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Molecular diagnosis and treatment of Anaplasmosis in cattle

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Abstract

Anaplasma marginale was identified from the blood samples of the clinical cases by PCR in the present study with an expected amplicon size of 458bp. Inappetence, pale mucous membranes, hyogalactia, pyrexia, jaundice, depression, brown coloured urine, enlargement of lymphnodes, loss of weight, ectoparasitic infestation, enteritis and blood in faeces are the most prominent clinical signs observed. Haemato-biochemical profile revealed anemia with normal leukogram, increased Serum total bilirubin and reduced albumin, glucose, calcium and phosphorus. However Total Protein (TP) and AST values are within normal range. Oxytetracycline (OTC) LA injection @ 20 mg/kg b.wt intra muscular single dose and Imidocarb dipropionate (Babimido) @ 3 mg/kg b.wt IM were found to be useful in the treatment of bovine anaplasmosis.

Keywords: PCR, *Anaplasma marginale*, Imidocarb, blood smear examination

1. Introduction

Rickettsial blood parasite *Anaplasma marginale* causes anaplasmosis in bovines is classified under the genus *Anaplasma*, family Anaplasmataceae under order Rickettsiales. Transmission of disease to cattle biologically through ticks and mechanically by biting flies and contaminated instruments or fomites (de Echaide *et al.*, 1998) [1]. Bovine anaplasmosis is clinically characterized by fever, loss of appetite, reduced milk yield, pale mucous membranes, progressive anaemia, jaundice, brown-colored urine, labored breathing (Belal *et al.*, 2014) [2]. Diagnosis of bovine anaplasmosis is usually based on Giemsa stained blood smear examination under microscope. This method can only detect infections when about 10⁶ infected red blood cells per ml are present during acute stages (Gale *et al.*, 1996) [3]. On microscopic examination of Giemsa stained blood smears *A. marginale* organisms appears as dense, spherical, blue intra erythrocytic bodies situated near the margin (Wahba *et al.*, 2017) [4]. Gold standard test for diagnosing bovine anaplasmosis in clinically suspected animals is Giemsa-stained thin blood smears. However, this method cannot detect carrier animals with subclinical infection. Carrier animals act as reservoirs for the spread of *Anaplasma marginale* and remain undetectable in chronic cases by microscopy (Eriks *et al.*, 1989) [5]. To identify *A. marginale* DNA Molecular methods have been developed with high sensitivity and specificity (Eriks *et al.*, 1989; Gale *et al.*, 1996; Molad *et al.*, 2006) [5, 3, 6]. PCR-based techniques are especially useful for diagnosing sub clinical infection in carrier animals. Detection limit of PCR is as low as 24 infected RBC per microlitre of blood, equal to 0.0001% of infection (Gale *et al.*, 1996) [3].

2. Materials and Methods

2.1 Selection of Animals

Six apparently healthy cattle were selected as control group for obtaining normal data for comparison of parameters under study cattle presented to the large animal ward, Veterinary Clinical Complex, NTR CVSc, Gannavaram, surrounding Veterinary Dispensaries and dairy farms in certain parts of coastal AP with clinical symptoms suspected for anaplasmosis were taken up for the present study.

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2.2 Haemato-biochemical parameters

5ml blood was collected from twelve animals before initiation of therapy and 14th day post treatment for haematology and serum biochemistry. 5 ml blood was collected once from six apparently healthy animals (Group I) for comparative study. Haematological parameters such as haemoglobin (Hb), PCV, TEC, TLC and DLC were estimated as per the standing procedures (Coles, 1986). Hb (g%) was estimated by acid hematin method, PCV (%) was estimated by microhematocrit method, TEC ($10^6/\mu\text{l}$) and TLC ($10^3/\mu\text{l}$) were carried out by haemocytometer method. Blood smear was stained with Leishman's method and DLC was carried out by battlement method. The diagnostic kits used in the present study for estimation of serum TP (Total protein), albumin, Total bilirubin, AST, Calcium, phosphorus and glucose were supplied by Erba (Trans Asia Bio-Medicals Ltd) and potassium by Monotech Diagnostic Systems (Asritha Diotech India Pvt Ltd).

2.3 Blood smear examination and PCR

Peripheral blood smears were fixed with methanol for one minute and air dried. 1:8 dilution Giemsa staining solution used for staining for a period of 45 minutes and washed under running tap water, air dried and examined under oil immersion objective of microscope for *Anaplasma marginale* organisms. DNA isolation was done for 130 blood samples from the suspected cases for the detection of *Anaplasma marginale* using DNA isolation kit (QIAamp DNA Mini kit Germany product (imported and marketed by QIAGEN indo (P) Ltd, New Delhi). DNA quantification by Nanodrop spectrophotometer (Thermo scientific Nanodrop 2000c, USA).

2.4 Treatment protocol

The Selection criteria for inclusion into therapeutic trials includes Clinical signs and PCR findings were suggestive of *A. marginale* infection. 12 cattle which were positive for *A. marginale* were randomly selected into 2 groups (Group II and Group III) each consisting of 6 animals and efficacy of different therapeutic agents were evaluated. A total 6 apparently healthy cattle were used as healthy control under Group I. The infected cattle in this group-II were treated with Oxytetracycline LA @ 20 mg/kg BW I/M as single injection (Radostiis *et al.*, 2006) [7]. The animals in group-III administered with Imidocarb dipropionate @ 3 mg/Kg BW, I/M as single dose. (Akhter *et al.*, 2010) [8]. The cattle in both groups (II, III) were treated with supportive therapy as 3D Red @ 50 ml/animal orally SID. Monitoring of The cattle under the therapeutic trails for a period of 14 days. The evaluation of treatment regimens at 14 days period based on clinical signs improvement, Haemato biochemical profile improvement and disappearance of *A. marginale* from the blood.

2.5 Statistical Analysis

Oneway ANOVA and correlation analysis were used for statistical analysis by using software called SPSS (Software Packages for Social Sciences).

3. Results and Discussion

Microscopic examination of 130 Giemsa stained thin blood smears showed 4 bovines were positive for *A. marginale* (3.07%) (Figure 1). This finding agrees with the reports of Hofmann-Lehmann *et al.*, (2004) [9] and Wahba *et al.*, (2017) [4]. Out of 130 blood samples, 15 were found positive for *A.*

marginale infection by PCR with an expected amplicon size of 458 bp (Figure 2). Diagnosis in all affected bovines was confirmed by PCR. These findings were in agreement with the studies of Hamid *et al.*, (2014) [10]. These results highlights the superiority of PCR over traditional blood smear examination for detecting *A. marginale* infection, which supports the observations of the present investigation. Common clinical findings noticed in bovines with Anaplasmosis were Anorexia, pale mucosal membranes, hyogalactia, pyrexia, icterus, brown colored urine, enlarged lymphnodes, ectoparasitic infestation, dyspnoea, enteritis and blood in faeces. The findings were consistent with the observations of Selcuk *et al.*, (2015) [11]. The clinical signs recorded, such as pale mucous membranes, jaundice, high fever, weakness, and weight loss in affected animals, may be due to the release of endogenous pyrogens by *A. marginale*. These pyrogens cause the destruction of red blood cells and stimulate various blood-forming and temperature-regulating centers of the body, as reported by Sharma *et al.* (2013) [12].

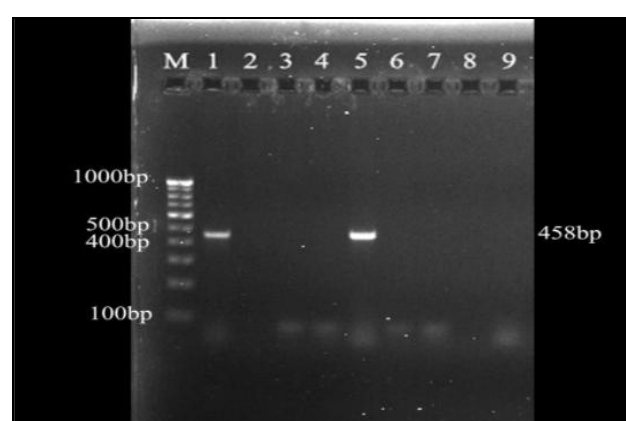


Fig 1: Giemsa stained blood smears showing *A. marginale* organisms at the margins of erythrocytes

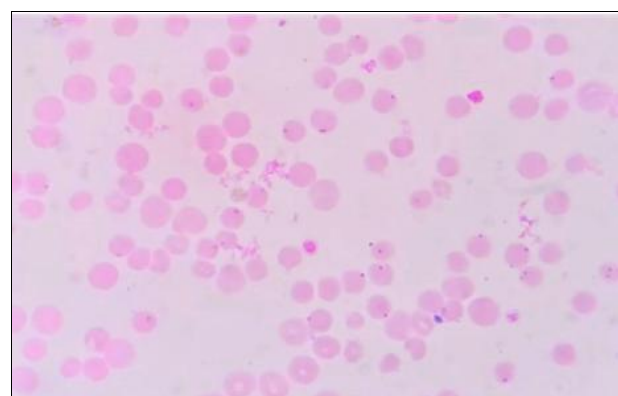


Fig 2: The PCR assay of 458 bp fragment of msp5 gene of *Anaplasma marginale*

Lane M-Molecular marker 100 bp, Lane 1-Positive control, Lane 2-Negative control, Lane 5-*A. marginale* positive sample, Lane 3, 4 and 6 to 9-*A. marginale* negative samples. The present study showed a significant decrease in haemoglobin, PCV, and TEC values in cattle affected with anaplasmosis when compared with the control group. These results were consistent with the findings of Ajayi *et al.* (1987) [13]. Although there was an increase in total protein, potassium, and AST values in bovine anaplasmosis, the values remained within the normal range. This observation agreed with the earlier reports of Allen and Kuttler (1981) [15]. A significant increase in total bilirubin was noted in bovine

anaplasmosis compared to the control group. Khan *et al.* (2011) ^[16] reported that hyperbilirubinemia in anaplasmosis occurs due to excessive destruction of red blood cells and liver dysfunction. A decrease in serum albumin, calcium, phosphorus, and glucose levels was also observed in bovine anaplasmosis, which may be due to prolonged reduced feed intake or decreased protein synthesis ability of the affected liver, as reported by Allen and Kuttler (1981) ^[15]. In the present study, clinical cases showed improvement after the 4th day of treatment in both groups. By the 14th day of treatment, clinical signs had resolved, and the animals showed overall improvement in condition, returning to normal feeding and rumination. A significant increase in haemoglobin, PCV, and TEC values was observed in both groups after 14 days of treatment. Post-treatment mean values of serum albumin, calcium, phosphorus, and glucose also increased significantly, while serum bilirubin levels significantly decreased in both groups. These findings agreed with the earlier reports of Akhter *et al.* (2010) ^[8].

In the present study, the survival rates in Group II and Group III were 100% each. Regarding haemoglobin, PCV, and TEC values, there was no significant difference between Group II and Group III after 14 days of treatment. The survival rate of Group II animals with bovine anaplasmosis was in agreement with the findings of Stewart *et al.* (1979). Similarly, the survival rate of Group III animals with bovine anaplasmosis was in agreement with the findings of Akhter *et al.* (2010) ^[8].

4. Conclusion

The PCR was found to be ideal for confirmatory diagnosis of anaplasmosis. Oxytetracycline LA and imidocarb dipropionate were found to be useful in the treatment of anaplasmosis in cattle.

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Conflict of Interest

Not available

Financial Support

Not available

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