



Therapeutic Management of Cattle Suffering From Anaplasmosis

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Abstract

The present clinical study highlights the therapeutic management of anaplasmosis in cattle. A total of 21 cattle presented to Veterinary Clinical Complex, LUVAS, Hisar, suspected to be suffering from tick-borne disease based on clinical signs, were considered for the present study. Blood samples were collected in EDTA vials and serum collecting vials for microscopic examination, PCR assay, and evaluation of haemato-biochemical alterations. Blood smear examination revealed nine samples (42.85%) positive for anaplasmosis, and among them, three were found to be of concurrent infection with theileria. A therapeutic regimen using long acting oxytetracycline alongwith supportive therapy was given to seven clinical cases and hematobiochemical parameters were tested on day 0 and day 5 of post-treatment by day 5th of treatment. Treated cases included six cases of single infection and one case of concurrent infection with Theileria annulata having low parasitemia, and the remaining two were treated with Buparvaquone in addition to oxytetracycline, hence not considered in the study. On day 5th, clinical remission of signs was observed in all the animals with the start of feed intake, and values of all hematobiochemical parameters either returned to normal or approached normal levels.

Keywords: Anaplasmosis, Cattle, Diagnosis, Oxytetracycline, Therapy.

Introduction

Anaplasmosis is an economically significant disease of cattle reported worldwide, including India. The prevalence of anaplasmosis in cattle in India ranges from 5.1 to 38.2%, depending on the region and the season (Patel et al., 2019). Animals affected with disease cause significant losses in terms of reduced milk production, decreased weight gain, and mortality, resulting in considerable economic losses to the dairy farmers (Patel et al., 2019).

The main cause of bovine anaplasmosis is *Anaplasma marginale*, which is an intra-erythrocytic rickettsial organism (Dumler et al., 2001). The clinical symptoms of the disease include anaemia, jaundice, decreased milk yield, labored breathing, constipation, abortion and sudden death (Richay and Palmer, 1990). The gold standard diagnosis of anaplasmosis relies on microscopic examination of blood smear, however, PCR assay is more advantageous due to high sensitivity and effectiveness in diagnosing active and carrier state infection (Ghosh and Nagar 2014). Oxytetracycline is the drug of choice for treating domestic animals with acute anaplasmosis (Kocanet al., 2010). A single intramuscular injection of long-acting oxytetracycline @ 20 mg/kg BW is recommended.

The present study highlights the incidence of clinical anaplasmosis in cattle and its therapeutic management.

Material and Methods

Sample Collection

A total of 21 cattle presented to veterinary Clinical complex suspected to be suffering from tick borne diseases on the basis of clinical signs and tick infestation were considered for the present study. Clinical signs were observed and recorded. Blood samples were collected in EDTA containing vial and serum collecting vial. EDTA containing blood was processed for microscopic examination, serum was separated and stored at -4°C, DNA was extracted from EDTA containing vial for PCR assay and evaluation of haemato-biochemical alterations using stored serum. Day 5th samples were collected and processed for haemato-biochemical alterations.

Microscopic Examination for Detection of *A. marginale*

Paired blood smear was prepared from each sample and air dried. The dried blood smears were immersed in absolute methanol for 5 to 10 minutes, until they were completely air-dried, then stained with 10% Giemsa's stain (Himedia Laboratories Pvt. Ltd, Mumbai, India). Blood smears were immersed for 30 minutes in Giemsa staining fluid. Then the slides were rinsed with distilled water and air dried. Blood smears were examined for dot-shaped organisms on the margins of erythrocytes and morulae within neutrophils under oil immersion objective (100x) of the microscope. A single parasitic form found on slide smear was considered as a positive sample.

Haemato-biochemical Alterations

Haematological analysis of blood samples was performed from the whole blood collected from the jugular vein of suspected cattle in EDTA vials. Estimation of haematological parameters was done using haematological analyzer (MS4s, Meletschlosing Lab, USA). Samples were analyzed for evaluation of Biochemical parameters by clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH, Germany) using standard procedures and assay kits according to the manufacturer's instructions

PCR Assay Confirmation for *Anaplasma marginale*

DNA extracted were subjected to PCR to confirm the *Anaplasma marginale* infection. Oligonucleotide primer set targeting msp4 gene of *Anaplasma marginale* from published source (M'ghirbi et al., 2016) was selected for standardization of PCR assay. Primer set (Forward: 5'-ATCTTTCGACGGCGCTGTG3' /Reverse: 5'ATGTCC TTGTAAGACTCATCAAATAGC-3') was used to amplify a 420 bp variable region by targeting msp4 gene.

The reaction mixture was optimized in 25 µl reaction mixture containing 12.5µl Dream Taq Green PCR Master Mix (2X) (Fermentas, USA), 1 µl of each primer of 10µM concentration, 3µl template and rest nuclease free water. PCR master mix was initial denaturation at 95°C for 2 minutes, followed by 34 cycles of denaturation (95°C for 30 s), annealing at 55°C for 30 s, extension at 72°C for 30 s and final extension at 72 °C for 5 minutes.

Therapeutic Management

A total of seven cattle (n=7) out of nine found positive for anaplasmosis based on clinical signs, blood smears examination and PCR assay were subjected to therapeutic management using long acting oxytetracycline @ 20 mg/kg BW intramuscularly and repeated after 48 hrs. In this six cases of single infection and one case of concurrent infection with theileria was considered with low parasitemia. Rest two positive cases were given buparvaquone alongwith long acting oxytetracyclin and excluded from the current study.

For supportive therapy, Inj. Analgin @ 8 ml/100 kg body weight and Inj. Chlorpheniramine maleate @ 30-50 mg were given intramuscularly, and iron supplementation were given per oral q24hr in all animals along with appropriate fluid therapy. For therapeutic trial, the haematobiochemical examinations were carried out in both pre-treatment (day 0) and post-treatment (day 5) periods. Blood samples were collected post therapy and examined for Haematobiochemical changes.

Statistical Analysis

For evaluation of treatment trial, data generated was subjected to paired t- test. In this, values of day 0 group were compared with those of day 5 group of treatment of clinically affected cattle. Mean and standard error mean were calculated from continue data. Test was employed using SPSS software version 22.0.

Results and Discussion

Anaplasmosis is an economic important disease affecting the cattle and is responsible for reduced milk production. Anamnesis and clinical examination of screened animals revealed fever was the most frequent clinical sign occurring in 52.38% of the cases, followed by pale mucous membrane in 47.61%, enlarged lymph nodes in 38.09% and icterus 4.76%. Many researchers have also reported similar clinical finding like fever (Shaukat *et al.*, 2019; Ashraf *et al.*, 2021), pale mucous membrane (Sharma *et al.*, 2013; Belal *et al.*, 2014), enlarged lymph nodes (Radwan *et al.*, 2013) and icterus (Belal *et al.*, 2014; Aktas and Olzubek, 2017).

Among the tested samples, 9 samples were found positive for anaplasmosis and among them three were found to be of concurrent infection with theileria. PCR assay confirmed the presence of *Anaplasma marginale* in blood. Therapeutic intervention in seven treated animal showed improvement in clinical picture. Remission of clinical signs observed in all the treated group shows the effectiveness of the drug in combating the ill-effect of parasite. Similar results are observed by Ganguly *et al.* (2017) and Nitture *et al.* (2020). Haematologically, the mean values of Hb (g/dL) and TEC (m/mm³) were improved significantly (P<0.001) on day '5' post-treatment as compared to day '0'. The mean values of PCV (%), eosinophils (%), basophils (%) and MCH (pg) were improved significantly (P<0.05) on day 5 post-treatment as compared to day 0'. Non significant improvement was seen in mean values of lymphocyte (%), MCV (fl) and MCHC (g/dl). Whereas a significant decrease in TLC after oxytetracycline treatment, which is consistent with the findings of Arun kumar and Nagarajan (2013) and Meenakshi sundaram *et al.* (2014), who also reported a significant reduction in TLC.

Parameter	Pre-treatment at day 0 (n=7)	Post-treatment at day 5 (n=7)	P value
TLC (m/mm ³)	5.79±0.53	4.52±0.71**	0.000
Lymphocyte(%)	51.77±1.62	54.60±2.59	0.084
Monocyte(%)	5.85±0.46	5.84±0.48	0.014
Neutrophils(%)	38.53±1.13	35.77±2.39	0.140
Eosinophils(%)	3.41±0.27	3.44±0.38*	0.009
Basophils(%)	0.42±0.08	0.34±0.13*	0.011
TEC(m/mm ³)	5.23±0.36	6.03±0.33**	0.001
MCV(fl)	47.72±3.22	46.74±3.81	0.081
PCV(%)	24.54±1.38	27.74±1.76*	0.033
MCH(pg)	12.63±0.92	11.59±0.76*	0.007
MCHC(g/dL)	26.40±0.41	25.00±0.81	0.252
Hb(g/dL)	6.51±0.46	7.12±0.45**	0.000

Mean (±SE) values of various haematological parameters in cattle affected with clinical anaplasmosis on day '0' (pre-treatment) and day '5' (post-treatment)

Parameter	Pre-treatment at day 0 (n=7)	Post-treatment at day 5 (n=7)	P value
AST (IU/L)	118.75±23.26	78.05±20.48	0.071
ALT (IU/L)	64.47±9.94	42.34±15.01*	0.006
ALP (IU/L)	130.14±14.20	67.42±14.20	0.209
Direct Bilirubin (mg/dL)	0.25±0.08	0.16±0.05*	0.003
Total Bilirubin (mg/dL)	0.26±0.02	0.16±0.01	0.481
BUN (mg/dL)	43.58±13.94	29.58±11.96**	0.000
Creatinine (mg/dL)	0.90±0.07	0.42±0.04	0.066
Total protein (g/dL)	4.66±1.76	5.81±1.18*	0.019
Albumin (g/dL)	1.15±0.13	0.81±0.14	0.269
Globulin (g/dL)	3.50±0.71	5.00±0.44	0.143
A:G ratio	0.62±0.32	0.17±0.03	0.958

Mean ($\pm$ SE) values of various biochemical parameters in cattle affected with clinical anaplasmosis on day '0' (pre-treatment) and day '5' (post-treatment)

Results of statistical analysis of biochemical parameters have been presented in Table 2. The mean values of BUN (mg/dL) were improved significantly ($P < 0.001$) on day 5 post-treatment as compared to day '0'. The mean values of direct bilirubin (mg/dL), total protein (g/dL) and ALT (IU/L) were improved significantly ($P < 0.05$) on day 5 post-treatment as compared to day '0'. Non significant improvement was seen in mean values of AST (IU/L), ALP (IU/L), total bilirubin (mg/dL), creatinine (mg/dL), albumin (g/dL) and globulin (g/dL). The findings of present study are in corroboration with the findings of Nittureet *al.* (2020).

Conclusion

Clinical signs, along with microscopic examination of stained blood smear can lead to diagnosis of clinically anaplasmosis. Oxytetracyclin, the drug of choice along with the supportive therapy is effective in combating the ill-effects of the pathogen.

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Contribution by Authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

Conflict of Interests

There is no conflict of interest.

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