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Haematology in the early diagnosis of cattle diseases - a review

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ABSTRACT

Laboratory blood tests are not only an important element of the assessment of the general condition of animals, but they are also important for the early diagnosis of many diseases. In modern buiatrics laboratory tests are most frequently limited to the analysis of metabolic profiles, using selected biochemical tests. This reductionist approach results from economic reasons and ignores the possibility of using haematological tests, often limiting the possibility of a certain diagnosis. Haematology (Greek αιμα [ema] - blood) concerns the issues related to blood and the haematopoietic system, which is the first to respond to any disruptions in intra-body homeostasis. These changes guide the veterinarian in the search for a diagnosis and are indispensable in monitoring the effectiveness of treatment. Haematology can also be useful in managing a herd of cattle. In this article, the most recent data regarding the use of blood screening in livestock and the prophylaxis and treatment of dairy cattle are presented. The article presents the possibilities of using haematology in diagnostics, therapy and in the raising of dairy cattle. The most common causes of changes in haematological parameters in cattle are also discussed.

Key words: haematology; cattle; RBC; WBC; PLT

Introduction

Modern cattle livestock production is based on well-balanced nutrition, appropriate zoohygienic care, animal well-being, as well as prophylaxis in the herd. The appropriate implementation of this goal requires comprehensive clinical diagnostics as well as laboratory testing of the feed. The availability of clinical assessment methods for a veterinary physician is often limited, not to mention the restrictions due to economic reasons. Very often a veterinary surgeon is forced to rely solely on his own professional experience and the limited results of biochemical blood tests, in the absence of comprehensive haematology. In recent times, more publications have been discussing

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the unquestionable diagnostic usefulness of these tests in detecting many diseases. An increasing number of bovine diseases are occurring in subclinical form or with unusual symptoms (which makes correct and rapid diagnosis difficult) are characterized by specific haematological symptoms. Changes in the white blood cell system and/or erythrocytes are observed, which after proper interpretation allow for the recognition of disease and for effective treatment to be undertaken. These changes alone or in combination with the biochemical results of body fluids analysis are also used in monitoring the well-being of the herd. The authors own observations of the last ten years confirm the decline in the incidence of typical clinical cases of alimentary diseases, and the increasing number of cases of atypical and subclinical course. The diagnosis of these diseases requires the use of a wide range of diagnostic tests, which results in additional costs of maintaining the herd. Relatively cheap hematological tests based on automatic analyzers can be a valuable aid in making a decision to conduct biochemical tests in a herd.

Already, at the initial stage of nutritional deficiency diseases, haematological disruptions may occur. Phosphorus deficiency - hypophosphataemia induces haemolytic anaemia in animals as well as humans (FORRESTER and MORELAND, 1989; MELVIN and WATTS, 2002; OK et al., 2009). Haemolysis occurs in cows during the perinatal period due to low phosphorus levels in the blood serum. A decrease in adenosine triphosphate (ATP) in red blood cells may occur, which results in the deformation of the erythrocyte cell membrane and an increase in its brittleness, leading to haemolysis and severe haemoglobinuria (FORRESTER and MORELAND, 1989; OK et al., 2009). VAJA et al. (2016), when describing ketosis reported a decrease in the number of neutrophils and an increase in the lymphocyte count as a consequence of the discharge of cortisol into the blood. Based on our own unpublished results, in the course of ketosis, leucopoenia was observed, which may be related to enhanced leukocyte catabolism (MARUTSOVA et al., 2015) in the perinatal period. In addition, the high concentration of ketone bodies inhibits the proliferation of cells in the bone marrow (HOEBEN et al., 1999). The inhibitory effect on the phagocytic activity of leukocytes *in vitro* has also been noted (SURIYASATHAPORN et al., 1999).

The article presents the possibilities of using haematology in diagnostics, therapy and in the raising of dairy cow breeding. The most common causes of changes in haematological parameters in cattle are also discussed.

The handling of the material for haematology. For carrying out haematological tests in dairy cattle, the blood sample is most often collected from the external jugular vein (Vena jugularis externa). In some cases, the blood sample is collected from the medial coccygeal vein (Vena coccygea mediana, s. Vena caudalis mediana) or from the milk vein (Vena epigastrica cranialis superficialis). These sampling methods are less common, due to the risk of the contamination of the sample with faeces present at the ventral surface of the tail and, when the blood sample is collected from the milk vein, there is a high risk of the formation of haematomata and abscesses (JONES and ALLISON, 2007; ROLAND et al. 2014). The feeding regime and the time of day are not of great importance when collecting a blood sample, but on farms with permanent tethering of the cows, it is best practice to collect a blood sample after morning milking and just before feeding. In turn, on free-range cattle farms, it is best to collect blood samples just after milking, when the animals pass through a corridor or a crowd gate and can be easily stopped without causing them additional stress. It is very important to collect blood samples before the cows are watered, because consuming a large volume of fluids may affect haematological parameters.

Blood for haematological examination is placed into tubes with an anticoagulant, most often dipotassium and tripotassium salts of ethylenediaminetetraacetic acid (K_2EDTA or K_3EDTA) (TWARDOCH, 2014). In addition, disodium edetate, sodium citrate and heparin are used. The blood sample should be collected rapidly, as well as accurately, using an appropriate needle size (1.6×40mm or 1.8×40mm are most commonly used). After the tested material is placed into the test tube, it should be carefully mixed with an anticoagulant in order to avoid erythrocyte damage. Also, there should be an adequate volume of blood in the test tube (attention should be paid to the volume marking line on the test tube), because the wrong blood/anticoagulant ratio may cause erroneous readings of the haematological parameters tested. Excess disodium edetate causes damage to erythrocytes and leukocytes, a decrease in the Ht parameter and an increase in MCHC, as well as damage to and the degradation of platelets, which may result in their overquantification. The blood should be delivered to the laboratory for examination, no more than 3 hours from the moment of sample collection. The blood samples may be stored for up to 24 hours at 4 °C, whereas a blood smear should be performed within 15 minutes from the time of sample withdrawal and sent to the laboratory along with the sample. When blood is stored at room temperature for over 12 hours, changes in the nuclei of neutrophils may occur (the patches of the nuclei may separate), and vacuoles appear in the cytoplasm. Analogous changes are observed for lymphocytes and monocytes. The prolonged storage of blood also results in changes in the erythrocyte volume, leukocyte and platelet count (MARIAŃSKA et al., 2006; JONES and ALLISON, 2007; ROLAND et al., 2014). If the platelet count is of clinical significance for the tested animal, the sample should be analysed within 4-6 hours from the time of collection. Haematological tests are most frequently performed by means of automated testing equipment, usually 3- and 5-Diff analysers. They differ by the number of leukocyte subpopulations that can be detected. 3-Diff analysers can distinguish between 3 types of white blood cell, according to their size (neutrophils, lymphocytes and other white blood cells), whereas 5-Diff analysers can detect 5 leukocyte subpopulations (neutrophils, lymphocytes, basophils, eosinophils and monocytes) based on two-dimensional blood cell analysis. It should be noted that the haematological analysers used for the blood testing of cattle must be adequately calibrated for these animals (dedicated to them), otherwise unreliable results may be produced or the measurements may be delayed.

The examination of the red blood cell system. Red blood cells (erythrocytes, RBC) are produced in the process of erythropoiesis in the red bone marrow located in the bone marrow cavities of spongiosa in the epiphyses of the long and flat bones. This process takes about 5 days and is stimulated by erythropoietin. Erythrocytes are oval, non-nuclear cells and have a relatively long life cycle of 120-160 days (HATTANGADI et al., 2011; KLINKON and JEŽEK, 2012; ADILI et al., 2014; ROLAND et al., 2014, ABRAMOWICZ et al., 2016). The average size of a red blood cell in cattle is 5-6 μm, erythrocytes are small compared to other animal species. When analysing the red blood cell system, the total count of erythrocytes, haematocrit (Ht), haemoglobin content (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are considered. The erythrocyte count, Ht, Hgb are classified as direct indicators of the erythrocyte system, whereas MCV, MCH and MCHC are indirect indicators of the erythrocyte system. These parameters provide information about the size (MCV) of the red blood cell (normal - normocyte, above normal - macrocyte, below the norm - microcyte) and the Hgb concentration in the erythrocyte (normal - normochromic, above normal - hyperchromic, below normal - hypochromic) (JONES and ALLISON, 2007; ADILI et al., 2014; ROLAND et al., 2014; ABRAMOWICZ et al., 2016).

Beef cattle have more erythrocytes than dairy cattle, similarly to males (bulls) compared to females (WEISS and WARDROP, 2010). In calves, the erythrocyte count and Ht may be higher, and the MCV and MCHC parameters may achieve lower values than in adult animals, while the normal levels of Hgb are in a similar range in both calves and adult animals (KLINKON and JEŽEK, 2012; ROLAND et al., 2014; PANOUSIS et al., 2018).

The most frequently observed change in the erythrocyte system is anaemia, when erythrocyte count, haematocrit index and haemoglobin concentration are below the accepted reference standards (ROLAND et al., 2014). According to the WHO, anaemia is a condition in which the number of red blood cells or their ability to carry oxygen is insufficient to meet physiological needs (ALLI et al., 2017). The classification of anaemia is made on the basis of the analysis of blood cell size and Hgb content in the erythrocyte (*e.g.*, microcytic, macrocytic, normocytic, hypochromic, hyperchromic, normochromic anaemia), as well as a decrease in erythrocyte production (bone marrow aplasia), or a blood loss due to haemorrhage or haemolysis (ALLI et al., 2017). Another type to be distinguished is pseudoanaemia, which may be encountered in conditions with high water retention by the body, *e.g.* in pregnancy, in iatrogenic overhydration of the body, in congestive heart failure or in the case of corticosteroid therapy (CALLAN and RENTKO, 2003).

In another classification, anaemia is divided into regenerative (haemorrhages and haemolysis) and non-regenerative types depending on the bone marrow response to blood loss. Regenerative anaemia is indicated by the release of juvenile red blood cells (reticulocytes) from the bone marrow and even erythroblasts. Regeneration in ruminants is only visible after 2 - 4 days, and the highest juvenile cell production is observed between 4 and 7 days after the activation of the pathogenetic factor. The characteristic features of regenerative anaemia, as evaluated from the manual smear test are: the presence of reticulocytes, basophilic stippling and Howell-Jolly cells (erythrocytes with pathological fragments of the cell nucleus). Furthermore, an increase in MCV and a decrease in MCHC are observed. In the case of prolonged bleeding, the reticulocyte production capacity may be exhausted and regenerative anaemia may progress into non-regenerative anaemia. As a result of acute haemorrhage, the red blood cell parameters do not change initially, because the animal loses whole blood, and the circulatory system reacts with reflexive vasoconstriction. Changes in the erythrocyte count, Ht and Hgb concentration can only be detected after a few hours and normocytic normochromic anaemia is usually observed (JONES and ALLISON, 2007; ROLAND et al., 2014; MENDEL et al., 2017). The most common causes of acute haemorrhage in cattle are: mechanical injuries, vascular damage during delivery, abomasal ulcers and haemorrhagic intestinal inflammation. The common causes of haemolysis are dietary components such as alfalfa (*Medicago sativa*) and clover (*Trifolium pratense*) plants that contain saponin and infectious agent (*Anaplasma* spp, *Babesia* spp., *Mycoplasma* spp.), and also hypophosphataemia and overhydration of the organism (JONES and ALLISON, 2007; RAUT et al., 2008; ROLAND et al., 2014; MENDEL et al., 2017).

Non-regenerative anaemia is caused by reduced erythropoiesis in the bone marrow or other diseases that have indirect effects on the bone marrow. In the case of chronic renal failure, there is a deficiency of erythropoietin, causing normochromic normocytic anaemia. Similar haematological results are observed in some endocrine disorders (diabetes), also in the case of the use of oestrogen preparations, as well as in lead poisoning and cancer (JONES and ALLISON, 2007; FRY, 2013; ROLAND et al., 2014). An inadequate composition of nutritional doses, low in macro- and microelements results in deficiencies, which can also be manifested by haematological disorders.

Iron deficiency is frequently observed in cattle. In the bovine gastrointestinal tract, the most important location of iron absorption is the duodenum (during absorption, Fe is partially stored in the intestinal epithelium), whereas the rumen-reticulum part and the abomasum are less important. The absorption of this microelement is positively influenced by low pH, an increased supply of high-energy compounds (especially glucose), normal levels of copper (Cu), calcium (Ca) and vitamin C in the body, as well as the physiological composition of the ruminal flora. Fe antagonists are phosphates, oxalates and phytates, and also vitamin E. To a lesser extent, however, the unfavourable effects on Fe absorption may be caused by high concentrations of cobalt, zinc, nickel and manganese in the rumen. The absorption of iron from the gastrointestinal tract is also adversely affected by: lack of appetite, diarrhoea (rapid passage of digestive content), administration of antibiotics in the feed and change of the rumen microenvironment (microbiomes facilitating the reduction of Fe^{++} to Fe^{+++}). Long-term disruptions in the absorption of iron and/or its deficiency in the body leads to the development of anaemia. In these cases, a decrease in MCV, MCH and MCHC is characteristic, which indicates the occurrence of hypochromic microcytic anaemia (RAHMAN et al., 2005; JONES and ALLISON, 2007; WEISS and WARDROP, 2010; ROLAND et al., 2014). It was also observed in these cases that the MCH decreases more severely than MCV and MCHC, which may be indicative of iron deficiency (MAZZULLO et al., 2014). Iron deficiency most often occurs in calves during the nursing period (anaemia of newborn calves), because its content in cow's milk is low. It is also believed that the transient decrease in haemoglobin and erythrocytes in newborn calves is a result of the adaptation to postnatal living conditions. A similar trend in the occurrence of anaemia is observed in the case of copper (Cu) deficiency (ABRAMOWICZ et al. 2019)). In contrast, if anaemia is triggered by deficiencies in vitamin B_{12}, folic acid and cobalt, then non-regenerative anaemia has a different clinical manifestation. An increase in the MCV parameter is detected, which suggests normochromic macrocytic anaemia (RAHMAN et al., 2005; JONES and ALLISON, 2007; ROLAND et al., 2014; VÁLKA and ČERMÁK, 2018).

One of the effective methods used to counteract the effects of anaemia is the transfusion of whole blood, which is intended to supplement lost blood components. The total blood volume of cows is 7-8% of body weight. Transfusion is performed for cows in life-threatening situations, when the haematocrit drops rapidly to values in the range of 10-15%. The most common indicators for this procedure are abomasal ulcers, acute massive bleeding for various reasons and endovascular haemolysis caused by hypophosphataemia (postpartum haemoglobinuria) or babesiosis (SOLDAN, 1999).

The examination of the white blood cell system. White blood cells (leukocytes) are nucleated cells that are produced and matured in the bone marrow and lymphatic system. They are divided into: granulocytes, which contain granules in the cytoplasm and agranulocytes - without granules. All leukocytes are part of the body's immune system. In young animals, the number of leukocytes, including neutrophils is higher than in adults. With age, this situation changes, the number of neutrophils decreases in favour of lymphocytes. In adult animals, the ratio of neutrophils to lymphocytes is approximately 1:2. Compared to other animal species, cattle have a lower reserve of granulocytes in the bone marrow. This results in leukopenia and neutropenia (reduced leukocyte count with a reduced neutrophil count in the blood) at the initial stage of an acute inflammation. Bone marrow mobilization is slower than in other animal species (*e.g.*, in dogs). Within 24 - 48 hours, an intensive production of juvenile neutrophils (with nonsegmented nuclei and less clumped chromatin, band neutrophil) takes place in the bone marrow and then these neutrophils are released into the peripheral blood. At this time, immature neutrophils-myelocytes and metamyelocytes (juvenile cells, not physiologically present in the blood) are also observed in the peripheral blood. As a result of acute inflammation, an intense granulopoiesis occurs and morphotic changes are observed in neutrophils. These changes can be described as toxic. They are characterized by the fact that on the edge of the cell basophilic cytoplasm occurs (that can be coloured blue or navy-blue with May-Grünwald Giemsa stain) and also azurophilic granules and vacuoles occur in the cytoplasm. In some of the toxic neutrophils in the cytoplasm one may also observe Döhle bodies (pale blue inclusions consisting of ribosomes and RNA). After the passage of 3-5 days after the activation of the infectious agent, a general increase in the number of leukocytes (leukocytosis), with granulocytes of both juvenile forms and mature nucleus (segmented) forms, are observed in cattle. The blood smear, at this time, as assessed using Schilling's index [which informed about an age classification of neutrophils, dividing them into juvenile forms (with the band nucleus) and mature ones (with the segmental nucleus)], is shifted to the left, indicating an increased release of juvenile neutrophilic granulocytes with a band nuclei into the blood (JONES and ALLISON, 2007; WEISS and WARDROP, 2010; ROLAND et al. 2014; WOOD, 2014; BOTEZATU et al., 2014; BRAUN et al., 2018; PANOUSISA et al. 2018).

Bovine leukocytosis is most often observed in bacterial infections, poisoning, anaphylactic shock, stress and traumatic reticuloperitonitis. The stress leukogram is characterized by neutrophilia, lymphopoenia and eosinopoenia, and occasionally by monocytosis. This state is short-lived and there is no shift to the left in Schilling's index. The stress leukogram often occurs in the displacement of the abomasum, perinatal period and ketosis (JONES and ALLISON, 2007; WOOD, 2014; BRAUN, 2018). The opposite condition to leukocytosis is leukopoenia, when the overall number of leukocytes decreases below the lower limit of physiological norms. It is observed in viral infections (*e.g.*, infectious inflammation of the nose and trachea), bacterial infections (inflammation of the peritoneum, uterus), bone marrow aplasia, liver diseases. It may also be observed in the first days of acute inflammation, due to the migration of circulating neutrophils to the focal point of inflammation, with the bone marrow response being reduced (ROLAND et al., 2014; BRAUN, 2018).

The most common causes of neutrophilia are chronic inflammation and stress. In the case of chronic diseases, it is observed in the inflammation of the udder, gastrointestinal tract, urinary tract and respiratory system. It also occurs due to cancer, abomasal displacement, poisoning, immunological anaemia, tissue injury, traumatic reticuloperitonitis, ketosis and also over the course of a difficult delivery (WEISS and WARDROP, 2010; ROLAND et al., 2014; BRAUN 2018). Neutropoenia often begins on the 1^{st}-2^{nd} day of acute and hyperacute inflammation in the course of sepsis caused by Gram-negative bacteria, mastitis, endometritis, pneumonia and peritonitis. It can accompany viral and fungal infections as well as bone marrow cancer. Neutropoenia lasting longer than 3-4 days indicates that granulopoiesis is blocked or that there is damage to the bone marrow (WEISS and WARDROP, 2010; ROLAND et al. 2014).

Eosinophilia is commonly observed in allergic reactions (mainly type I) and parasitic infections. It should be noted that cattle have a greater number of eosinophils than other animal species. Eosinophilia may also occur during the passage of parasites through the body, in cancer, certain infections (*e.g.*, atypical interstitial pneumonia), and in the case of acute emphysema in cattle. Eosinopoenia is rarely observed but may occur in the early stages of infection, uraemia, acute haemolysis and in response to stress. Calves have a lower number of eosinophils than adult individuals (JONES and ALLISON, 2007).

The number of basophils is low in healthy ruminants, they take part in allergic and inflammatory processes through the release of heparin, histamine and other inflammatory mediators. Their number may increase in the case of allergic skin diseases and in cattle, which have suffered from tick bites (JONES and ALLISON, 2007).

Lymphocytosis is rather rare in cattle. It may be diagnosed over the course of a chronic viral disease, purulent peritonitis, purulent pericarditis, chronic inflammation of the kidneys or liver and mastitis. Bovine leukosis virus (BLV) causes persistent lymphocytosis, with an increased number of B-lymphocytes and morphological changes to lymphocytes. Lymphopoenia may occur during acute stress, viral and bacterial infections, chronic renal failure, and after corticosteroid use (ROLAND et al., 2014).

Monocytosis is observed in stressed animals as well as during the treatment of acute and chronic infections. In addition, it occurs during haemolysis, haemorrhage, inflammation, necrosis or tissue ulceration, cancer and during corticosteroid therapy. A low number of monocytes are present during endotoxaemia, viraemia and other inflammatory states with acute and hyperacute manifestation (ROLAND et al., 2014).

Platelets. Platelets (thrombocytes), in mammals, are small, round, nuclei-free, morphotic elements that are produced in the bone marrow from megakaryocytes cytoplasm. In cattle they are smaller compared to other species of animals, their average volume is 4.0-4.8 femtolitres. The average survival time for thrombocytes in peripheral blood is 10 days. About 30% of platelets are stored in the spleen, but they are also stored in the liver and bone marrow and released into the blood in response to epinephrine (ROLAND et al., 2014).

The main function of thrombocytes is their participation in blood haemostasis. Calves have a higher number of platelets than adults. Thrombocytosis (an increase in the number of thrombocytes) is usually a secondary condition that occurs under stress, during chronic blood loss, inflammation, cancer and iron deficiency. The increase in platelet count may be associated with an increased risk of thrombosis. Thrombocytopoenia (a decrease in the number of thrombocytes) occurs due to increased platelet consumption (*e.g.*, blood loss, DIC - disseminated intravascular coagulation syndrome), with a reduced production in bone marrow (bone marrow hypoplasia), toxic bone marrow damage (due to toxins, drugs, infections, cancer). Reduced platelet count (thrombocytopoenia) is also detected during bacterial infections (salmonellosis, leptospirosis), in babesiosis and in BVD virus infections. It is also diagnosed in animals fed with poor quality fodder containing mycotoxins. (JONES and ALLISON, 2007; KOCATÜRK et al., 2010; WEISS and WARDROP, 2010; BELL, 2011; ROLAND et al., 2014)

In clinical practice, the haematological examination is essentially performed for two purposes. Firstly, for a diagnostic purpose, and secondly, to monitor the patient's health. Carefully selected diagnostic parameters allow for an effective assessment of both the state of homeostasis of the body, as well as possible disruptions in the health status of a single patient or herd. It is necessary to refer the obtained results to recognized reference standards developed for a given species, and for specific objectives of livestock production. In addition, for each monitored farm it is useful to create internal clinical standards, taking into account the specificity of animals and herd management.

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SAŽETAK

Laboratorijski krvni testovi važni su ne samo za procjenu općega stanja životinje nego i u dijagnostici mnogih bolesti. U modernoj su bujatrici laboratorijski testovi najčešće ograničeni na analizu metaboličkog profila primjenom biokemijskih testova. Takav redukcionistički pristup, koji postoji iz ekonomskih razloga, zanemaruje mogućnosti upotrebe hematoloških testova, često ograničujući mogućnost dobivanja određene dijagnoze. Hematologija uključuje krv i hematopoetski sustav koji prvi reagiraju na bilo kakav poremećaj u homeostazi. Promjene u krvnim pokazateljima veterinare usmjeruju u potrazi za dijagnozom i nužne su u praćenju učinkovitosti liječenja. Hematologija može biti korisna i u upravljanju stadom. Ovaj rad donosi najnovije podatke o primjeni krvnih testova u stočarstvu te profilaksi i liječenju mliječnih pasmina. Članak nudi mogućnosti upotrebe hematologije u dijagnostici, liječenju i uzgoju mliječnih pasmina. Također su razmotreni najčešći uzroci promjena u krvnim pokazateljima u goveda.

Ključne riječi: hematologija; goveda; RBC; WBC; PLT