The Utility of the Blood Smear in Bovine Medicine

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INTRODUCTION

Most bovine practitioners regularly screen blood smears for intra-erythrocytic parasites and recently L. Roland, M. Drillic and M. Iwersen highlighted the utility of haematology in bovine medicine and suggested that it may often be overlooked for more direct or specific diagnostic methods and tests. There is a lot more information that can be obtained from a blood smear itself, let alone a full blood count (FBC) or Complete Blood Count (CBC), in addition to whether there is a parasitaemia or not. This article is a review of practical haematology and will also present some new work on the genetic classification of some blood parasites

HAEMATOLOGY

The quality of any laboratory result is directly related to the quality of the sample submitted. Good quality blood smears will yield more information than poorer smears. It is advisable for veterinarians to train their farmer clients on how to prepare reasonable smears because they are able to collect smears earlier and in the case of a dead animal, as soon after death as possible. Post-mortem changes occur quickly in the peripheral blood and include red cell crenation, neutrophil hypersegmentation and karyolysis, monocyte and lymphocyte nuclear swelling and mononuclear cell degeneration.

Ear capillary smears are most often used, but if blood is collected from a central vein a smear should be made from the ethylenediamine tetra-acetic acid (EDTA) anticoagulated tube. There are two types of EDTA tubes: potassium-ethylenediamine tetra-acetic acid (EDTA/K3) with 1.27mg EDTA/K3 per milliliter of blood and the disodium-ethylenediamine tetra-acetic acid tubes with 1.5mg of EDTA/Na₂ per
milliliter of blood. The concentrations of these EDTA salts highlight the importance of the ratio of blood to EDTA salt and the correct filling of tubes in order to achieve not only satisfactory anticoagulant effect, but also limit the morphological alterations that can occur to white cells and platelets when the EDTA salt is present in too a high concentration. Heparin is neither a good anticoagulant for morphological analysis nor a good sample type for molecular diagnostic techniques.

It is important to note that there are sometimes quite striking, differences between the central venous and peripheral ear blood smears. These include red cell morphological changes, slight shifts in the differential count and sizes of the mononuclear cells.

When evaluating blood smears it is important to develop a systematic approach and follow it with every smear. Starting with a general scan at a lower magnification to actually assess the quality of the smear in terms of the distribution of white cells, presence of a decent monolayered area in the body of the smear where the red cells are almost touching, a good fairly uniform ‘feather-edge’ and platelet clumping at the periphery of the smear. Then one should mentally focus on the erythrocytes and find the monolayered area in the body of the smear. Assessing various fields in this region one can evaluate cell size, cell staining characteristics and make note of any unusual red cell shapes or wall anomalies. Then concentrating on the leukocytes one should assess their distribution, morphology and relative proportions. Search for platelets evaluating their size and looking for platelet clumping. Then lastly one can search for intra-erythrocytic and white cell inclusions. Parasitized red blood cells are heavier than normal cells and tend to be more prominent in the feather-edge of the smear but there should be some parasitized cells in the monolayer area.

THE ERYTHROCYTES
Roland et al in their article mention that bovine erythrocytes are quite small compared to other species and that the average size is 5-6 um. Bovine red cells have a life span of roughly 130-160 days.

The erythrocyte series in cattle:

<table>
<thead>
<tr>
<th></th>
<th>RANGE</th>
<th>AVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>5 to 10</td>
<td>7</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>8 to 15</td>
<td>11</td>
</tr>
<tr>
<td>PCV</td>
<td>24 to 46</td>
<td>35</td>
</tr>
<tr>
<td>MCV</td>
<td>40 to 60</td>
<td>52</td>
</tr>
<tr>
<td>MCH</td>
<td>11 to 17</td>
<td>14</td>
</tr>
<tr>
<td>MCHC microhaematocrit</td>
<td>30 to 36</td>
<td>32.7</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ESR 1 hour</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ESR 8 hours</td>
<td>0 to 3</td>
<td></td>
</tr>
<tr>
<td>RBC diameter</td>
<td>4 to 8</td>
<td>5.8</td>
</tr>
<tr>
<td>Resistance to hypotonic saline MIN</td>
<td>0.52 to 0.66</td>
<td></td>
</tr>
<tr>
<td>Resistance to hypotonic saline MAX</td>
<td>0.44 to 0.52</td>
<td></td>
</tr>
<tr>
<td>M:E Ratio</td>
<td>0.31 to 1.85: 1.0</td>
<td>0.71:1.0</td>
</tr>
<tr>
<td>Erythrocyte life span</td>
<td>160 days</td>
<td></td>
</tr>
</tbody>
</table>
Anaemia is classically defined by a low haematocrit and red cell count but most practitioners are adept at diagnosing anaemia clinically and when armed with that knowledge, important clues can be obtained from evaluating the blood smear even before the FBC results are available.

Regenerative anaemia is most often due to haemorrhage or haemolysis. Clinically apparent haemorrhage would be self-explanatory but blood loss that is not clinically visible might be more challenging to investigate and possible causes include abomasal ulcers, haemorrhagic enteritis, severe verminosis, vena cava syndrome and foreign body trauma.

Haemolytic anaemia may suggest exposure to certain antibiotics, plants such as *Brassica* species, onions, rye grass or red maple, possibly ingested toxins or intake of excess minerals such as copper. There are many other causes of haemolysis not mentioned here including intra-erythrocytic parasites.

In cattle regenerative responses begin after roughly two days and take weeks to be fully established. Actual reticulocytosis is only moderate compared to other species and not that visible microscopically.

Non-regenerative anaemia can also be recognized at blood smear examination. There is a lack of anisocytosis along with clinical anaemia. It can be classified according to red cell morphology, visible in the smear, as macrocytic, normocytic or microcytic; and according to staining of the erythrocytes as normochromic or hypochromic. Normocytic normochromic anaemia is often associated with non-specific chronic inflammation. It is seen in chronic renal failure, endocrine disorders, bone marrow suppression due to drugs (estrogens, chloramphenicol), toxins like lead, abscesses and neoplasia. Microcytic hypochromic anaemia is commonly seen with iron deficiency due to chronic blood loss or parasitaemia. It can be seen in calves raised solely on milk, copper deficiency, lead toxicosis and pyridoxine deficiency. Macrocytic normochromic anaemia is seen in deficiencies such as cobalamin, folate and cobalt. Polled Herefords suffer from a congenital dyserythropoiesis that results in a macrocytic normochromic anaemia.

THE LEUKOCYTES
A complete white blood cell count (WBC) includes the total number of leukocytes, the relative differential white cell count and the absolute differential blood counts. Although this requires an automated analyser, estimates can be made from assessing a set number of microscopic fields in a well-made blood smear. By examining 5 low-power (20X) fields in the monolayer area of the smear 7-15 white cells is probably a normal count. Alternatively the average number of white cells counted in several high-power/oil immersion fields can be multiplied by 2000 to estimate the WCC reasonably reliably in cattle. The relative differential count of any laboratory-generated WBC will have been made microscopically anyway. Thus, although leukocyte numbers may be an estimate the relative differential that a vet makes from a good quality blood smear should be reliable. Therefore it is important to try maximizing the information that is available from the smear itself and put it together with the history and clinical findings. One does not have to wait for the automated haematology results.
The Lekocyte series in cattle

<table>
<thead>
<tr>
<th>LEUKOCYTE</th>
<th>RANGE</th>
<th>AVE count</th>
<th>PERCENTAGE</th>
<th>AVE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil band</td>
<td>4000 to 12000</td>
<td>8000</td>
<td>0 to 2</td>
<td>0.5</td>
</tr>
<tr>
<td>Neutrophil mature</td>
<td>0 to 120</td>
<td>20</td>
<td>15 to 45</td>
<td>28</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>2500 to 7500</td>
<td>4500</td>
<td>45 to 75</td>
<td>58</td>
</tr>
<tr>
<td>Monocyte</td>
<td>25 to 840</td>
<td>400</td>
<td>2 to 7</td>
<td>4</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0 to 2400</td>
<td>700</td>
<td>2 to 20</td>
<td>9</td>
</tr>
<tr>
<td>Basophil</td>
<td>0 to 200</td>
<td>50</td>
<td>0 to 2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

It is important to understand the kinetics of the different white cell types peculiar to the bovine and what physiologic and pathologic conditions that will cause deviations from the norm.

Bovine lymphocytes basically have 3 size groupings. Small lymphocytes are round with a small round, densely-staining nucleus chromatin elliptically-positioned in a small amount of clear to pale blue cytoplasm. This sparse cytoplasm and dense chromatin have led to the theory that these small lymphocytes may be metabolically-dormant. The medium-sized lymphocytes have larger, lighter staining nuclei that might be indented or oval with a greater volume of cytoplasm encircling the nucleus. These make up the majority of circulating lymphocytes in the bovine compared to the average circulating lymphocytes in other species. The largest lymphocytes are morphologically very similar to monocytes with more central, even lighter-staining chromatin and round, oval to deeply cleaved nuclei. All lymphocytes can have magenta granules of varying size and shape in their cytoplasm. It is not uncommon for nucleolar rings and even nucleoli to be seen in reactive lymphocytes and these features must not be confused as a malignant change.

Antigenic stimulation often causes lymph node enlargement but this rarely translates into circulating lymphocytosis. In fact, localised infections may cause lymphocyte entrapment in lymph nodes resulting in a lymphocytopenia. Besides the stress leukogram, other causes of lymphocytopenia include viral infections (more so than bacterial infections), endotoxic bacterial infections, granulomatous disease disrupting lymph node architecture, loss of afferent lymph in gastrointestinal or respiratory tracts and acquired T-lymphocyte deficiencies such as thymic atrophy or destruction. In pregnant cows the lymphocyte count decreases in the week before calving, reaching its lowest value the day before calving.

Lymphocytosis may be physiological, reactive or proliferative. The physiological or excitement lymphocytosis is transient and mostly due to an easily-accessible pool of lymphocytes that are rapidly mobilized into circulation by epinephrine release. This pool in calves is estimated to be seven times the size of the circulating lymphocyte pool. The other two mechanisms of lymphocytosis are usually persistent. Persistent lymphocytosis is a sub-clinical, non-neoplastic manifestation of bovine leukaemia virus infection. The lymphocytes mostly have normal morphology but in about 10%
of cases the disease may progress to lymphosarcoma or lymphocytic leukaemia and atypical lymphocytes may be seen in circulation. Deep fungal infections, some protozoal infections and other viral infections can occasionally result in a lymphocytosis.

At birth calves have a greater proportion of neutrophils than lymphocytes mostly attributed to the stress at birth and a stress leukogram profile. However this changes quickly with lymphocyte numbers rising and neutrophils staying the same and the N:L ratio may actually reverse in the first week post-partum. Calves that are delivered by cesarean have N:L ratios similar to those of adult cattle while dystocia calves can have severe neutropenia and lymphopaenia. The cow also goes through similar stress at parturition and neutrophil numbers increase, sometimes with a left shift (a high number of young, immature white blood cells present), and lymphocyte numbers decline to varying degrees depending on the stress endured and status of the foetal membranes. These changes are visible within 12-24 hours post-partum and then subside over the next few days. Since the storage pool of neutrophils is quite small in the cow conditions such as retained placenta or metritis quickly result in leukopaenia, neutropenia, a left shift to bands and even metamyelocytes and a monocytosis within 2-5 days. Physiological stress of any kind especially at weaning or during transport will lead to a similar leukogram profile. In early inflammatory disease in the bovine the WCC may not reflect the seriousness of the process because the lymphocytes, which predominate in health, decline due to the stress effect along with eosinophil numbers. Simultaneously neutrophils and monocytes are leaving the vasculature to the site of inflammation. Although the bone marrow releases the neutrophil storage pool the cells are also attracted to the inflammatory site and the WCC can drop dramatically. Immature neutrophil stages such as bands and metamyelocytes also enter the circulation and are easily recognizable as a left-shift microscopically. In the leukopaenic stage this is regarded as a degenerative left-shift but as neutrophil production is up-regulated it becomes a regenerative left-shift and ultimately as a mature neutrophilia, often with a monocytosis, and the WCC may rise to 20000/ul-30000/ul, only rarely rising higher. Thus a marked left-shift is not unusual in cattle with severe inflammatory disease but it should not persist for longer than 4-5 days.

Leukopaenia is a function of decreased production, increased tissue demand and consumption combined with marginalisation. A wide variety of conditions can lead to leukopaenia including viral infections, circulatory shock, peracute inflammation, cytotoxic substances, as well as haematopoietic stem cell disorders and bone marrow atrophy. In cattle, leukopaenia often occurs with metabolic disorders, liver disease and infectious disease (Bovine Viral Diarrhoea virus, Theileriosis, paratuberculosis or Salmonellosis). Panleukopaenia a depression of all WBC subpopulations, is observed in viral disease (BVD, infectious bovine rhinotracheitis), rickettsiosis, bacterial septicaemia and purulent splenitis.

Neutrophils develop in the bone marrow and take about 4-9 days from myeloblast to mature segmented or polymorphonuclear neutrophil. Broadly speaking neutrophilia has three causes – inflammation, excitement (or epinephrine) response and lastly, the stress or steroid response. It is easiest to differentiate these responses once the white cell differential is available but early clues can be gained from the blood smear examination. The first step in this
differentiation is looking for a left-shift which is visualized by neutrophil morphology examination. If present, an inflammatory process is indicated. If no left-shift is evident, then one focuses on the lymphocytes. A lymphopaenia is indicative of a stress leukogram but normal numbers or a lymphocytosis indicates an epinephrine response. It is important to note that an inflammatory leukogram may often be superimposed on one of the other responses. The most common causes for neutrophilia are chronic inflammation and stress. Chronic inflammation has been reported with many infections such as mastitis, urogenital tract, gastrointestinal tract, liver, respiratory tract, heart and central nervous system infections. In bovines, neutrophilia is also commonly observed with acute purulent processes, such as endometritis, retained placenta and foreign body peritonitis. Inflammatory neutrophilia is seen in viral, bacterial, protozoal, parasitic and fungal infections. Additionally neutrophilia is observed with non-infective inflammation (traumatic injuries, necrosis, infarction, burns and thrombosis etc), neoplasia, intoxication, endocrine disorders, haemorrhage and haemolysis. In cattle, stress-induced neutrophilia is also associated with abomasal displacement, ketosis, bloat and dystocia. Severe leukocytosis exceeding 40 000 cells/ul and sometimes even 100 000 cells/ul, due to a marked increase in neutrophils may be a sign of BLAD in Holstein Friesians. BLAD is a disease of dysfunctional adhesion molecules leading to an inability to migrate out of the vasculature and it is usually diagnosed in calves and most affected calves die within 1 year of age.

As in other species there is a storage pool of neutrophils in the bone marrow to cope with a sudden peripheral demand but the bovine storage pool is quite small compared to other species. Once released from the bone marrow, the neutrophils join the vascular pool. The vascular pool is then divided into the circulating pool and the marginal pool. In the vasculature, especially within post-capillary venules, the neutrophils travel more slowly than red blood cells and plasma mainly due to adhesion molecules on the neutrophils and endothelial cells. Hence the concentration of neutrophils is greater in these venules than in larger vessels. Thus if only a few neutrophils are seen in an ear smear, a neutropaenia is probable. Since the storage pool of bovines is smaller than other species it is not unusual for a neutropaenia to develop in the first 1-2 days of an inflammatory response in cattle and then rising back into reference range with a persisting left-shift. If the animal survives, the numbers rise later leading to a neutrophilia. Neutropaenia can be caused by viral (BVDV, Bluetongue virus, Border disease, Ehrlichia ruminantium), protozoal (Theileria species) and fungal infections as well as bone marrow disease, toxins, neoplasia or idiosyncratic drug reactions.

THE PLATELETS

Bovine platelets are generally small, without projections and occur singly or in small clumps. Platelets or thrombocytes occur at a rate between 1.0 and 8.0 ×10^5 averaging about 5.

Signs of regeneration include giant platelets and pseudopodia. Platelet numbers increase significantly during the first 2 weeks of age and more slowly thereafter during the first 3 months. Platelet counts in calves might be within or above adult reference intervals. Thrombocytosis occurs physiologically with the epinephrine-
induced contraction of the spleen. Reactive or secondary thrombocytosis is triggered by cytokine release and is observed in connection with stress, chronic blood loss, inflammation, neoplasia or iron deficiency. Primary thrombocytosis is a myoproliferative disorder that is rare.

If platelet clumping is visible in the smear it becomes impossible to make a judgement about platelet numbers. Thrombocytopenia is found in excessive consumption in blood loss, decreased platelet production (bone marrow toxic suppression), destruction (due to infections, toxins, drugs, neoplasia or immune-mediated) or distribution anomalies (splenomegaly).

THE COMMON PARASITES AND THE HAEMIC MYCOPLASMAS
The Anaplasmal and Babesial parasites are well known to veterinarians and most farmers and will not be discussed further. However, the careful examination of blood smears from suspected cases of such diseases may reveal some other parasites that may have some significance. See figure 1

The Theilerioses are less well recognized in blood smears and should be mentioned here because they are more likely to be confused with *Mycoplasma* species. The benign Theilerial species occurring in southern Africa include *Theileria mutans*, *Theileria velifera*, *Theileria taurotragi* and *Theileria orientalis* groups. These species mainly replicate through their intra-erythrocytic piroplasmal stages rather than schizogony of the schizonts in the white cells as occurs with the highly pathogenic *T. parva* and *T. annulata* that cause East Coast Fever and Tropical Theileriosis (not in southern africa) respectively. The benign Theilerioses generally cause a mild pyrexia when cattle are infected and their major importance is confusion for *T. parva* piroplasms in blood smears. They have varied piroplasmic forms which tend to be larger than *T. parva*. They vary from round or oval to more comma-shaped and even tadpole-like forms may be seen. Some strains of *T. mutans* and *T. taurotragi* cause more severe clinical disease with anaemia and icterus. Turning sickness is a condition that is associated with *T. taurotragi* in young cattle that have a partial immunity but are re-infected. There is a proliferation of schizont-infected lymphoblasts in the meninges, brain, spinal cord and sometimes spleen leading to thrombosis and infarction. The exact pathogenesis has not been fully elucidated.

The genus *Eperythrozoon* was established in 1928 by Schilling when he described parasites in mice blood. In 1934 Neitz et al described *Eperythrozoon ovis* in sheep in South Africa and in the same year Adler and Ellenbogen described *Eperythrozoon wenyonii* in a splenectomised calf. Hoyte then described organisms in cattle as *Eperythrozoon teganodes* and commented that the organisms are so highly pleomorphic that future diagnostics may identify mixed infections of more than one species. *Eperythrozoon tuomii* was identified as a platelet-bound organism in Madagascar and then Finland. All three species have been identified in Germany, Argentina and South Africa. They are considered non-pathogenic in bovines but disease outbreaks have been reported and even fatal cases documented elsewhere in the world. Interestingly *E. teganodes* does not appear in Bergy’s Manual of Systematic Bacteriology and *E. tuomii* does not seem to have earned taxonomic status.

With the advent of molecular biology and genotyping the eperythrocytic parasites previously known as *Eperythrozoon* and *Haemobartonella* (formerly classified as
rickettsial organisms) are now understood to be more closely related to the order Mycoplasmatales. This affiliation is based on their lack of a cell wall, use of the codon UGA to encode tryptophan, and 16S rRNA gene sequences. Although the reassignment of Eperythrozoon and Haemobartonella to the genus Mycoplasma is still under debate, referral to this genus has been widely accepted by the scientific community, and they are commonly referred to as haemotropic mycoplasmas or haemoplasmas. Several of these have been renamed Mycoplasma, whereas newly described haemoplasmas are given the designation "Candidatus." The haemoplasmas infect a wide variety of vertebrates throughout the world, including several reports of human infection. They share similar characteristics and morphologic features such as rod, coccoid, and ring-shaped structures found individually or in chains on the red cell and gram-negative staining because of the lack of a cell wall; none of the haemoplasmas have been cultured outside their hosts. It is well established that the haemoplasmas attach to the surface of the red cell but may under certain conditions penetrate the host cell.

Haemotrophic Mycoplasmas have been isolated from cattle in various parts of the world. Mycoplasma (formerly Eperythrozoon) wenyonii and Candidatus Mycoplasma hemobos have been identified in cattle in Switzerland, Germany, Japan, China, Brazil, United Kingdom and United States of America.

**CONCLUSION**
The careful evaluation of a blood smear is a useful and practical tool that is possibly underestimated in its importance in general practice. This adds great value to a clinical diagnosis when noticeable changes support a diagnosis and should be part of every practitioner’s routine procedure.

REFERENCES