Int.J.Curr.Microbiol.App.Sci (2020) *9*(3): 1440-1449

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 3 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.903.168

Clinico, Haemato-Biochemical Changes and Therapeutic Management of Anaplasmosis

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Keywords

Anaplasmosis, haematology, imidocarb dipropionate, oxytetracycline

Article Info

Accepted:
12 February 2020
Available Online:
10 March 2020

A B S T R A C T

The present study was undertaken to know the clinical, haemato-biochemical and therapeutic management of anaplasmosis in bovines. A total of 150 animals showing signs of tick infestation, anorexia, jaundice, anaemia with varied pyrexia were included in the present study. Around 5ml blood was collected from jugular vein aspectically for haemato-biochemical estimation. For therapeutic study, Twelve anaplasmosis affected animals were randomly selected and divided into two groups consisting of six animals in each group. Animals in group I were treated with Oxytetracycline hydrochloride @ 20 mg/kg body weight intravenously in normal saline daily for 5 consecutive days along with supportive therapy whereas, animals of group II were given Imidocarb dipropionate @ 5 mg/kg body weight deep intramuscularly as a single dose. Partial to complete anorexia, high fever (104 to 106 ˚F), anaemia, jaundice, debility, decrease in milk yield, hurried respiratory rate, tachycardia and presence of ticks on the body were the consistent clinical signs shown by the affected animals. There was a significant reduction ($P<0.05$)in the haemoglobin, packed cell volume, total erythrocyte count of affected animals on day 0 as compared to healthy control group. Biochemical examination revealed significant elevation($P<0.05$) of SGOT, SGPT and total bilirubin concentration on day 0 as compared to the animals of control group. Among Group I animals treated with Oxytetracycline, five animals recovered completely suggestive of 83.33 per cent efficacy whereas, all animals of Group II (06) treated with Imidocarb dipropionate recovered completely by seventh day with cent per cent efficacy rate.

Introduction

Bovine anaplasmosis formerly known as gall sickness is an infectious, non-contagious, tick borne disease of domesticated and wild ruminants caused by obligate intra-erythrocytic parasites of family *Anaplasmataceae* and genus *Anaplasma* (Radostitis *et al.*, 2000). *A. marginale* is primarily a pathogen of bovine anaplasmosis (Rymaszewska and Grenda 2008) which is a small, coccoid to ellipsoidal, often pleomorphic, non-motile Alpha-proteobacteria that resides and replicate in membrane-bound vacuoles within the cytoplasm of eukaryotic host cells (Dumler *et al.*, 2006). Routes of transmission are mainly by biological, mechanical and transplacental

(Zaugg, 1985). Infection is characterised by progressive haemolytic anaemia associated with high fever, jaundice, decreased milk production, abortion, loss of appetite coupled with dullness/depression, rapid deterioration of the physical condition (Al Saad 2007). Demonstrations of the infective stages in thin blood smears stained with Giemsa, a traditional laboratory method, newer diagnostic aids such as Polymerase chain reaction- based method has been developed which is capable of detecting low levels of infection in infected as wellas carrier animals. Use of oxytetracycline and imidocarb (Richey, 1981) appears to be more promising in the treatment of bovine anaplasmosis. Keeping in view the economic losses due to anaplasmosis, the present study was designed to know the clinical, haemato-biochemical changes and therapeutic management of anaplasmosis.

Materials and Methods

A total of 150 animals showing signs of tick infestation, anorexia, jaundice, anaemia with varied pyrexia were included in the present study. Around 2ml blood was collected from jugular vein in vacutainers containing EDTA as an anticoagulant for haematological studies using BD Vacutainer Eclipse blood collection needle (BD, Franklin, USA).

For biochemical estimations 3ml blood was collected in vials coated with clot activator and blood was allowed to coagulate. Serum was separated by centrifugation at 2500 rpm for 10 minutes and serum was separated in Eppendorf tubes.

Haematological parameters

Total Erythrocyte Count (TEC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were estimated using fully automatic blood cell counter model PCE-210 (ERMA INC®.)

Biochemical Parameters

Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetate transaminase (SGOT) (Reitman and Frankel, 1957) and Serum total bilirubin (Tietz, 1976) were analyzed using ARTOS Elita® semi-automatic biochemical analyser.

Clinical parameters

Clinical parameters namely temperature, respiration rate, heart rate, appearance of conjunctival mucous membrane and ruminal contractions were recorded.

Therapeutic management

Twelve animals suffering from Anaplasmosis were randomly selected and divided into two groups consisting of six animals in each group. Animals in group I were treated with Oxytetracycline hydrochloride @ 20 mg/kg body weight intravenously in normal saline daily for 5 consecutive days along with supportive therapy whereas, animals of group II were given Imidocarb dipropionate @ 5 mg/kg body weight deep intramuscularly as a single dose.

Both the groups received uniform supportive therapy with Iron tonics (Inj. Ferritas, Intas pharma) @ once in two days for a week @ 1ml/50kg body weight intra muscularly, antipyretic like meloxicam (Inj. Melonex, Intas pharma) @ 0.5mg/kg body weight daily for 3-5 days intramuscularly. B-complex with liver extract (Inj.Bivinal Plus, Alembic pharmaceuticals) @ 10ml every day for 3-5 days deep intramuscularly.

Statistical analysis

The haematological, biochemical and vital parameter values obtained before treatment and after treatment were subjected to statistical analysis by one-way ANOVA using Statistical Package for Social Sciences (SPSS) version 2.0. Differences between means were tested using Duncan's multiple comparison test and significance was set at 5 per cent ($p<0.05$) (Snedecor and Cochran 1994).

Results and Discussion

Partial to complete anorexia, high fever (104 to 106 ˚F), depression, anaemia, jaundice, debility, decrease in milk yield, hurried respiratory rate, tachycardia and presence of ticks on the body were the consistent clinical signs shown by the affected animals used for the study. Similar clinical signs in anaplasmosis were observed by earlier workers Kumar *et al.,* (2015) and Szabara *et al.,* (2016) in dairy cattle.

The affected animals showed high rectal temperature at 0 day (105.80 ± 0.28 and 105.66 ± 0.20) and as therapy progressed it showed reduction in rectal temperature and returned to normal by 7th day post treatment (100.22 ± 0.34 and 100.22 ± 0.34). Significant ($P<0.05$) increase in the rectal temperature on 0 day when compared to healthy control group indicative of pyrexia in the present investigation was in accordance with Kumar *et al.,* (2015) in dairy cattle. Parasitized erythrocytes removed by phagocytosis in the reticular endothelial system, with release of acute phase inflammatory reactants has been attributed for development of fever in anaplasmosis (Radostits *et al.*, 2000).

The significant increase in the respiratory rate on 0 day (37.67 ± 1.76 and 49.33 ± 5.44) was observed in anaplasmosis affected animals of Group I and Group II, respectively, when compared to healthy control group, which returned to normal on 7th day post treatment in both study groups. Similar observations were reported by Jaswal *et al.,* (2014) in dairy cattle. The increased respiratory rate observed in present study could be attributed as compensatory mechanism in order to make up oxygen requirements due to decreased oxygen carrying capacity of RBC's due to lowered haemoglobin concentration in anaplasmosis affected animals.

The affected animals of Group I and II showed increased heart rate on the day of presentation (86.83 ± 3.61 and 90.83 ± 1.72). However, it returned to normal on 7th day post treatment. This increase was significantly ($P<0.05$) higher when compared to healthy control group. Similar observations were made by Vatsya *et al.,* (2013) in anaplasmosis affected animals. The increased heart rate in anaplasmosis has been attributed to severe anaemia of the affected animals in order to compensate demand by peripheral organs.

Decreased ruminal contractions in affected animals of Group I and II returned to normal (2.33 ± 0.19 and 2.33 ± 0.21) on 7th day post treatment which did not differ significantly ($P<0.05$) from values of control group. Anaplasmosis is also named as gall sickness and anorexia has been observed as important clinical signs of the disease (Radostits *et al.*, 2000).

In present study, all the cattle suffering from anaplasmosis showed pale to icteric conjunctival mucous membrane on the day of presentation, however visible mucous membrane was found to be light pink in group I and II on 7th day post treatment. Similar results were opined by Vetrivel *et al.,* (2018) in dairy cattle and Szabara *et al.,* (2016) HF cross cows. Pallor conjunctival mucous membrane has been attributed to anaemia

whereas icteric conjunctival mucous membrane could be attributed to intravascular haemolysis and increased amount of indirect bilirubin in the anaplasmosis affected animals (Radostits *et al.,* 2000). Haematinics along with liver extract in treatment regimen might have helped to regain normalcy after treatment.

Haematological parameters

Total erythrocyte count (millions/µl)

The anaplasmosis affected animals in Group I and II on 0^{th} day significantly showed lower values (4.35 ± 0.76 and 4.28 ± 0.16 respectively) when compared with that of control healthy group (7.25± 0.14). However, it returned towards normalcy (5.58 ± 0.12 and 5.91 ± 0.11) on 7^{th} day post treatment in both study groups which did differ significantly (P<0.05) from values of control group(Table 2). On the day of presentation animals were anaemic indicating severe reduction in TEC and were apparently normal on 7^{th} day post treatment. Similar results are observed by Ganguly *et al.,* (2017) in crossbred cows and Maharana *et al.,* (2016) in dairy animals. Considerable increase in the haemoglobin concentration was noticed in all the treated animals and similar results were noted by Szabara *et al.,* (2016) and Ganguly *et al.,* (2017) in crossbred cows.

Haemoglobin (g/dL) and packed cell volume (%)

The anaplasmosis affected animals in Group I and II on 0^{th} day showed lower haemoglobin values (5.58 ± 0.06 and 5.67 ± 0.07 respectively) and differed significantly (P<0.05) from the control healthy group (9.93 ± 0.27). The mean haemoglobin values in group I and II on 7^{th} day post treatment were 8.15 ± 0.76 and 8.35 ± 0.13 respectively which did differ significantly (P<0.05) from values of control group. However, showed trend of returning towards normalcy on 7^{th} day post treatment (Table 2).

In the present study, Group I and II on the day of presentation showed lower PCV values (24.32 ± 0.80 and 23.73 ± 0.80 respectively) and differed significantly (P<0.05) from the control healthy group (37.75± 0.99). The mean PCV values in group I and II on 7^{th} day post treatment (28.02 ± 0.59 and 29.65 ± 0.61 respectively) which did differ significantly (P<0.05) from values of control group. However, showed trend of returning towards normalcy on 7^{th} day post treatment (Table 2).

The present findings of significant reduction in haematocrit values on 0 day when compared to healthy control group are in accordance with observations reported by Maharana *et al.,* (2016) in dairy cattle and Kumar *et al.,* (2015) in crossbred cattle. Anaemia due to intravascular haemolysis and phagocytosis of parasitized erythrocytes is a hallmark observation of bovine anaplasmosis (Radostits *et al.,* 2000). Diminished values of haemogram in the present investigation could be attributed to these mechanisms. The appearance of anti-erythrocyte antibodies late in the acute stage has also been attributed to exacerbate the anaemia (Richeyand Palmer, 1990) in cases of anaplasmosis.

Erythrocytic indices

Mean corpuscular volume (fl)

The mean corpuscular volume on 0 day in group I and group II were 55.94 ± 1.79 and 55.58 ± 1.78 respectively and did not differ significantly (P<0.05) from the control group (52.09 ± 0.98) mean and SE value. The mean corpuscular volume values in group I and II on 7^{th} day post treatment were 50.21 ± 0.84 and 50.21 ± 0.84. They did not differ significantly (P<0.05) from values of control

group, but showed a trend to return towards normalcy on 7th day post treatment (Table 2).

Mean corpuscular haemoglobin (pg)

The values of mean corpuscular haemoglobin on 0 day in group I and group II were 12.87 ± 0.36 and 13.30 ±0.42 respectively and did not differ significantly (P<0.05) from the control group (13.70 ± 0.19) mean and SE value. Though, the mean corpuscular haemoglobin values in group I and II on 7th day post treatment were 14.61 ± 0.17 and 14.12 ± 0.20 which differ significantly (P<0.05) from values of control group, they show a trend of returning towards normalcy on 7th day post treatment (Table 2).

Mean corpuscular haemoglobin concentration (g/dL)

The mean corpuscular haemoglobin concentration on 0 day in group I and group II were 23.11 ± 0.95 and 24.03 ± 0.96 respectively and did not differ significantly (P<0.05) from the control group (26.37 ± 0.79) mean and SE value. Though the mean corpuscular haemoglobin concentration values in group I and II on 7th day post treatment were 29.14 ± 0.46 and 28.22± 0.69 which did not differ significantly (P>0.05) from values of control group, it returned towards normalcy on 7th day post treatment (Table 2).

As the erythrocytic indices are arithmetic representatives of haemogram, corresponding decrease has been observed in MCH and MCHC. Mean MCH and MCHC values before treatment (0 day) were significantly (P<0.05) lower when compared to healthy control group. The values of MCH and MCHC improved on 7th day (post treatment) when compared to before treatment in both study groups. Similar decrease in MCH and MCHC values has been observed by Ashuma *et al.,* (2013) and Maharana *et al.,* (2016) in dairy cattle. Though the mean values of MCV were significantly increased on 0th day when compared to healthy control group, MCV concentrations were within normal physiological limits on 7th day post treatment.

Macrocytic hypochromic anaemia in cases of bovine anaplasmosis reported by Ashuma *et al.,* (2013) is in close agreement with present investigation which suggest normocytic hypochromic anaemia.

Biochemical parameters

Serum transaminases (SGOT and SGPT) (IU)

The serum glutamic oxaloacetic transaminase on 0 day in group I and group II were 167.13±21.98 and 158.33±14.03 respectively and differed significantly (p<0.05) from the control group (67.92 ±3.08). The mean serum glutamic oxaloacetic transaminase values in group I and II on 7th day post treatment were 114.43±13.24 and 79.44±10.97 which did not differ significantly (p<0.05) from values of control group, it returned towards normalcy on 7th day post treatment (Table 3).

Serum glutamate pyruvate transaminase on 0 day in group I and group II were 90.56 ±25.48 and 76.63 ± 8.89 respectively with significant (p<0.05) difference from the control group (18.86±3.71). Though, the mean serum glutamate pyruvate transaminase values in group I and II on 7th day post treatment were 55.68±11.75 and 35.53 ± 6.59 though these values did not differ significantly (p<0.05) from values of control group, they returned towards normalcy on 7th day post treatment (Table 3).

Mean SGOT concentration before treatment (0 day) was significantly higher when

compared to after treatment (7th day) in both the study groups. The two-fold increase in the SGOT concentration in affected animals on 0 day when compared to healthy control group was indicative of severe hepatic insufficiency.

Mean SGPT concentration before treatment (0 day) was significantly higher when compared to after treatment (7th day) in both the study groups. The increase in the SGPT concentration in affected animals on 0 day when compared to healthy control group was indicative of hepatic damage.

SGOT and SGPT have been reported to be liver specific enzyme for bovine and its elevation has been linked with hepato-cellular damage (Kaneko *et al.,* 2008). Elevated SGOT and SGPT in the anaplasmosis affected animals in the present study could be attributed to hepatic injury due to increased load of phagocytosed parasitized erythrocytes in hepatic RES as reported by Radostitis *et al.,* (2000) and Kataria and Bhatia, (1991).

Serum bilirubin (total bilirubin) mg/dl

The mean values of total bilirubin were significantly higher before (2.88 ± 0.14 and 3.75 ± 0.68) treatment when compared to 7th day post treatment in both the study groups. The increase in bilirubin on 0 day when compared to healthy control group was indicative of hyperbilirubinemia (Indirect bilirubin) in affected animals suggestive of prehepatic jaundice. The observations of present investigation are corroborated with earlier reports of Ashuma *et al.,* (2013) in 320 cattle.

Therapeutic studies

Out of six animals in Group I treated with Oxytetracycline at 20 mg per kg body weight intravenously in 500 ml normal saline for 5 days daily, five animals recovered completely as revealed by clinical, haematological, biochemical improvement and absence of intracellular Anaplasma organisms by seventh day, suggestive of 83.33 per cent (Table 4) efficacy, all animals of Group II (06) treated with Imidocarb dipropionate at 5mg per kg body weight deep intra muscularly single dose, recovered completely by seventh day with the efficacy rate of 100 per cent (Table 4).

Table.1 Clinical observation at 0 day and 7th day after treatment in Different groups of cattle with Anaplasmosis

Parameters	Control group	BT Mean ± SE (0 day)		AT Mean ± SE (7th day)	
		Group I	Group II	Group I	Group II
Rectal temperature	100.30 ± 0.16[a]	105.80 ± 0.28[b]	105.66 ± 0.20[b]	100.20 ± 0.15[a]	100.22 ± 0.34[a]
Respiration rate per min	22.83 ± 1.56[a]	37.67 ± 1.76[b]	49.33 ± 5.44[c]	23.50 ± 1.33[a]	24.66 ± 0.88[a]
Heart rate beats/min	62.50 ± 2.18[a]	86.83 ± 3.61[b]	90.83 ± 1.72[b]	67.50 ± 1.31[a]	68.00 ± 1.06[a]
Ruminal contractions per 3 minutes	2.33 ± 0.21[a]	0.33 ± 0.21[b]	0.33 ± 0.19[b]	2.33 ± 0.19[a]	2.33 ± 0.21[a]

*Means bearing different superscripts differ significantly (P < 0.05)

Table.2 Haematological parameters at 0 day and 7th day after treatment in different groups of cattle with Anaplasmosis

Parameters	Control group	BT Mean ± SE		AT Mean ± SE	
		Group I	Group II	Group I	Group II
TEC (10^6/µl)	7.25 ± 0.14^a	4.35 ± 0.76^b	4.28 ± 0.16^b	5.58 ± 0.12^c	5.91 ± 0.11^c
Hb (g/dL)	9.93 ± 0.27^a	5.58 ± 0.06^b	5.67 ± 0.07^b	8.15 ± 0.76^c	8.35 ± 0.13^c
PCV (%)	37.75± 0.99^a	24.32 ± 0.80^b	23.73 ± 0.80^b	28.02 ± 0.59^c	29.65 ± 0.61^c
MCV (fl)	52.09 ± 0.98^{ab}	55.94 ± 1.79^a	55.58 ± 1.78^a	50.21 ± 0.84^b	50.21 ± 0.84^b
MCH (pg.)	13.70 ± 0.19^{ab}	12.87 ± 0.36^a	13.30 ±0.42^{ab}	14.61 ± 0.17^c	14.12 ± 0.20^c
MCHC (%)	26.37 ± 0.79^a	23.11 ± 0.95^b	24.03 ± 0.96^b	29.14 ± 0.46^c	28.22± 0.69^{ac}

* Means bearing different superscripts differ significantly (P < 0.05)

Table.3 Biochemical parameters at 0 day and 7th day after treatment in different groups of cattle with Anaplasmosis

Parameters	Control group	BT Mean ± SE		AT Mean ± SE	
		Group I	Group II	Group I	Group II
SGOT (IU/L)	67.92 ±3.08^a	167.13±21.98^c	158.33±14.03^c	114.43±13.24^b	79.44±10.97^{ab}
SGPT (IU/L)	18.86±3.71^a	90.56 ±25.48^b	76.63 ± 8.89^b	55.68±11.75^{ab}	35.53 ± 6.59^a
Serum bilirubin (Total bilirubin) mg/dl	1.23 ± 0.30^a	2.88 ± 0.14^{bc}	3.75 ± 0.68^c	2.02 ± 0.09 ^{ab}	1.81 ± 0.17^{ab}

* Means bearing different superscripts differ significantly (P < 0.05)

Table.4 Therapeutic efficacy of different drugs

Groups	No. of cattle	Treatment	No. of animal recovered completely by 7th day	Efficacy (%)
I	06	Oxytetracycline 50 mg/ml	05	83.33
II	06	Imidocarb dipropionate 12%	06	100

Table.5 Cost - Efficacy of different drugs

Treatment	Cost of drug (Rs)	Cost incurred in treatment (Rs)	Cost of supportive therapy (Rs)	Total cost (Rs)	Efficacy (%)
Oxytetracycline HCl	66	396	530	926	83.33
Imidocarb dipropionate	160	192	530	722	100

Oxytetracycline - a broad spectrum tetracycline group of antibiotic acts by interfering the ability of protozoa to produce essential proteins by inhibiting 30s ribosome without which a protozoon cannot grow, multiply and increase in number which aids host immune system to take upper hand and kill the protozoa. (Srivastava, 1989).

The mode of action of imidocarb is to interfere with the production and/or utilization of polymers and prevention of entry of inositol parasitized erythrocytes (Neill *et al.*, 2010). Therapeutic trial clearly indicated superiority of imidocarb with cent percent efficacy as compared to oxytetracycline (83.33%).

The haemoprotozoan diseases pose an increased production loss as well as treatment cost to the poor farmers and in order to reduce the losses, cost effective treatment is also a necessary. The cost incurred for the treatment with oxytetracycline for 5 days for one cattle was Rs. 396 with the efficacy of 83.33 per cent whereas treatment with imidocarb dipropionate needed Rs. 192 with efficacy of 100 per cent (Table 5).

Cent per cent efficacy along with cost effective economy (Rs. 192) of imidocarb was found superior to oxytetracycline hydrochloride and hence is recommended for treatment for bovine anaplasmosis.

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How to cite this article:

Baswaraj Nitture, Vivek R. Kasaralikar, S. C. Halmandge, B. G. Ravindra, Shrikant Kulkarni and Patil. N. A. 2020. Clinico, Haemato-Biochemical Changes and Therapeutic Management of Anaplasmosis. *Int.J.Curr.Microbiol.App.Sci.* 9(03): 1440-1449.
doi: https://doi.org/10.20546/ijcmas.2020.903.168