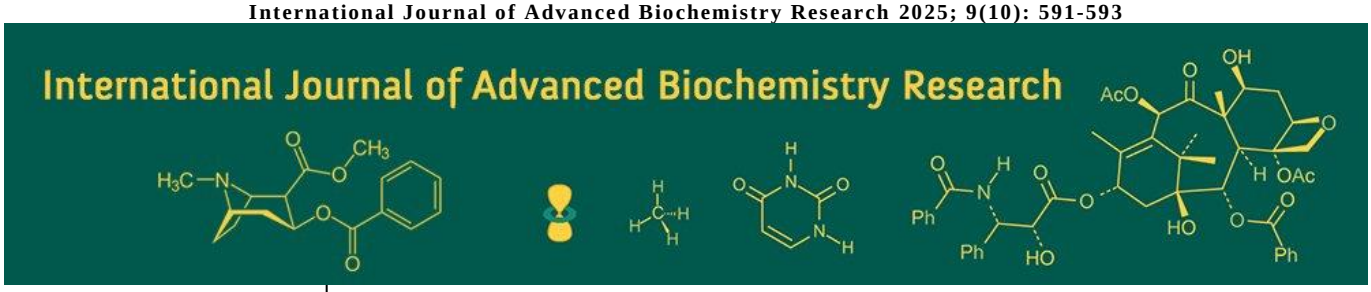

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Molecular identification and clinico-therapeutic study of Babesiosis in dairy cattle

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Abstract
A total of 60 suspected crossbred cows were screened for *babesia bigemina*. Out of which 40 were positive by molecular study in and around Namakkal district. Animals with the history of fever, weakness, hemoglobinuria anaemia, and jaundice, pale mucus membrane were observed. Haematological and biochemical analysis were done for assessment of infection status and therapeutic efficacy. Giemsa-stained blood smear revealed numerous *B bigemina* is paired parasites lying almost parallel or at an acute angle to each other. The blood picture and serum biochemistry showed anemia with leucopenia, leukocytosis, neutrophilia and eosinophilia, hypoproteinemia, hypoalbuminemia and elevated liver enzyme (AST). For further confirmation PCR was performed in whole blood using the primer specific for Bb18S rRNA gene (504 bp). The animals were treated with inj. diminazene aceturate (Berenil) is administered at 3.5 mg/kg, i/m (once) and Inj. Oxytetracycline @ 22 mg/kg i/v with Dextrose normal saline and along with supportive for 4 days. Topical synthetic pyrethroids (Flumethrin 1%) also recommended as tick control measures. The animal recovered uneventfully after 7 days of treatment.

Keywords: PCR, hemoglobinuria, diminazene aceturate, cow

Introduction
Cattle is an important dairy and meat producing animal playing an important role in the economy (Suarez and Noh 2011) [6]. Babesia is a protozoan parasite belonging to the genus piroplasmida, causes a worst disease affect livestock and farm animals and is transmitted by the ticks. Because direct economic effects like decreased milk yield, loss of body weight and animal death (Menshawy 2020). It causes anorexia, hyperthermia, anemia, absence of rumination and increases in heart and respiratory rates. In later stages, it may lead to hemoglobinuria a yellowish mucous membrane and mortality. Ixodidae tick can transmit the babesiosis infection to several animal species. *B. bovis* and *B. bigemina* are the two important *Babesia* species in cattle (Zintl *et al.* 2003) [9]. This organism mainly transmitted through a tick as a vector. B. *bigemina* is transmitted through a Rhipicephalus *spp* of ticks. Tropical countries like India are most vulnerable for the vector propagation. Babesiosis is classified as the second most widespread blood-borne disease among animals (Homer *et al.*, 2000) [1] This study describes the clinical and molecular identification of Babesia infected cattle with successful therapeutic management.

Materials and Methods
The study was conducted on sixty cross-bred cows aged between three and six years. During clinical examination, the affected animals appeared dull and depressed, with emaciated high fever, hemoglobinuria, anemia, yellow mucous membranes, anorexia, and tick infestation. The vital clinical parameters recorded included rectal temperature (103°F-106°F), pale conjunctival mucous membranes, hemoglobinuria, and mild to moderate tick infestation. Based on the general clinical examination, the case was tentatively diagnosed as a haemoprotozoan disease. For further confirmation, peripheral blood smear whole blood abd serum samples were collected. Complete blood count (CBC) and serum biochemical analyses were performed before treatment and on post-treatment. The peripheral blood smear were stained with Giemsa for 30 minutes after methanol fixation (Souls by, 1982) [5] and screened

For blood parasites under microscopic examination (100X). For molecular identification, the DNA was extracted from whole blood using extraction kit (Qiagen). The affected animal was treated with a single dose of inj. Diminazene aceturate at 3.5 mg/kg intramuscularly. Supportive therapy included inj. Oxytetracycline at 20 mg/kg intravenously along with 500 ml of dextrose normal saline, inj. Chlorpheniramine maleate at 0.5 mg/kg intramuscularly and inj. Tribivet (10 ml I/M) for four consecutive days. Hematinic supplementation and an ethnoveterinary formulation consisting of *Moringa* leaves, curry leaves, and *Keezhanelli* (Phyllanthus niruri) leaves mixed with jaggery were administered for ten days. For tick control, topical application of a synthetic pyrethroid (Flumethrin 1%) was recommended.

Results and Discussion

Peripheral blood smear revealed numerous *B bigemina* was lying almost parallel or at an acute angle to each other more in erythrocytes (Fig.1). However, this method is insensitive and not suitable for carrier animals because the pathogen level is usually low in the blood stream. Hematology revealed anaemia, leucopenia and neutrophilia. Serum biochemistry showed hypoproteinemia, hypoalbuminemia and hyperbilirubinemia. Treated animals recovered uneventfully. Post treatment, results showed negative for causative organism and remarkable improvement was observed in haematology and serum biochemistry values (Table 1). The levels of Hb, RBC, PCV, total protein, albumin, calcium, phosphorus, and glucose showed a significant (p<0.01) increase were noticed in recovered animals. In contrast, the values of WBC, MCV, MCH, ALT, and AST were significantly (p<0.01) decreased in recovered animals. Parameters such as PCV, Hb and RBC were decreased in clinically affected dairy cattle, whereas increased values of WBC, MCV, and MCH were observed, as reported by Yadi *et al.* (2017) [8]. Similarly, serum biochemical values including TP, albumin, calcium, phosphorus, and glucose were significantly reduced, while ALT and AST levels were elevated in clinically infected animals. These findings are in agreement with those of Saber *et al.* (2008) [4].

Polymerase chain reaction analysis confirmed the presence of the *Babesia bigemina* 18S rRNA gene, with an amplicon size of approximately 540 bp, indicating infection with *B. bigemina*. PCR has largely superseded diagnostic methods and is now widely employed as a species-specific molecular diagnostic assay to identify piroplasm-carrier animals. This technique is highly sensitive and reliable for the detection of pathogens in carrier animals compared to traditional diagnostic approaches.

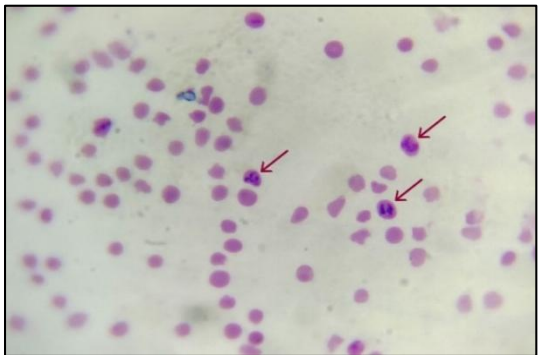

Fig 1: Blood smear showing piroplasms of *B. bigemina*

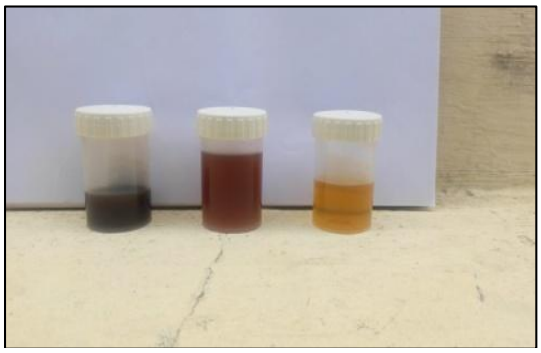

Fig 2: Haemoglobinuria from *B. bigemina* infected animal

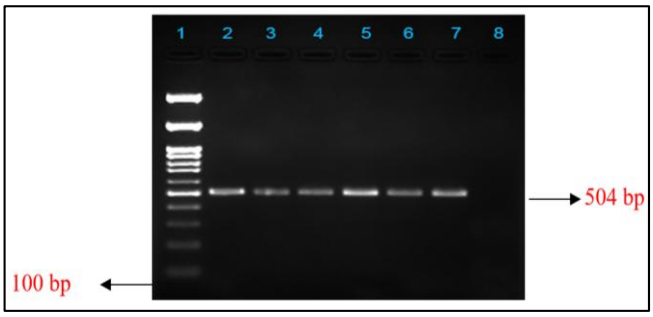

Fig 3: Picture showing amplified PCR product of *B. bigemina* Bb18S rRNA gene (504 bp) clinically infected cases.
Well 1-Ladder
Well 2-Positive control
Well 3 to 7-Positive samples
Well 8-Negative control

Table 1: Mean (± S.E) of haemato biochemistry parameters in *B. bigemina* infected cattle before and after treatment

Parameters	*B.bigemina*			
	0th day	5th day	10th day	F value
Hb (g/dl)	4.73^{a}±0.146	5.9^{b}±0.13	6.97^{c}±0.12	188.237**
PCV (%)	18.54^{a}±0.56	22.16^{b}±0.57	26.29^{c}±0.56	265.250**
RBC (x10^{6}/µl)	3.50^{a}±0.126	4.741^{b}±0.15	5.96^{c}±0.18	155.552**
WBC (x10^{3}/µl)	12.3^{c}±0.24	9.998^{b}±0.203	8.41^{a}±0.141	329.427**
MCV (pg)	58.3^{c}±0.72	54.75^{b}±0.754	48.0^{a}±0.75	147.205**
MCH (pg)	24.38^{c}±0.319	21.62^{b}±0.267	1.31^{a}±0.31	151.924**
TP (g/dl)	5.23^{a}±0.089	5.59^{b}±0.03	6.03^{c}±0.04	58.268**
ALB (g/dl)	2.68^{a}±0.034	2.2^{b}±0.028	3.15^{c}±0.08	165.650**
ALT (U/L)	53.58^{c}±1.250	47.95^{b}±1.130	40.04^{a}±1.34	222.341**
AST (U/L)	137.33^{c}±2.557	123.08^{b}±2.511	102.91^{a}±2.12	262.459**
CA (mg/dl)	8.137^{a}±0.11	8.508^{b}±0.87	9.05^{c}±0.01	62.000**
P (mg/dl)	6.53^{a}±0.10	7.20^{b}±0.13	7.78^{c}±0.12	35.805**
GLU (mg/dl)	50.4^{a}±0.65	60.20^{b}±0.86	70.1^{c}±0.66	32.581**

Conclusion

Microscopic examination remains the cheapest and fastest method for identifying *Babesia* parasites; however, its sensitivity and specificity are limited (Mosqueda *et al.*, 2012) [3]. When tick populations are high, the disease may become acute and can cause death within a few days. During such cases, the packed cell volume (PCV) often falls below 20%, and parasitaemia—usually detectable once clinical signs appear—may involve 0.2% to 45% of the red blood cells, depending on the *Babesia* species involved (Urquhart *et al.*, 1996) [7]. For the treatment of babesiosis, imidocarb and diminazene aceturate are the most commonly used drugs. In recent years, several pharmacological compounds have been developed and evaluated for the control of this disease (Mosqueda *et al.*, 2012) [3]. Diminazene aceturate, which consists of an organic base and organic acid that dissociate upon dissolution in water, is usually adinistered intramuscularly at a dose of 3-5 mg/kg body weight. Long-acting oxytetracycline has also been shown to have a prophylactic effect against *Babesia divergens* infection (Urquhart *et al.*, 1996) [7]. Supplementation with B-complex vitamins and oral hematinics was continued for three weeks until the animals fully recovered from anemia. PCR assays demonstrated a significantly higher sensitivity compared to conventional blood smear examination. Notably, all animals suspected of clinical and subclinical infection were confirmed by PCR. Early diagnosis combined with an appropriate treatment protocol effectively minimized the disease consequences. In endemic areas, babesiosis can be controlled through the implementation of vaccination programs and integrated tick control measures to reduce economic losses.

Declaration of competing interest

The authors declare that they have no conflicts of interest related to this study.

Acknowledgements

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