RESEARCH ARTICLE

Anaemia in Bovines: Haematobiochemical Alterations with Diagnostic and Clinical Significance

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Abstract

Anaemia in bovines is a multifactorial disorder influenced by nutritional deficiencies, parasitism, infectious agents, and metabolic imbalances. The present study was conducted to evaluate haematological and biochemical alterations associated with anaemia in bovines. Blood samples were collected from clinically suspected animals and compared with healthy controls. Haematological evaluation revealed significant reductions in haemoglobin concentration, packed cell volume, and erythrocytic indices in anaemic animals, confirming impaired erythropoiesis. Biochemical analysis demonstrated alterations in protein, iron, and related metabolic parameters, reflecting the underlying nutritional and pathological disturbances. These findings emphasize the complex etiology of anaemia in bovines and underline the importance of integrated diagnostic approaches for timely detection and effective management. The outcomes of this study contribute to a better understanding of bovine anaemia and provide a baseline for field diagnosis and preventive strategies.

Key words: Bovine anaemia, Nutritional deficiency, Parasitism, Iron metabolism, Veterinary diagnostics.

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Introduction

Anaemia is defined as a reduction in haemoglobin concentration, red blood cell count, or packed cell volume below the reference range, resulting in impaired oxygen transport and subsequent tissue hypoxia (WHO, 1972). In domesticated animals, particularly bovines, anaemia poses a significant challenge as it leads to both direct economic losses, through increased susceptibility to infections and mortality, and indirect losses due to reduced productivity (Chavai et al., 2001; Zahid et al., 2005).The condition arises from diverse etiological factors, which are broadly categorized into blood loss, hemolysis, and impaired erythrocyte production. Hemorrhagic anaemia is commonly associated with gastrointestinal and ectoparasite infestations, while hemolytic anaemia is frequently linked to tick-borne infections such as *Theileria* and *Anaplasma* spp. (Omer et al., 2002; Henniger et al., 2013). Nutritional deficiencies, particularly of iron, copper, cobalt, and selenium, are also recognized as significant contributors. Among these, iron deficiency is the most prevalent in calves owing to the inherently low iron content of bovine milk, which contains only about 0.5 mg/kg of iron (Matrone et al., 1957; Mann et al., 2013). Calves born in an iron-deficient state are more susceptible to diarrhoea, and studies have demonstrated significantly reduced haemoglobin, haematocrit, and red blood cell counts, along with decreased serum iron concentrations, in diarrhoeic calves compared to healthy ones (Prodanovic et al., 2019).

Clinically, anaemia in bovines is characterized by pale mucous membranes, tachyarrhythmia, dullness, exercise intolerance, bounding peripheral pulses, reduced ruminal contractions, and abdominal pain (Radostits et al., 2006). In cases of severe hemorrhage, additional findings such as haemoglobinuria, jaundice, petechiae, ecchymosis, and bleeding tendencies may also be observed (Dabak et al., 2007). Diagnosis is based on a combination of case history, clinical examination, and laboratory tests. Complete blood count remains fundamental, with haemoglobin concentration, red blood cell count, and packed cell volume serving as the primary parameters for grading anaemia. Red blood cell indices, including mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), further aid in classifying anaemia as microcytic/macrocytic and hypochromic/hyperchromic (Abutarbush, 2015). Reticulocyte counts help determine whether anaemia is regenerative or non-regenerative, and serial monitoring is important for evaluating the response to treatment. Management strategies depend on the underlying cause

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and may include fluid therapy to restore circulatory volume in cases of hemorrhage, etiology-specific interventions such as antiparasitic treatment, and supplementation of deficient micronutrients. Given the multifactorial nature of bovine anaemia and its substantial economic implications, the present study was undertaken to investigate its causes and the associated biochemical alterations in calves.

Materials and Methods

Selection of animals for study

Screening for anaemia was carried out in cattle and buffaloes maintained at the Livestock Production and Management Section, ICAR–Indian Veterinary Research Institute, Izatnagar, over a period of six months. Animals were included irrespective of age, breed, or sex. The screening protocol comprised detailed clinical examination along with the estimation of haematological and serum biochemical parameters. Based on the results, animals were categorized into three groups according to the severity of anaemia, and subsequent investigations were conducted accordingly.

Clinico-diagnostic aspects of anaemia in bovine

Clinical signs

1.2.1.1 Pale mucous membranes, dullness, exercise intolerance, and abdominal pain (tormina) are common clinical indicators of anaemia in bovines (Radostits et al., 2006).

During screening, both ocular and vulval mucous membranes were examined as an initial step. The overall health status of the animals was also assessed, with particular attention to conditions such as emaciation, reduced feed intake, exercise intolerance, diarrhoea (associated with sepsis), and external parasitic infestations like ticks. All observed findings were systematically recorded for each animal screened.

1.2.1.2 Tachyarrhythmia

The normal heart rate is approximately 60 beats per minute (bpm) in adult cattle and 120 bpm in calves (Stober, 1979). During screening, heart rate was recorded by auscultation behind the left elbow against the chest wall.

1.2.1.3 Bounding pulses (peripheral) normally 60 to 90 per minute (Abdisa and Tagesu., 2017). Pulse rate was assessed in all animals by palpating the coccygeal artery on the ventral aspect of the tail.

1.2.1.4 Ruminal Motility

Normal ruminal contractions occur at a frequency of 0.5–2 per minute (Wyburn, 1980; Constable et al., 1990; Cunningham, 1997). Ruminal movements in heifers and adult cattle were evaluated by placing a clenched fist into the left paralumbar fossa and monitoring the contractions.

Haematology

Blood samples (approximately 2 mL) were collected aseptically from the jugular vein into K_3-EDTA vacutainer tubes. Haematological analysis included haemoglobin concentration, haematocrit, total erythrocyte and leukocyte counts, differential leukocyte count, platelet count, reticulocyte count, and erythrocytic indices (MCV, MCH, MCHC).

Haemoglobin (g/dL):

Haemoglobin concentration was estimated in K_3-EDTA anticoagulated blood using the Acid Haematin method (Jain, 1986). Briefly, 20 µL of blood was added to 0.1 N HCl in a haemoglobin tube, mixed, and allowed to stand for 10 minutes. Distilled water was then added dropwise until the colour matched the Sahli's standard, and the reading was recorded in g/dL

Haematocrit (%) or Packed Cell Volume (PCV)

Haematocrit was determined from K_3-EDTA anticoagulated blood using the Wintrobe tube method (Jain, 1986). Blood was filled into the Wintrobe tube up to the 100 mark and centrifuged at 2300 rpm for 7–10 minutes. The packed red cell column was then measured and expressed as a fraction of the total blood volume.

Total Leukocyte Count(103/µL)

Total erythrocyte count was determined in K_3-EDTA anticoagulated blood using a Neubauer's counting chamber (Jain, 1986). Blood was diluted 1:200 with Hayem's fluid, loaded into the chamber, and erythrocytes were counted in the designated area. The final count was calculated by applying the dilution factor and expressed as 10^6/µL.

Differential Leukocyte Count

Differential leukocyte count was performed on thin blood smears fixed in methanol for 45–60 seconds and stained with Giemsa for 45 minutes. After washing and air-drying, smears were examined under oil immersion (100×), and 100 leukocytes were classified based on morphology and expressed as percentages (Jain, 1986).

Platelet count

Platelet count was estimated from thin blood smears fixed in methanol (45–60 seconds) and stained with Giemsa for 45 minutes. After washing and air-drying, smears were examined under oil immersion (100×). The number of platelets in 10 oil immersion fields was averaged and multiplied by 15,000 to obtain the platelet count/µL (Jain, 1986).

2.3.6. Erythrocytic indices (Adamson and Longo, 2001)

2.3.6.1 Mean Corpuscular Volume

MCV values are obtained by the formula; MCV (fl) = HCT(%)/RBC (millions/µL) × 10

2.3.6.2 Mean Corpuscular Haemoglobin

MCH values are obtained by the formula; MCH (pg) = Hb(g/dL)/RBC (millions/µL) × 10

2.3.6.3 Mean corpuscular Haemoglobin Concentration

MCHC values are obtained by the formula; MCHC (g/dl) = Hb(g/dL)/HCT (%) × 10

Enumeration of Reticulocytes (Deiss and Kurth, 1970)

Reticulocytes were identified using the supravital stain Brilliant Cresyl Blue (BCB). Equal volumes of K_3-EDTA anticoagulated blood and stain were mixed in a clean tube and incubated at 37 °C for 15–30 minutes. After incubation, thin smears were prepared, air-dried, and examined under oil immersion (100×). A total of 1,000 erythrocytes were counted, and reticulocytes were expressed as a percentage.

Blood Smear Examination for Haemoprotozoa (Jain, 1986)

Thin blood smears were prepared, fixed in methanol (45–60 seconds), and stained with Giemsa for 45 minutes. After washing and air-drying, smears were examined under oil immersion (100×) for the presence of haemoprotozoa across multiple fields.

Serum Biochemistry

Total Protein

Serum total protein was estimated by the Biuret–Damas method using commercial diagnostic kits, and results were expressed in g/dL

Albumin

Serum albumin concentration was measured by the Biuret–Damas method with commercial kits and expressed in g/dL

Creatinine

Serum creatinine was determined by the modified Jaffe's kinetic method using commercial kits and expressed in mg/dL (Frankel et al., 1970).

Urea Nitrogen

Serum urea was estimated by the modified Berthelot method and expressed in mg/dL. Blood urea nitrogen (BUN) values were obtained by multiplying urea concentration by 0.467.

Potassium

Serum potassium concentration was determined by the tetraphenylboron method with commercial kits and expressed in mEq/L.

Chloride

Serum chloride concentration was measured by the thiocyanate method with commercial kits and expressed in mEq/L.

Aspartate Aminotransferase (AST)

Serum AST activity was estimated by the modified IFCC method using commercial kits and expressed in IU/L.

Iron

Serum iron concentration was measured by the Ferrozine method with commercial kits and expressed in µg/dL.

Total Iron Binding Capacity (TIBC)

Serum TIBC was determined by the Ferrozine method using commercial kits and expressed in µg/dL.

Copper

Serum copper concentration was estimated by the colorimetric method using commercial kits and expressed in µg/dL.

Serum Ferritin

Serum ferritin concentration was estimated by sandwich ELISA using a commercial bovine ferritin (FTL) kit (Bioelsa). Standards were prepared by serial dilution to obtain four working standards, along with a negative control (blank). Samples, standards, and controls (100 µL each, in duplicate) were added to the antibody-precoated wells and incubated for 80 minutes at 37 °C. After incubation, wells were washed three times with diluted wash buffer.

Subsequently, 100 µL of biotinylated antibody working solution was added to each well and incubated for 50 minutes at 37 °C, followed by three washes. Then, 100 µL of streptavidin–HRP conjugate was added and incubated for 50 minutes at 37 °C, followed by five washes. Color development was achieved by adding 90 µL of TMB substrate, incubating for 20 minutes (protected from light), and stopping the reaction with 50 µL of stop solution. Optical density was measured at 450 nm using an ELISA reader. Serum ferritin concentration was determined by plotting absorbance values of the standards against their respective concentrations to generate a standard curve.

Statistical interpretation of the collected data:

Relationship in parameters between healthy & anaemic animals were analysed via multiple analysis of variance and t test, using IBM SPSS software as forwarded by Snedecor and Cochran, (1994).

Result and Discussion

Selection of animal

The incidence and etiology of anaemia in bovines were investigated at the Cattle and Buffalo Farm, ICAR–Indian Veterinary Research Institute, Izatnagar, over a six-month period. Out of 862 animals screened, 40 (4.64%) were identified as anaemic based on haemoglobin <8 g/dL and pale mucous membranes. From these, 18 calves were selected for the study along with 6 healthy controls. Haematological and serum biochemical parameters were assessed to evaluate the changes associated with anaemia, irrespective of etiology.

Clinical examination of animals

The major clinical signs observed in anaemic animals (n = 40) included pale mucous membranes (97.5%), dullness (72.5%), tachyarrhythmia (70%), anorexia (62.5%), increased pulse rate (57.5%), diarrhoea (20%), weight loss (15%), reduced rumen motility (7.5%), melena (5%), haemoglobinuria (5%), and recent antibiotic use (2.5%) (Table 1).Pale mucous membranes, the most consistent finding, reflect reduced erythrocyte or haemoglobin levels, in agreement with earlier reports (Katsoulos et al., 2017). Tachyarrhythmia and abnormal heart sounds are linked to decreased blood viscosity and turbulence in circulation (Radostits et al., 2006). Melena was observed in two cases, both positive for coccidiosis, supporting previous associations of haemorrhagic anaemia with parasitic infections (Ok et al., 2001; Xu, 1992). Chronic cases presented with weakness, anorexia, and poor weight gain, commonly seen in anaemia due to endoparasitism or micronutrient deficiencies (Giannitti et al., 2014). Notably, oxytetracycline use was identified in one animal, consistent with reports of drug-induced haemolysis (Chi et al., 2010). Haemoglobinuria, although rare, may indicate haemolytic pathology, often accompanied by abnormal urine colour or jaundice (Braun et al., 2008; Giannitti et al., 2014).(Fig 1)

Table 1: Clinical expositions of anaemia in bovine

Sl	Clinical signs	No. of cases	Percentage(%)
1.	Pallor of Mucus Membrane	39	97.5
2.	Anorexia	25	62.5
3.	Dullness	29	72.5
4.	Diarrhoea	08	20
5.	Melena	02	5
6.	Haemoglobinuria	02	5
7.	Weight loss	06	15
8.	Reduced Rumen motility	03	7.5
9.	Tachyarrhythmia	28	70
10.	Increased pulse rate	13	57.5
11.	Recent use of Antibiotics	01	2.5

Calf with septicaemic Diarrhoae

Pale mucous membrane

Fig.1: Clinical signs noticed in anaemic bovine

Etiology

The predominant causes of anaemia in bovines were tick infestation (52.5%; 21/40), iron deficiency (37.5%; 15/40), and sepsis associated with neonatal conditions such as joint ill, navel ill, and diarrhoea (10%; 4/40) (Table 2). Tick infestation and sepsis-related haemorrhagic anaemia were more common in calves than in adults, with a higher prevalence of tick infestation observed in males. The principal species identified was *Rhipicephalus* spp. Iron deficiency, particularly in calves fed exclusively on whole milk, has been well documented (Matrone et al., 1957; Andrews, 2004), which supports the present findings. Seasonal influence may also explain the higher tick incidence, as noted by Gasparin et al. (2007), with infestations peaking during summer (March–July), coinciding with the study period. Previous reports also indicate *Rhipicephalus* as the predominant tick species in cattle, consistent with the present observation (Singh et al., 2013).

Table 2: Aetiology based classification

Aetiology	No. of anaemic animals	Percentage (%)
Tick infestation	21	52.5%
Micronutrient deficiency	15	37.5%
Sepsis	04	10%

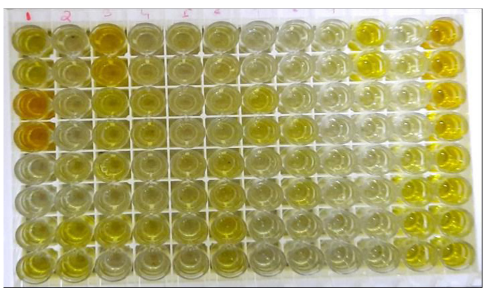

Fig. 2: Reticulocyte staining by Brilliant Cresyl Blue staining

Haematology

Haemoglobin

Anaemic animals showed a significantly lower haemoglobin concentration (5.80 ± 0.21 g/dL) compared to healthy controls (9.88 ± 0.26 g/dL) ($P < 0.05$) (Table 7; Fig. 10). A decline in haemoglobin concentration is a key diagnostic feature of anaemia (WHO, 1972), commonly arising from impaired erythropoiesis or increased haemolysis. Iron deficiency, particularly in calves fed exclusively on whole milk, is a major contributor due to the inherently low iron content of milk (Matrone et al., 1957; Andrews, 2004). Tick-borne haemoparasites such as *Theileria* spp. and *Anaplasma* spp. are well-recognized causes of haemolytic anaemia in bovines (Omer et al., 2002; Henniger et al., 2013). Additionally, toxicities (e.g., copper or nitrate), cruciferous plant ingestion, and certain drugs like oxytetracycline have been implicated in haemoglobin destruction and haemoglobinuria (Chi et al., 2010).

Haematocrit (PCV)

The packed cell volume (PCV) of anaemic animals was significantly lower (19.87 ± 0.69%) compared to healthy controls (30.60 ± 1.81%) ($P < 0.05$) (Table 3). PCV is a reliable indicator of anaemia severity, with values below 20% considered clinically anaemic and requiring therapeutic intervention (Weiss and Wardrop, 2010; Hunt and Moore, 1990). When PCV falls below 15%, tissue hypoxia due to inadequate oxygen delivery is expected (Divers, 2005). The present findings therefore reflect a clinically relevant anaemic state, consistent with previous reports in calves where lower haematocrit values are common during the early growth phase (Katsoulos et al., 2017).

Table 3 : Haematological parameters of anaemic bovine

Parameters	Healthy Control	Anaemic animals	t value	p
Haemoglobin (g/dl)	9.88±0.26	5.80±0.21	10.1	0.00
PCV (%)	30.60±1.81	19.87±0.69	6.8	0.00
TEC (*106/µL)	8.51±0.44	4.93±0.32	5.8	0.00
TLC (*103/µL)	10.40±0.52	14.11±0.96	2.1	0.021
Lymphocytes (%)	59.90±3.98	49.30±3.62	2.07	0.025
Neutrophils (%)	36.15±3.63	45.79±2.69	1.87	0.037
Monocytes (%)	3.18±0.59	3.23±0.34	0.811	0.468
Eosinophils (%)	0.66±0.21	1.75±0.95	0.64	0.262
Platelets (*103/µL)	442±41.92	446.38±43.15	0.55	0.47
MCV (fl)	44.10±1.64	36.46±1.32	3.06	0.03
MCH (pg)	12.53±0.29	11.20±0.36	2.00	0.02
MCHC (g/l)	320.50±6.29	277.72±8.02	2.93	0.04
Reticulocyte count (%)	0.40±0.05	1.14±0.09	3.69	0.02

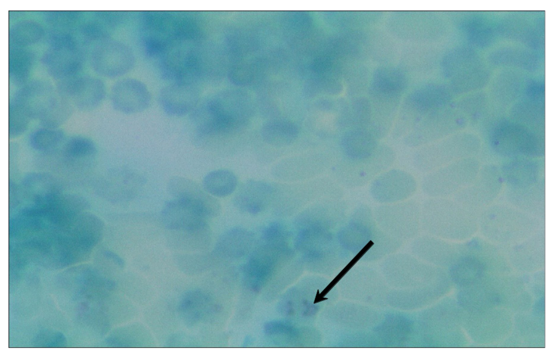

Fig. 3: Quantitative Estimation of Serum Ferritin by Sandwich ELISA

Total erythrocyte count

The total erythrocyte count (TEC) was significantly lower in anaemic animals (4.93 ± 0.32 × 10^6/µL) compared to healthy controls (8.51 ± 0.44 × 10^6/µL) ($P < 0.05$) (Table 3). Reduced erythrocyte counts in bovines are commonly associated with blood loss due to gastrointestinal parasites, ectoparasites, or haemoprotozoan infections (Van Aken et al., 1997; Favero et al., 2016). In the present study, haemorrhagic anaemia from tick infestation and coccidiosis appeared to be the primary contributors, particularly in calves under one year of age (Fox et al., 1991). Haemolytic conditions, oxidative injury to erythrocytes, and nutritional deficiencies such as iron, copper, or cobalt may further exacerbate the decline in TEC (Yagi et al., 2002; Shiono et al., 2003; Nazifi et al., 2008).

Total leucocyte count

The total leukocyte count (TLC) was significantly higher in anaemic animals (14.11 ± 0.96 × 10^3/µL) compared to healthy controls (10.40 ± 0.52 × 10^3/µL) (P < 0.05) (Table 3). Variations in leukocyte counts are often influenced by the underlying cause of anaemia (Katsoulos et al., 2017). The elevated TLC observed in this study may reflect inflammatory or parasitic etiologies, consistent with earlier reports. Anaemia-induced tissue hypoxia may further contribute to leukocytosis through stress-mediated glucocorticoid release and catecholamine sensitivity (Singh, 2010).

Differential leukocyte count

Neutrophil count was significantly higher in anaemic animals (45.79 ± 2.69%) compared to healthy controls (36.15 ± 3.63%), while lymphocyte count was significantly lower (49.30 ± 3.62% vs. 59.90 ± 3.98%) (P < 0.05) (Table 3). This reciprocal shift in neutrophil-to-lymphocyte ratio has been previously noted in anaemic animals (Hegde & Puranik, 2021). Such alterations are commonly associated with iron deficiency anaemia, where systemic inflammation and hepcidin expression promote iron sequestration and recycling, aggravating iron deficiency (Lopez et al., 2016). Monocyte and eosinophil counts showed no significant difference between groups.

Thrombocytes

Thrombocyte count did not differ significantly between anaemic (446.38 ± 43.15 × 10^3/µL) and healthy control animals (442 ± 41.92 × 10^3/µL) (P < 0.05) (Table 3). Anaemia associated with coagulation defects or persistent viral infections such as BVD has been linked to thrombocytopenia (Dabak et al., 2007; Katsoulos et al., 2017), but no such etiologies were evident in the present study. The observation of normal platelet levels agrees with reports that iron deficiency anaemia is typically associated with normal or elevated platelet counts (Morris et al., 2010).

Mean Corpuscular Volume (MCV)

MCV was significantly lower (P < 0.05) in anaemic animals (36.46 ± 1.32 fL) compared to healthy controls (44.10 ± 1.64 fL) (Table 3). Reduced MCV is generally associated with chronic conditions or micronutrient deficiencies, particularly iron (Abutarbush, 2015). The present findings, where iron deficiency anaemia was prominent, are consistent with these reports.

Mean Corpuscular Haemoglobin (MCH)

MCH was significantly reduced (P < 0.05) in anaemic animals (11.20 ± 0.36 pg) compared to healthy controls (12.53 ± 0.29 pg) (Table 3). Reduced MCH is a feature of microcytic, hypochromic anaemia, most commonly associated with iron deficiency, where all erythrocytic indices (MCV, MCH, MCHC) tend to decline (Carlos et al., 2018). Mohri et al. (2010) further reported that in calves, MCH and MCHC remain low up to one month of age and increase after three months, supporting the developmental variation observed in young animals (Table 3).

Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC was significantly lower (P < 0.05) in anaemic animals (277.72 ± 8.02 g/dL) compared to healthy controls (320.50 ± 6.29 g/dL), indicating microcytosis consistent with iron deficiency anaemia (Green et al., 1997). (Table 3).

Reticulocyte count

Reticulocyte count was significantly higher (P < 0.05) in anaemic animals (1.14 ± 0.09%) compared to healthy controls (0.40 ± 0.05%), indicating a regenerative response by the bone marrow (Katsoulos et al., 2017; Wood and Quiroz-Rocha, 2010). **(Table 3 and fig.4)**

Biochemical parameters

Serum ferritin

Ferritin concentration was significantly lower (P < 0.05) in anaemic animals (11.51 ± 0.25 µg/L) compared to healthy controls (17.79 ± 1.20 µg/L), indicating iron-deficient anaemia. Ferritin serves as a sensitive marker of body iron stores, declining early in iron deficiency even before changes in haemoglobin, PCV, or serum iron occur (Walters et al., 1973; Miyata and Furugouri, 1984). Reduced ferritin may result from insufficient dietary iron, rapid growth and erythropoietic demand in young calves, or chronic blood loss, whereas serum iron may remain unaltered in early deficiency or influenced by inflammation, malabsorption, or neoplastic disorders (Jain, 1986).**(Table 4 and fig.5).**

Table 4: Biochemical parameters of anaemic bovine

Parameters	Healthy Control	Anaemic animals	t value	p
Ferritin (µg/l)	17.79±1.20	11.51±0.25	7.8	<0.001
Iron (µg/dl)	90.98±5.52	56.54±3.67	4.81	<0.001
TIBC (µg/dl)	151.22±12.87	185.12±10.30	1.7	0.04
Copper (µmol/l)	20.33±1.47	16.94±0.54	2.71	0.006
Total protein (g/dl)	6.55±0.40	6.52±0.24	0.57	0.47
Albumin (g/dl)	3.06±0.10	2.69±0.11	0.58	0.19
Potassium (mEq/l)	3.92±0.19	3.62±0.21	0.74	0.32
Chloride (mEq/l)	101.59±2.04	103.13±2.00	0.41	0.34
BUN (mg/dl)	17.13±0.66	18.46±1.16	0.63	0.26
AST (IU/L)	60.32±7.25	60.26±2.95	0.06	0.49
Creatinine (mg/dl)	0.95±0.05	0.88±0.05	0.63	0.26

Serum iron

Iron concentration was significantly lower ($P < 0.05$) in anaemic animals (56.54 ± 3.67 μg/dL) compared to healthy controls (90.98 ± 5.52 μg/dL), indicating iron deficiency anaemia. In young calves, exclusive feeding on dam's milk, which contains only ~0.5 mg/kg of iron, is insufficient to meet the high demands of rapid growth and active erythropoiesis, predisposing them to iron deficiency (Matrone et al., 1957; Andrews, 2004; Mann et al., 2013). In the present study, 55% of calves aged 0–3 months were affected, supporting this observation. Additional contributing factors may include prenatal iron deficiency (Bostedt et al., 1990) and blood-feeding helminths (Ramin et al., 2012) (Table 4).

Total Iron Binding Capacity (TIBC)

TIBC concentration was significantly higher ($P < 0.05$) in anaemic animals (185.12 ± 10.30 μg/dL) compared to healthy controls (151.22 ± 12.87 μg/dL), indicating iron deficiency. Total iron-binding capacity reflects the amount of unbound transferrin in circulation; when body iron is low, transferrin remains largely unbound, elevating TIBC (Delpeuch et al., 1980; Graziadei et al., 1993) **(Table 4).**

Serum copper

Copper concentration was significantly lower ($P < 0.05$) in anaemic animals (16.94 ± 0.54 μmol/L) compared to healthy controls (20.33 ± 1.47 μmol/L), though values remained within the normal reference range for cattle (16–32 μmol/L). Copper plays a critical role in bone marrow haematopoiesis and iron metabolism; deficiency can impair iron absorption and its oxidation from Fe^{2+} to Fe^{3+}, reducing haemoglobin synthesis and potentially contributing to anaemia (Abutarbush & Radostits, 2003; Myint et al., 2018).(Table 4).

Serum electrolytes (K & Cl)

Serum potassium and chloride concentrations showed no significant difference ($P > 0.05$) between anaemic and healthy animals. Mean potassium levels were 3.62 ± 0.21 mEq/L in anaemic animals and 3.92 ± 0.19 mEq/L in controls, while chloride levels were 103.13 ± 2.00 mEq/L and 101.59 ± 2.04 mEq/L, respectively. Although potassium is essential for iron absorption and acidification in the gut (Salsbury, 2014; Radostitis et al., 2006), values in the present study were within normal limits, suggesting that anaemia likely arose from iron deficiency, sepsis, or ectoparasite infestation rather than electrolyte imbalance. Chloride levels remained normal, possibly due to prompt treatment of diarrhoeic animals preventing significant electrolyte loss (Table 4).

Serum Total protein, Albumin & Aspartate aminotransferase (AST)

Serum total protein and albumin concentrations did not differ significantly ($P > 0.05$) between anaemic and healthy animals in the present study. Mean total protein was 6.52 ± 0.24 g/dL in anaemic animals and 6.55 ± 0.40 g/dL in controls, while albumin was 2.69 ± 0.11 g/dL and 3.06 ± 0.10 g/dL, respectively. Although previous studies have reported hypoproteinemia and hypoalbuminemia in anaemic cattle (Katoch & Mandial, 2003; Tufani et al., 2009), such reductions are typically associated with decreased dietary protein intake (Morris & Johnston, 2001), which was not evident in the present study Serum AST activity did not differ significantly ($P > 0.05$) between anaemic and healthy animals, with mean values of 60.26 ± 2.95 IU/L and 60.32 ± 7.25 IU/L, respectively. Measurement of AST, along with total protein and albumin, indicated that the anaemic condition in the present study did not cause hepatic damage, suggesting that hypoxia associated with anaemia had not progressed to compromise liver function (Radostits et al., 2006) (Table 4).

Blood urea nitrogen (BUN) & Creatinine

Blood urea nitrogen (BUN) and creatinine levels did not differ significantly ($P > 0.05$) between anaemic and healthy animals, with mean values of 18.46 ± 1.16 mg/dL vs. 17.13 ± 0.66 mg/dL for BUN, and 0.88 ± 0.05 mg/dL vs. 0.95 ± 0.05 mg/dL for creatinine, respectively. Assessment of renal function indicated no evidence of kidney injury or hypoxia-induced damage. This aligns with findings by Shetty et al. (2018), where pre-renal azotaemia occurs only under conditions of shock or reduced renal perfusion, which was not observed in the present study due to timely intervention. (Table 4).

Heart rate and Pulse rate

Heart rate was significantly higher ($P < 0.05$) in anaemic animals compared to healthy controls, with mean values of 116.83 ± 1.19 bpm versus 100.66 ± 2.40 bpm, respectively. This increase is likely due to hypoxia-induced stimulation of chemoreceptors, activating the sympathetic nervous system (Metivier et al., 2000). Pulse rate showed no significant difference, averaging 104 ± 1.50 bpm in anaemic animals and 101.16 ± 1.16 bpm in controls, although bounding pulses were occasionally observed, reflecting the cardiovascular response to increased oxygen demand and peripheral circulation (Radostitis et al., 2006). (Table 5).

Blood smear examination in anaemic animals

Blood smear revealed absence of haemoprotozoa but anaemic changes were noticed. Anisocytosis and poikilocytosis,

Table. 5: Clinical parameters (HR, PR) in anaemic bovine

Parameters	Healthy Control	Anaemic animals	t value	p
Heart rate (bpm)	100.66±2.40	116.83±1.19	6.49	<0.001
Pulse rate (bpm)	101.16±1.16	104±1.50	1.03	0.15

central pallor, hypochromic erythrocytes was observed during examination.

Conclusion

This study demonstrates that bovine anaemia is primarily associated with iron deficiency, ectoparasite infestation, and sepsis. Haematological and biochemical analyses confirmed iron deficiency as the major contributing factor, with secondary alterations in red and white blood cell parameters. The study underscores the importance of early detection and targeted management of underlying causes such as micronutrient deficiency, ectoparasite control, and sepsis to prevent anaemia in calves. These findings provide critical insights for improving herd health and productivity through effective preventive and therapeutic strategies.

Author's Contribution

AS carried out the experimental work, data collection, and research investigations. PM was responsible for drafting, editing, and finalizing the manuscript. KM provided overall supervision, technical guidance, and critical revision of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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