

Treatment effects on Hematological Parameters and Serum Biochemical Profile of Lulu Cattle Infected with Bovine Anaplasmosis

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ABSTRACT

Anaplasmosis is a vector-borne, infectious blood disease of cattle caused by the rickettsial parasites, *Anaplasma marginale* and *Anaplasma centrale*. This parasite infects the red blood cells and causes severe anemia. Ticks are a common vector of this disease. The estimates of biochemical constituents are the prerequisites to diagnose patho-physiological disorders in cattle. This study aimed to compare the hematological and serum biochemical parameters after effect of treatment of antibiotics on infected Lulu cattle [*Bos taurus*]. This study further investigated the effectiveness of antibiotics with different routes of administration: intramuscular and intravenous. Hematological and serum biochemical profiles were taken from 31 Lulu cattle infected with Bovine Anaplasmosis and 14 treated Lulu cattle. Due to blood parasitic infestation, white blood cells increased to 17.8 and dropped significantly to normal value 12.7 after treatment. Lymphocyte levels significantly decreased after treatment from a diseased condition of 13.3 down to 7.92. Other hematological parameters such as Neutrophil, Eosinophil and Basophil were not significantly different before and after treatment. Serum biochemical profiles including glucose, total protein, albumin, calcium and phosphorous were affected by Bovine Anaplasmosis but values were not significantly different before and after treatment. Further, we found that intravenous administration of antibiotics is more effective over intramuscular administration route as blood smear showed clear up parasites after 10 days treatment by administration of drug by I/V mode. Our results indicate that hematological values return to normal levels after effective treatment using antibiotic.

KEYWORDS

Bovine Anaplasma

Lulu cattle

Hematology

Serum biochemical indices

I/V

I/M

1. INTRODUCTION

Livestock is an integral part of nearly all rural livelihoods in Nepal. High proportions of poor and marginalized farmers depend on livestock as their main or supplementary source of income. The Nepalese agriculture system is subsistence mixed farming where majority of small farmers rear cattle along with crops. Only 13% of the total cattle are improved, the rest are indigenous breeds, which can withstand very harsh climatic conditions [Neopane 2006]. Lulu cattle are one of these breeds having high potential to adapt to a wide range of altitudes and climates. This leads to the breed being at low risk from the effects of climate change due to its adaptability [Gorkhali et al. 2017]. Lulu cattle are claimed to be resistant to internal and external parasites [Neopane 2006]. A case of Lulu cattle heavily infested with *A. marginalis* was found at the Animal Breeding Division [ABD] farms under the Nepal Agricultural Research Council [NARC] at Khumaltar, Lalitpur, Nepal, as these infected Lulu cattle were not exhibiting any signs of infestation [Joshi 2018].

Among tick-borne diseases, Bovine Anaplasmosis is considered to be the most important disease in ruminants worldwide, causing significant economic losses in tropical and subtropical areas [Kocan et al. 2000]. The socio-economic impact of the disease and restrictions on trading infected animals internationally led the Office International

des Epizooties [OIE] Animal Health Code to categorize Anaplasmosis as a disease that required a notification of its presence [OIE 2008]. Cattle can be infected by several *Anaplasma* species, like *A. marginale*, *A. phagocytophilum*, *A. centrale* and *A. bovis* [Kuttler 1966]. *Anaplasma marginale* is one of the most prevalent tick-transmitted rickettsial diseases of cattle in the world [Kocan et al. 2003]. This species is highly pathogenic, especially in cattle up to two years old and causes progressive anemia and icterus [Kuttler 1984]. This tick-borne disease is known to cause high fever, coughs, miscarriages, decreased milk production and loss of appetite in cattle [Woldehiwet and Scott 1982]. Disease treatment and prevention strategies focus on using reliable diagnostic tests to accurately and precisely identify infected cattle. Bovine Anaplasmosis is diagnosed by identifying *Anaplasma* in Giemsa-stained blood smears from clinically suspected animals during the acute phase of the disease. Even though this method is not useful for detecting pre-symptomatic and carrier animals, it is cost effective and is practical for developing countries. A more reliable screening test for infected cattle can be performed using ELISA [Knowles et al. 1996] or highly sensitive and specific molecular methods such as identifying *A. marginale* and *A. phagocytophilum* DNA [M'ghirbi et al. 2016].

Bovine Anaplasmosis, a rickettsial disease caused by *A. marginale* is responsible for great economic losses in developing countries [Rodriguez et al. 2009]. A study was conducted with two main objectives. The first objective was to compare the hematological and serum biochemical parameters before and after the treatment of antibiotics on infected Lulu cattle. The second objective was to evaluate the effectiveness of antibiotics' different route of administration, including intravenous and intramuscular.

2. Materials and Methods

2.1 Study design and sampling approach

This study was conducted at the ABD Farm in Khumaltar, Lalitpur where cattle shed was found to be infested with ticks. Five ticks were collected each from five Lulu cattle [25 ticks altogether] from ABD-farm, using chloroform in the cotton in order to prevent the mouth part. Mouth part is considered for the identification of the tick. The mouth part was observed under compound microscope and the ticks were identified as *Boophilus microplus* [Figure 1].

Blood samples were collected from 31 Lulu cows. Blood was collected from the jugular vein for hematological and biochemical profiles, primarily having a history of tick infestation in the shed but animals were apparently healthy with normal body temperature and with no signs and symptoms of Bovine Anaplasmosis. These individuals had high infestation by Bovine Anaplasmosis, which was confirmed by making blood smears. From each animal, 10 ml blood was drawn from the jugular vein, using an 18-gauge needle with syringe and 5 ml kept in vacutainer with EDTA vial and 5 ml in clot vial for hematological and serum biochemical profile, respectively. Cold chain was maintained while transporting samples to laboratory.

Out of 31 Lulu cows sampled, 14 infected cattle were treated with LA OTC @ 20 mg kg⁻¹ body weight intramuscular injection three times with 72 hours intervals. After one month of treatment, blood samples from those 14 treated individuals were collected. Blood smears were made and results showed that *A. marginale* was still prevalent in blood [Figure 2]. This indicated the animals had not fully recovered. Animals were treated again with LA OTC @ 20 mg kg⁻¹ body weight intravenous injection for three days with 72 hours intervals. Oxytetracycline was diluted with physiological saline prior to injecting intravenously. After 10 days of treatment, all animals [n = 14] were found to have fully recovered as *A. marginale* was not present in the smears. This proved that intravenous administration of LA OTC was more effective than intramuscular. Hematological and biochemical profiles were studied in the treated animals as well to compare the values before and after treatment for Bovine Anaplasmosis.

Sample Processing

2.2.1 Hematological parameters

Hematological parameters were determined by automatic hematology analyzer. The hematological parameters such as Total erythrocyte count [TEC], Total leukocyte count [TLC], Packed cell volume [PCV], Haemoglobin [Hb] content, Erythrocyte sedimentation rate [ESR], Mean corpuscular volume [MCV], Mean corpuscular haemoglobin [MCH] and Mean corpuscular haemoglobin concentration [MCHC] were determined with the automatic hematology analyzer.

2.2.2 Serum Biochemical profile

Serum samples obtained by centrifugation were used to determine total protein, albumin, phosphorus, and calcium using the automatic biochemical analyzer Spectrophotometer. For serum glucose, Randox assayed method was used.



Figure 1: *Boophilus microplus*.

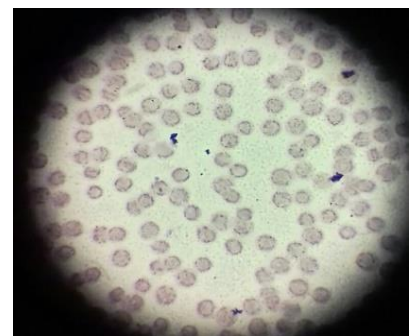


Figure 2: Microscopic examination of Giemsa stained blood smear showing piroplasm of *A. marginale* parasite in erythrocyte.

2.3 Statistical Analysis

Data on hematological parameters and serum biochemical profiles before and after treatment were compiled in Microsoft Excel 2010 and the differences in parameters before and after treatment were calculated. All Statistical analyses were performed using R [v.3.4.1, R Core Team 2013] with GUI RStudio [v1.0.143]. We conducted paired t-tests to test the significant differences between means post and prior to treatment for each hematological parameter and serum biochemical profile. To determine significance, α value was set at 0.05 and any p-values less than α were concluded as being significant indicating that there is a difference between two means.

3. RESULTS

The change in hematological and biochemical parameters of indigenous Lulu cattle [*Bos taurus*] before and after treatment with antibiotics are shown in Table 1 and 2. Reference values for Lulu cattle [Joshi 2018] are shown in Table 1. With regards to hematological parameters, lymphocyte levels significantly decreased by an average of 4.87 ± 3.66 [$t=4.7$, $p=0.0003$].

3.1 Variation in Red Blood Cell [RBC] parameters

RBC count in cattle increased on average by 0.33 ± 1.5 [mean \pm SD] after treatment. The mean value of RBC before treatment was 6.5 ± 0.23 and after treatment the value increased to 7.06 ± 0.23 . However, the results from the paired t-test indicated that this difference was not significant [$t=0.788$, $p=0.44$, $\alpha=0.05$].

3.1.1 Sex wise

The mean RBC count in females increased by 0.482 ± 1.48 . Prior to treatment, the RBC count in females was 5.75 ± 0.5 , which increased to 6.52 ± 0.4 after treatment. Paired t-test showed this difference was not significant [$t=1.027$, $p=0.328$, $\alpha=0.05$]. In males, RBC count increased by 1.53 ± 1.5 . The mean value of RBC before treatment was 6.0233 ± 0.68 and after treatment the value increased to 7.54 ± 0.6 . Paired t-test indicated that this difference in males before and after treatment was not significant [$t\text{-value} = -0.21$, $p\text{-value} = 0.85$, $\alpha=0.05$].

3.1.2 Age wise

The mean value of RBC in individuals under four years old increased by 0.26 ± 1.40 . In the younger individuals, before treatment RBC count was 6.20 ± 0.7 and after treatment it increased to 7.9 ± 0.7 . This difference, however, was not significant as indicated by paired t-test [$t=0.3$, $p=0.7$, $\alpha=0.05$]. In cattle above 4 years old, the mean value of RBC increased by 0.3 ± 1.58 . In these older individuals, RBC counts before treatment was 5.89 ± 0.8 and after the treatment the count increased to 6.38 ± 0.74537 . This difference in older individuals was not significant [$t=0.660$, $p=0.52$, $\alpha=0.05$].

3.2 Variation in White Blood Cell [WBC] parameters

The mean value of WBC count decreased by 4.82 ± 3.7 after the use of antibiotics [Table 1]. WBC counts before treatment was 16.2 ± 2.5 and after treatment the count decreased to 11.4 ± 3.3 . The data were analyzed by t-test and indicated that this difference was statistically significant [$t=4.68$, $p=0.0004$, $\alpha=0.05$].

3.2.1 Sex wise

The mean value of WBC in female decreased by 4.6 ± 3.9 after treatment. Before treatment, WBC in females was 15.4 ± 2.1 and decreased to 10.8 ± 3.3 . This difference was shown to be significant by paired t-test [$t=3.74$, $p=0.004$, $\alpha=0.05$]. In males, the WBC mean value decreased by -5.60 ± 2.78 . The WBC count before treatment was 19.28 ± 1.35 and decreased to 13.5 ± 1.3 . Paired t-test results indicated that this difference was, however, not significant in males.

3.2.2 Age wise

For cattle under 4 years old, the WBC decreased by 5.07 ± 5.0 . For these young individuals, WBC before treatment was 17.8 ± 2.7 and decreased to 12.78 ± 3.48 after treatment. This difference, however, was not significant [$t=2.03$, $p=0.112$, $\alpha=0.05$]. For individuals older than 4 years of age, the mean WBC decreased by -4.69 ± 2.7 . Before treatment, WBC was at an average of 15.38 ± 2.01 and decreased to 10.6 ± 3.0 after treatment. In the older cattle, the difference in WBC before and after treatment was significant [$t=4.83$, $p=0.001$, $\alpha=0.05$].

3.3 Variation in Differential Leukocyte Count

The mean value of leukocyte count decreased by 4.87 ± 3.6 after the use of antibiotics [Table 1]. Leukocyte count before treatment was 12.1 ± 2.5 and after treatment it decreased to 7.26 ± 2.4 . The data were analyzed by paired t-test, which indicated that this difference was statistically significant [$t=4.68$, $p=0.0004$, $\alpha=0.05$].

3.3.1 Sex wise

The mean value of leukocyte in females decreased by 4.51 ± 4.0 . In females, leukocyte count before treatment was 11.1 ± 2.6 and decreased to 6.97 ± 2.1 after treatment. Results from paired t-test support that this difference was statistically significant [$t=3.54$, $p=0.005$, $\alpha=0.05$]. The mean value of leukocyte in males decreased by -6.21 ± 1.0 .

Average leukocyte count before treatment was 14.57 ± 0.568 and decreased to 8.36 ± 1.48 after treatment. Paired t-test results showed significant difference in leucocyte counts before and after treatment [$t = 8.57$, $p = 0.013$, $\alpha = 0.05$].

3.3.2 Age wise

For cattle under 4 years of age, leukocyte count decreased by 5.07 ± 5.0 . In these young individuals, the average before treatment was 13.3 ± 2.5 and leukocyte count decreased to 7.92 ± 2.6 after treatment, although not significant [$t = 2.44$, $p = 0.071$, $\alpha = 0.05$]. For cattle above 4 years old, leukocyte count decreased by an average of 4.54 ± 2.7 . Paired t-test results showed significant difference before and after treatment [$t = 4.177$, $p = 0.003$].

Table 1: Mean hematological parameters [HP] of Lulu cow before [n=31] and [n=14] after treatment [BT=Before Treatment, AT=After Treatment] of Bovine Anaplasmosis with LA OTC @ 20 mg kg⁻¹ along with sex wise and age wise differences. SD=Standard Deviation.

HP	BT		AT		Females		Males		Age wise [Under 4 years]		Age wise [above 4 years]	
	Mean ± SD	Mean ± SD	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
WBC	16±2	11±3	15±2	10±3	19±1	13±2	17±2	12±3	15±2	10±3		
LYM	12±2	7.2±2	11±2	6.9±2	6.2±2	10±2	13±2	7.9±2	6.2±2	10±2		
MON	0.9±0	0.8±0	0.3±0	0.5±1	0.7±0	0.6±0	0.3±0	0.5±1	0.6±0	0.7±0		
NEU	2.7±0	2.9±1	2.3±0	2.4±1	2.0±0	2.6±0	2.2±0	2.2±1	2.4±0	2.9±0		
EOS	0.4±0	0.3±0	0.4±0	0.4±0	0.4±0	0.4±0	0.5±0	0.6±0	0.5±0	0.3±0		
BAS	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0		
LY	72±8	70±11	71±8	71±0	60±11	63±0	71±8	71±0	60±11	63±0		
MO	6.5±4	5.6±3	5.5±4	6.5±0	5.8±3	5.3±0	5.4±4	6.4±0	5.5±3	5.2±0		
NE	17±6	15±11	16±6	15±0	14±11	13±0	16±0	14±0	12±10	12±0		
EOS.1	2.8±1	2.0±1	2.8±1	2.6±0	2.0±1	2.1±0	2.4±1	2.4±0	2.0±1	2.1±0		
BA	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0	0.0±0		
RBC	6.5±0	7.0±0	5.7±0	6.5±0	6.0±0	7.5±0	4.7±0	6.4±0	6.2±0	7.4±0		
HGB	10±1	10±1	10±1	9.5±0	11±1	10±0	10±1	9.5±0	11±1	10±0		
HCT	36±0	34±4	35±4	34±0	12±4	13±0	35±4	34±0	12±4	13±0		
MVC	52±6	48±6	53±6	50±0	45±6	50±0	53±6	50±0	45±6	50±0		
PLT	146±2	176±155	143±6	132±0	173±155	173±0	143±62	132±0	173±155	173±0		
PCT	0.1±0	0.1±0	0.1±0	0.1±0	0.2±0	0.3±0	0.1±0	0.1±0	0.2±0	0.3±0		
MPV	7.8±0	8.3±1	4.8±0	3.5±0	7.3±1	3.4±0	4.8±0	3.4±0	7.3±1	3.4±0		

3.4 Serum Biochemical Changes Findings

3.4.1 Serum Glucose

The mean value of serum glucose before treatment was 46.6 ± 4.8 and after treatment it increased to 49.0 ± 2.1 . Results from paired t-test illustrate that this difference was not significant.

3.4.2 Sex wise

The mean value of serum glucose before treatment in females was 44.5 ± 2.3 and after treatment it increased to 50.7 ± 3.4 . Results from paired t-test showed this difference was not significant [$t = -0.75$, $p = 0.452$, $\alpha = 0.05$]. The mean value of serum glucose [gm dl⁻¹] in males before treatment was 54.6044 ± 3.4043 and after treatment it decreased to 54.5 ± 0.0 .

3.4.3 Age wise

The mean value of serum glucose for cattle under 4 years old was 52.5 ± 8.4 and decreased to 45.3 ± 6.3 after treatment. Paired t-test showed this result was not significant [$t = -1.75$, $p = 0.145$, $\alpha = 0.05$]. The mean value of serum glucose for cattle above the age of 4 was 45.3 ± 6.3 and decreased to 40.3 ± 4.3 , which was not significant.

3.5 Total protein

The mean value of total protein before treatment was 5.29 ± 0.1 and after treatment it decreased to 5.0 ± 0.1 . Results from paired t-test indicated that there was no significant difference.

3.5.1 Sex wise

The mean value of total protein in females before treatment was 5.28 ± 0.1 and after treatment the value was 5.12 ± 0.1 . Paired t-test indicated no significant difference [$t = 0.162$, $p = 0.370$, $\alpha = 0.05$]. In males, the mean value before treatment

was 5.31 ± 0.3 and decreased to 5.00 ± 0.2 after treatment. Paired t-test indicated no significant difference [$t = -0.515$, $p = 0.754$, $\alpha = 0.05$].

3.5.2 Age wise

The mean value of total protein in cattle under 4 years old was 5.04 ± 0.1 before treatment and it decreased to 4.04 ± 0.1 after treatment. Paired t-test did not show significant difference [$t = -0.033$, $p = 0.0867$, $\alpha = 0.05$]. The mean value of total protein in cattle above 4 years old was 5.2 ± 0.2 and after treatment it was 5.00 ± 0.2 . Results from paired t-test indicated no significant difference [$t = -2.80$, $p = 0.120$, $\alpha = 0.05$].

3.6 Albumin

The mean value of albumin before treatment was 1.52 ± 0.2 and it increased to 1.63 ± 0.2 . Results from paired t-test indicated no significant difference [$t = 0.166$, $p = 0.122$, $\alpha = 0.05$].

3.6.1 Sex wise

The mean value of albumin in females before treatment was 3.82 ± 0.2 and after treatment it decreased to 3.48 ± 0.2 . Paired t-test results showed significant difference in female albumin levels after treatment [$t = -0.841$, $p = 0.522$, $\alpha = 0.05$]. In males, albumin level before treatment was 3.53 ± 0.3 and it decreased to 2.71 ± 0.1 after treatment.

3.6.2 Age wise

The mean value of albumin in cattle under 4 years of age was 3.02 ± 0.2 before treatment and it decreased to 2.89 ± 0.1 after treatment. The mean value of albumin in cattle above 4 years of age was 3.9 ± 0.2 before treatment and decreased to 3.00 ± 0.1 .

3.7 Globulin

The mean value of globulin before treatment was 1.52 ± 0.8 and increased to 1.63 ± 0.9 after treatment. Results from paired t-test indicated no significant difference [$t = -0.335$, $p = 0.743$, $\alpha = 0.05$].

3.7.1 Sex wise

The mean value of globulin in females before treatment was 1.46 ± 0.2 and after treatment it increased to 1.48 ± 0.3 . Paired t-test indicated no significant difference [$t = 0.159$, $p = 0.174$, $\alpha = 0.05$]. In males, globulin levels before treatment was 1.77 ± 0.1 and after treatment it increased to 2.30 ± 0.2 . Results from paired t-test did not show significant difference [$t = -1.48$, $p = 0.130$, $\alpha = 0.05$].

3.7.2 Age wise

The mean value of globulin in cattle under 4 years of age was 3.0 ± 0.2 and after treatment it increased to 3.90 ± 0.1 . Paired t-test results did not show significant difference [$t = -2.09$, $p = 0.0783$, $\alpha = 0.05$]. Globulin levels in cattle above 4 years old increased from 1.45 ± 0.1 before treatment to 1.74 ± 0.1 after treatment. Paired t-test showed no significant difference [$t = -1.03$, $p = 0.621$, $\alpha = 0.05$].

3.8 Serum calcium

The mean value of serum calcium before treatment was 2.24 ± 0.0609 and increased to 5.44 ± 0.2 after treatment. Results from paired t-test indicated that this difference was not significant.

3.8.1 Sex wise

The mean value of serum calcium in female before treatment was 4.00 ± 0.0 and increased to 5.43 ± 0.2 after treatment. Results from paired t-test indicated that this difference in females before and after treatment was not significant [$t = -1.4$, $p = 0.430$, $\alpha = 0.05$]. In males, serum calcium before treatment was 3.9 ± 0.09 and increased to 5.47 ± 0.5 after treatment. Results of paired t-test indicated that this difference in males before and after treatment was not significant.

3.8.2 Age wise

The mean value of serum calcium in cattle under 4 years old was 4.0 ± 0.3 and after treatment the value was 5.8 ± 0.5 . Results from paired t-test showed this difference in young individuals was not significant [$t = -2.48$, $p = 0.786$, $\alpha = 0.05$]. In cattle above 4 years old, the mean serum calcium before treatment was 3.50 ± 0.1 and after treatment the mean was 5.50 ± 0.5 . Paired t-test indicated this difference was not significant.

3.9 Serum phosphorous

The mean value of serum phosphorous before treatment was 2.30 ± 0.1 and increased to 3.46 ± 0.2 after treatment. Paired t-test results showed this difference was not significant.

3.9.1 Sex wise

The mean value of serum phosphorous in female before treatment was 2.58 ± 0.1 and after treatment the value was 3.38 ± 0.3 . In males, serum phosphorous before treatment was 3.32 ± 0.1 and after treatment it was 3.75 ± 0.3 . Paired t-test showed significant difference [$t = -2.25$, $p = 0.0457$, $\alpha = 0.05$].

Table 2: Comparative biochemical profile of Lulu cattle before and after treatment of Bovine Anaplasmosis via antibiotics.

	Biochemical Profiles	Before treatment	After treatment
		Mean \pm SD	Mean \pm SD
Total	Serum Glucose[gm/dl]	46.6 \pm 18.2	49.0 \pm 8.10
	Total protein[gm/dl]	5.29 \pm 0.60	5.09 \pm 0.60
	Albumin[gm/dl]	3.35 \pm 0.73	3.31 \pm 0.75
	Globulin[gm/dl]	1.52 \pm 0.82	1.63 \pm 0.93
	Serum Calcium[mmol/dl]	2.23 \pm 0.22	5.44 \pm 0.74
	Serum Phosphorous[mmol/dl]	2.30 \pm 0.30	3.46 \pm 0.13
Female	Serum Glucose[gm/dl]	44.5 \pm 2.35	44.53 \pm 2.4
	Total protein[gm/dl]	5.28 \pm 0.16	5.11 \pm 0.18
	Albumin[gm/dl]	3.82 \pm 0.23	3.48 \pm 0.22
	Globulin[gm/dl]	1.45 \pm 0.27	1.48 \pm 0.39
	Serum Calcium[mmol/dl]	4.00 \pm 0.06	4.43 \pm 0.21
	Serum Phosphorous[mmol/dl]	2.58 \pm 0.14	3.38 \pm 0.33
Male	Serum Glucose[gm/dl]	54.6 \pm 0.00	54.5 \pm 0.00
	Total protein[gm/dl]	5.31 \pm 30.0	5.00 \pm 0.00
	Albumin[gm/dl]	3.53 \pm 0.33	2.70 \pm 0.13
	Globulin[gm/dl]	1.77 \pm 0.21	2.30 \pm 0.21
	Serum Calcium[mmol/dl]	3.90 \pm 0.09	5.47 \pm 0.54
	Serum Phosphorous[mmol/dl]	3.32 \pm 0.14	3.75 \pm 0.37
Under 4 years	Serum Glucose[gm/dl]	42.2 \pm 9.23	40.3 \pm 11.0
	Total protein[gm/dl]	4.34 \pm 0.67	3.50 \pm 0.67
	Albumin[gm/dl]	3.25 \pm 0.76	4.32 \pm 0.78
	Globulin[gm/dl]	1.34 \pm 0.21	1.82 \pm 0.45
	Serum Calcium[mmol/dl]	2.98 \pm 0.34	2.70 \pm 0.56
	Serum Phosphorous[mmol/dl]	3.40 \pm 0.69	4.20 \pm 0.78
Above 4 years	Serum Glucose[gm/dl]	45.3 \pm 4.34	41.5 \pm 9.56
	Total protein[gm/dl]	5.43 \pm 0.78	3.22 \pm 0.56
	Albumin[gm/dl]	4.39 \pm 0.87	2.58 \pm 0.68
	Globulin[gm/dl]	1.40 \pm 0.56	1.21 \pm 0.54
	Serum Calcium[mmol/dl]	3.21 \pm 0.67	2.49 \pm 0.32
	Serum Phosphorous[mmol/dl]	4.26 \pm 0.56	4.52 \pm 0.57

3.9.2 Age wise

In cattle under 4 years of age, the mean value of serum phosphorous before treatment was 2.50 ± 0.1 and increased to 3.50 ± 0.3 after treatment. Paired t-test results showed this difference was not significant [$t = -0.838$, $p = 0.453$, $\alpha = 0.05$]. In cattle above 4 years of age, the mean value of serum phosphorous before treatment was 3.5 ± 0.1 and increased to 4.00 ± 0.4 after treatment. Results of paired t-test showed significant difference [$t = 3.34$, $p = 0.034$, $\alpha = 0.05$].

4. DISCUSSION

A. marginale, known to be highly pathogenic to cattle and ranked among the most important pathogens of cattle hosts [Soulsby 1986], was detected in Lulu cattle. These infected Lulu cattle did not show any symptoms of *A. marginale*, which is evident to its resistance to blood parasites. Increase in monocytes observed in this study confirms the blood parasites infestation. Testing of monocyte level in blood can be an easy method to detect blood parasites infestation. Due to blood parasitic infestation, white blood cells significantly increased and dropped significantly to normal value after treatment. Lymphocyte levels significantly decreased after treatment from a diseased condition. In case of Lulu cattle, the means of eosinophils and neutrophil in the infected cattle did not increase as reported in infected exotic cattle [Zaugg et al. 1996]. Similarly, other hematological parameters such as RBC, Hb, PCV, and MCHC did not show any significant

decrease in Lulu cattle as reported in infected exotic cattle [Arslan and Shukur 1994]. Temperatures of the sampled animals were normal and did not show any signs and symptoms of disease. Above all, scientific evidences indicate that Lulu cattle are resistant to blood parasites caused by ticks, whereas exotic animals show increase in body temperature and evidence of icterus and decrease of RBC and haemoglobin, supported by anemic mucus membranes [Splitter et al. 1956]. Long acting OTC for 3 times at an interval of 72 hours by the route of I/V was more effective as no blood parasites were observed after 10 days of treatment. Faster recovery might be attributed to the early diagnosis and higher dose of Oxytetracycline used in the present study. This is in agreement with the findings of Ananda et al. [2009].

4. CONCLUSIONS

Among *Anaplasma spp.*, *A. marginale* is found in Lulu cattle, caused by tick *Boophilus multiplus*. Lulu cattle are resistant to blood parasites, caused by ticks. Testing of monocyte level in blood can be an easy method to detect blood parasites infestation and complete recovery. Long acting OTC for 3 times at an interval of 72 hours by the route of I/V is more effective.

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REFERENCES

- Ananda KJ, D'Souza PE and Puttalakshamma GC [2009]. Prevalence of Haemoprotozoan diseases in crossbred cattle in Bangalore north. *Veterinary World*, 2[1]:15–16.
- Arslan SH and AA Shukur [1994]. Clinical and haematological studies on theileriosis and anaplasmosis in local breed cattle in Mosul region. *Iraqi Journal of Veterinary Sciences*, 7[2]: 93-100.
- Gorkhali NA, Sapkota S, Shrestha BS, Joshi BR and Shrestha YK [2017]. High adaptation potential exhibited in Lulu cattle, a dwarf cattle breed of Nepal. *Proceeding of the 10th Workshop on Livestock and Fisheries Research*.
- Joshi D [2018]. Hematological analysis and biochemical examination of serum in the Lulu cattle. B.V.Sc and A.H, internship thesis, Himalayan College of Agricultural Science and Technology, Nepal
- Knowles D, Torioni de Echaide S, Palmer G, McGuire T, Stiller D, and McElwain T [1996]. Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. *Journal of Clinical Microbiology*, 34: 2225–30.
- Kocan KM, Blouin EF, and Barbet AF [2000]. Anaplasmosis control. Past, present, and future. *Ann N Y Acad Sciences* 916:501–9. doi: 10.1111/j.1749-6632.2000.tb05329.x.
- Kocan KM, de la Fuente J, Guglielmone AA, and Melendez RD [2003]. Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. *Clinical Microbiology Review*. 16: 698–712. doi: 10.1128/CMR.16.4.698-712.2003.
- Kuttler KL [1966]. Clinical and hematologic comparison of *Anaplasma marginale* and *Anaplasma centrale* infections in cattle. *American Journal of Veterinary Research*, 27: 941–6.
- Kuttler KL [1984]. *Anaplasma* infections in wild and domestic ruminants: a review. *J Wildl Dis*. 20:12–20. doi: 10.7589/0090-3558-20.1.12.
- M'ghirbi Y, Bèji M, Oporto B, Khrouf F, Hurtado A and Bouattour A [2016]. *Anaplasma marginale* and *A. phagocytophilum* in cattle in Tunisia. *Parasites Vectors*, 9: 556.
- Neopane SP [2006]. Characterization of Indigenous Animal Genetic Resources of Nepal. *Proceedings of the 6th National Workshop on Livestock and Fisheries Research*. Nepal Agricultural Research Council. Pp 1-11.
- Rodriguez SD, Qurtiz MA, Ocampo RJ and Murguía CA [2009]. Molecular epidemiology of Bovine Anaplasmosis with Particular focus in Mexico. *Intect-Genet Evol*, 9: 1092-1101.
- Sattar A and Mirza RH [2009]. Hematological parameters in exotic cows during gestation and lactation under subtropical conditions. *Pakistan Veterinary Journal*, 29[3]: 129-132.
- Soulsby EJJ [1986]. *Helminths, Arthropods and Protozoa of Domesticated animals*. 7th edition. London: Baillière Tindall.
- Splitter E, Castro E and Kanawyer W [1956]. Feline infectious anemia. *Vet Med Small Anim Clin*, 51: 17-22.
- World Organisation for Animal Health [OIE] [2008]. *Manual of standards for diagnostic tests and vaccines for terrestrial animals*, 6th edition, pp 599–10.
- Woldehiwet Z and Scott GR [1982]. Stages in the development of *Cytoecetes phagocytophila*, the causative agent of tick-born fever. *J Comp Path*, 92: 469–74. doi: 10.1016/0021-9975[82]90033-0.
- Zaugg JL, Goff WL, Foreyt W and Hunter DL [1996]. Susceptibility of elk [*Cervus elaphus*] to experimental infection with *Anaplasma marginale* and *A. ovis*. *Journal of Wildlife Diseases*, 32: 62.