

Finding the answer in that mess of numbers

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Abstract

A complete blood count (CBC) can be an important extension of the physical examination in ruminants, and may be used to suggest certain disease processes when exam findings are vague, and is useful for establishing a prognosis in many cases. Because of the high prevalence of inflammatory diseases in cattle, the CBC and plasma proteins are often the most valuable set of parameters to gather. Common abnormalities seen in ruminants include regenerative and nonregenerative anemias, blood parasites, inflammatory leukograms with left shifts, panhypoproteinemia, panhyperproteinemia, and transient or persistent lymphocytosis.

Key words: complete blood count, total protein, anemia

A complete blood count (CBC) can be an important extension of the physical examination in ruminants, and may be used to suggest certain disease processes when exam findings are vague, and is useful for establishing a prognosis in many cases. Whole blood in EDTA is the preferred sample, with the vacuum volume of the tube fully replaced by the sample. An unstained, air-dried blood film should be made within 15 minutes of collection and submitted with the remaining sample in the tube for laboratory analysis if the time to analysis is greater than 2 hours. An accurate CBC can be achieved in properly stored samples in tubes up to 24 hours. If platelet counts are of particular importance, those counts should be made within 4 to 6 hours. If samples are to be shipped to an outside laboratory, they should be wrapped in packing material to protect from breakage and shipped on ice. Automated analyzers must be properly calibrated for the species of interest and stained smears should undergo manual examination.

Erythron

Red Blood Cell Parameters

A complete evaluation of RBCs should include a PCV and/or hematocrit (HCT), RBC count, hemoglobin (Hgb), MCV, and MCHC, RBC morphology, and parasites.

The PCV is determined by centrifugation of a microhematocrit tube and determination of the percentage of RBCs per volume of blood. This is easily performed in-clinic. The HCT is a calculated value based on RBC size and number.

The MCV, MCH, and MCHC are characteristics of the RBCs, indicating average cell size, average cell hemoglobin content, and average cell hemoglobin concentration, respectively. MCV

may be calculated, but most instruments directly measure red cell volume, reporting the mean. A decreased MCV is termed microcytosis; increased MCV is macrocytosis and normal MCV is normocytosis. Note that many microcytes or macrocytes must be present to change the MCV since it is a mean value.

There are also 2 cellular hemoglobin measurements reported by many automated analyzers, MCHC and MCH. The MCHC is considered the more useful of the 2 cellular hemoglobin measurements, and is a calculated value. Descriptive terms relative to MCHC are normochromic, hypochromic or hyperchromic for normal, decreased and increased cellular hemoglobin concentrations, respectively. The most common cause of a slightly decreased MCHC is a strongly regenerative anemia, because immature red cells (polychromatophils) contain less than a full complement of hemoglobin. Iron deficiency anemia may also cause a decreased MCHC, and hypochromasia (pale red cells) may be visible on the blood film.

Red Blood Cell Parasites

A variety of hemoparasites may be observed on the blood film. The rickettsia *Anaplasma* spp, bacteria *Mycoplasma* spp (formerly *Eperythrozoon*), and 3 protozoal parasites, *Babesia* spp, *Theileria* spp, and *Trypanosoma* spp, may affect ruminants. *A marginale* (cattle) and *A ovis* (sheep and goats) appear as small, round basophilic inclusions on the periphery of RBCs. A significant hemolytic disease is seen in adult cattle infected by this parasite through tick transmission in the southeastern and western US.

Mycoplasma organisms, *M wenyonii* in cattle, *M ovis* in sheep and goats, and *M haemolamae* in camelids, may appear on the surface of RBCs or free in the background on the blood film. They typically cause anemia only in immunocompromised animals. A syndrome in heavily parasitized cattle has been described with signs including hind-limb edema and lymphadenopathy.

Anemia

Anemia is a common abnormality seen in the blood profiles of ruminants. Physical examination findings may include pale mucous membranes, weakness, exercise intolerance, and mental depression or aggression. Anemia is subdivided into regenerative or non-regenerative anemia using RBC morphology and the reticulocyte count, limiting the list of differential diagnoses.

In ruminants, the presence of any polychromasia or reticulocytes indicates some degree of regeneration. Other common findings in a regenerative response include basophilic stippling, Howell-Jolly bodies, nRBCs, an increased MCV,

and decreased MCHC. In the absence of polychromasia and reticulocytosis, the anemia is considered non-regenerative; however the clinician must remember that ruminants typically take 2 to 4 days to mount a bone marrow response, with the peak response occurring at about 4 to 7 days.

Causes of Regenerative Anemia

Hemorrhage/ Whole Blood Loss – low PCV with proportionately low TPP

- Internal – Whole blood into a body cavity
 - Hemoabdomen, hemothorax – Erosion of vessel by abscess or neoplasia, rupture of middle uterine artery
- External – Whole blood exits the body
 - Abomasal ulcers
 - Parasites – *Haemonchus*, sucking lice
 - Hemorrhagic enteritis
 - Caudal vena cava syndrome
 - External trauma

* Abnormalities in the PCV and TPP are not noted after acute blood loss for 24 to 48 hours and it may take 2 to 4 weeks after the insult has ended for the PCV to return to its original level.

Hemolysis – low PCV, normal to increased TPP

- RBC parasites: *Anaplasma* spp, others
- Infectious: *Clostridium novyi*, *Leptospira* spp
- Toxins: copper, onion, *Brassica* spp, red maple leaves, water intoxication

Causes of Non-regenerative Anemia

Anemia of Inflammatory (Chronic) Disease – normocytic, normochromic

- Gastrointestinal disorders
 - Lymphosarcoma
 - Chronic BVDV infection
 - Johne's disease
- Chronic abscess
- Hepatic diseases
 - Liver abscesses
- Endocrine diseases

Anemia of chronic renal failure – normocytic, normochromic

Chronic Nutrient Deficiencies

- Iron – microcytic, hypochromic anemia
- Copper – microcytic, hypochromic anemia
- Cobalt- normocytic, normochromic

Intrinsic bone marrow disease – often have neutropenia, thrombocytopenia

- Bracken fern toxicity
- Myelofibrosis
- Myelophthisis

Polycythemia/Erythrocytosis

Polycythemia, or increased PCV, is another frequent finding on the ruminant CBC. The most common is a relative

polycythemia, caused by dehydration. This can be confirmed clinically by detecting prolonged skin turgor, tacky mucous membranes, recession of the globe, and an elevated TPP and albumin.

Absolute polycythemias, in which the red cell mass is truly increased, rarely occur in ruminants, but may result from systemic hypoxia seen in animals living at high altitudes, chronic pulmonary disease, or right-to-left cardiovascular shunting. Blood gas analysis and consideration of the animal's geographic origin confirm this etiology.

Leukon

A differential count by cell type is more important than is the total white blood cell count, as increases and decreases in individual cell types may occur simultaneously, leaving the total WBC count unchanged.

Neutrophils

Neutrophils function by migration into damaged tissue within 2 hours of an insult for phagocytosis of foreign material and bacteria. Neutrophils are the dominant WBC in young ruminants, but with age, the lymphocyte becomes the dominant WBC, with a normal neutrophil:lymphocyte ratio of 1:2 in adult animals. Goats may have equal or slightly increased neutrophil numbers when compared to lymphocytes.

Two types of neutrophils exist: mature, segmented ruminant neutrophils and immature, band neutrophils. Bands are frequently released into circulation with acute inflammation. Toxic changes may be noted on manual differential counts and indicate changes to the neutrophil morphology from severe inflammation, usually associated with gram negative organisms and septic shock. Toxic changes occur in the bone marrow and include Döhle bodies in the cytoplasm, toxic granulation, diffuse cytoplasmic basophilia, bizarre giant forms, and cytoplasmic vacuolation or foamy cytoplasm. It is important to note that cytoplasmic vacuolation may be artifactual from prolonged exposure to EDTA which can be avoided by prompt blood film preparation. Hypersegmentation of neutrophils may occur as an artifact of aged EDTA samples and as a result of corticosteroid treatment.

Neutrophilia is primarily caused by the presence of mild to moderate inflammation or during recovery from more severe inflammatory conditions, such as infectious processes, tissue injury, neoplastic diseases, and non-inflammatory conditions. Bovine Leukocyte Adhesion Deficiency (BLAD) is a significant genetic cause of neutrophilia in Holstein cattle, which, with a monocytosis, results in WBC counts in excess of 40,000 cells/ μ L. With this condition, leukocytes fail to express adhesion receptors on their surface that are required for movement from the vasculature to tissue sites of inflammation. Affected calves may have recurrent bacterial infections, diarrhea, pneumonia, lymphadenopathy, dermatosis, and stunted growth.

Neutropenia is caused by acute, severe inflammatory diseases in cattle including gram negative sepsis, metritis,

mastitis, peritonitis, pneumonia, *Salmonella* infection, and many others. It may also be seen with bone marrow injury such as that caused by bracken fern poisoning, often accompanied by non-regenerative anemia and thrombocytopenia.

Lymphocytes

Lymphocytes are predominantly B cells or T cells, functioning in antibody and cell-mediated immunity, respectively. Normal and reactive large lymphocytes in the bovine may have atypical features often associated with malignancy in other species, and should be interpreted by experienced pathologists.

Pathologic lymphocytosis is unusual in ruminants, but may be associated with chronic viral infections, chronic pyogenic conditions or autoimmune diseases. Approximately 30% of cattle infected with bovine leukemia virus (BLV) will exhibit a benign persistent lymphocytosis of B cells. This does not indicate the presence of a tumor; however BLV-infected cattle with persistent lymphocytosis are at increased risk for developing a lymphoid malignancy.

Lymphopenia may be seen with stress or corticosteroid administration, acute viral or bacterial infections, endotoxemia, BVD virus infection, and rare immunodeficiencies.

Eosinophils

Eosinophils function in the immune response to parasites, allergens, and other inflammatory processes. Cattle normally have higher numbers of eosinophils than other species, but eosinophilia may result from parasite migration, atypical interstitial pneumonia, acute bovine pulmonary emphysema, and milk autoantibody formation in dairy cattle.

Basophils

Basophils are present in very small numbers in normal ruminants, functioning in allergic and inflammatory processes by releasing heparin, histamine and other inflammatory mediators in immediate hypersensitivity reactions. Their numbers may be increased with allergic dermatoses and hypersensitivity reactions.

Monocytes

Monocytes enter the tissues from the circulation to become macrophages, capable of phagocytizing infectious organisms, particulates, and cell debris. Their numbers are quite variable in cattle and are not sensitive indicators of disease processes. Increases may accompany chronic inflammation, tissue necrosis, hemolysis, or a stress response. Low monocyte numbers have been associated with endotoxemia and viremia.

Inflammatory Leukogram

Inflammatory diseases occur commonly in ruminants and it is important to note the changes that occur in the leukogram with inflammation at various stages of the disease

process. The lack of a significant segmented (mature) neutrophil bone marrow storage pool in adult cattle results in an initial neutropenia for 24 to 48 hours in the face of severe inflammation, decreasing the neutrophil:lymphocyte ratio. Stress associated with the disease process may also cause lymphopenia and eosinopenia. A left shift, signified by the appearance of numerous immature neutrophils (bands or earlier forms) in circulation, typically appears within 24 hours with acute inflammation. A degenerative left shift refers to the situation when immature forms outnumber segmented neutrophils, or to a left shift with concurrent neutropenia. While this is considered a poor prognostic sign if persistent, it is not unusual in ruminants due to their initial neutropenic response to severe inflammation. A regenerative left shift occurs as the bone marrow responds to the inflammation with increased production, resulting in increased mature neutrophil numbers in addition to bands, with mature neutrophils outnumbering immature forms.

The bone marrow is normally able to replenish the bone marrow pool of neutrophils in 4 to 5 days, returning counts to normal if the inflammation is resolved. A neutrophilia with or without a left shift may appear if the inflammation is ongoing. As inflammation becomes chronic, lasting for a few days to weeks, the WBC and differential counts will often return to normal, making the diagnosis of chronic inflammation difficult in ruminants. In some cases, there may be slight increases in the neutrophil, lymphocyte and monocyte numbers.

Stress Leukogram

The stress leukogram results from glucocorticoid administration or release, generally from a non-inflammatory disease, such as a displaced abomasum or indigestion. Typically, there is a normal or increased total WBC count, mature neutrophilia, lymphopenia, eosinopenia, and mild monocytosis. The neutrophil:lymphocyte ratio may be 2 to 3:1, but there is no left shift.

Physiologic Leukocytosis

Physiologic leukocytosis results from epinephrine release during excitation or exercise. An increase in blood pressure and splenic contraction leads to a transient, mild leukocytosis, mature neutrophilia, and a lymphocytosis. This phenomenon is not as prevalent in ruminants as in other species.

Platelets

Platelets form the initial hemostatic plug to damaged vasculature and maintain vascular integrity. Petechia and mucosal bleeding tendencies are clinical signs that warrant evaluation of platelets. Thrombocytosis is usually secondary (reactive thrombocytosis), and may occur with exercise, stress, or inflammatory conditions. In ruminants, a false elevation in the platelet count can occur due to the counting of small RBCs as platelets by some automated analyzers.

Thrombocytopenia is defined as a platelet count less than 100,000 cells/ μ L, with prolonged bleeding occurring when platelets number less than 40,000 cells/ μ L, and spontaneous bleeding at less than 10,000 cells/ μ L. Extensive hemorrhage may result in mild thrombocytopenia due to platelet loss in whole blood. Specific causes of thrombocytopenia in ruminants include septicemia, endotoxemia, vasculitis, bracken fern and trichloroethylene toxicities, *Salmonella* infection, mastitis, metritis, neoplasia, disseminated intravascular coagulation, bovine viral diarrhea virus infection, and bluetongue virus infection in sheep.

Plasma Proteins

A TPP level can be achieved in-house by use of a hand-held refractometer. In order to interpret alterations in the TPP, one should consider the differential values of albumin and globulins. This will require additional testing than what is typically included on a CBC. If TPP is elevated, it must be determined if albumin or globulins are elevated, or both (panhyperproteinemia). Conversely, decreases in TPP may be due to low albumin, low globulins or both (panhypoproteinemia). The normal albumin:globulin (A:G) ratio is 0.84-0.94 in cattle.

- Panhyperproteinemia
 - Dehydration
- Panhypoproteinemia
 - Nematode parasitism, Johne's disease, salmonellosis
 - Hemorrhage
- Albumin
 - Hyperalbuminemia
 - Dehydration
 - Hypoalbuminemia
 - Inadequate production: severe, chronic liver disease, poor intake
 - Loss: renal, GI disease, hemorrhage, exudation

- Globulins
 - Hyperglobulinemia
 - Chronic inflammatory disease: traumatic reticuloperitonitis, liver abscess, chronic pneumonia
 - Hepatic disease
 - Hypoglobulinemia
 - Not common alone in mature cattle
 - Neonates with failure of passive transfer

Fibrinogen is an acute phase protein, readily analyzed by most laboratories. Fibrinogen increases over a period of 2 days after initiation of inflammation of bacterial, viral or chemical origin, or trauma. Dehydration may cause a relative hyperfibrinogenemia in addition to a relative hyperproteinemia. To correct an increased fibrinogen for hydration status a TPP:Fibrinogen ratio should be considered. If the ratio is <10:1, there is an absolute increase in fibrinogen, indicating inflammation. A potential problem with this analysis is that inflammation may also increase globulins, causing an absolute increase in the TPP, so clinical hydration status should always be considered when interpreting plasma proteins. A decreased fibrinogen is unusual, but may be caused by liver disease and disseminated intravascular coagulation.

Suggested Readings

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