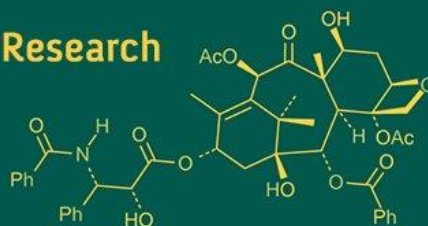


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
 ISSN Online: 2617-4707  
 NAAS Rating (2025): 5.29  
 IJABR 2025; 9(12): 622-627  
[www.biochemjournal.com](http://www.biochemjournal.com)  
 Received: 02-11-2025  
 Accepted: 04-12-2025

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## Study of hemato-biochemical changes in cases of anaplasmosis in cattle

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**DOI:** <https://www.doi.org/10.33545/26174693.2025.v9.i12h.6598>

### Abstract

Bovine anaplasmosis is one of the economically important haemoprotozoan diseases in India. The disease is a major constraint in dairy industry and causes great economic losses to livestock farmers through mortality and loss of productivity. The present research work was carried out to study haemato-biochemical changes in cattle affected with anaplasmosis. A total of 66 cattle suspected for anaplasmosis were screened based on clinical signs and blood smear examination. Out of them, 22 were found positive for anaplasmosis. Out of them total 18 positive cases of anaplasmosis were selected for study. while group 1 of six healthy cattle was kept for comparison of various haemato-biochemical parameters. The haematological examination revealed that the mean $\pm$ SE values of haemoglobin, packed cell volume, total erythrocytes count and mean corpuscular haemoglobin concentration (MCHC) were significantly decreased ( $p < 0.01$ ) in anaplasmosis affected cattle as compared to healthy cattle. However, lymphocyte and platelets count were decreased non-significantly. Furthermore, there was significant ( $p < 0.05$ ) increase in mean corpuscular volume (MCV), while total leucocytes count (TLC), mean corpuscular haemoglobin (MCH), neutrophils, monocytes and eosinophils increase non-significantly. The serum biochemical examinations revealed that the mean $\pm$ SE values of total protein were decreased non-significantly, while albumin and A/G ratio were decreased highly significantly ( $p < 0.01$ ) in anaplasmosis affected cattle as compared to healthy control group. Globulin and alanine aminotransferase were increased significantly ( $p < 0.05$ ), whereas aspartate aminotransferase and bilirubin were increased highly significantly ( $p < 0.01$ ). However, serum creatinine was increased non-significantly in anaplasmosis affected cattle as compared to healthy cattle.

**Keywords:** Bovine anaplasmosis, *A. marginale*, *Rhipicephalus* spp, Haemoglobin, A/G Ratio

### 1. Introduction

In Indian economy livestock plays an important role and contributes up to 4.11 per cent in Indian GDP. Total of 20.5 million rural residents depend on cattle for their livelihood. (DAHDF, 19<sup>th</sup> Livestock Census 2012 report, 2014). Many viral, metabolic, and parasitic diseases concern India due to climate differences in tropical and subtropical countries. The global loss due to Ticks and Tick-Borne Diseases (TTBDs) is estimated to be between US\$ 13.90 and 18.70 billion annually (De Castro, 1997) [9], while in India the cost of controlling TTBDs has been estimated as US\$ 498.7 million/annum (Minjauw and McLeod, 2003) [16].

The most common haemoprotozoan infections in animals are trypanosomiasis, theileriosis, babesiosis, and anaplasmosis. Bovine anaplasmosis is considered as one of the economically important rickettsial diseases affecting ruminants. In India, *A. marginale*, *A. centrale*, and *A. bovis* are the prevalent species in cattle (Nair *et al.*, 2013) [17] and they are principally transmitted by a tick of *Rhipicephalus* spp. (Constable *et al.*, 2017) [7].

Bovine anaplasmosis, formerly known as gall sickness, is an infectious, non-contagious, tick-borne disease of domesticated and wild ruminants caused by *Anaplasma* species characterized by fever, icterus, anorexia, anaemia, constipation, weakness, dehydration, laboured breathing, depression, abortion, and often death (Richey and Palmer, 1990) [20]. Diagnosis can be done by Giemsa stained blood smear examination (Maharana *et al.* 2016) [14]. Diagnosis based on microscopic examination is not reliable for detecting pre-symptomatic or carrier animals. In these cases, the infection is usually detected by presence of antibodies on a serologic test with confirmation by molecular detection methods such as Complement Fixation Test (CFT), Capillary Agglutination Assay, Card Agglutination Test (CAT),

Indirect Fluorescent Antibody (IFA) Enzyme Linked Immunosorbent Assays (ELISA) and PCR (Aubry and Geale, 2011)<sup>[5]</sup>, (Carelli *et al.*, 2007)<sup>[6]</sup>.

## 2. Materials and Methods

The present investigation was carried out at the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Anand with the objectives to study haemato-biochemical changes in cattle affected with anaplasmosis. Total 66 Giemsa's-stained thin blood smears were examined from cattle suspected for anaplasmosis. Out of them, 22 (33.34%) cattle were found positive by microscopic examination seen as compact spherical masses, inside the red blood cells near the periphery of RBCs wall. Out of 66 samples 34 (51.52%) samples were found positive for anaplasma species by PCR. Out of those 18 animals were selected for hematobiological examination.

### 2.1 Sample Collection and Storage

Whole blood samples were collected from the jugular veins of the suspected animals in the vacutainers containing an anticoagulant, tripotassium ethylene diamine tetra-acetic acid (K<sub>3</sub>EDTA) and serum clot activators on day '0' from selected cases. The similar samples were also collected from cattle under healthy group. These blood samples were kept in ice box, transported to the lab within 2-3 hours, and processed on the same day. Blood samples taken in serum clot activator vials were centrifuged at 3000 rpm for 10 minutes. The sera samples were separated/aspirated with sterile pipette and were stored at -20°C until final use for estimation of various biochemical parameters.

### 2.2 Haematological Examination

Haematological analysis of samples was performed from the whole blood collected from the jugular vein of healthy and infected cattle in a K<sub>3</sub>EDTA vacutainers. Estimation of haematological parameters was done by using auto-haematological analyzer machine (Abacus junior Vet-5) at

Veterinary Clinical Complex, which included following parameters.

- Haemoglobin (g/dl)
- Total Erythrocyte Count ( $10^6/\mu\text{l}$ )
- Total Leukocyte Count ( $10^3/\mu\text{l}$ )
- Differential Leukocyte Count (%)
- Packed Cell Volume (%)
- Platelets count ( $10^3/\mu\text{l}$ )
- Mean Corpuscular Volume (fl)
- Mean Corpuscular Haemoglobin (pg)
- Mean Corpuscular Haemoglobin Concentration (g/dl)

### 2.3 Serum Biochemical Examination

All the biochemical parameters were analyzed by using standard procedures and assay kits on clinical chemistry analyzer (CKK 300) at Veterinary Clinical Complex as per manufacturer's instructions. The serum samples obtained from blood collected on day 0 from cattle affected with anaplasmosis and stored deep frozen were used for the estimation of following biochemical parameters.

- Total Protein (g/dl)
- Albumin (g/dl)
- Bilirubin (mg/dl)
- Creatinine (mg/dl)
- Alanine Aminotransferase (U/L)
- Aspartate Aminotransferase (U/L)
- Globulin (g/dl)
- Albumin/Globulin (A/G) Ratio

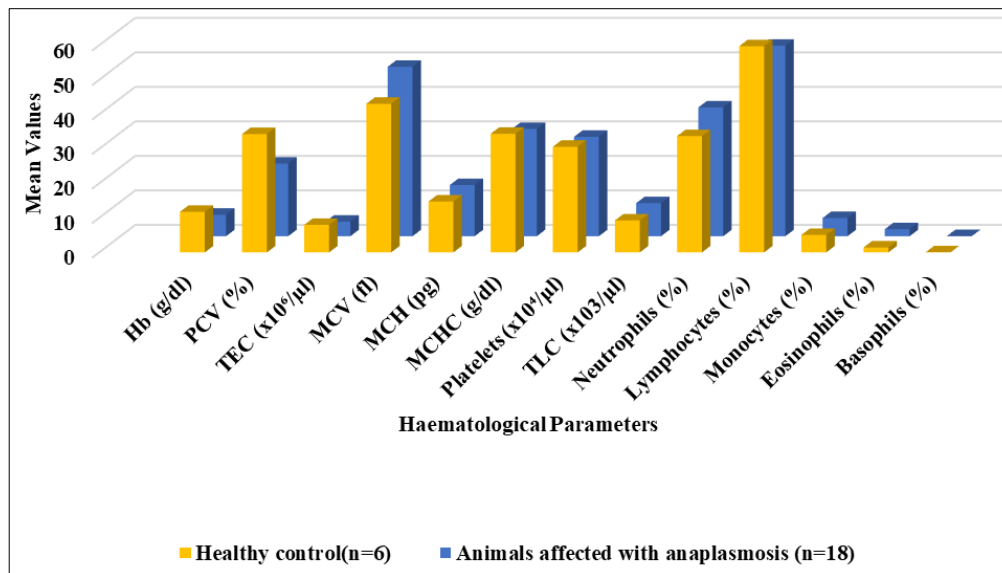
## 3. Result and Discussions

### 3.1 Hematological Examination

In present study, whole blood samples were collected from all 18 cattle affected with anaplasmosis and a healthy group (n=6) on day 0 to determine the haematological profile. The mean ( $\pm$ SE) values of various haematological parameters and indices of cattle affected with anaplasmosis are shown in Table 3.1 and Figure 3.2 and are discussed in detail as follows.

**Table 3.1:** The mean ( $\pm$ SE) values of haematological parameters of healthy and anaplasmosis affected cattle

Parameters	Healthy (n=6)	Anaplasmosis infected animals (n=18)	"p" value
Hb (g/dl)	11.75 $\pm$ 0.76	6.23 $\pm$ 0.20**	0.001
PCV (%)	34.30 $\pm$ 2.44	21.06 $\pm$ 0.77**	0.002
TEC ( $\times 10^6/\text{dl}$ )	7.97 $\pm$ 0.52	4.23 $\pm$ 0.16**	0.000
MCV (fl)	43.07 $\pm$ 1.62	49.09 $\pm$ 0.97*	0.011
MCH (pg)	14.75 $\pm$ 0.31	14.82 $\pm$ 0.35	0.870
MCHC (g/dl)	34.37 $\pm$ 0.74	31.08 $\pm$ 0.55**	0.004
Platelets ( $\times 10^3/\mu\text{l}$ )	306.16 $\pm$ 22.07	288.16 $\pm$ 19.74	0.553
TLC ( $\times 10^3/\mu\text{l}$ )	9.27 $\pm$ 0.51	9.59 $\pm$ 0.31	0.603
Neutrophils (%)	33.71 $\pm$ 2.53	37.32 $\pm$ 3.05	0.375
Lymphocytes (%)	59.75 $\pm$ 2.61	55.21 $\pm$ 2.96	0.275
Monocytes (%)	5.06 $\pm$ 0.69	5.28 $\pm$ 0.58	0.810
Eosinophils (%)	1.43 $\pm$ 0.20	2.03 $\pm$ 0.16*	0.041
Basophils (%)	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.511



\* $p < 0.05$  = significant, \*\* $p < 0.01$  = highly significant

**Fig 3.1:** Mean values of haematological observations of healthy and anaplasmosis affected cattle

### 3.1.1 Haemoglobin (Hb; g/dl)

In present study, the mean value of haemoglobin recorded in healthy cattle was  $11.75 \pm 0.76$  g/dl and in cattle with anaplasmosis it was  $6.23 \pm 0.20$  g/dl. The level of haemoglobin was highly significantly ( $P < 0.001$ ) decreased in anaplasmosis affected cattle (Table 3.1, Figure 3.1).

Present findings were in accordance with Shaukat *et al.* (2019) [23]. Similar observations were also reported by Coskun *et al.* (2012) [8], Sharma *et al.* (2013) [22], Singh *et al.* (2014) [24], Meenakshisundaram *et al.* (2014) [15], Maharana *et al.* (2016) [14], Ganguly *et al.* (2018) [11], Subramanian *et al.* (2019) [25], and Ola fadunsin *et al.* (2021) [19].

A significant reduction in the haematological parameters (Hb, PCV, RBC, and MCHC) might be due to the intravascular haemolysis of erythrocytes, increased erythrocyte phagocytosis by the reticuloendothelial system, and restricted erythropoietic activity in the bone marrow, which is a result of *A. marginale* infection (Sharma *et al.*, 2013) [22].

### 3.1.2 Packed cell volume (PCV; %)

In present study the mean  $\pm$  SE value of PCV recorded in healthy cattle was  $34.30 \pm 2.44\%$  and in cattle with anaplasmosis  $21.06 \pm 0.77\%$ . It was highly significantly ( $P < 0.002$ ) decreased in anaplasmosis affected cattle (Table 3.1, Figure 3.1).

Shaukat *et al.* (2019) [23] also reported significantly ( $P < 0.05$ ) decreased packed cell volume in anaplasmosis affected cattle as compared to healthy animals. Similar findings were also reported by many previous workers (Ajayi *et al.*, 1987; Singh *et al.*, 2014; Meenakshisundaram *et al.*, 2014; Szabara *et al.*, 2016; Maharana *et al.*, 2016; Ganguly *et al.*, 2017; Ganguly *et al.*, 2018; Subramanian *et al.*, 2019; Ola fadunsin *et al.*, 2021) [24, 14, 15, 25, 10, 11].

The decreased value of PCV might be due to intravascular haemolysis of erythrocytes by *Anaplasma* spp. which invade and cause erythrophagocytosis.

### 3.1.3 Total erythrocyte count (TEC; $\times 10^6/\mu\text{l}$ )

In present study, the mean  $\pm$  SE value of TEC recorded in healthy cattle was  $7.97 \pm 0.52 \times 10^6/\mu\text{l}$  and in cattle with anaplasmosis  $4.23 \pm 0.16 \times 10^6/\mu\text{l}$ . It was highly significantly

( $P < 0.001$ ) decreased in anaplasmosis affected cattle (Table 3.1, Figure 3.1).

This observation was in accordance with Shaukat *et al.* (2019) [23], who also reported that there were significantly ( $P < 0.05$ ) decreased level of total erythrocyte count in anaplasmosis affected cattle as compared to healthy animals. Present findings were also in accordance with Coskun *et al.* (2012) [8], Arunkumar and Nagarajan (2013) [4], Sharma *et al.* (2013) [22], Meenakshisundaram *et al.* (2014), Singh *et al.* (2014) [24], Maharana *et al.* (2016) [14], Szabara *et al.* (2016), Ganguly *et al.* (2017), Ganguly *et al.* (2018), Subramanian *et al.* (2019), and Ola-fadunsin *et al.* (2021) [10, 11, 25].

The decrease level of total erythrocyte might be due to erythrophagocytosis by reticuloendothelial system because of intravascular haemolysis of erythrocyte which was initiated by parasitic damage to erythrocytes and that leads to decrease in total erythrocyte count (Sharma *et al.*, 2013) [22].

### 3.1.4 Mean corpuscular haemoglobin concentration (MCHC; g/dl)

The mean  $\pm$  SE value of mean corpuscular haemoglobin concentration (MCHC) recorded in healthy cattle was  $34.37 \pm 0.74$  g/dl and in cattle with anaplasmosis it was  $31.08 \pm 0.55$  g/dl. The level of mean corpuscular haemoglobin concentration was decreased highly significantly ( $P < 0.004$ ) in anaplasmosis affected cattle (Table 3.1, Figure 3.1).

The present findings agreed with Ola-Fadunsin *et al.* (2021) [19], who reported significantly decreased mean value of MCHC. Present findings were also in accordance with Arunkumar and Nagarajan (2013), Sharma *et al.* (2013) [22], Maharana *et al.* (2016), and Shaukat *et al.* (2019) [14, 23].

The significant decrease in MCHC might be due to intravascular haemolysis of erythrocytes, increased erythrophagocytosis by reticuloendothelial system and subsequently rapid release of immature erythrocytes into circulation (Ola-fadunsin *et al.*, 2021) [19].

### 3.1.5 Mean corpuscular volume (MCV; fl)

The mean  $\pm$  SE value of mean corpuscular volume (MCV) recorded in healthy cattle was  $43.07 \pm 1.62$  fl and in cattle

with anaplasmosis it was  $49.09 \pm 0.97$  fl. The level of mean corpuscular volume was increased significantly ( $P < 0.011$ ) in anaplasmosis affected cattle (Table 3.1, Figure 3.1).

The present findings agreed with Shaukat *et al.* (2019) [23], who reported significant ( $P < 0.05$ ) increase in value of MCV in anaplasmosis affected cattle as compared to healthy cattle. Similar observations were also reported by Arunkumar and Nagarajan (2013) [4], Sharma *et al.* (2013) [22], Maharana *et al.* (2016) [14], Ola-Fadunsin *et al.* (2021) [19]. Whereas Ganguly *et al.* (2018) [11] reported a non-significant decrease (MCV of cattle affected with anaplasmosis).

The increase in MCV might be due to rapid haemolysis of erythrocytes which leads to release of immature macrocytic erythrocytes into the circulation.

### 3.1.6 Eosinophils (%)

The mean  $\pm$ SE value of eosinophils recorded in healthy cattle was  $1.43 \pm 0.20\%$  and in cattle with anaplasmosis it was  $2.03 \pm 0.16\%$ . The level of eosinophils was significantly

( $P < 0.041$ ) increased in anaplasmosis affected cattle as compared to healthy cattle (Table 3.1, Figure 3.1).

Ganguly *et al.* (2018) [11] reported that the mean value of eosinophils was increased non-significantly in anaplasmosis affected cattle as compared to healthy cattle, while Hussain *et al.* (2017) [12] observed a significantly increased mean value of eosinophils in anaplasmosis affected cattle as compared to healthy cattle.

However, lymphocytes and platelets count were decreased non-significantly. Furthermore, total leucocytes count (TLC), mean corpuscular haemoglobin (MCH), neutrophils, monocytes and eosinophils increased non-significantly in anaplasmosis affected cattle.

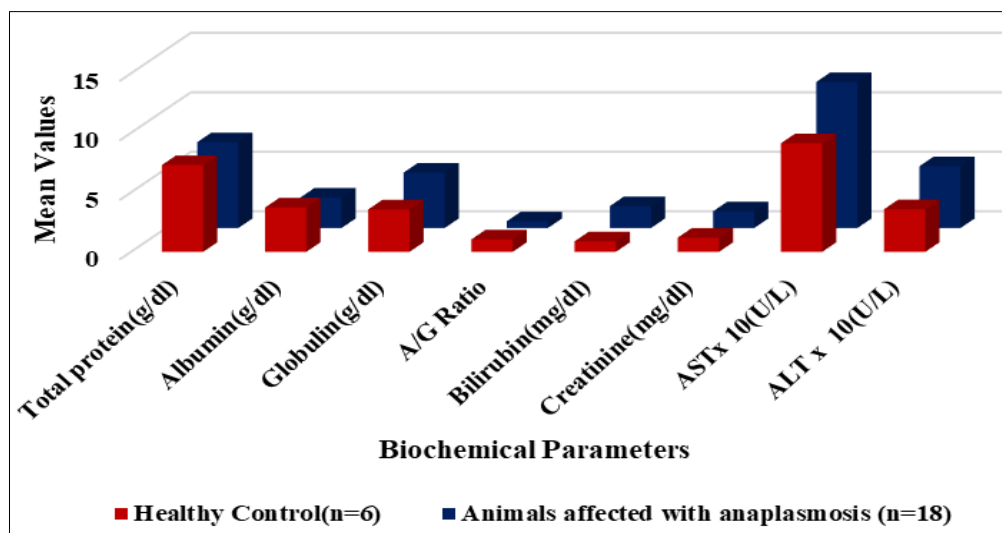
### 3.2 Serum Biochemical Profile

In present study, whole blood samples were collected from all 18 cattle affected with anaplasmosis and a healthy group ( $n=6$ ) for estimation of various serum biochemical constituents. The mean ( $\pm$ SE) values of different serum biochemical parameters studied are shown in Table 3.2 and Figure 3.2, and are discussed in detail as follows

**Table 3.2:** The mean ( $\pm$ SE) serum biochemical profile of healthy and anaplasmosis affected cattle

Parameters	Healthy	Anaplasmosis infected animals (N=18)	"p" Value
Total protein (g/dl)	$7.30 \pm 0.23$	$7.21 \pm 0.07$	0.356
Albumin (g/dl)	$3.73 \pm 0.19$	$2.56 \pm 0.10^{**}$	0.001
Globulin (g/dl)	$3.57 \pm 0.15$	$4.65 \pm 0.14^{**}$	0.000
A/G Ratio	$1.05 \pm 0.085$	$0.56 \pm 0.03^{**}$	0.001
Bilirubin (mg/dl)	$0.89 \pm 0.04$	$1.84 \pm 0.08^{**}$	0.000
Creatinine (mg/dl)	$1.20 \pm 0.11$	$1.40 \pm 0.08$	0.185
AST (U/L)	$91.31 \pm 3.38$	$122.72 \pm 2.76^{**}$	0.000
ALT (U/L)	$35.94 \pm 4.24$	$51.92 \pm 2.15^{**}$	0.010

\* $p < 0.05$  = significant, \*\* $p < 0.01$  = highly significant



**Fig 3.2:** Mean values of serum-biochemical observations of healthy and anaplasmosis affected cattle

#### 3.2.1 Albumin (g/dl)

The mean  $\pm$ SE value of serum albumin recorded in healthy cattle was  $3.73 \pm 0.19$  g/dl and in cattle with anaplasmosis  $2.56 \pm 0.10$  g/dl. It was highly significantly ( $P < 0.001$ ) decreased in anaplasmosis affected cattle (Table 3.2 and Figure 3.2).

The present findings were in accordance with Ganguly *et al.* (2018) [11] and Ola-Fadunsin *et al.* (2021) [19] who observed significantly decreased albumin level of cattle affected with anaplasmosis. However, Szabara *et al.* (2016) observed mild hypoalbuminemia in cattle affected with anaplasmosis as

compared to healthy group. Furthermore, Hussain *et al.* (2017) [12] reported no changes in biochemistry in cattle affected with anaplasmosis.

#### 3.2.2 Globulin (g/dl)

In present study, the mean  $\pm$ SE value of serum globulin recorded in healthy cattle was  $3.57 \pm 0.15$  g/dl and in cattle with anaplasmosis it was  $4.65 \pm 0.14$  g/dl. The level of globulin was highly significantly increased ( $P < 0.000$ ) in anaplasmosis affected cattle as compared to healthy ones (Table 3.2 and Figure 3.2).



The present findings were in accordance with Nasreldin *et al.* (2020) <sup>[18]</sup>, who observed significant increase in the level of globulin in cattle affected with anaplasmosis. Similar finding was also reported by Sharma *et al.* (2013) <sup>[22]</sup>. The elevated serum globulin level observed in the *A. marginale* infected animals could be due to activation of a defensive immune response that leads to an increase in circulating immunoglobulin in the serum (Sharma *et al.*, 2013) <sup>[22]</sup>.

### 3.2.3 Albumin/Globulin (A/G) ratio

The mean  $\pm$ SE value of serum A/G ratio recorded in healthy cattle was  $1.05 \pm 0.085$  and in cattle with anaplasmosis  $0.56 \pm 0.03$ . It was highly significantly decreased ( $P < 0.001$ ) in anaplasmosis affected cattle (Table 3.2 and Figure 3.2).

The present findings are in accordance with Nasreldin *et al.* (2020) <sup>[18]</sup> who observed significantly decreased level of A/G ratio in female cattle affected with anaplasmosis.

### 3.2.4 Bilirubin (mg/dl)

In present study, the mean  $\pm$ SE value of serum bilirubin recorded in healthy cattle was  $0.89 \pm 0.04$  mg/dl and in cattle with anaplasmosis it was  $1.84 \pm 0.08$  mg/dl. The level of bilirubin was highly significantly ( $P < 0.000$ ) increased in anaplasmosis affected cattle than in healthy cattle (Table 3.2 and Figure 3.2).

The present findings are in consistent with Nasreldin *et al.* (2020) <sup>[18]</sup> who reported significant increase in the level of total bilirubin in the anaplasmosis affected cattle in comparison to healthy group. Similar observations were also reported by Coskun *et al.* (2012) <sup>[8]</sup>, Sharma *et al.* (2013) <sup>[22]</sup>, Jaseem *et al.* (2015), Szabara *et al.* (2016), Ganguly *et al.* (2018) <sup>[11]</sup>, and Ola-Fadunsin *et al.* (2021) <sup>[19]</sup>.

The elevated serum bilirubin levels could be due to haemolysis of parasitized erythrocytes. Additionally, higher serum bilirubin levels have been linked to haemolytic anaemia and hepatic dysfunction (Coskun *et al.*, 2012) <sup>[8]</sup>.

### 3.2.5 Aspartate aminotransferase (AST; U/L)

In present study, the mean  $\pm$ SE value of serum aspartate aminotransferase activity recorded in healthy cattle was  $91.31 \pm 3.38$  U/L and in cattle with anaplasmosis it was  $122.72 \pm 2.76$  U/L. The level of aspartate aminotransferase was highly significantly ( $P < 0.000$ ) increased in anaplasmosis affected cattle (Table 3.2 and Figure 3.2).

Similar findings were reported by Aleksh and Iamail (2019) in cattle affected with anaplasmosis. Our findings were also in conformance with those reported by Riond *et al.* (2008), Coskun *et al.* (2012) <sup>[8]</sup>, Jaseem *et al.* (2015), Szabara *et al.* (2016), Ganguly *et al.* (2017), Ganguly *et al.* (2018) and Nasreldin *et al.* (2020) <sup>[10, 11, 18]</sup>.

The increase in AST is related to a hepatic damage or muscular trauma induced by prolonged clinical recumbency in animals. Massive haemolysis may also occur, which when combined with hypoxia, can result in hepatic cell degeneration and glomerular dysfunction, resulting in a rise in AST and ALT enzymes.

### 3.2.6 Alanine Aminotransferase (ALT; U/L)

The mean  $\pm$ SE value of serum alanine aminotransferase activity recorded in healthy cattle was  $35.94 \pm 4.24$  U/L and in cattle with anaplasmosis it was  $51.92 \pm 2.15$  U/L. The level of alanine aminotransferase was significantly ( $P < 0.010$ ) increased in anaplasmosis affected cattle (Table 3.2 and Figure 3.2).

Our findings were in accordance with Jaseem *et al.* (2015), Szabara *et al.* (2016), Ganguly *et al.* (2017) <sup>[10]</sup> and Ganguly *et al.* (2018) <sup>[11]</sup> and Nasreldin *et al.* (2020) <sup>[18]</sup>, who reported significant ( $P < 0.05$ ) increase in the serum alanine aminotransferase in cattle affected with anaplasmosis. However, Aleksh and Iamail (2019) observed non-significantly increased alanine aminotransferase in anaplasmosis affected cattle.

The present finding is conceivable since it is related to a hepatic damage or muscular trauma induced by prolonged clinical recumbency. These tissues might contain a large amount of enzymes that could be released into the bloodstream. (Coskun *et al.* 2012) <sup>[8]</sup>. According to the researchers, the rise in enzyme activity might be due to severe anaemia, which causes hypoxia and liver damage. Massive haemolysis may also occur, which, when combined with hypoxia, can result in hepatic cell degeneration and glomerular dysfunction, resulting in a rise in AST and ALT enzymes (Sharma *et al.* (2013) <sup>[22]</sup>).

However non-significant changes were observed in the level of total protein and creatinine.

## 4. Conclusion

The haematological examinations revealed that the Mean  $\pm$ SE values of haemoglobin, packed cell volume, and total erythrocytes count were decreased highly significantly ( $p < 0.01$ ) and mean corpuscular haemoglobin concentration (MCHC) was decreased significantly ( $p < 0.05$ ) in anaplasmosis affected cattle as compared to healthy cattle. The serum biochemical examinations revealed that the Mean  $\pm$ SE values of total protein were decreased non-significantly, while albumin and A/G ratio were decreased highly significantly ( $p < 0.01$ ) in anaplasmosis affected cattle as compared to healthy control group. Globulin and alanine aminotransferase were increased significantly ( $p < 0.05$ ), whereas aspartate aminotransferase and bilirubin were increased highly significantly ( $p < 0.01$ ).

## 5. Acknowledgement

The authors are grateful to the Dean of College of Veterinary Science and Animal Husbandry, and authorities of Kamdhenu University for the facilities and encouragement provided for this work.

Conflict of Interest: Not available

Financial Support Not available

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