

**EFFECT OF ANAPLASMA INFECTION ON INDUCTION OF
STRESS CONDITION IN DAIRY COW**

A THESIS

BY

MD. ZAKER HOSSAIN

STUDENT ID: 1604061

SEMESTER: JAN – JUN, 2024

SESSION: 2022-2023

MASTERS OF SCIENCE (MS)

IN

PHARMACOLOGY



**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
DINAJPUR-5200**

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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
DINAJPUR-5200

JUNE, 2024

**Dedicated
To My
Beloved Parents**

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The Author

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ABSTRACT

Present study was aimed to examine the role of Anaplasma infection as stress factor in dairy cow. A total of 20 blood samples were collected from cyclic cow. During blood collection BCS, body temperature were recorded. Blood samples were analyzed for protozoa (Anaplasma) identification. Others were considered as control. Blood samples were further examined to determine the ferritin and C – Reactive Protein (CRP) values as a stress indicator. Results showed that the body temperature and BCS were significantly affected ($p < 0.01$) by the presence of Anaplasma infection. Ferritin as a stress indicator were found significantly elevated and C – Reactive Protein (CRP) level were slightly increased in the Anaplasma positive group but the result was not significant.

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CHAPTER I

INTRODUCTION

Livestock and Dairy industry is facing a lot of challenges in the form of various diseases which ultimately affect the economy of any country (Carroll, 2008). Parasitic diseases in animals cause production losses and several critical issues across the globe (Mehmood *et al.*, 2017). The disease has been placed recently in B type infection category by Office of International des Epizooties, Terrestrial Animal Health Code (OIE, 2012). An annual estimated loss of \$100 million in USA which includes 50,000 to 100,000 deaths of cattle (Ashuma *et al.*, 2013) was due to Bovine anaplasmosis.

Cattle have more susceptibility to *Anaplasma* than buffalo (Rajput *et al.*, 2005). Bovine anaplasmosis occurs in tropical and sub-tropical regions of the world including 40 states of the USA, south and Central America, Asia, Africa, southern Europe, Middle East and Australia (Cynthia and Susan, 2008). Bovine anaplasmosis can be seen at any age group in cattle, however, the severity of disease and death rate increase with the advance in age, with clinical anaplasmosis being more commonly in cattle older than 1 year of age (Kocan *et al.*, 2003).

Anaplasmosis is previously known as gall sickness in cattle and it is a tick-borne disease caused by obligate intra-erythrocytic rickettsial microorganisms, i.e. *Anaplasma marginale* and *Anaplasma centrale* of the order Rickettsiales (Rajput *et al.*, 2005). It is transmitted by ticks, biting flies, and blood-contaminated fomites including used needles, ear tagging, dehorning, and castration equipment (Aubry *et al.*, 2011). Anaplasmosis is found on six continents and is responsible for high morbidity and mortality in temperate, subtropical, and tropical regions (Kocan *et al.*, 2010). The cycle of *A. marginale* in ticks is very complex and associated with the tick feeding cycle. Infected erythrocytes ingested by ticks with the blood meal provide the source of *A. marginale* infection for tick gut cells. After the development of the pathogen in tick gut cells, many other tick tissues become infected, including the salivary glands, the site from which *A. marginale* is transmitted to cattle during feeding. Clinically, the disease is characterized by hemolytic anemia, fever, jaundice, decreased milk production, abortions, hyper-excitability, and sudden death (Radostits *et al.*, 2010). The recovered animals act as carriers and remain as a patient source of infection to other susceptible animals. The present communication puts a

record on *Anaplasma* infection in a cattle herd, haemato-biochemical changes, and its therapy.

Giemsa stained thin blood smear (GSTBS) microscopic examination and serological test, are the main methods used for diagnosis of anaplasmosis. The diagnosis of bovine anaplasmosis is performed routinely by Giemsa stained blood smears which can indeed be used as a suitable method to detect *Anaplasma* in the animals clinically suspected for acute diseases, but it is not applicable to the determination of pre-symptomatic and carrier animals (Carelli *et al.*, 2007). Therefore, several serological tests have been used to measure *Anaplasma* specific antibodies, including the complement fixation test, card agglutination test, indirect fluorescent antibody test and enzyme-linked immunosorbent assay (ELISA) (Goff *et al.*, 1990). For the detection of clinically suspected animals' conventional parasitological techniques like microscopic examination of GSTBS are always a gold standard test for the diagnosis of anaplasmosis while in subclinical (serve as reservoirs) and in chronic infections GSTBS is not applicable (Eriks *et al.*, 1989; Ashuma *et al.*, 2013). For confirmatory diagnosis, PCR is used for detection even in case of low level of parasitemia (Ge *et al.*, 1995; Torina *et al.*, 2008).

Oxidative stress may result from an imbalance between reactive oxygen species (ROS) and antioxidants levels (Lightboy *et al.*, 2001). It is well known that ROS are produced by several pathological conditions and cause cellular damages such as lipid peroxidation and protein oxidation. The biological oxidative effects of free radicals on lipids and proteins are controlled by a spectrum of antioxidants (Serdar *et al.*, 2006). The antioxidant status of tissues can be described by the analysis of single components in the defense systems against ROS, as well as by the determination of total antioxidant capacity (TAC). In contrary, the TAC measurement does not represent the sum of activities of antioxidants; it could be used for clinical diagnosis, as it is an easy and less time consuming procedure (Kankofer *et al.*, 2005).

Studies have shown that Procalcitonin (PCT) levels are high in the patients with malaria (Uzzan *et al.*, 2006.). As with malaria caused by *Plasmodium falciparum*, babesiosis can also be classified as protozoal sepsis (Matijatko *et al.*, 2010). In another study, significant differences in PCT levels were found between healthy dogs and dogs with babesiosis (Brkljačić *et al.*, 2014). It is stated that PCT has a diagnostic and prognostic value, and may also help to assess therapeutic efficacy, as it increases in accordance with the

severity of the inflammatory response to the infection (Karzai *et al.*, 1997). It has been stated that the prognosis of patients whose PCT levels continuously increase is poor, and the prognosis of patients whose PCT levels decrease rapidly is good (Harbarth *et al.*, 2001). C reactive protein (CRP) is an acute phase protein synthesized in the liver; it increases in a way similar to PCT in infections, and is a biomarker that is also used to monitor the progress of the disease. Unlike PCT, CRP levels can also increase in slight inflammatory reactions and viral infections (Günel, 2009). A marked elevation in ferritin with or without other features of hemophagocytic/macrophage activation syndrome has been occasionally reported in patients with *A. phagocytophilum* infection. A case of HGA is described in which the patient had an elevated ferritin of 1964 µg/L. For patients presenting with features of hemophagocytic syndrome including a markedly elevated ferritin, infection with *A. phagocytophilum* should be considered in the differential diagnosis in the appropriate clinical context (i.e., at-risk geographic location, season, tick exposure) as this distinction has management implications (Andrew *et al.*, 2019).

Considering the facts mentioned above the objectives of this study were:

- To observe the Body condition score and body temperature of *Anaplasma* affected cows.
- To evaluate the values of stress indicators (C-Reactive Protein and Ferritin) in blood samples.

CHAPTER II

REVIEW OF LITERATURE

Abdela et al. (2018) performed this cross-sectional study was therefore undertaken from November 2016 to April 2017 with the objectives to assess the prevalence and potential risk factors associated with bovine anaplasmosis and babesiosis and also to identify the vectors involved in transmission of these diseases in and around Jimma town, south western Ethiopia. A simple random sampling technique was employed for selecting a sampling unit and logistic regression was used to determine the association of hypothesized risk factors with positivity for bovine anaplasmosis and/or babesiosis. A total of 408 bovine blood samples were examined for the presence of either anaplasmosis or babesiosis by Giemsa staining technique and overall prevalence of 11.7% babesiosis and 6.1% anaplasmosis were determined. Two babesia species (2.2% *Babesia bovis* and 9.8% *B. bigemina*) and two anaplasma species (5.1% *Anaplasma marginale* and 1.2% *A. centrale*) were identified. Even though risk factors like age, body condition, management system, sex and presence of ticks were considered, only age ($p=0.006$) and body condition ($p=0.039$) were found to be significantly associated with anaplasmosis. Moreover, multivariable logistic regression analysis showed statistically significant association of babesiosis with age ($p=0.003$), body condition ($p=0.012$) and presence of ticks ($p=0.005$). For both infections the mean PCV of infected animals was significantly ($p<0.05$) lower than non-infected animals. Similarly the mean body temperature of infected animals was significantly ($p<0.05$) higher than non-infected animals.

Abdullah et al. (2020) found that *Anaplasma* infected sheep displayed the following clinical signs: paleness of the mucous membrane, bloody diarrhea, emaciation, pyrexia, jaundice, nasal discharge, coughing, loss of wool, nervous signs, hemoglobinuria, and lacrimation. The prevalence of *Anaplasma* infection was 66.19%, and female sheep were significantly ($p<0.05$) more infected than male sheep. The hematological and biochemical parameters were significantly different between *Anaplasma* positive and *Anaplasma* negative sheep.

Altug et al. (2018) investigated haematological parameters, serum concentrations of antioxidant vitamins, minerals and trace elements as well as serum glutathion peroxidase (GSH-Px) and superoxide dismutase (SOD) activities before treatment and 7

days after in infected animals and compared to control values from 10 healthy cattle. In the theileriosis affected cattle, remarkable decreases in haematocrit values, haemoglobinaemia and platelet counts were associated to decreased serum α - / γ -tocopherol, vitamin C, vitamin B12, Ca, Mg, Zn, Na, GSH-Px and SOD levels and to increased sideraemia. The iron concentrations remained elevated and some antioxidants (α - / γ -tocopherol and vitamin C concentrations and GSH-Px and SOD activities) decreased in all treated cattle. Haematocrit values, haemoglobinaemia and vitamin B12 concentrations also remained depressed but maximal increases were recorded in cattle receiving vitamins B as supportive therapy (group 2). In parallel, platelet numeration was significantly restored in all groups, but specifically in group 2. On the other hand, the maximal increases in α - tocopherol and Zn concentrations recorded in groups 3 and 4, respectively, were not associated with cure of anaemia.

Arslan et al. (2018) carried out this study to reveal the importance of procalcitonin, C reactive protein (CRP), nitric oxide levels, and adenosine deaminase activity in the diagnosis and prognosis of the disease in naturally infected sheep with *Babesia ovis*. Thirty sheep diagnosed clinically and parasitologically as having *Babesia ovis* were allocated to 2 groups. The first group was treated only with imidocarp dipropionate and the second group with imidocarp dipropionate and flunixin meglumin. On the seventh day after treatment, blood samples were collected again from the sheep in the babesiosis-infected group and the treatment responses were assessed. Serum PCT (1.72 ± 0.34 ng/mL, $P < 0.01$), CRP (101.42 ± 11.73 μ g/mL, $P < 0.001$), NO (15.77 ± 2.75 μ mol/L, $P < 0.01$), and ADA (13.92 ± 0.88 IU/L, $P < 0.01$) were higher in sheep with babesiosis than in the healthy sheep (0.49 ± 0.04 ng/mL, 49.46 ± 4.57 μ g/mL, 8.15 ± 0.63 μ mol/L, 9.34 ± 1.19 IU/L, respectively). When PCT, CRP, NO, and ADA before treatment and after treatment in the infected sheep were compared, the levels of these parameters except for ADA in the second group were determined to have statistically decreased after the treatment. As a result, it has been concluded that the measurements of PCT, CRP, NO, and ADA in sheep with babesiosis may be useful for the diagnosis and prognosis of the disease when assessed in association with clinical examination.

Das et al. (2022) recorded an overall prevalence of *A marginale* infection in cattle was 15.71%. The prevalence of *A. marginale* infection was highly associated with age, sex, breed and tick infestation status of animals, floor system and management of farms, and season. The mean values of total erythrocyte count (TEC), haemoglobin (Hb), packed

cell volume (PCV), total platelet count, total protein, albumin, superoxide dismutase (SOD), glutathione (GSH), total antioxidant capacity (TAC), copper (Cu) and zinc (Zn) were significantly ($P < 0.05$) lower, whereas the mean values of mean corpuscular volume (MCV), aspartate aminotransferase (AST), total bilirubin, indirect bilirubin, blood urea nitrogen (BUN), creatinine and lipid hydroperoxide (LPO) were significantly ($P < 0.05$) higher in cattle infected with *A. marginale*.

Hamid et al. (2014) performed this experiment on 100 cows aged from (1-6) years. These animals suffered from the major symptoms of anaplasmosis as fever (41°C), enlargement lymph node and drop in milk yield, emaciation and anemia. Blood samples were collected from all animals on EDTA for microscopic examination and PCR for diagnosis of anaplasmosis. *Anaplasma marginale* (*A. marginale*) was detected by PCR in blood samples obtained from cattle supposed to be infected. The assay employs primers specific for the gene encoding *A. marginale* specific primers derived from major surface protein 5 (msp5) gene. The PCR products for 26 positive samples were subjected to sequence (Labtechnology, Egypt) and BLAST analysis was used for identification of the genomic DNA of these parasites. Changes associated with *A. marginale* in these cattle particular emphasis to the oxidative stress the reduce TAC level may reflect a decrease in antioxidant capacity and CBC change.

Jassem et al. (2015) designed this study to investigate the prevalence of bovine anaplasmosis among cattle from various areas in Wassit governorate; the investigation was performed on 184 blood samples collected from suspected cattle suffering from fever (41°C), severe anemia, pale mucus membrane, progressive emaciation and drop in milk yield, including 85 male and 99 female cattle, aged from < 1 year to > 2 years, the samples were collected during the period of October 2012 - April 2013 from AL-Kut, AL-hayy, AL-Bashair, AL-Moufaqia and AL-Noamania areas to investigate antibodies against *Anaplasma* parasite by using indirect enzyme-linked immunosorbent assay (ELISA) test and to determine the species of genus *Anaplasma* by using RFLP PCR technique and by also measuring some biochemical parameters to indicate the effect of the disease on liver function. The results of ELISA test showed that the rate of infection was 13.04%, the rate of infection was different between age groups and were 8, 11.25 and 16.45% in ages < 1 , 1-2 and 2 - 3 years, respectively. The study revealed that females were given higher percent of infection 14.14% than males 11.7%, there is no significant differences under $p > 0.05$ according to age groups and sex. The highest rate

of infection was recorded in AL-Kut, 17.14% followed by AL-hayy, 14% and AL-Bashair, 10% and the lowest rate was recorded in AL- Noamania, 8.33% and AL-Moufaqia, 5%; the study showed significant differences in incidence of disease between study districts and area in Wassit governorate at $p > 0.05$. The most sensitive method for the diagnosis of anaplasmosis is the method of polymerase chain reaction, DNA extraction was performed only on 24 blood samples which were positive for *Anaplasma spp.* by ELISA test, the extracted DNA from blood cells were analyzed by PCR and PCR-RFLP technique using primers derived from 16S rRNA gene and restriction endonuclease Bst1107I enzyme which can recognize the sequence (GTATAC) in corresponding PCR product of *Anaplasma marginale* and cut it in the position 68 and 509, whereas the used restriction enzyme cannot cut the corresponding PCR product of other *Aanaplasma spp.* and the result was 20 from 24 which were positive for *Aanaplasma spp.* by PCR and 18 from 20 was positive for *A. marginale*. The results of liver enzymes activity showed significant increase in serum AST, ALT, CK and TBIL level in infected cattle (96.8 ± 0.97 , 42 ± 0.52 , 406 ± 2.06 and 0.95 ± 0.24 , respectively) as compared to the control (65.5 ± 1.26 , 21.4 ± 0.45 , 142 ± 14.17 and 0.27 ± 0.05 , respectively).

Khaki et al. (2018) determined the biochemical and acute phase proteins changes in sheep experimentally infected with *Anaplasma ovis* (*A. ovis*). One Iranian sheep naturally infected with *A. ovis* (parasitemia 0.02%) but with no other blood parasites based on blood smear and polymerase chain reaction methods was selected as donor, and it was splenectomized to induce high level of parasitemia. Then, three weeks after splenectomy when parasitemia was 6%, donor's blood was intravenously administered to each recipient animal. Five 5-6 months old Iranian male sheep without any blood parasites were selected as recipient animals. The percent of parasites, packed cell volume, serum biochemical parameters (urea, creatinine, bilirubin, aspartate aminotransferase activity, cholesterol, total protein, albumin, globulin, Fe), acute phase proteins (haptoglobin, total iron binding capacity, fibrinogen), were evaluated in sheep before and after being experimentally infected with *A. ovis* (until day 38). In addition, body weights of sheep were measured on days 0, 20 and 38. In recipient sheep, microscopic examination of erythrocytes revealed a significant rise of parasitemia on days 12 and 15. The lowest level of packed cell volume in sheep was seen on day 15 post infection. A significant rise existed in mean urea and bilirubin (total, direct and indirect) on days 15 and 20. The increase of indirect bilirubin level was higher than direct

bilirubin. Furthermore, serum Fe significantly increased on days 20 and 23. The mean total protein concentration significantly increased on day 38. A significant increase was found in the serum globulin concentration from days 20 and 27 to 38. Maximum values of haptoglobin were observed on days 27 and 30. Moreover, aspartate aminotransferase activity (from days 20-30) and cholesterol concentration (on day 20) significantly decreased. However, no significant changes were found in other parameters. Experimental ovine anaplasmosis caused by *A. ovis* could be associated with some changes in measured parameters, which presumably could be helpful for evaluation on staging of disease.

Koebel *et al.* (2012) Human granulocytic anaplasmosis (HGA) is a tick-borne infection characterised by an acute, nonspecific febrile illness. To date, few clinical cases have been supported by both a positive polymerase chain reaction (PCR) assay and subsequent seroconversion against *Anaplasma phagocytophilum* antigen all over Europe. We report here 3 consecutive cases of Human granulocytic anaplasmosis (HGA) that occurred during the summer of 2009 which fulfilled the epidemiologic, clinical, and biological criteria for HGA. These data highlight PCR assay on ethylenediaminetetraacetic acid blood rather than serology as the diagnostic test of choice during the acute phase of the disease. In endemic areas, HGA should be investigated in patients presenting an undifferentiated febrile illness with cytopenia, elevated rates of liver enzymes, and increased C- Reactive Protein values.

Markos *et al.* (2018) determined the overall prevalence of bovine anaplasmosis and its associated risk factors in and around Wolaita Sodo town. A total of 384 blood samples were collected from randomly selected cattle in the study area. Age, sex, origin, breed, body condition, tick infestation and previous treatment were considered as risk factors in the study. Giemsa stained thin blood smears were prepared and examined under oil immersion lens (100x) of microscope for the presence of anaplasma based on morphology and position within RBC. An overall prevalence of bovine anaplasmosis was 11.46% and *A. marginale* was relatively most prevalent species (7%), then *A. centrale* (2.6%) and co-infection of both species (1.8%). The variation of animal origin with the prevalence of disease was not statistically significant ($P>0.05$). In addition the association of breeds, sex, age and body condition with the prevalence of anaplasmosis was not significant ($P>0.05$). Animal infested with tick was more exposed to the infection of the disease than that of non-infested animals. As a result tick infestation was

significantly ($p < 24$) was also significantly correlated with incidence of bovine anaplasmosis compared with non-anemic animals ($PCV \geq 24$). In conclusion, bovine anaplasmosis is one of the important tick borne diseases of cattle that present in the study area. Therefore, appropriate disease control measures should be done to improve livestock production.

Ola-Fadunsin et al. (2021) aimed at determining the prevalence, patterns of infections and risk factors associated with haemoparasitic infections of cattle in Ilorin, Nigeria. It is also aimed at determining the effect of *Anaplasma marginale* infection on the haemato-biochemical profiles of White Fulani cattle. Blood samples were collected from 478 apparently healthy cattle from both farms and slaughterhouses. The collected blood samples were subjected to parasitological, haematological, and biochemical analyses. The study revealed the presence of six haemoparasites: *Anaplasma marginale* (25.3%), *Babesia bigemina* (22.6%), *Babesia bovis* (12.1%), *Trypanosoma sp.* (8.6%), *Theileria sp.* (7.1%), and *Anaplasma centrale* (5.2%). The haemoparasite species co-infections rate decreased with increase in the number of co-infections. Breed, body condition score, level of packed cell volume (PCV) and presence of ticks were the risk factors significantly associated ($p < 0.05$) with the occurrence of *A. marginale* infection, while breed, age, body condition score, physiological status, PCV and presence of ticks were significantly associated ($p < 0.05$) with the occurrence of *B. bigemina* infection. Haemoglobin concentration, PCV, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), albumin, blood urea nitrogen, calcium, glucose, total bilirubin, and total protein were the haematological and biochemical parameters significantly associated ($p < 0.05$) with *A. marginale* infection in the White Fulani breed of cattle.

Sajid et al. (2014) conducted this study in 836 cattle [*Bos (B.) indicus* and *B. taurus* x *B. indicus*] and 700 buffaloes (*B. bubalis bubulus*) ($n=1536$) of district Khanewal, Punjab, Pakistan, in order to determine the epizootiology of anaplasmosis through conventional optical microscopy of Giemsa's stained blood films. With an overall prevalence of 4.17% (64/1536); the distribution of anaplasmosis was higher in buffaloes than cattle, calves than adults, females than males. Anaplasmosis in selected animal population was found statistically associated with the breed, season, animal keeping, housing system and hygienic management. The collected information provides the first report of anaplasmosis in the study population of the region. The data will not only be helpful for

the dairy farmers to modulate farming practices but also for the policy and decision makers to control the nuisance in the livestock population of the district. However, use of modern molecular tools is suggested for differential diagnosis of *Anaplasma* from other haemoprotozoa like *Babesia* and *Theileria* on a wider geographical spectrum.

Sharma et al. (1986) Changes in histamine, serotonin, cortisol and cortisol binding globulin (CBG) levels were observed in *Anaplasma marginale*-infected bovine calves. In addition, packed cell volume (PCV), platelet number and percentage parasitaemia were also recorded. The whole blood histamine and serotonin values rose significantly ($P < 0.01$ and $P < 0.025$, respectively) during the acute stage of *Anaplasma* infection. Higher serum cortisol and CBG levels ($P < 0.05$) were observed in acute and carrier infections, respectively. A sharp drop in thrombocyte count (59%) and PCV (33%) was also noticed in clinical anaplasmosis. The results suggest that the higher levels of biogenic amines which are known to produce increased vasodilation, capillary permeability and tissue anoxia and hypercortisolaemia to protect animals from stress and cell damage may play a similar role in the pathogenesis of acute anaplasmosis.

Shaukat et al. (2019) investigated the prevalence related associated risk factors and hematological changes (due to bovine anaplasmosis) were studied in Sahiwal cattle and crossbred cattle of the district Faisalabad of Punjab province, Pakistan. Therapeutic efficiency of Oxytetracycline- Imidocarb dipropionate combination was compared with Enrofloxacin-Imidocarb dipropionate against *Anaplasma marginale* (*A. marginale*). For this study, the blood samples of three hundred and sixty nine cattle were collected. Giemsa stain of blood smears showed 10.84% infection of cattle with *Anaplasma*. *A. marginale* positive cases were further confirmed through PCR. Among risk factors, age ($\chi^2 = 19.35$, P value=0.001), breed ($\chi^2 = 29.08$, P value=0.000), frequency of acaricidal treatment ($\chi^2 = 18.56$, P value=0.001), number of cleaning times ($\chi^2 = 16.11$, P value=0.002), feeding system ($\chi^2 = 23.41$, P value=0.001), floor pattern ($\chi^2 = 17.98$, P value=0.000) and hygienic measures ($\chi^2 = 25.79$, P value=0.001) significantly influenced the incidence of disease in the cattle of district Faisalabad. *A. marginale* induced statistically significant reduction was observed in RBC count, Hb, MCV, PCV and MCHC in infected cattle compared to healthy animals ($P < 0.05$). Anaplasmosis infected animals more effectively treated with combination of Oxytetracycline (22mg/kg, I/V, once/day for 5 days) and Imidocarb dipropionate (5mg/kg, I/M, twice 7 days apart) compared to Enrofloxacin (12.5 mg/kg, I/V, once/day for 5 days) and Imidocarb

dipropionate (5mg/kg, I/M, twice 7 days apart) combination.

Silva et al. (2013) observed that pregnancy and lactation influenced significantly ($p < 0.05$) in the seropositivity of the animals. *Bos indicus* animals had 5.21 times more chances of being seropositive than *B. taurus* animals. Primiparous female had 88% more chances of being seropositive than pluriparous female. Animals with high milk production were 63% more positive than low production animals. When infested by ticks the animals had 39% more chance of being seropositive to *A. marginale*. *Bos indicus* animals presented 5.21 times more chance of being seropositive than *B. taurus* animals. Primiparous females presented 88% more chance of being seropositive than the pluriparous ones. High milk production animals were 63% more positive than the low production ones. When infested by ticks the animals had 39% more chance of being seropositive to *A. marginale*. High density grazing provided for the animals 3.2 times more chances of being seropositive than low density ones. The herd was classified as erratic to *A. marginale*, even being placed in a steady enzootic area.

Silva et al. (2014) investigated the relationship between some risk factors and the seroprevalence of *A. marginale* in dairy cows during peripartum in Rio de Janeiro, Brazil. The risk factors analyzed in association with the prevalence of antibodies against *A. marginale* in dairy cows were calving season, reproductive experience, breed standard, tick infestations, stocking density, and milk yield. The antibodies against *A. marginale* were tested in indirect enzyme-linked immunosorbent assays. A primary screening using a $2 \times k$ contingency table of the exposed variables with the outcomes was performed. All variables for which $p < 0.20$ were included in a fixed effects log regression. The risk factors investigated to anaplasmosis were calving (OR 2.61, IC 1.08–7.63), breed standard (OR 3.83, IC 0.08–0.28), reproductive experience (OR 33.7, IC 2.14–5.16), milk yield (OR 3.9, IC 2.24–7.03), *Rhipicephalus microplus* infestations (OR 10.3, IC 0.05–0.17), and stocking density (OR 22.3, IC 0.05–0.17). Low titers of antibodies against *A. marginale* during peripartum had been characterized as a period previous to development of clinical anaplasmosis. Thus, studies on anaplasmosis should consider each farm as an epidemiological unit, where environmental and immunological factors may influence the endemic status of the pathogen.

Sivajothi and Reddy (2022) screened cattle with fever, weakness, reduced milk yield, loss of body weight, constipation followed by diarrhea and pale mucous membrane with

loss of milk yield for haemoprotozoan diseases during a herd visit. The cattle were subjected to Giemsa stained peripheral blood smear microscopic examination. Out of 31 cattle examined 14 were found to be positive for *Anaplasma marginale*. Clinical examination revealed high rectal temperature, tachycardia, tachypnea, anorexia, pale mucous membrane, weakness, reduced rumen motility, enlarged lymph nodes, and yellowish mucus membranes. Hematology revealed low levels of hemoglobin, packed cell volume, total erythrocyte count, and leucocytosis. Serum biochemical analysis showed elevated levels of ALT, ALP, BUN, total bilirubin and reduced levels of serum albumin.

Dumler *et al.* (2007) described Human granulocytic anaplasmosis (HGA) is a tick-borne disease caused by the intracellular bacterial pathogen *Anaplasma phagocytophilum*. Patients typically present with non-specific symptoms, including high fever, headache, malaise, and myalgias. Laboratory investigations often reveal leukopenia, thrombocytopenia, and an elevation in serum hepatic aminotransferases. A marked elevation in ferritin with or without other features of hemophagocytic/macrophage activation syndrome has been occasionally reported in patients with *A. phagocytophilum* infection. A case of HGA is described in which the patient had an elevated ferritin of 1964 µg/L. For patients presenting with features of hemophagocytic syndrome including a markedly elevated ferritin, infection with *A. phagocytophilum* should be considered in the differential diagnosis in the appropriate clinical context (i.e., at-risk geographic location, season, tick exposure) as this distinction has management implications.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area and study period

This study was conducted in Dinajpur sadar upazila of Dinajpur district during the period of January 2023 to December 2023. A total of 20 dairy farms were selected for this study. The laboratory work was performed in the laboratory of Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.2 Grouping of animal

The selected animals for this study were grouped into two groups. In Group A, total 10 healthy cows were taken those were tested negative to microscopic examination. On the other hand, a total of 10 cows were tested positive to microscopic examination placed into Group B.

3.3 Diagnostic procedures

3.3.1 History taking from the farmer

- A detailed history was taken including cow factors such as age, breed, lactation no., stage of lactation, daily milk production, treatment, etc.
- Records of treatments and outcomes were observed (If present).

3.3.2 General Examination

- General appearance were carefully observed such as Alertness, posture and gait, skin and hair, body condition, Chewing cud or not.
- Head, eyes, ears, muzzle, legs, tail, udder, mucous membranes etc. were also examined.

3.3.3 Clinical Examination

- Suffered from fever,
- Progressive emaciation and loss of body weight,
- Reduced milk yield,
- Constipation followed by diarrhea,
- Pale mucous membrane.

3.4 Laboratory Examination

3.4.1 Sample collection

About 2.5 ml of peripheral blood samples were collected from different dairy farms Dinajpur Sadar Upazila, Dinajpur. All the blood samples were collected aseptically within Ethylene Diamine Tetra Acetate (EDTA) tube and clot activator tube. After collection, the samples were immediately shifted to the laboratory of Physiology and Pharmacology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.4.2 Transportation and Preservation of Samples

The samples were immediately carried out to the laboratory for microscopic examination and biochemical analysis.



Figure 01: Collection of blood samples

3.4.3 Identification of blood protozoa through Giemsa's stain method

Giemsa stained blood smear microscopy was used to evaluate blood samples. In this study, the effects of geography and season were kept in consideration.

3.4.3.1 Equipment and appliances

- Blood
- 10% Giemsa stain
- Alcohol
- Tape water
- Distilled water
- Clean slides
- Cover slips
- Microscope

3.4.3.2 Procedure of Giemsa Staining Method

Giemsa staining was used to create thick and thin blood smears.

- On a clean dry microscopic glass slide, a thin film of collected blood sample was made and air dried.
- Dipped the smear (2-3 dips) into pure methanol for fixation of the smear, air dried for 30seconds.
- Flooded the slide with 5% Giemsa stain solution for 20-30 minutes.
- Flush with tap water and leave it to dry. The blood protozoa were identified microscopically using the methods given by Benjamin (1978) and Soulsby (1982).

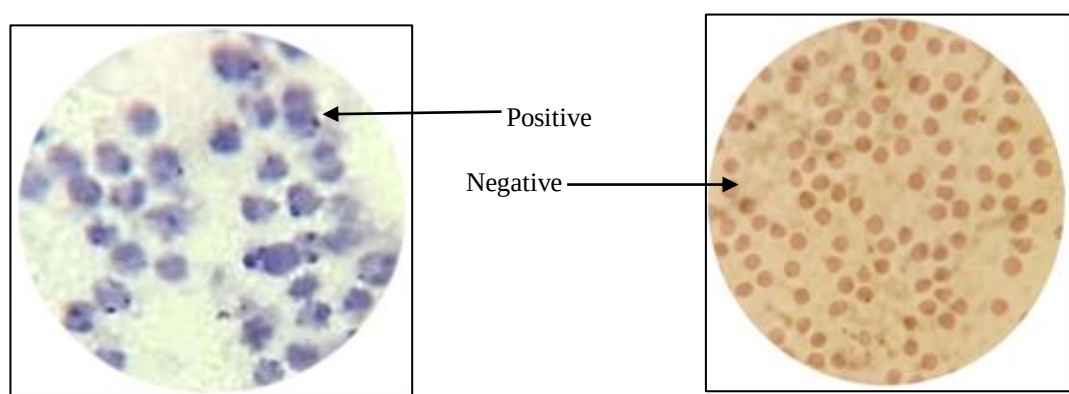


Figure 02: Identification of blood protozoa

3.5 Body Condition Score

Body condition score of the control and affected animal were determined by observing different physical parameters of the cow.

Body Condition Score = 2.0

This cow is too thin and is hopefully rarely seen on a farm. This cow will not milk well or reproduce. This cow probably isn't healthy. The vertebrae, short ribs, hooks, pins, and tail head are very sharp and visible. One-half of the length of the transverse processes is visible. The ligaments are easily seen and the thurl area is very dished. The area around the tail head is very dished and there are folds of skin seen between the tail head and pins.

Body Condition Score = 3.0

This cow is very thin, causing low milk production and poor reproduction. Health may be okay. The spine and short ribs can be easily seen, but the individual vertebrae are not real apparent. The short ribs appear scalloped. The upper surfaces of the short ribs can be felt. One-half to 1/3 of the length of the transverse processes is visible. The hooks and pins stand out and the thurl area is very dished. No fat can be felt on the pin bones. The ligaments are sharp and easily seen. The area around the tail head is very dished and there are folds of skin seen between the tail head and pins.

Body Condition Score = 4.0

It is a reasonable goal not to have more than 10% of the herd scoring 2.5 or less. This is the lowest acceptable condition score. A cow with a score of 2.5 has vertebrae showing but they cannot be seen as individual bones. The short ribs can be counted but are not scalloped. One-third to ¼ of the length of the transverse processes is visible. The ligaments are easily seen but not as sharp as with a BCS of 2.0. Both the hooks and pins are angular but some fat can be felt on the pin. The thurl area is dished. The area around the tail head is dished.

Body Condition Score = 5.0

This cow could be a healthy, high-producing cow. But, if a cow calves in at a score of 3.0 or less, she may not have enough body fat to use for high peak milk production and to carry her through until dry matter intake increases. At this score the dish of the rump (thurl area) is at the transition between looking like a “U” and looking like a “V”. Any cow under a BCS of 3.0 has a thurl area which looks like a “V”. The backbone is seen but the individual vertebrae are rounded. Covering the short ribs is ½ to 1-inch of flesh. Less than ¼ of the length of the transverse processes is visible. There is fat covering the ligaments but they are still obvious. The hooks and pins have some fat that can be felt. The area around the tail head is dished but no folds of skin are seen.

Body Condition Score = 6.0

Dry cows and calving cows should have a body condition score of 3.5. On this cow, fat can be felt on the backbone, short ribs, and ligaments. The hooks and pins are rounded. No individual transverse processes can be seen. The thurl is somewhat dished. The coccygeal (tail head) ligament is barely visible but the sacral ligament can still be seen. The area around the tail head is rounded and filled in but not fat.

Body Condition Score = 7.0

Cows calving in at this condition will eat less, lose more weight, and have more metabolic problems. This cow's back is flat because of the fat that has filled in. The short ribs cannot be seen as individuals but they can just barely be felt. The hooks and pins are obviously fat. The "U" between the hooks and pins is very flat with no depression. The ligaments cannot be seen. The area around the tail head is filled in and folds of fat are seen.

Body Condition Score = 9.0

This cow is extremely fat and will have metabolic and breeding problems. The backbone and short ribs cannot be seen and are hard to feel. The hooks and pins are buried in fat and hard to feel. The thurl is totally filled in. The tail head is buried in fat. (Klopčičet *al.*, 2011).

3.6 Body Temperature Measurement

Rectal temperature of the cows were taken by using Mercury thermometers.

3.6.1 Equipment

- Rectal thermometer,
- Watch,
- Vaseline.

3.6.2 Procedure

- The cows were properly restrained.
- A small amount of lubricant were placed on the end of the thermometer,
- Elevated the tail and the thermometer was inserted into the rectum,
- The thermometer was placed at 45°
- Kept it in the rectum for approximately 2 minutes,
- Removed the thermometer and wiped off lubricant and any feces,
- The results were recorded.

3.7 C-Reactive Protein (CRP) Test

The quantification of serum C - Reactive Protein (CRP) was undertaken using an ultrasensitive CRP commercial kit (Thermo Fisher Scientific Oy: Ratastie 2, FI-01620 Vantaa, Finland) following the manufacturer's protocol. All assay steps were performed automatically on anIndiko™ Plus Clinical Chemistry Analyzer with results expressed as mg/dL.

3.8 Ferritin Test

The concentrations of ferritin was undertaken using a commercial kit (BioMérieux India Private Limited43A, Okhla Phase-3, New Delhi – 110020, INDIA) following the manufacturer's protocol. All assay steps were performed automatically on amini VIDAS® Compact multiparametric immunoanalyzerwith results expressed as ng/mL.

3.9 Data analysis

All the collected data were analyzed by using statistical analytical software SPSS (version 29.0.1.0). Associations between explanatory variables i.e. body temperature, body condition score, C-Reactive protein (CRP) and Ferritin were done by paired sample T test. All significant differences were considered at 1% level of significance.

CHAPTER IV

RESULTS

The present study was conducted to determine the changes in the blood concentration of Ferritin and CRP in the blood of *Anaplasma* affected dairy cows compared to healthy cows considering their body temperature and BCS. A total of 20 cows from different dairy farms were observed during the study period of January, 2023 to December, 2023. For the detection of *Anaplasma* from collected blood samples were taken into the laboratory of Physiology and Pharmacology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur. Ferritin and C - Reactive Protein (CRP) test were performed at Check Up Diagnostics and Hospital, Dinajpur.

4.1 Body condition score (BCS) in *Anaplasma* affected cows

Body condition score is a very important indicator of physical and reproductive health of a cow. In this study we found significant association, the cows affected with *Anaplasma* progressively lose their body weight with a very poor BCS.

Table 01: Body condition score (BCS) in *Anaplasma* affected cows

	Mean $\pm$ Standard Error
<i>Anaplasma</i>	2 $\pm$ 0.447
Control	3 $\pm$ 0.316
Level of significance	*

* means significant at 1% level of significance ($P < 0.01$).

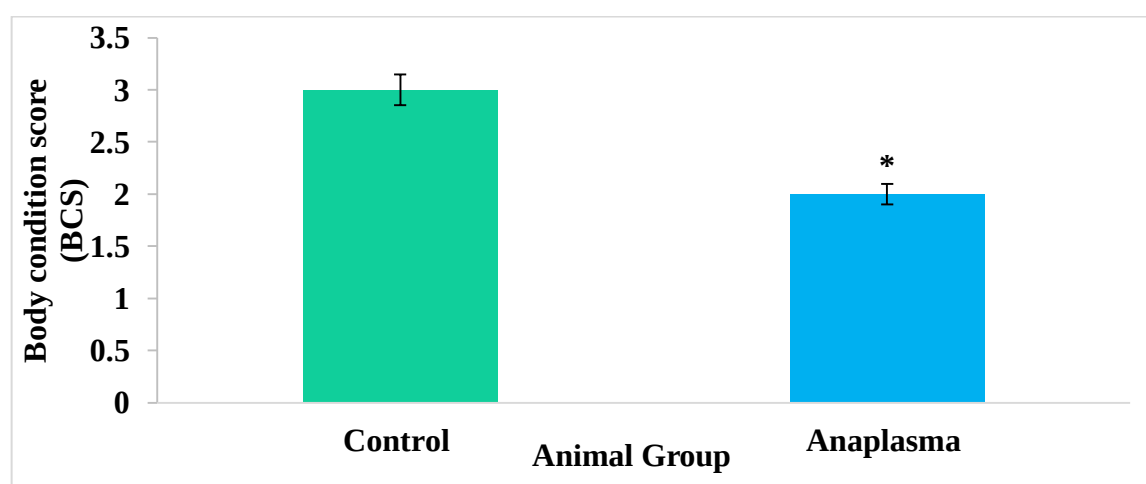


Figure 01: Body condition score in *Anaplasma* affected cows

Figure 01 the average Body condition score in control cow was 3 ± 0.316 and in *Anaplasma* affected cow was 2 ± 0.447 . There was significant variation in Body condition score.

4.2 Body temperature changes in *Anaplasma* affected cows

The body temperature of *Anaplasma* affected cattle were significantly higher ($p < 0.01$) than the temperature of healthy cows.

Table 02: Body temperature changes in *Anaplasma* affected cows

	Mean $\pm$ Standard Error
<i>Anaplasma</i>	103.04 $\pm$ 0.436
Control	101.84 $\pm$ 0.265
Level of significance	*

* means significant at 1% level of significance ($P < 0.01$).

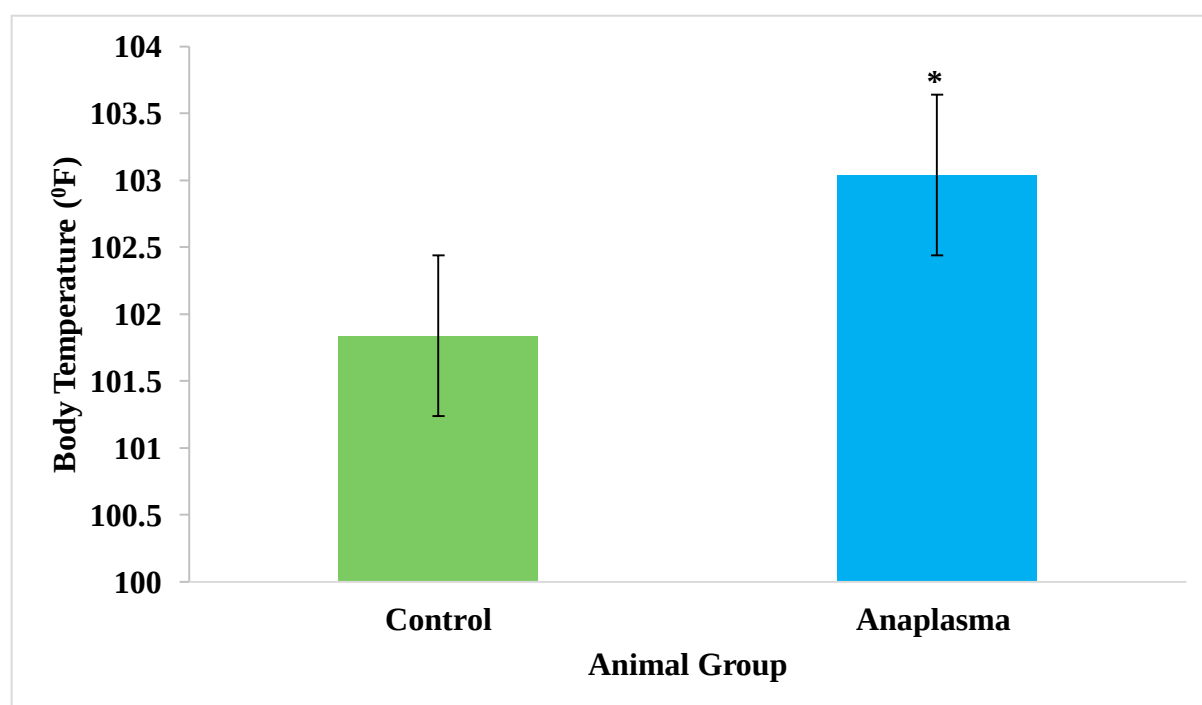


Figure 02: Body temperature changes in *Anaplasma* affected cows

Figure 02 the average temperature in control cow was 101.84 ± 0.265 and in *Anaplasma* affected cow was 103.04 ± 0.436 . There was significant variation in body temperature

4.3 Ferritin Test

The table no. 04 shows that the Ferritin level in the blood of *Anaplasma* affected cows were increased significantly than the non-affected healthy cows.

Table 03: Ferritin Test

	Mean $\pm$ Standard Error
<i>Anaplasma</i>	6.88 $\pm$ 0.34
Control	5.866 $\pm$ 0.22
Level of significance	*

* means significant at 1% level of significance ($P < 0.01$).

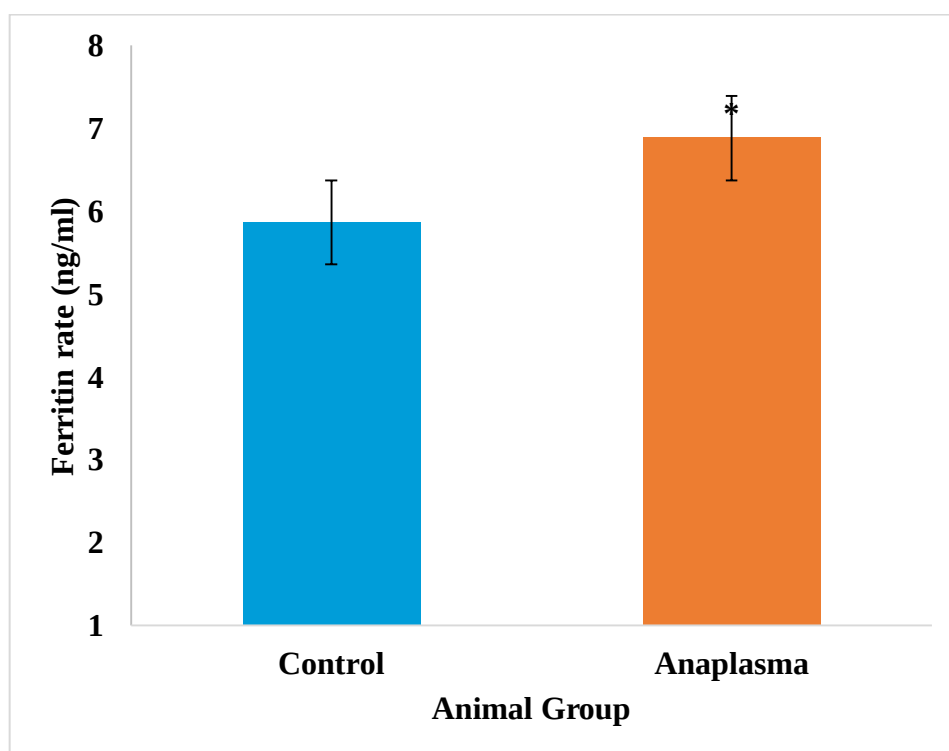


Figure 03: Ferritin Test

Figure 03 the average Ferritin rate in control cow was 5.866 $\pm$ 0.22 and in *Anaplasma* affected cow was 6.88 $\pm$ 0.34. There was significant variation in Ferritin test.

4.4 C – Reactive Protein (CRP) Test

The recorded CRP concentration of this study clearly showed that the CRP value were slightly higher in *Anaplasma* affected cattle but the result was not significant at 1% level of significance.

Table 04: C – Reactive Protein (CRP) Test

	Mean $\pm$ Standard Error
<i>Anaplasma</i>	1.6 $\pm$ 0.083
Control	1.5 $\pm$ 0.032
Level of significance	NS

NS = Non-significant

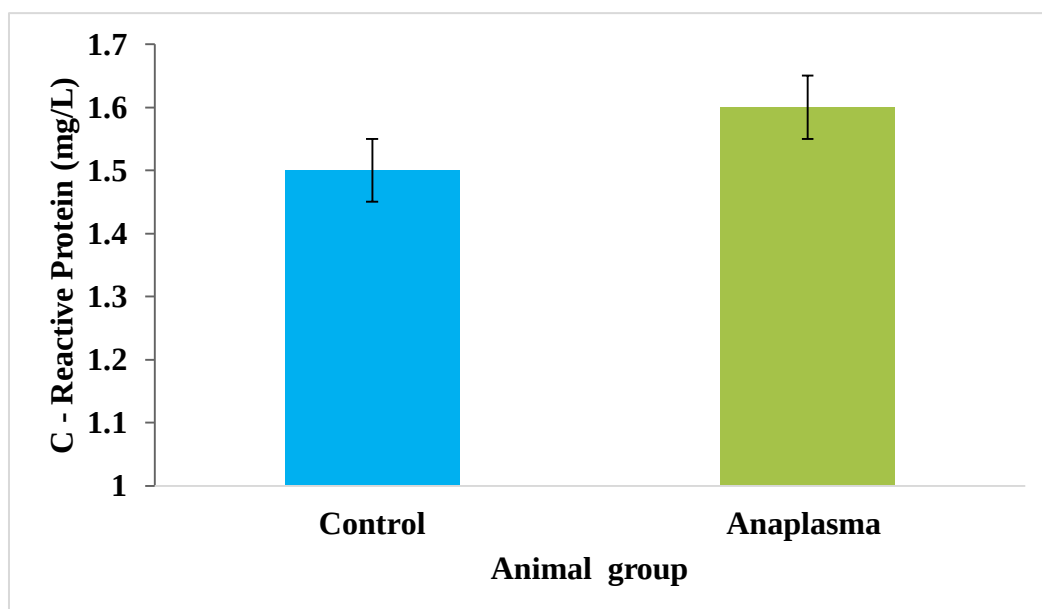


Figure 04: C – Reactive Protein (CRP) Test

Figure 04 the average CRP in control cow was 1.5 $\pm$ 0.032 and in *Anaplasma* affected cow was 1.6 $\pm$ 0.083. There was non-significant variation in C – Reactive Protein test.

CHAPTER V

DISCUSSION

The present study was aimed to investigate the body temperature and body condition score (BCS) as well as the concentration of C – Reactive Protein (CRP) as a stress indicator and level of ferritin in the blood of healthy and *Anaplasma* positive cows.

The present study showed that the body temperature was higher (>103 °F) in *Anaplasma* infected cows than that of healthy cows. Similar observation were reported by several authors Hamid *et al.* (2014); Jassem *et al.* (2015). Both of them reported 105.8 °F body temperature in *Anaplasma* infected cows along with other clinical signs and symptoms such as loss of body weight, enlargement of lymph node and drop in milk yield, emaciation and anemia.

In this study, the body condition score of *Anaplasma* infected cattle was very poor to moderate as compared with the healthy animals. These findings were in agreement with Abdullah *et al.* (2020); Jassem *et al.* (2015); Subronto (2007). These authors reported that the cows infected with *Anaplasma* progressively lose their body weight and emaciated which results into poor BCS.

The results of Ferritin test showed that the blood ferritin level is increased in case of *Anaplasma* infected cows compared to control. Walkty *et al.* (2019) found marked elevation of serum ferritin associated with *Anaplasma phagocytophilum* infection in human. Dumler *et al.* (2007) investigated a possible relationship between macrophage activation and infection with *A. phagocytophilum*. They determined the concentration of Ferritin and several cytokines in serum samples from 29 patients with HGA compared with 29 control subjects. The median serum Ferritin was 548 µg/L among patients versus 251 µg/L among controls ($p = 0.018$), and the serum ferritin level correlated with disease severity.

The CRP test reveals that C – Reactive Protein level is slightly higher but not significant as compared with the CRP level in the blood of control group containing healthy cows. These findings were supported by Koebele *et al.* (2012). He observed three patients affected with Human granulocytic anaplasmosis (HGA) and reported elevated C-reactive protein (CRP) values.

CHAPTER VI

CONCLUSION

From the present study it was concluded that the presence of *Anaplasma* infection in dairy cows increased the body temperature. Body condition score of *Anaplasma* infected cows were also poor as compared with the control group consists of *Anaplasma* negative healthy cows. The concentration of C – Reactive Protein were increased in blood. Marked elevation in the serum Ferritin level were also found in this present study. From the findings of the present study, it can be concluded that the presence of *Anaplasma* infection leads to stress in cows.

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