Interpretation of Alterations in the Horse Erythrogram

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Abstract: The interpretation of erythrogram is pivotal to assist clinicians in diagnosis, prognosis, patient management and control of equine diseases. Relative erythrocytosis associated to dehydration and blood splenic mobilization are common in horses. Absolute erythrocytosis appears less often in the horse and it can be related to increased erythropoietin concentrations, as happen in chronic hypoxic situations, neoplasias and paraneoplasic syndromes. Even less common, primary absolute erythrocytosis or polycythemia vera has been also described in horses. Anemia is a very common equine hematological disorder. The classification between regenerative and non-regenerative is difficult in this species, because peripheral signs of regeneration are not common in horses, despite having an intense regenerative anemia. This classification would need in many cases a bone marrow biopsy. The most common causes of anemia in horses are acute and chronic blood loss, hemolytic anemia and anemia of chronic disease. Assessment of peripheral blood smears is also an important tool for diagnose a hematological disorder in a horse. It should be taken into consideration that rouleaux formation and echinocytes (spiculated regular erythrocytes) are physiological characteristics of equine blood, in opposite to what happen in other animal species. Abnormal erythrocyte shapes described in horses are spherocytes, target cells, leptocytes, acanthocytes, schistocytes and leptocytes. The most common erythrocyte inclusions are, Howell-Jolly bodies (nuclear remnants, sometimes associated to rapid bone marrow maturation), Heinz bodies (indicative of oxidative damage) and hemoprotozoan parasites, such as *Babesia caballi* and *Theileria equi*.

Keywords: Anemia, Erythrocyte, Hematology, Horse, Polycythemia.

1. INTRODUCTION

The term erythron refers to red cell precursors, the tissues in which production takes place, mature erythrocytes themselves and its functional unit, the red blood cell (RBC) [1, 2]. The erythron is assessed from peripheral blood samples, by calculating the number of circulating RBC, hemoglobin concentration (HB), packed cell volume (PCV), volumetric indices, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), microscopic morphological examination [1, 2] and sometimes, examination of the bone marrow [3].

For the interpretation of hematological data in horses, it must take into account that the PCV is unstable. PCV in horses is highly variable, due to the significant innervation of the spleen and its performance as a reservoir of blood, which could store more than one third of the blood volume [4]. Therefore any adrenergic stimulation, as occurs during exercise and/or in response to excitation, causes a splenic contraction and releases a large amount of blood cells into the peripheral circulation. Then, the PCV in the horse at rest should be carefully evaluated according to the excitation level [4]. For this reason, the most practical and reliable method of assessing an erythrocyte regenerative response in an anemic horse is the bone marrow analysis of iliac crest, sternum and ribs. A myeloid erythroid (M/E) ratio less than 0.5 is considered an evident sign of erythrocyte regeneration (normal ratio is 0.5:1.0 to 1.5:1.0) [5, 6]. Reticulocyte counts may also be performed on marrow aspirates to determine the erythropoietic response in anemic horses. Values greater than 5% are consistent with accelerated erythropoiesis [3]. Furthermore, in cases where anemia results from decreased erythropoiesis, bone marrow examination may identify the cause and enable a definitive diagnosis [3].

The correct interpretation of equine hematology values is dependent upon whether the individual animal is considered to be one of the so-called "hot-blooded" or "cold-blooded" breed [3, 7]. The "hot-blooded" horses are those of Arabian ancestry, including Arabian, Quarter Horse, Appaloosa, Standardbred and Thoroughbred, whereas cold-blooded horses are basically draft type animals including Clydesdale, Percheron, hack and Shire. In general, cold-blooded has lower reference limits for RBC parametesr than hot-blooded [3, 7].

For a proper interpretation of the erythrogram is necessary to measure total plasma proteins (TPP) and

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fractions (albumin, fibrinogen and globulins). The assessment of these parameters allows differentiate between absolute or relative hematological changes, associated with changes in plasma volume [8].

Reference data for erythrocyte parameters in horses are presented in Table **1**.

Modern automated analyzers also determine RBC distribution width (RDW). RDW is a coefficient of variation of RBC volume distribution [10]. This index indicates the degree of anysocitosis in the RBC population, modifications in MCV, and might be increased in anemia with significant macrocytosis and/or microcytosis. In healthy horses, RDW ranges between 14 and 25%. It is well known that equine erythrocytes exhibit a mild degree of naturally occurring anysocitosis [2].

2. INTERPRETATION OF THE ERYTHROGRAM

Hematologic alterations often reflect the condition of the individual or an overall response to a pathological situation. The results should be interpreted taking into account different patient data such as age, race, gender, venipuncture method, season, reproductive status, feeding, training, exercise, administration of sedatives and tranquilizers, circadian biological rhythms, altitude… and the information provided by the clinical examination [1, 11-17].

2.1. Pathological Changes of the Erythrogram in the horse

Pathological changes that can be found in the erythrogram are erythrocytosis and anemia.

2.1.1. Erythrocytosis

Erythrocytosis is defined as the absolute or relative increase in the number of circulating RBCs. It is represented by an increase in PCV, HB and RBC counts [5, 9]. The erythrocytosis is classified into two main groups, relative and absolute. In the relative erythrocytosis there is not a real increase in RBC. It is caused by dehydration and splenic contraction [5, 9]. Dehydration leads to a decreased plasma volume in relation to the cellular component of the blood. Therefore, it refers to a hemoconcentration, which is accompanied by an increase in TPP concentrations, unless there are additional losses. Moreover, splenic contraction can substantially increase PCV, HB and RBC, as explained before. In both cases, the total number of erythrocytes is not modified [5, 8, 18].

Absolute erythrocytosis occurs with the addition of newly formed cells to the peripheral circulation, and it can be subdivided into primary and secondary absolute erythrocytosis. Primary erythrocytosis is considered a myeloproliferative disorder of the bone marrow. This type of erythrocytosis occurs in some neoplasia or functional disorders of the bone marrow [19, 20]. This condition might be accompanied by thrombocytosis or leukocytosis, and erythropoietin (EPO) concentrations are within normal limits [5, 18, 21].

By contrast, secondary erythrocytosis is due to the action of the EPO on the bone marrow, since the concentrations of this hormone raise significantly. According to some authors, it can be distinguished between physiologically correct secondary erythrocytosis and physiologically incorrect secondary erythrocytosis. The physiologically correct secondary erythrocytosis occurs in cases of systemic chronic hypoxia, as happens in patients with cardiovascular and pulmonary disease or in adaptation to high altitude. Hypoxemia (PaO_2 < 80 mm Hg and O_2 saturation < 92%) is diagnostic for secondary appropriate erythrocytosis [22]. The most common anomalies associated with erythrocytosis could be of cardiac origin (complex defects such as tetralogy or pentalogy of Fallot, and other defects, including ventricular septal

Table 1: Reference Values for the Erythrocyte Parameters in Adult Healthy Horses (Adapted from Jain [7]; Modified from Lassen and Swardson [9])

PARAMETERS	Units	Hot-blooded	Cold-blooded
RBC *(red blood cell)*	10^6/µL	8.2-12.2	5.5-9.5
HB *(hemoglobin concentration)*	g/dL	13.0-17.0	8.0-14.0
PCV *(packed cell volume)*	%	32-48	24-44
MCV *(mean corpuscular hemoglobin)*	fl	36-50	40-48
MCH *(mean corpuscular hemoglobin)*	pg	13-19	12-17
MCHC *(mean corpuscular hemoglobin concentration)*	g/dL	33-39	32-38
TPP *(total plasma protein)*	g/L	6.0-8.0	6.0-8.0

defect, eventually may result in right to left shunting and secondary erythrocytosis), or respiratory origin (chronic pneumonia and pleuropneumonia). Horses with this condition frequently exhibit cyanotic mucous membranes [5].

The physiologically incorrect secondary erythrocytosis refers to the production of EPO exacerbated either directly, as occurs in renal tumors (renal carcinoma) or indirectly as part of paraneoplasic syndrome, as in hepatocellular carcinoma or hepatoblastoma [23, 24]. These horses have persistently elevated PCV that does not response to intravenous fluid therapy, normal plasma protein concentration and mild to moderate elevations in hepatic enzymes [5].

2.1.2. Anemia

Anemia is defined as an absolute or relative decrease in PCV, HB and circulating RBC [1]. In the relative anemia, there is not a reduction in the total number of RBC. Hemodilution or erythrocyte sequestration makes the three variables mentioned decline [25].

Absolute anemia is clinically relevant because there is a decrease in the total number of RBC and PCV. HB is also decreased, except in cases of intravascular hemolysis. Anemia may be classified as regenerative and non-regenerative based on bone marrow response to the decrease in circulating RBC mass. Regenerative anemia results from either acutely or chronically loss of intact RBCs from circulation (hemorrhage) or accelerated destruction of RBCs (hemolysis). Regenerative anemia is characterized by an increase in effective erythropoiesis in the bone marrow. Non-regenerative anemia occurs following systemic abnormalities or because of intrinsic bone marrow disease and results from a lack of appropriate marrow erythropoiesis in response to normal or accelerated RBC senescence or destruction [5].

Clinical signs of severe anemia are related to decreased tissue oxygenation and to physiological compensatory mechanisms developed in order to alleviate hypoxia. Signs include paleness of the mucous membranes, tachycardia, tachypnea, weakness, lethargy and a systolic heart murmur caused by decreased viscosity and increased turbulence of the blood in the heart and great vessels in cases of severe anemia [5]. Horses with slight to moderate anemia may have no obvious clinical signs or may have only lethargy and slightly pale mucous membranes. Other clinical signs, including fever, icterus and hemoglobinuria may be present in anemic horses, depending on the primary cause of the anemia [5].

The types and causes of regenerative anemia described in equines are [5, 9, 21]:

<u>Blood loss, acute or chronic</u>:

- Epistaxis produced in clinical pathologies as guttural pouch mycosis, pulmonary abscesses, exercise induced pulmonary hemorrhage, ethmoidal hematoma, paranasal sinus abscess or infection, upper respiratory tract neoplasm, pneumonia or pleuritis [26, 27].
- Hemothorax related with fractured ribs, lacerated heart or vessels, neoplasia or coagulopathy among others [28, 29].
- Hematuria produced in pyelonephritis, cystitis, urolithiasis, urethral ulceration, coagulopathy, idiopathic or neoplasias [30, 31].
- Hemoperitoneum related with splenic or hepatic rupture, mesenteric or uterine vessel rupture, ovarian neoplasias, verminous arteritis or neoplasia [32-34].
- Gastrointestinal conditions as ulcerations, non-steroidal anti-inflammatory drug toxicity, parasites, granulomatous intestinal disease, neoplasia such as squamous cell carcinoma, endoparasites as *Strongylus vulgaris*, trichostrongyles or lymphoma [35-38].
- External conditions as trauma, surgical complication, coagulopathy or external parasites [39, 40].

<u>Hemolysis, which could be intravascular and/or extravascular</u>:

1. Hemolysis caused by infectious and parasitic diseases, such as anemia associated with piroplasmosis (*Theileria equi or Babesia caballi*), clostridiosis and equine infectious anemia [41, 42].
2. Hemolysis caused by oxidative injury, in cases of phenothiazine, onion, garlic and red maple leaf toxicosis [42, 43].
3. Immune-mediated hemolytic anemia (IMHA), which could be primary or secondary. The primary IMHA appears in the neonatal

isoerythrolysis and incompatible blood transfusions [44]. Secondary IMHA has been described in association with bacterial infections (*Clostridium perfringens*, streptococcal infections), viral infections (equine infectious anemia) or neoplasias (lymphoma) [45].

4. Hemolysis caused by iatrogenic conditions (hypotonic solutions, ionophores, trimethoprim-sulphamethoxazole, human erythropoietin or penicillin), anemia secondary to systemic therapy with organophosphorus compounds or deficiency in glucose-6-P dehydrogenase [46, 47].

5. Hemolysis caused by miscellaneous conditions (hepatic disease, hemolytic uremic syndrome or disseminated intravascular coagulation) [48, 49].

6. Microangiopathic hemolysis, as happen in hemangiosarcoma and in disseminated intravascular coagulation [39].

Acute blood losses are related with disseminated intravascular coagulation, trauma and surgery, angiopathic, plasmopatic and trombopatic hemorrhagic diathesis, rodenticide poisoning, guttural pouch mycosis, equine purpura hemorrhagic and progressive ethmoid hematoma. Chronic blood losses are related to the digestive system, parasitism, neoplasms and gastric ulcers, blood losses on the respiratory system (tumors and pulmonary hemorrhage) and blood losses on the genitourinary system (cystitis, urolithiasis, bladder tumors...) [26, 30, 33, 37, 39, 50].

Internal hemorrhage (into body cavities) permits the body to reuse blood components. Approximately two thirds of the erythrocytes lost into the abdomen or thorax are auto transfused back into the circulation within 24 to 72 hours. The other one third of erythrocytes is lysed or phagocytized and the iron and protein are reused. Accelerated bone marrow erythropoiesis is usually evident by 3 days after acute hemorrhage and is maximal by 7 days [5].

Initially, RBC count appears normal because all blood components have been lost in equal volumes. Physiologic compensatory mechanisms induce redistribution of interstitial fluid into the vasculature and decreased RBC and TPP in peripheral blood. This redistribution of interstitial fluid into the vasculature 24 hours after acute hemorrhage makes impossible to assess the severity of the blood loss [5]. In addition, many erythrocytes stored in the spleen can be released into the circulation as a consequence of endogenous catecholamine release. After vascular equilibration, hematologic parameters should reveal a decrease in PCV, RBC and HB without changes in RBC indices (MCV, MCH and MCHC). This type of anemia usually is accompanied by panhypoproteinemia through proteins loss. A neutrophilic leukocytosis is apparent by 3 hours after hemorrhage and platelets (PLT) may increase if have not been consumed by excessive coagulation [5].

In relation to hemolytic anemia, it can be produced by intravascular or extravascular causes. During intravascular hemolysis, HB released from destroyed erythrocytes combines with plasma haptoglobin, and the tissue mononuclear phagocytes remove the haptoglobin-hemoglobin complex. As a consequence, plasma haptoglobin levels decrease as intravascular hemolysis increases [42]. When plasma haptoglobin binding is exceeded, free HB accumulates in the plasma and is eliminated by kidneys. Thus, intravascular hemolysis is characterized by hemoglobinemia and hemoglobinuria. Hemolysis induces a more regenerative response than blood loss [5].

IMHA (Immune Mediated Hemolytic Anemia) develops by type II hypersensitivity mechanisms with antibodies that attach to the surface of RBCs. Primary IMHA is an autoimmune process in which antibodies act directly against the membrane surface antigens of RBC. Secondary IMHA is more common than autoimmune disease. Antibodies attach to the surface of erythrocyte for one or more reason: 1.-Alterations in the RBC membrane produced by a primary viral, bacterial or neoplastic process [45, 52]; 2.-Antigen-antibodies complex deposition on the surface of RBCs; 3.-Drugs that cause immunoproteins to react indirectly with RBCs. Drug-induced immune-mediated hemolysis may occur *via* three mechanisms: 1.-The drug might combine with RBC membranes and might be recognized as foreign by the body. An antibody to this new antigen develops and destroys the drug-coated RBC. These animals have a positive direct Coombs´ test; 2.-The drug can bind to a carrier molecule in the blood and induce an immune response and the drug-carrier complex-antibody binds erythrocyte membranes in a process mediated by the complement leading to hemolysis; 3.-Drug may induce true autoantibody production and causes RBC destruction [46].

Antibodies-coated RBCs are unable to pass through the microcirculation of the spleen, become sequestered

and destroyed or phagocytized. If RBC membrane is lost in excess, spherocytes with an increase of osmotic fragility can be formed. Most of the IMHAs are extravascular, but if the antibody fixes and activates complement, intravascular complement-mediated hemolysis may result [3, 5].

The types and causes of non-regenerative anemia described in equines are the following:

1. **Iron deficiency** (chronic hemorrhage, nutritional deficiency….) [53, 54].

2. **Anemia of chronic disease** (chronic infection / inflammation: pleuritis, pneumonia, peritonitis, enteritis, bacterial endocarditis, internal abscessation or chronic viral disease as equine infectious anemia). This is one of the most common cause of anemia in horses [36, 57, 58, 59, 60].

3. **Bone marrow failure** (myelophthisis, myeloproliferative disease, bone marrow toxins: phenylbutazone, chloranphenicol…), radiation, Standardbred horse family hypoplasia or idiopathic pancytopenia among others [61-63].

4. **Miscellaneous conditions** (chronic hepatic o renal diseases, endocrine diseases….) [5].

Dietary factors as folic acid or cobalamin (vitamin B12) deficiencies are rare, but severe protein deprivation and iron deficiency by chronic external blood loss and chronic disease may result in decreased erythropoiesis in horses. The mechanisms implicated in anemia of chronic diseases and bone marrow failure are selective erythroid hypoplasia, block of iron release from reticuloendothelial storage (ferritin and hemosiderin) resulting in an unavailability of iron for heme synthesis and production of antibodies that cross-react with EPO, that can interfere with erythropoiesis, among others [5].

2.2. Evaluation of Erythrocyte Morphology in the Horse

Examination of the peripheral blood smear should be considered, along with peripheral blood counts and red blood cell indices, an essential component of the initial evaluation of all equine patients with hematologic disorders. The evaluation of erythrocyte morphology includes assessment of RBC shape, size, color, inclusions, and arrangement. Abnormalities of RBC shape and other RBC features can provide key information in establishing a differential diagnosis of anemia. In addition, RBC findings can suggest specific etiologies [5].

For the interpretation of hematological data in horses, it must take into account the following characteristics of this species that are different from others, as rouleaux formation and absence of peripheral signs of regeneration [1, 18].

Rouleaux formation is a characteristic of the blood of horses, with a marked tendency to form stacks. This implies a rapid separation of the formed elements of plasma with a high erythrocyte sedimentation rate [4]. Rouleaux formation can be accentuated by some diseases associated with hyperproteinemia, because high concentrations of plasma proteins, particularly fibrinogen and immunoglobulins, have an insulating effect that reduces the RBC surface membrane charge, promoting RBC aggregation [64].

Equine erythrocytes remain in the bone marrow during the process of formation and they are not discharged into the blood circulation until maturation is completed. For this reason the morphological characteristics related to regeneration, described in other species, such as polychromasia or reticulocytosis, macrocytosis, or other signs of peripheral regeneration, are rarely found in horse blood smears. Life span of equine RBC in the circulation is approximately 140 to 150 days. RBCs are released from bone marrow as mature cells and the horse is unique in failing to release reticulocytes into peripheral blood when there is a regenerative response to hemorrhage or hemolysis [65]. The reticulocyte count can be performed on marrow aspirates in anemic horses. Normal equine bone marrow contains approximately 3% reticulocytes, then, values greater than 5% are consistent with accelerated erythropoiesis, and an increase as high as 66% has been described in response to severe blood loss [6]. Increases in MCV are inconsistent but slightly more common after hemolysis than after acute blood loss [42]. The only change in equine peripheral blood after acute hemorrhage or hemolysis may be a slight anisocytosis that is quantitatively assessed through changes in RBC distribution [5, 66]. However, nucleated erythrocytes are observed more often during severe anemia, suggesting intense stimulation of marrow due to severe hemolysis, hemorrhage, or hypoxia [67].

The red cell indices, MCV, MCH and MCHC are particularly useful in assessing and categorizing

anemic processes. Clinically, the most used are the MCV and MCHC, reporting the average size of the red cells and the amount of HB that they have respectively [1, 21]. In relation to MCV, erythrocytes can be classified as normal or normocytic (mean diameter of normal equine RBCs: 5.7 μm) [5, 6], smaller than normal or microcytic or larger than normal or macrocytic. According to the MCHC, erythrocytes are classified as normochromic, if they have a normal amount of HB, or hypochromic, if the amount of hemoglobin is below normal [3]. Normocytic normochromic anemia accompanies many chronic systemic diseases, including renal and hepatic failure, endocrine abnormalities, neoplastic conditions and chronic infections [31]. Microcytes are small erythrocytes that retain a variable degree of central pallor. Microcytosis, with hypochromia (increased central pallor of erythrocytes) suggests iron deficiency related with chronic blood loss from parasitism, gastrointestinal neoplasia, or severe gastrointestinal ulceration [55]. Macrocytic anemia (increased MCV) occasionally occurs in horses after a severe hemolytic or hemorrhagic crisis and less commonly, in other regenerative causes of anemia. Microcytosis is a normal finding in foals up to 1 year of age, and is attributable to a physiologic iron deficiency [5].

From a diagnostic standpoint, poikilocytosis or abnormal shape, has no specificity, but the recognition of specific forms of poikilocytes (irregularly shaped cells) often points to specific disorders. ***Spherocytes*** are round, densely staining red cells that lack central pallor and have a smaller than normal diameter. Spherocytes result from partial removal of red cell membrane by the monocyte-macrophage system, while the cytoplasmic volume remains unchanged and are present in immune-mediated hemolysis. However, identification of spherocytes in this specie may be difficult because equine RBCs generally lack central pallor. These cells are also common in foals with neonatal isoerythrolysis [67].

Other sign of immune-mediated hemolysis is the ***auto-agglutination*****,** although it can be seen in some horses without hemolysis as a result of cold antibodies, with a maximal activity at 4-20ºC or as a result of unfractionated heparin treatment [68], induced by the administration of drugs, infections caused by *Clostridium* spp. or be idiopathic. Macroscopically, agglutination has a granular appearance and microscopically, appears as grape-like clusters of RBCs. It should be differentiate from rouleaux by using the saline dilution test. Typically a 1:2 dilution will disperse rouleaux but not the auto agglutinated RBCs. Infrequently, a higher dilution (up to 1:10) may be needed to disperse rouleaux. Agglutination causes erroneous MCV values and RBC numbers determined by impedance, because the aggregates may interfere with the electronic or optical evaluation of the erythrocytes. Pre-treating cell suspensions from agglutinated heparin-treated horses with trypsin might reverse the agglutination, improving the accuracy of cell counts [8].

Target cells **(codocytes)** have a centrally located disk of hemoglobin surrounded by an area of pallor with an outer rim of hemoglobin adjacent to the cell membrane giving the cell the appearance of a target. Codocytes may be associated with hypochromic anemia or hepatic disease [67].

Leptocytes (or wafer cells) are thin, flat cells with the hemoglobin at the periphery of the cell. The two primary mechanisms causing this abnormal morphology are either an upset in the cholesterol: phospholipid ratio in plasma resulting in lipid loading (as might be seen with liver disease and other metabolic disorders) or a decrease in cytoplasmic content compared with normal (as might be seen with iron deficiency typically due to chronic blood loss). With iron deficiency, RBC hemoglobin content decreases, and in addition to the leptocytosis, hypochromasia is often observed [67].

Elliptocytes (ovalocytes) range from slightly oval to elongated cigar-shaped forms and are present in animals with iron deficiency or myelophthisic anemia [2, 3, 5, 8, 10].

Dacryocytes are red cells with one end round and the other end more pointed [2, 3, 5, 8, 10].

Acanthocytes have several (usually 3 to 7) irregularly spaced blunted projections from the margin of the cells. Acanthocytes may be associated with changes in the concentration of phospholipid-cholesterol level of erythrocyte membrane. Acanthocytosis is most frequently associated with liver disease, splenic hemangiosarcoma or poor gastrointestinal absorption [67].

Crenation can be confused with RBC changes such as acanthocytosis. Crenation is a shrinking artifact most commonly seen when less than optimal amounts of blood are collected into the EDTA tube and

the peripheral blood film is not made relatively quickly after the anticoagulation process. Some less common causes of crenation include electrolyte disturbances, uremia, and rattlesnake envenomation. A useful differentiating feature for identifying crenation is that crenation typically affects large numbers of cells in a particular area of the slide, whereas true poikilocytosis typically affects relatively lower numbers of cells throughout the peripheral blood film. Crenation can be minimized by preparing blood films immediately after blood samples are collected and properly anticoagulated [5, 67].

Echinocytes are also cells with cytoplasmic projections, but in contrast to acanthocytes, the projections are typically evenly spaced on the cell surface, more numerous (often 10 to 15), and frequently have sharper points. Its formation may be artifactual or associated with renal disease, lymphoma, or snakebite, and it might occur in response to exercise [69] apparently associated with electrolyte disturbances, especially hyponatremia.

Schistocytes are fragmented erythrocytes appearing in a variety of morphologic forms such as small triangular erythrocytes, helmet cells, and normal-size erythrocytes with 2 to 3 pointed surface projections (keratocytes, or "horn cells"). Schistocytes are indicative of systemic disorders, such as changes in the microcirculation, disseminated intravascular coagulation, neoplasms, inflammatory processes in highly vascularized organs such as liver, lung, spleen, bone marrow or placenta. ***Leptocytes*** are thin, flat RBC frequently associated with hepatic disease or iron deficiency [5, 67].

Morphologic identification of inclusion bodies within erythrocytes can be helpful clinically. ***Howell–Jolly bodies*** are basophilic nuclear remnants in the cytoplasm of erythrocytes. Although these structures may be observed in normal horses (approximately 10 in 10.000 erythrocytes contain Howell-Jolly bodies), Howell-Jolly bodies are observed more often during severe anemia, suggesting erythrocyte regeneration [42, 70]. An increase in concentration also occurs in cases of splenectomy and splenic function suppression.

Babesia caballi and ***Theileria equi*** may cause from mild to severe anemia, but only a small proportion of red blood cells may be parasitized. Organisms are teardrop shaped, with one to four organisms per cell and can be observed when 0.1% or more of the erythrocyte population is infected [67].

Oxidative Damage: Oxidative damage to erythrocytes may be associated with membrane lipid peroxidation, denaturation of hemoglobin, or a change in the valence state of the iron atom secondary to administration of phenothiazine, anthelmintics or ingestion of wild onions or red maple leaves. The ***eccentrocytes*** are red cells with a ragged appearing, poorly hemoglobinized fringe of cytoplasm along one side of the cell. Also known as "hemi-ghost" cells, this appearance results from an opposition and adherence of opposite inner surfaces of the cell membrane in this area (which variably excludes hemoglobinized cytoplasm to an "eccentric" location in the cell). Eccentrocytes are indicative of exposure to oxidizing agents and are thought to form by alterations of the erythrocyte membrane [71].

The ***Heinz bodies*** are denatured aggregates of hemoglobin project from the cell margin. Extensive Heinz body formation may contain erythrocyte “ghosts,” which result from erythrolysis, with preservation of the cellular membrane but loss of HB [5]. ***Ghost cell*** may sometimes be seen in intravascular hemolysis. When stained with a vital dye such as new methylene blue, appear blue, more evident than in Romanowsky staining. They can be formed by the intake of plants of the Allium family (onions and wild garlic), dried leaf maple (*Acer rubrum*), or following administration of phenothiazine and methylene blue [72].

3. CONCLUSIONS

The erythrogram, including red blood cell numbers, hemoglobin concentration, packed cell volume or microhematocrit, erythrocyte indices (mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration) and the assessment of peripheral blood smears, is one of the most important tool to diagnose hematological diseases as well as to evaluated systemic consequences in many other diseases in horses. Secondary erythrocytosis and anemia associated to acute or chronic blood loss, hemolysis and chronic diseases are the most common erythrocyte disorders in the horses.

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