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Investigation of Anaemia in the Horse

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I. Introduction

Anaemia is a common clinical syndrome seen in equine practice and is defined as a decrease in the circulating RBC mass caused by an imbalance in the rate of loss or destruction of erythrocytes and the rate of their production in the bone marrow.\(^1\) Anaemia is a hematologic abnormality resulting from an underlying disease process, and should not be considered as a primary diagnosis.\(^1\) Unfortunately practitioners will often 'treat' anaemia without determining the underlying cause. This leads to therapeutic failures and frustration for the client and veterinarian involved. A systematic approach to investigation of anaemia will enable the veterinarian to make a definitive diagnosis and will facilitate rational therapy aimed at the underlying disease process.

The primary presenting clinical sign in horses with anaemia is mucous membrane pallor (Figure 1), however other clinical signs directly related to decreased tissue oxygenation and the physiologic compensatory mechanisms intended to alleviate this hypoxia may be seen. Horses with mild to moderate anemia often have no obvious clinical signs or may only have lethargy and slightly pale mucous membranes. In cases of severe anaemia, the clinical signs will vary depending upon the cause and rate at which anaemia develops. Horses with chronic anaemia are able to compensate for the reduced oxygen transport from the lung to the tissues of the body, and often the only presenting clinical sign is exercise intolerance or lethargy. With acute anaemia, the clinical signs are far more dramatic, and will become evident at a much higher red cell mass, as affected horses are unable to compensate for the reduction in oxygen carrying capacity of the blood. Affected horses present with tachycardia, tachypnoea and weakness. A systolic heart murmur can often be identified in these horses due to decreased viscosity and increased turbulence of the blood. With acute anaemia due to severe blood loss, the clinical signs are attributable to hypovolaemic shock, and include tachycardia, tachypnoea, pale mucous membranes, prolonged capillary refill time, hypothermia, muscle weakness and eventual cardiovascular collapse. Other clinical signs, including fever, icterus, haemoglobinuria, petechial haemorrhages and weight loss may be present in anemic horses and reflect the primary pathophysiological process or underlying disease process involved.

Anaemia should be confirmed by laboratory demonstration of reductions in packed cell volume (PCV, % or L/L), decreased red blood cell count (RBC) and hemoglobin concentration (g/dl). There are some features unique to the equine erythron that may complicate interpretation of anaemia however, and it must be remembered that the PCV should always be interpreted in light of the horses' age, breed, use and level of hydration and excitement.

- Normal PCV in the horse varies with age, breed and use of the animal. Foals tend to have a lower PCV than adult horses. Cold blooded breeds (e.g. Clydesdales and ponies) have a lower PCV than hot blooded breeds (e.g. Standardbreds and Thoroughbreds). Fit racehorses have a significantly higher PCV than sedentary horses.
- The equine spleen is an important reservoir for erythrocytes and splenic contraction in response to adrenergic stimulation can result in as much as 50% increase in the PCV. Because of this feature, anaemia in the horse can be masked by temporary elevations in PCV due to exercise or excitement, and for this reason it is advisable to assess PCV in the rested horse and avoid blood collection immediately after exercise. Similarly, horses with dehydration will have a relative increase in PCV that can mask anaemia, and PCV should always be interpreted in conjunction with the hydration status of the horse.
- Horse erythrocytes show marked tendency for rouleaux formation which causes rapid sedimentation (Figure 2). Blood must therefore be thoroughly mixed before sampling and rouleaux...
formation must be differentiated from autoagglutination.

- Erythrocyte indices are not very useful in horses, as equine erythrocytes are maintained in the bone marrow until hemoglobin synthesis is complete. Therefore, some of the usual signs of regenerative anemia (polychromasia, reticulocytosis, and macrocytosis) are not observed in the horse.

**Figure 2:** Horse erythrocytes demonstrating rouleaux formation (arrow)

### II. Classification of Anaemia

Once anaemia is confirmed, the veterinarian should attempt to classify the anaemia into one of three pathophysiological mechanisms.

1. Blood loss
2. Increased red cell destruction (haemolysis)
3. Decreased red cell production

Anaemia caused by blood loss or haemolysis is generally regarded as regenerative, while anaemia caused by decreased red cell production is non-regenerative. Unlike other domestic species and humans, horses do not release immature red cells into the peripheral circulation, and thus it is impossible to differentiate between regenerative and non-regenerative without performing a bone marrow biopsy or aspirate. Consequently, the initial diagnostic approach in the horse is aimed at determining if the anaemia is caused by blood loss or haemolysis. If there is no evidence of blood loss or haemolysis, then a bone marrow biopsy or aspirate should be obtained in order to rule out decreased red cell production. The diagnostic approach to anaemia in the horse is outlined in figure 8.

**1. Blood Loss Anaemia**

The first step in the investigation of anemia is to determine if the horse has hypoproteinaemia. Horses with anaemia and concurrent hypoproteinaemia are likely to have blood loss, and every attempt should be made to try and identify the source of blood loss.

Blood loss in the horse can be broadly classified as acute or chronic and external or internal. Acute blood loss is usually associated with surgery, trauma or rupture of a large vessel. If severe (> 30% of blood volume), affected animals will present with signs of progressive haemorrhagic shock and require emergency medical treatment. External blood loss is visible on physical examination, and is not difficult to identify. Internal blood loss presents more of a diagnostic challenge however, as it is often insidious and may be difficult to identify. Diagnosis in these cases is facilitated by thoracic and abdominal ultrasonography, endoscopy, abdominocentesis, pleurocentesis and rectal examination. It is important to remember that the PCV and total protein in these cases will not be representative of the true volume of blood loss until approximately 24 hours after the haemorrhagic insult has occurred. This is because of the dilutional effect caused by a net movement of fluid from the interstitial space and increased water intake to compensate for the loss in intravascular blood volume. The PCV will usually stabilize within 24 hours after the haemorrhage has been controlled and will begin to increase in 4 to 6 days due to a regenerative bone marrow response.

**Figure 4:** 2 year old Thoroughbred colt with severe EIPH. Remember that in cases of acute blood loss, PCV and total protein will not be representative of the true volume of blood loss until approximately 24 hours after the haemorrhagic insult has occurred

Chronic blood loss can be caused by a plethora of disorders and results in a slowly developing anaemia because the bone marrow has a chance to compensate...
for the lost red cells. Anaemia only occurs once the rate at which red cells are lost exceeds the rate at which they produced in the bone marrow. Common causes of chronic blood loss in the horse include bleeding gastrointestinal lesions, haematuria and haemostatic disorders. Diagnosis is facilitated by gastroscopy, faecal occult blood tests, urinanalysis, thrombocyte counts and clotting times.

Figure 5: Immune mediated thrombocytopaenia in a 13 year old mare. Haemostatic disorders should be ruled out as a cause of chronic blood loss

2. Increased Red Cell Destruction (Haemolysis)

If the horse has normal plasma protein and there is no obvious source of blood loss, the next step is to determine if there is any evidence of haemolysis. This can be determined by demonstrating the presence of icterus (increase in total and indirect bilirubin), haemoglobinemia or haemoglobinuria (Figure 6). Haemoglobinemia can be identified by the presence of pink plasma and elevations in MCH and MCHC. Haemoglobinuria will cause red urine and a positive for blood on a urine dipstick, but be sure to differentiate haemoglobinuria from myoglobinuria and haematuria. Myoglobinuria will be accompanied by elevations in muscle enzymes, while haematuria can be confirmed by the presence of whole erythrocytes on urine sediment analysis. It is also important to remember that icterus in the horse can be caused by other pathophysiological processes, including inappetance. Furthermore, horses with extravascular haemolysis will not have haemoglobinuria or haemoglobinemia.

If haemolysis is likely, a blood smear should be made and examined for the presence of Heinz bodies, which would be indicative of oxidative erythrocyte damage. Heinz bodies can be identified as round bluish structures within the erythrocyte when stained with new methylene blue. Haemoglobinuria is rare, and the most likely cause is ingestion of wild or domestic onions. Diagnosis is based upon a history of exposure, a characteristic onion odour on the breath, and the demonstration of Heinz bodies on blood smears. Other less likely causes of Heinz body anaemia include ingestion of leaves from the red maple tree and rarely, administration of phenothiazines.

A blood smear can also be used to identify erythrocyte or granulocyte cytoplasmic inclusions, which would be indicative of piroplasmosis or equine granulocytic ehrlichiosis respectively. These diseases are transmitted by specific tick vectors that spread the organisms from horse to horse. Transmission is also possible through use of veterinary needles/instruments shared between horses or by the use of infected blood products. Piroplasmosis is caused by the protozoan parasites *Babesia caballi* or *Theileria equi*. Clinical signs are variable. Horses with acute infection present with fever, inappetence, depression, labored or rapid respiration, petechiation, icterus, anaemia and haemoglobinuria. Subacute cases have similar but less severe clinical signs. In chronic cases, common clinical signs include mild inappetance, poor exercise tolerance, weight loss, transient fevers and an enlarged spleen. The paired pyriform parasites can easily be identified on blood smears stained with Giemsa, however it is important to remember that parasitaemia precedes haemolysis, and thus the absence of parasitized
Erythrocytes does not rule out piroplasmosis. If there is a high index of suspicion for the disease in the absence of parasitaemia, then serology should be performed. A complement fixation test will detect babesia antibodies 5 to 8 days after parasitaemia. Equine granulocytic ehrlichiosis is a self-limiting disease caused by the obligate intracellular bacterium *Anaplasma phagocytophilum*. Anaemia is usually mild and is caused by the suppressive effects of the parasite on the bone marrow and in some cases immune mediated destruction of erythrocytes. Severity of signs varies with age of the animal and duration of the illness. Adults are most severely affected, and usually present with fever, inappetance, depression, reluctance to move, limb edema, petechiation, icterus and anaemia. Laboratory abnormalities include leukopaenia, thrombocytopaenia, anaemia and icterus. The fever is highest during the first 1-3 days of infection (39.5-40°C), and persists at for 6-12 days. Characteristic morula can be identified in eosinophils and neutrophils during the pyrexic phase of the disease and diagnosis can be confirmed with PCR or serology.

If there is no evidence of oxidative erythrocyte damage or parasitaemia, then haemolysis is most likely secondary to immune mediated haemolytic anaemia (IMHA), and an EDTA blood sample should be examined for evidence of red cell agglutination. Agglutination occurs when antibodies and/or complement binds to the erythrocyte membrane, and when present, it confirms a diagnosis of IMHA. Occasionally red cell agglutination can be seen with the naked eye, and this is termed autoagglutination (Figure 7). This finding should be interpreted with caution however, as equine erythrocytes have a tendency for rouleaux formation, which can be misinterpreted as autoagglutination. Autoagglutination can be confirmed by performing a simple ‘in-saline agglutination’ test. A small drop of blood is placed on a slide, diluted 1:4 with 0.9 % saline, and then examined under magnification for the presence of agglutination. Normal red blood cells will disperse evenly throughout the slide, whereas true autoagglutination can be seen as red cell clumping.

True autoagglutination confirms IMHA, however some affected horses will not demonstrate autoagglutination, and in these cases, a direct antiglobulin test (Coombs test) should be done to rule out IMHA. The Coombs test detects membrane bound Ig and/or C3 on erythrocytes, but it is important to remember that the end point of this test is agglutination, so it is pointless to perform this test on autoagglutinated blood. The most well recognized form of IMHA is neonatal isoerythrolysis (NI), which occurs when the foal ingests colostrum containing alloantibodies directed against the foal’s erythrocytes. Alloantibodies responsible for NI are usually directed against the Aa or Qa alloantigens, and because these erythrocyte antigens are common in the equine population, the prevalence of NI is low. In order for NI to occur, a series of co-ordinated events need to occur: 1) the stallion needs to have alloantigens that are incompatible with the mare; 2) the mare needs to be exposed to these alloantigens and produce alloantibodies; 3) the foal needs to inherit the alloantigens from the stallion; 4) the mare needs to concentrate alloantibodies against the foals erythrocytes in her colostrum; and finally, 5) the foal needs to ingest these antibodies. Foals with NI are usually born normal, and then develop progressive clinical signs compatible with haemolytic anaemia 12-24 hours after ingestion of colostrum. Affected foals have pale mucous membranes, marked icterus and are generally weak, lethargic and unwilling to suckle. The severity of the clinical signs are dependent upon the quantity of colostrum that was ingested, and in severe cases, death can occur due to cerebral anoxia. The diagnosis is not difficult, as acute onset anaemia, icterus and weakness in a neonatal foal is highly suggestive of NI, however the diagnosis should be confirmed by demonstrating autoagglutination or performing a Coombs test. Alternatively, a haemolytic cross match using washed foal erythrocytes and mare serum can be used to confirm the diagnosis.

IMHA in the adult horse can be primary or secondary. Primary IMHA represents true autoimmune disease, whereas secondary IMHA is caused by prior sensitization with a drug, infection or neoplasia. Often the underlying cause is not easy to elucidate and many cases remain ‘idiopathic’ despite a thorough diagnostic investigation. Perhaps the most common recognized cause of IMHA is drug therapy, and in particular the administration of penicillin. IMHA has also been described in horses with lymphoma, *Clostridium perfringens* infection, and equine infectious anaemia (EIA). EIA should be considered as a possible cause of anaemia in any horse that presents with concurrent fever, icterus, depression, anorexia, ventral oedema, weight loss or petechial haemorrhages, particularly if they have a history of recent travel. Haemolysis is thought to occur as a consequence of viral immune complexes that are deposited on the erythrocyte membrane, leading to subsequent removal of the affected erythrocytes from the circulation by the MPS. Diagnosis is based upon......
identification of viral antibodies in an agar gel immunodiffusion test (Coggins test).

Other rare causes of haemolysis that can be considered as a last resort include microangiopathic haemolysis, end-stage liver disease, and DIC.

3. Decreased Red Cell Production

If blood loss and haemolysis have been ruled out, a bone marrow aspirate or biopsy should be performed in order to determine if the bone marrow is responsive or not. When evaluating bone marrow specimens, the clinical pathologist will usually report the myeloid:erythroid ratio (M: E) and reticulocyte count as an indication of the regenerative response of the bone marrow. The normal M: E ratio in horses ranges from 0.5 to 1.5 and the reticulocyte count ranges from 0.5 to 2%. In order for the anaemia to be classified a regenerative, the M: E ratio should be < 0.5 and the reticulocyte count > 2%. The overall cellularity of the sample and the presence or absence of abnormal cells will also be reported; and an indirect assessment of bone marrow iron stores will be made by determining the haemosiderin content on slides stained with Prussian blue. If the M/E ratio or reticulocyte count is inadequate, then the anaemia is considered to be non-regenerative, and attempts should be made to determine the underlying cause.

Anaemia of chronic disease (ACD) is the most common cause of non-regenerative anaemia in the horse and occurs because the bone marrow is unable to compensate for the marginal decrease in erythrocyte lifespan due to damage while passing through inflamed tissues or destruction by the MPS. Impaired erythropoiesis is due to sequestration of iron in the reticuloendothelial system in a form that is not readily mobilized and thus unavailable for compensatory erythropoiesis. Horses with ACD have mild anaemia, and the predominant clinical signs can be attributed to the primary disease process (e.g. intraabdominal abscess). Total serum iron, total iron binding capacity (TIBC) and % saturation of transferrin are all decreased, however bone marrow iron stores are adequate and thus administration of iron is not therapeutic in these cases.

Iron deficiency anaemia is very rare in horses and only develops when the rate of iron loss from the body exceeds absorption via the diet. The most common cause of iron deficiency anaemia is chronic blood loss (e.g. coagulopathies and severe gastrointestinal parasitism). Affected horses usually have a microcytic, hypochromic anaemia and total serum iron and % saturation of transferrin are decreased. Unlike ACD, however, horses with iron deficiency anaemia have a normal or elevated total iron binding capacity (TIBC) and bone marrow iron stores are depleted.

Myelophthisic anaemia is a rare cause of non-regenerative anaemia in which the bone marrow is obliterated by neoplastic or inflammatory tissue (e.g. myelogenous leukaemia). Affected individuals usually have a pancytopaenia and clinical signs include bleeding diathesis (e.g. epistaxis, mucosal petechiae, haematomas' etc.), generalized or localized infections and severe mucosal pallor. Other clinical signs are attributable to the primary disease process, and include weight loss, lethargy and anorexia. Diagnosis is based upon identification of atypical cells on a bone marrow aspirate or biopsy.

Aplastic anaemia is a rare cause of non-regenerative anaemia in which the bone marrow stem cells fail to undergo differentiation, leading to reduced production of all blood components. There is no evidence of a primary disease infiltrating or suppressing the bone marrow. Some cases of aplastic anaemia are idiopathic, but the majority of cases arise secondary to exposure to drugs, chemical or infection. There is also some evidence in humans that aplastic anaemia may have an autoimmune component. In horses, the most common cause of aplastic anaemia is due to administration of recombinant human erythropoietin (EPO) as a performance enhancer in racehorses. EPO induces immune interference with endogenous erythropoietin leading to impaired erythropoiesis and selective erythroid bone marrow suppression. The clinical features of aplastic anaemia are directly related to the pancytopenia. Diagnosis is based upon demonstration of hypoplastic bone marrow together with a concomitant peripheral pancytopenia. If the bone marrow is responsive, then the horse should be re-evaluated and further attempts should be made to try and identify a source of increased red cell loss or increased red cell destruction.

III. Conclusion

Anaemia is common in horses and generally indicates the presence of an underlying disease process. In order for appropriate therapy to be given, the cause of the underlying disease must be determined. Accurate diagnosis is based upon identifying the pathophysiological mechanism responsible for anaemia and systematic rule out of likely causes based upon information obtained from a complete history, physical examination, and appropriate clinicopathological and diagnostic testing.

IV. Selected References


**Figure 8:** Diagnostic approach to Anaemia