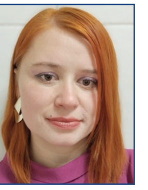
REVIEW ARTICLE / PREGLEDNI ČLANAK

Hemogram in cattle - understanding normal values and variations



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Abstract

Interpreting bovine hematology is essential for diagnosing, monitoring, and prognosing various diseases. One of the most commonly used diagnostic tool is the complete blood count (CBC), which helps identify a wide range of organ and systemic diseases. The purpose of this review article is to provide veterinarians, especially field veterinarians, with as much useful information as possible, about bovine hematological tests. Bovine complete blood count can be of great assistance in diagnosing, further testing and predicting the prognosis of bovine diseases. However, it should be noted that the diagnosis of bovine diseases based solely on a complete blood count is only occasionally possible. In most cases, a complete blood count in bovine serves as an important adjunctive tool in diagnosing diseases. In this paper, we will focus on the specificities of bovine erythrocytes, leukocytes, and thrombocytes.

Key words: *hemogram; blood cells; normal values; variation; bovine*

Introduction

Interpreting bovine hematology is crucial for diagnosing, monitoring, and prognosing diseases (Wood and Quiroz-Rocha, 2010). A complete blood count (CBC) is a standard diagnostic tool used to identify a wide range of organ and systemic diseases. While a complete blood count alone may not always lead to a definitive diagnosis, it can offer valuable insights aiding in proper diagnosis, surveillance, and prognosis of diseases in individual animals (Roland et al., 2014). This review aims to furnish bovine veterinarians and laboratory personnel with an encompassing understanding of sample collection, reference values, anemia classification, characteristics of bovine hemograms, and diseases linked with hematological abnormalities in bovines.

Many bovine veterinarians routinely submit blood samples for CBC analysis to local or remote diagnostic laboratories. These laboratories typically employ automated analyzers that generate comprehensive CBC data, including information on red blood cells (RBC) and platelet (PLT) count and size, as well as total and differential white blood cell (WBC) count (Warren et al., 2013). The values of various blood parameters in bovines fluctuate depending on age (Klinkon, 2000). Calves exhibit altered values shortly

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after birth due to colostrum intake, the short lifespan of RBCs, and a decline in fetal hemoglobin concentration (Kramer, 2006). Feeding and rearing practices significantly influence hematological parameters in growing calves (Reece and Hotchkiss, 1987). During this phase, RBC count, hemoglobin concentration, and hematocrit values increase. Calves predominantly fed with milk may experience decreased values of these parameters, leading to anemia due to iron deficiency (Reece, 1980). The WBC count in calves is typically higher compared to adult animals. Different leukocyte types have varying lifespans, resulting in rapid changes in their numbers, with blood serving as a mere transport medium.

Sampling of bovine blood and complete blood count

During blood sampling, the veterinarian must collect, store, and transport blood samples according to all professional standards to avoid potential changes in the results that may occur after sampling, thus affecting the blood analysis. Improper sampling can lead to *in vivo* cellular changes that may sometimes be misinterpreted as pathological changes. In bovine, blood is usually collected from the tail (coccygeal vein) or the neck (jugular vein). The sampling area is shaved, cleaned, and disinfected with an antiseptic solution.

For a complete blood count, the blood sample is collected with an EDTA (ethylene-diamine-tetraacetic acid) anticoagulant tube up to the marked line. The blood sample is gently and carefully mixed a few times to prevent clotting and cell damage. To ensure interpretability, the blood samples must be delivered to the laboratory as soon as possible. In case immediate delivery is not possible, the blood samples should be stored in a refrigerator at +4°C to minimize the possibility of result alterations (Kessell, 2015.).

If automated cell counters are used to obtain a CBC count, results should be interpreted with care to avoid false-positive or false-negative results. In case of doubt, manual microscopic evaluation of the blood sample is recommended. Changes in the bovine CBC count, especially in the leukogram, might not be as pronounced as in other species even during severe illness (Roland and al., 2014).

For example, hematological values for bovine at the Laboratory of hematology of the Clinic for Internal Medicine at the Faculty of Veterinary Medicine University of Zagreb are analyzed using a hematological analyzer (Horiba ABX, micros, France) with settings for bovine. The parameters measured include red blood cell count, hemoglobin concentration, packed cell volume, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red cell distribution width), platelet count, MPV (mean platelet volume) and total leukocyte count (Table 1). The differential blood count (relative numbers of segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils and basophils) is determined from blood smears stained with May – Grünwald Giemsa stainings and examined under the microscope with immersion magnification (Olympus BX421, Japan).

Bovine red blood cells

During erythropoiesis, red blood cells are produced in the bone marrow, stimulatated by erythropoietin, primarily produced in the kidneys (Brun-Hansen et al., 2006). Erythropoiesis takes about 5 days, and bovine erythrocytes have a relatively long lifespan, lasting between 130 and 160 days (Wood and Quiroz-Rocha, 2010). The main function of erythrocytes is to transport oxygen bound to hemoglobin to tissues. All the energy expended by erythrocytes is focused on optimizing the delivery of oxygen to the tissue (Olver et al., 2010).

Red blood cell morphology and structure

Erythrocytes are typically biconcave in shape, lacking a nucleus or organelles (Figure 1a), and thus incapable of protein synthesis. They consist of 61% water, 32% protein (mainly hemoglobin), 7% carbohydrates, and 0.4% lipids (Pennel, 1974). The diameter of erythrocytes in cattle is around 5 – 6 μm, which is smaller compared to the diameter of erythrocytes in other species of domestic animals (Wood and Quiroz-Rocha, 2010). Morphological abnormalities of erythrocytes can be detected during microscopic examination of blood smears (Jones and Allison, 2007). Poikilocytosis is a general term for variation in the shape of erythrocytes. In ruminants, poikilocytosis is a common occurrence and is considered a physiological finding. Anisocytosis represents variation in the size of erythrocytes (Barger, 2010). Mild to moderate anisocytosis is sometimes present in healthy ruminants, while more pronounced anisocytosis can appear in cases of regenerative anemia.

Polychromasia in erythrocytes represents a variation in the degree of staining and is indicative of reticulocytes that stain more blue due to the remaining DNA. In ruminants, polychromasia signifies a sign of regenerative anemia.

Howell-Jolly bodies are small, dark, round structures composed of nuclear material within erythrocytes. In cattle, Howell-Jolly bodies are similar to *Anaplasma marginal*e and can pose challenges in differentiation. Erythrocytes with nuclei are not normally seen in cattle blood smears. A small number of such cells may be observed in regenerative anemias, but they must be accompanied by a high degree of polychromasia. Damage to the bone marrow, intoxication, hypoxia, and neoplasia can also lead to an increase in the number of erythrocytes with nuclei. In ruminants, they most commonly appear in cases of lead poisoning (Sharkley and Overman, 2015).

Agglutination of erythrocytes in blood smears is the most commonly observed as clustered forms of erythrocytes. The presence of agglutinates indicates immune-mediated hemolytic anemia (IMHA). A frequent cause of agglutination and IMHA are hemoparasites (Jones and Allison, 2007).

Basophilic stippling of erythrocytes is seen as numerous small basophilic inclusions within erythrocytes, originating from the staining of ribosome aggregates. It is most often observed in regenerative anemias in ruminants.

Heinz bodies form within erythrocytes as a result of oxidative damage, leading to the deposition of hemoglobin. They appear as round, pink structures protruding from the edge of erythrocytes in Wright's staining or as round dark structures connected to the erythrocyte membrane in methylene blue staining. They can occur when animals are fed maple leaves, onions, garlic, phenothiazines or in cases of copper poisoning. Erythrocytes containing Heinz bodies are more susceptible to intravascular and extravascular hemolysis.

Rouleaux formation is visible as linear stacking of erythrocytes on a blood smear. It rarely occurs in healthy ruminants but can be seen with elevated levels of plasma proteins such as fibrinogen and immunoglobulins (Sharkley and Overman, 2015).

Anemias in cattle

Anemia is defined as a reduced ability to transport oxygen in the blood. There are three pathophysiological mechanisms of anemia: blood loss, increased erythrocyte destruction (hemolysis), and insufficient erythrocyte production. In the first two mechanisms, the bone marrow functions and responds normally with increased erythropoiesis (regenerative anemia). In ruminants, regenerative anemias are characterized by the presence of immature forms of erythrocytes in the peripheral blood. Inadequate erythrocyte production is caused by abnormalities in the bone marrow, resulting in a type of anemia known as non-regenerative anemia (Morris, 2002).

Hematocrit values less than 24%, total erythrocyte count less than 5.0 x 10^6 /μL, or hemoglobin concentration less than 8 g/dL in ruminants is defined as anemia. The presence of polychromasia or reticulocytes in ruminants indicates a regenerative anemia. Other signs include Howell-Jolly bodies, basophilic stippling of erythrocytes, erythrocytes with nuclei, elevated mean corpuscular volume (MCV), and decreased mean corpuscular hemoglobin concentration (MCHC). It is important to note that bone marrow response in ruminants can take from 2 to even up to 30 days (Harvey, 2001.).

Causes of regenerative anemias in cattle

Regenerative anemia is caused by bleeding or hemolysis. In the case of reduced hematocrit and normal or elevated total protein values, the animal is most likely experiencing a hemolytic process. On the other hand, if hematocrit is reduced while total protein values in the blood are proportionally decreased, we can talk about hemorrhagic changes (Jones and Allison, 2007).

The most common causes of bleeding in cattle include trauma, abomasal ulcers, hemorrhagic enteritis, vena cava syndrome, endo- and ectoparasites, hemostatic disturbances, and vascular erosions or ruptures (Jones and Allison, 2007; Brockus, 2011).

Hemolysis is caused by erythrocytic parasites such as *Anaplasma*, *Babesia*, *Mycoplasma*, and others, bacteria like *Clostridium haemolyticum* and *Leptospira* sp., toxins like copper, onions, red maple leaves, cruciferous plants, or phosphorus deficiency.

Causes of non-regenerative anemias in cattle

Non-regenerative anemias in cattle are most commonly caused by so-called anemias of inflammatory or chronic diseases (such as lymphoma, gastrointestinal disorders, chronic abscesses, endocrine diseases, hepatopathies, BVDV infections), anemias of chronic kidney failure, nutritional deficiencies (such as iron, copper, cobalt) and bone marrow diseases (Jones and Allison, 2007).

Polycythemia (erythrocytosis)

Polycythemia (erythrocytosis) is an elevated number of erythrocytes, hematocrit and hemoglobin concentration (Brockus, 2011). It can be relative or absolute. Relative is more common, where there is no actual increase in the total mass of erythrocytes; rather, hemoconcentration or spleen contractions cause an apparent rise (Jones and Allison, 2007). Hemoconcentration is often caused by dehydration and loss of plasma volume. In endotoxic shock, hemoconcentration can also occur due to increased vascular permeability. Absolute erythrocytosis occurs when there is a real increase in the mass of erythrocytes as a result of enhanced erythropoiesis and can be primary or secondary (Roland et al., 2014). Primary absolute erythrocytosis is caused by chronic myeloproliferative disease where erythropoiesis occurs independently of erythropoietin (EPO). Secondary absolute erythrocytosis occurs in response to elevated levels of EPO, which can be physiologically appropriate due to hypoxia or physiologically inappropriate due to tumor-produced EPO (Sharkley and Overman, 2015).

Cattle leukogram

Leukocytes (white blood cells) play an important role in the immune defence of the body and comprise various subpopulations, including neutrophils, eosinophils, basophils, monocytes, and lymphocytes (Roland et al., 2014). The neutrophil-to-lymphocyte ratio in adult cattle is approximately 1:2, which differs from the neutrophil-to-lymphocyte ratio in other domestic animals. Cattle have a small reserve of granulocytes in the bone marrow, resulting in a more neutropenic than neutrophilic response in the early stages of inflammatory processes (Wood and Quiroz-Rocha, 2010).

The complete leukocyte profile involves the total leukocyte count, relative differential blood count, and absolute differential blood count. The total leukocyte count in cattle decreases with age of the animal. The predominant leukocyte subpopulation in cattle are lymphocytes, whose ratio varies throughout their lifespan. Newborn calves have more granulocytes than lymphocytes in their bloodstream. During the first month of the calf's life, there is a decreasing total leukocyte, neutrophil and lymphocyte count, followed by their increase. Within three months, the lymphocyte percentage rises to 80% of all circulating leukocytes (Roland et al., 2014).

Neutrophils

Neutrophils in the blood are relatively large cells, with a diameter of 10-15 μm (Calamai and Spitznagel, 1982). They have a large and multilobed nucleus, and three types of granules are present in the cytoplasm (Figure 1b). Neutrophils in cattle possess larger granules compared to neutrophil granules in other domestic animal species (Bertram, 1985). In cattle, we categorise the granules into azurophilic or primary granules formed during the promyelocyte stage, and specific or secondary and large or tertiary granules formed during later myelocyte and metamyelocyte stages (Baggiolini et al., 1985; Paape et al., 2003). Compared to humans, bovine neutrophils play a stronger bactericidal role, featuring higher lactoferrin levels, B12-binding protein, acid and alkaline phosphatases, glutathione peroxidase, and glutathione reductase. However, they have a lower concentration of lysozyme, myeloperoxidase, catalase, beta-glucuronidase, and beta-galactosidase (Gennaro et al., 1978).

Toxic changes in neutrophils occur within the bone marrow and include the presence of Döhle bodies in neutrophil cytoplasm, toxic granules, diffuse cytoplasmic basophilia, bizarre giant forms, cytoplasmic vacuolisation, and foamy cytoplasm (Jones and Allison, 2007).

Pelger-Huët anomaly in cattle appears as an acquired form in animals with inflammatory processes such as mastitis, metritis, and traumatic reticuloperitonitis. The hereditary form of this anomaly has not been documented in cattle. Affected cells display immature nucleus forms, but their chromatin is mature and differentiates them from true non-segmented granulocyte forms (Osburn and Glenn, 1968).

Chediak-Higashi syndrome is an inherited disorder that can occur in Hereford, Brangus, and Japanese Black cattle, characterised by the presence of large and abnormal granules in neutrophils and eosinophils, and causing platelet abnormalities (Meyers, 2000).

Neutrophilia can occur due to mild to moderate inflammation or during recovery from severe inflammation. There are three leukocyte response patterns causing neutrophilia: the inflammatory leukogram, stress leukogram and physiological leukocytosis. Holstein cattle are known to have a lack of adhesion molecules in their leukocytes which prevents neutrophils from adhering to activated endothelium. Affected animals typically have very high leukocyte count and persistent or repeted infections.

An elevated neutrophil count most commonly arises from stress and chronic inflammation caused by infections such as mammary, urogenital, respiratory, and gastrointestinal, as well as infections affecting the liver, heart, or central nervous system.

Additionally, neutrophilia develops during acute purulent processes like endometritis, retained placenta, acute bacterial mastitis, and peritonitis triggered by a foreign body. Inflammatory neutrophilia can also be seen in viral, protozoal, parasitic, and fungal infections (Tornquist and Rigas, 2010). However, neutrophilia can also be present in non-infectious inflammation such as traumatic injuries, necrosis, thrombosis, burns, etc. (Weiser, 2006.). Stress can lead to mature neutrophilia with lymphopenia and eosinopenia, but a left shift and toxic changes are not seen. The total WBC count may be within normal reference limits because the degree of increase in absolute neutrophil numbers is less than in other species with stress conditions (Kessell, 2015).

Lymphocytes

Lymphocytes are unique cells, originating from the bone marrow cell but maturing and proliferating in other lymphoid tissue. Additionally, lymphocytes are the only subpopulation of leukocytes that recirculate between blood and tissues (Kramer, 2000).

Lymphocytes mostly consist of B cells and T cells. In cattle, we find small, medium, and large lymphocytes (Figure 1c), and it's not uncommon to encounter lymphocytes containing azurophilic cytoplasmic granules.

Pathological lymphocytosis in cattle is not a common occurrence, but it can be associated with chronic infections caused by viruses, chronic suppurative conditions, or autoimmune diseases (Joned and Allison, 2007). Enzootic bovine leukosis, caused by RNA virus from the *Deltaretrovirus* genus, *Retroviridae* family, results in persistent leukocytosis with an increased number of B lymphocytes and abnormal lymphocyte morphology, with more than 30% of such cells in affected cattle (Esteban et al., 1985).

Eosinophils

The main role of eosinophils is manifested in the immune response to parasites, allergens, and other inflammatory stimuli (Figure 1f). Eosinophils are stored in the bone marrow, and their half-life in circulation ranges from 30 minutes to 10 hours. Eosinophils can be found in the stomach, subcutaneous tissue, uterus, respiratory system, and many other organs, where their half-life is 12 days (Morris, 2002).

Eosinophilia in cattle most commonly occurs as a result of parasite migration (Tornquist and Rigas, 2010) and type I hypersensitivity reactions (Tizard, 2013). However, causes such as neoplasia, infections, and drug reactions can also lead to eosinophilia (Webb and Latimer, 2011).

Basophils

Basophils are very rarely found in the blood of large animals. They are formed in the bone marrow through mitosis of basophilic promonocytes and go through all stages of development like neutrophils (Figure 1d). Their half-life in circulation is 6 hours, after which they enter tissues where they can survive for 10 to 12 days. They play an important role in inflammatory processes and allergies by releasing inflammatory mediators such as heparin and histamine in immediate hypersensitivity reactions (Morris, 2002).

Monocytes

Monocytes (Figure 1e) are formed in the bone marrow, and once released into circulation, they can circulate for one to three days before entering tissues where they become macrophages. In tissues, they can survive from several weeks to a couple of years, and they are capable of phagocytizing infectious organisms, particles, and cellular debris. An increased number of monocytes in the blood may indicate chronic inflammation, tissue necrosis, hemolysis, or a stress response, while a decreased number of monocytes may be associated with endotoxemia or viremia (Jones and Allison, 2007).

Thrombocytes (platelets)

Platelets are non-nucleated cytoplasmic fragments of megakaryocytes (Figure 1a), and their most important role is in regulating hemostasis (Russell, 2010). With an average platelet volume (MPV) of 4.0 - 4.8 fL, bovine platelets fall into the category of small platelets when compared to those of other species (Boudreaux and Ebbe, 1998). About 30 – 40% of platelets are found in the spleen and are released into circulation stimulated by the release of the hormone epinephrine. Apart from the spleen, platelets are stored in the liver and the bone marrow (Boudreaux et al., 2011).

Brun-Hansen et al. (2006) found a higher total platelet count in calves up to 19-21 weeks of age compared to the total platelet count in adult cattle. They observed a pronounced increase in platelet count during the second week of calf's life.

In cases with established clinical signs such as petechiae and mucosal bleeding, platelet assessment is indicated. Platelet count should be done from blood samples within the first 4 to 6 hours after blood collection. Platelet aggregates are a common occurrence in cattle blood and can arise due to prolonged exposure to EDTA anticoagulants or the use of lithium heparin as anticoagulant. The appearance of platelet aggregates in some hematological counters can falsely lead to a decrease in the total platelet count (Jones and Allison, 2007).

Thrombocytopenia in cattle is considered when the total platelet count is less than 100,000 cells/μL and is caused by mechanisms such as reduced platelet production, short platelet lifespan, and platelet sequestration. The most common causes of thrombocytopenia in ruminants include conditions and diseases like disseminated intravascular coagulation (DIC), fern poisoning, and septic mastitis or metritis (Morris, 2002).

Conclusions

In this review article, physiological and pathological characteristics of bovine hemogram and leukogram were discussed as well as morphological characteristics of

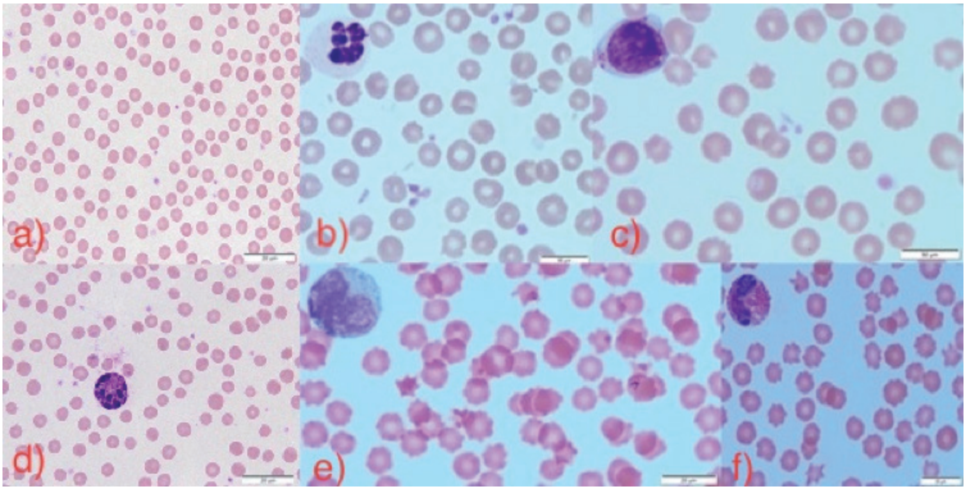

Figure 1. Bovine blood smear (a-f blood cells; May-Grünwald Giemsa staining)
a) erythrocytes and thrombocytes, x 1000; b) neutrophil with multilobed nucleus, x 600; c) lymphocyte with deeper smooth blue cytoplasm, cytoplasmic granules, round nucleus and clumped chromatin (heterochromatin), x 600; d) basophil, x 1000; e) monocyte with kidney shape nuclei and uniformly grainy blue-gray cytoplasm), x 1000 f) eosinophil with eosinophilic granules, x 1000

individual leukocytes. The results of a CBC are often very useful tool in the diagnosis, monitoring and prognosis of a different disease that can affect cattle. If automated cell counters are used to obtain a CBC, results should be interpreted with care to avoid false-positive or false-negative outcomes. A manual microscopic evaluation of a stained blood smear should be carried out. In cattle, changes in the CBC, especially in the leukogram, might not be as pronounced as in other species even during severe illness. Therefore, a diagnosis or prognosis should not be based only on hematological findings, but should also take findings from the clinical examination or other diagnostic procedures into consideration.

Table 1. Reference values of bovine hematological parameters from several literary sources

PARAMETER	UNIT	Merck*	Smith	Schalm
Hematocrit	**%**	24-46	22-33	21-30
Hemoglobin	**g/dL**	8-15	8.5 – 12.2	8.4-12.0
Erythrocytes	**x 10^6/µL**	5.0-10.0	5.1-7.6	4.9-7.5
Reticulocytes	**x 10^3/µL**	-	-	0
MCV	**fL**	40-60	38-50	36-50
MCH	**pg**	11-17	-	14-19
MCHC	**g/dL**	30-36	36-39	38-43
Platelets	**x 10^3/µL**	100-800	100-800	160-650
MPV	**fL**	3.5-6.5	-	4.6-7.4
Total leukocytes	**x 10^3µ/L**	4.0-12.0	4.9-12	5.1-13.3
Segmented neutrophils	**%** **x 10^3/µL**	15-33 0.6-4.0	- 1.8-6.3	- 1.7-6.0
Band neutrophils	**%** **x 10^3/µL**	0-2 0-0.1	- rare	- 0.0.-0.2
Lymphocytes	**%** **x 10^3/µL**	62-63 2.5-7.5	- 1.6-5.6	- 1.8-8.1
Monocytes	**%** **x 10^3/µL**	0-8 0-0.9	- 0-0.8	- 0.1-0.7
Eosinophils	**%** **x 10^3/µL**	0-20 0-2.4	- 0-0.9	- 0.1-1.2
Basophils	**%** **x 10^3/µL**	0-2 0-0.2	- 0-0.3	- 0.0-0.2

**The authors of the Merck Veterinary Manual stated that they used data from multiple sources, including Latimer, K. S., , Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th ed., Wiley-Blackwell, 2011; Weiss D. J., Wardrop, K. J., Schalm's Veterinary Hematology, 6th Ed., Wiley-Blackwell, 2010.*

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Hemogram goveda - razumijevanje normalnih vrijednosti i varijacija

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Interpretacija hemograma goveda ključna je za dijagnosticiranje, praćenje i prognozu različitih bolesti. Jedna od najčešće korištenih dijagnostičkih metoda je kompletna krvna slika (KKS) koja pomaže u prepoznavanju širokog spektra organskih i sistemskih bolesti. Svrha je ovog preglednog članka pružiti veterinarima, posebice terenskim veterinarima, što više korisnih informacija o hematološkim promjenama u goveda. Kompletna krvna slika goveda može biti od velike pomoći u dijagnostici, daljnjim ispitivanjima i predviđanju prognoze bolesti goveda. Međutim, pritom treba naglasiti da je dijagnostika bolesti goveda isključivo na temelju kompletne krvne slike rijetko moguća. U većini slučajeva, kompletna krvna slika služi kao važan dopunski alat u dijagnostici. U ovom smo radu obratili posebnu pozornost na specifičnosti eritrocita, leukocita i trombocita u goveda.

Ključne riječi: *hemogram, krvne stanice, normalne vrijednosti, odstupanja, govedo*