction and sequestration of platelets by splenic macrophages may also be connected to spleen inflammation, which is associated with a number of parasitic tick-borne diseases. (Pantanowitz, 2002).

In *Theileria* infected animals exhibit significant reduction in total leukocytes count and neutrophils while lymphocytosis and monocytosis were observed in comparison with apparently healthy control animals. These leukogram alterations may be caused by *Theileria* toxic metabolites, which have long-lasting negative effects on hematological tissues, particularly bone marrow, and interfere with neurogenesis. Ibrahim *et al*., (2009), observed the relative rise in lymphocyte and monocyte numbers serves as a defense mechanism for target cells against *Theileria* protozoan invasion. The significant increase in eosinophils in babesiosis and theileriosis may be caused by allergic reactions brought on by parasitic tick infestation on skin (Coles, 1986).

Data analysis showed that the mean activity of AST and ALT in serum elevated significantly in *Babesia* and *Theileria* cases. These findings coincide previous work of (Schneider *et al*., 2011, Zulfiqar *et al*., 2012) who revealed elevated serum ALT and AST levels in cattle infected with *Babesia* may be an indicator of hepatic dysfunction. Moreover, increased ALT and AST levels point to a negative effect of toxic *Babesia* metabolites, which affect hepatocyte function and cause the release of liver enzymes (Sharma *et al*., 2016). Alternately, elevated liver enzymes during *Babesia* infection might be the consequence of liver lesions and damage brought on by the parasite during blood-stage growth, which would indirectly induce hepatic dysfunction (Alam and Nasr, 2011). With babesiosis, elevated liver enzymes may also occur after RBC lysis or hyperbilirubinemia (Nasreldin *et al*., 2020).AST and ALT levels in blood of *Theileria* cases were significantly increased than healthy animal. Similar findings were observed by Sandhu *et al*., (1998); Singh *et al*. (2001); Omer *et al*. (2003)and Devadevi *et al*. (2018). Infection with T .annulata lead to damage in liver tissue characterized by distortion of hepatic cords, coagulative necrosis, and lymphocytic infiltration in the periportal areas (Sandhu *et al*., 1998).

Serum urea and creatinine level in *Babesia* infected cattle found to be higher than parasitic free animal, these findings are in similarly with those of Otsuka *et al*. (2002); Hamoda *et al*. (2014); Hashem *et al*. (2018) and Charaya *et al*. (2021). This result may be caused by the presence of globin catabolites released during hemoglobin breakdown which indicate indirect renal tis-

sue damage (Ismael *et al*., 2016). This diminution in renal function could be brought on by hemoglobin casts, necrotizing, and the separation epithelial cells of renal tubule in the proximal convoluted tubules (Solano-Gallego *et al*., 2008), hypoxia, and hemoglobinuria, which may have caused toxic and hypoxic renal tissue damage (Hamoda *et al*., 2014, Ganguly *et al*., 2017).

Serum urea and creatinine of cows infected with *Theileria* showed marked increased than healthy animal and treated one, these findings are in coordinate with Sandhu *et al*. (1998); Singh *et al*. (2001); Col and Uslu (2007) and Somu and Mani (2021). Sandhu *et al*. (1998) suggested elevated levels of urea and creatinine in *Theileria* infected cattle probably due to liver and renal damage. On other hand (Omer *et al*., 2003) revealed a non-significant rise in *T. annulata*-infected cattle.

In the current study, results show total protein and albumin levels of diseased animals with either *Babesia* or *Theileria* decreased significantly in comparison to non-infected animals. These data are in line with the earlier research, which shows that hypoalbuminemia in B.bigemina infected cows could be due to not getting enough protein from diet due to malnutrition and fever-accompanied illness; impairment of liver function during babesiosis that reduces albumin synthesis (Salem *et al*., 2016). The decrease in albumin levels can be attributed to the impaired protein synthesis capacity of the affected liver and the excretion of albumin in the urine as albuminuria. The decrease in albumin is accompanied by malnutrition during the disease, as mentioned by Henley and Vaitukaitis, (1985). These notable alterations likely indicate inflammatory changes in the cells of the liver and glomeruli, which consequently impact their functions. These findings align with the results reported by Sandhu *et al*., (1998); Singh *et al*. (2001). The results revealed significantly reduce blood glucose level in infected animal could be ascribed to a severe loss of glycogen reserves, persistent feverish condition brought on by babesiosis, and theileriosis, which causes anorexia, which in turn causes hypoglycemia (Col and Uslu, 2007; Hussein *et al*., 2007; Alam and Nasr, 2011; Ganguly *et al*., 2015; Aziz *et al*., 2020; Khan *et al*., 2011 and Nasreldin *et al*., 2020). Utilization of glucose by the parasite and the injured liver both have the capability of lowering blood glucose levels (Zulfiqar *et al*., 2012). Hypoglycemic *Theileria* infected cows are probably due to reduce feed intake and dysfunction of the intestine (Forsyth *et al*., 1999; Ganguly *et al*., 2015).

The cooperative actions of tightly regulated antioxidant enzymes safeguard tissues and cells against oxidative damage caused by reactive oxygen species (ROS) and oxidants (Nazarizadeh and Asri-Rezaie, 2016). Infections with various parasites elevate ROS levels, resulting in cellular and tissue damage (Deger *et al*., 2008; Ince, *et al*., 2010). ROS-induced lipid peroxidation, measured by malondialdehyde (MDA), is a significant biomarker, often elevated in infected cattle, reflecting increased free radical activity and enzyme depletion (Chaudhuri *et al*., 2008; Halliwell and Gutteridge, 2015).

The current study found substantial alterations in MDA, Catalase, and GPx levels in babesiosis and theileriosis cases. Infected cattle exhibited lower GPx and CAT activity alongside higher MDA levels, indicating impaired enzyme function and heightened oxidative stress (Esmaeilnejad *et al*., 2020). GPx, essential for ROS detoxification, may undergo peroxide-mediated deactivation (Lubos *et al*., 2011). Catalase activity increased, potentially as a compensatory response. Parasites damage cells responsible for antioxidative agent synthesis, consistent with decreased glutathione (GSH) levels seen in diseased animals (El-Sokkary *et al*., 2002; Kolodziejczyk *et al*., 2005; Ince *et al*., 2010) depleting host antioxidant reserves.

Due to the fact that the medications used in the current investigation were intended to kill the parasites, the treatment of infected cows to reduce blood parasite complications revealed significant changes to the hematobiochemical profile as well as oxidative stress, It blocks the signals necessary for the induction of the receptors and the growth factor-coding genes. Imidocarb is the medicine of choice for treating bovine babesiosis brought on by *B. bigemina*, *B. bovis*, *B. divergens*, and *B. caballi*. It is effective at a suggested dose of 1-3 mg/kg (Kuttler, 1980). Imodocarb dipropionate therapy caused the *Babesia* parasitaemia to diminish between 24 and 48 hours later (Patarroyo *et al*., 1982). Improvements in biochemical markers following imidocarb dipropionate delivery to *Babesia*-infected cows suggest recovery from potential damage of liver and kidney. The current findings were consistent with (Sevinc *et al*., 2013). Buparvaquone is effective against theileriosis (Abdela and Bekele, 2016). Buparvaquone was successfully administered as a single deep intramuscular injection to cows that had tropical theileriosis (Azhahianambi *et al*., 2021). Buparvaquone is an effective drug for treating and preventing all types of theileriosis (Verma and Singh, 2016).

## CONCLUSION

There is a genuine danger to the cattle industry in Egypt due to the existence of babesiosis, theileriosis, and tick as their vector. Modern techniques like PCR should be utilized for precise monitoring and to prevent spread of such diseases. Furthermore, adverse effect of *Babesia* and *Theileria* on hematological, biochemical, and antioxidant parameters can be eliminated through the appropriate use of medications such as Imidocarb dipropionate for babesiosis and Buparvaquone for theileriosis.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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