Neonatal isoerythrolysis, an immunologic disorder causing red blood cell destruction, seen in newborn horse and mule foals and other species, infrequently occurs in the equine population. It is preventable with an understanding of the pathogenesis of the disease, allowing the veterinarian and foal manager to modify breeding and management practices in order to decrease clinical significance of the disease. Clinical signs present in affected foals are due to anemia and are dependent upon the severity of that anemia.

Pathogenesis of Disease
Eight major blood group systems are present in horses, A, C, D, K, P, Q, T and U. Within each group system there are allelic factors responsible for inheritance and expression of red blood cell antigens. These allelic factors are represented as lowercase letters ie Aa, Ab, Ac. Horses rarely produce naturally occurring alloantibodies to red blood cells (RBC) antigens (factors) they lack, and those produced have very weak lysin or agglutinin activity [1]. A mare must lack a particular antigen, and at some point become exposed to that factor, in order to produce anti red blood cell antibodies. A foal is at risk for neonatal isoerythrolysis (NI) if the foal inherits an RBC factor from its sire for which the mare has pre-formed antibodies. The mare’s colostrum will contain antibodies against the factor the foal has inherited. If the foal consumes this colostrum, it will ingest antibodies capable of destroying the foal’s own red blood cells.

There are numerous ways in which a mare may be exposed to red blood cell antigens she does not posses. Transfusions from incompatible blood donors, leakage of her foal’s erythrocytes through an abnormal placenta, normal microhemorrhage occurring in the placenta during late gestation, or exposure to the her own blood at a previous delivery, leakage of foal’s erythrocytes through an abnormal placenta, normal microhemorrhage occurring in the placenta during late gestation, or exposure to a previous foal’s blood during delivery are all possible means of exposure. Mares are most likely to be exposed to a novel red blood cell antigen late in gestation or at parturition. Because of this, it is unlikely that the foal she is carrying at the time of first exposure will be affected. Foals of subsequent pregnancies are at greater risk.

In most reported cases of NI the Qa or Aa antigens are the cause of clinical illness as they are considered to be the most antigenic [2]. Pa, Ab, Qrs, Dc, Ua, Qb, Qc, Da, Ka, and Db have rarely been reported to cause the disease [3-5]. The most common red blood cell antibody found in horses is the anti-Ca antibody, and is often a naturally occurring alloantibody. NI has not been associated with Ca antigen in the horse. Interestingly, it has been reported that mares lacking the Aa and Ca groups will spontaneously develop Ca antibody. In doing so the production of anti-Aa antibody is suppressed as fetal red blood cells are removed from the circulation by the anti Ca antibody before the mare is sensitized to Aa [6] All mares bred to donkeys are at risk of developing NI as all horses lack the donkey factor [7]. NI reportedly has a prevalence of 1% in Thoroughbred horses and a 2% prevalence in Standardbred horses [7]. The percentage of Thoroughbred mares at risk for NI because they lack the Aa factor is 2%. Lack the Qa factor confers a greater risk of 16%. The percentage of Standardbred mares at risk for NI because they are Aa negative is 22-25%. One hundred percent of Standardbred mares are at risk as they are Qa factor negative [7]. However, one must also view the likelihood of a foal obtaining a particular blood group factor from the stallion and the frequency of which antibodies are actually made in the mare for a particular factor. The likelihood of a Thoroughbred foal inheriting the Aa antigen from the sire is 85%. However, only 50% of all thoroughbred mares at risk will produce antibodies. Even though the foal is likely to inherit the antigen, the prevalence of disease will be low as few mares make antibody [7]. Similarly, of 16% of Thoroughbred mares at risk for NI due to lack of Qa factor, only 3% will develop antibody, lowering the incidence despite a likely inheritance [7].

Clinical Signs of Disease
Aa and Qa antibodies act as lysins, as the Aa and Qa antibodies are accountable for 90% of all NI cases, agglutinating antibodies assume a less important role in the pathogenesis of disease. Anti-erythrocyte antibodies are concentrated in the colostrum prior to birth. NI as a result of the Aa antibody often presents as peracute, severe cases, and Qa antibody usually
produces a milder disease [8]. Consumption and absorption of the colostrum leads to hemolysis of the foal’s red blood cells. Extravascular hemolysis and removal of red blood cells via the reticuloendothelial system is the main means of red blood cell destruction in the affected foal, intravascular hemolysis also occurs [9]. Clinical signs are variable and may be peracute, acute, subacute, or subclinical. Usually foals are normal at birth and clinical signs do not develop until five hours to five days after birth [10]. Some foals may be found dead within the first 24 hours of life if severely affected. Severity of clinical signs is dependent upon the severity of the anemia, which in turn is dependent on the type and quality of colostrums ingested by the foal. Foals with complete failure of passive transfer, for example, will not develop NI. Foals may demonstrate any or all of the following signs: weakness, lethargy, pallor, icterus, hemoglobinuria, tachycardia, tachypnea, fever and variable cardiovascular stability, some presenting in shock. Kernicterus has also been reported as a complication of NI in foals resulting in severe neurologic signs [11].

Clinical hematology indicates anemia, with a decreased packed cell volume and red blood cell count. Anemia is defined as a PCV less than 25%. Free hemoglobin levels may increase. Foals who have not been nursing may be hypoglycemic. Total bilirubin values and unconjugated bilirubin values generally increase in response to hemolysis. Metabolic acidosis is found in foals with severe disease in which severe tissue hypoxia is present. Azotemia may be present as kidneys, overloaded by toxic heme pigments and experiencing some level of tissue hypoxia, are damaged. Primarily renal tubular cells are affected. Mule foals may develop thrombocytopenia [12]. Alloantibody associated thrombocytopenia and neutropenia have been reported in horse foals as well. Increased fibrinogen, leukocytosis, or leukopenia may be seen in foals with concurrent sepsis. Differential diagnosis for anemic foals include those that have sustained severe blood loss during foaling due to umbilical remnant bleeding, or traumatic injury resulting in rib fractures and hemоторax. Hemoperitoneum may also occur. Icterus in foals may be secondary to infection with equine herpesvirus I, or sepsis. An attempt must be made to rule out all potential causes of anemia and icterus in the newborn foal.

**Treatment**

Treatment of NI is variable and dependent upon the severity of clinical signs. There are a percentage of foals that have very mild signs of NI, often not even noted by the owner. These foals require careful monitoring but no treatment. Severely anemic foals often require a blood transfusion. If signs of renal failure exist, fluid therapy may be indicated. If sepsis is suspected from hematology findings and increased fibrinogen concentration, treatment with appropriate antimicrobial therapy is recommended. Extremely weak foals require caloric supplementation and tube feeding of milk is appropriate if the foal will not nurse. Blood transfusion is often recommended in foals whose PCV is less than 12% and in foals showing significant clinical signs of disease. Options for blood donors include the dam whose red blood cells have been washed aseptically at least 2-3 times, or an unrelated gelding. The mare’s red blood cells are washed in order to remove the anti-foal RBC antibody. If an unrelated gelding donor is chosen the ideal candidate would not possess the Qa or Aa blood type. Depending upon the severity of the anemia up to 4 liters of blood may be needed to see an improvement in the foal’s condition. The volume of blood required to increase the PCV to a desired value can be calculated by the following equation:

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\text{Body weight (kg)} \times \text{Blood volume (ml/kg)} \times \frac{\text{PCV desired} - \text{PCV observed}}{\text{PCV of donor}}
\]

*The blood volume of a 2 day-old foal can be estimated as 150 ml/kg.

The half-life of transfused erythrocytes in foals is suspected to be nearly five days. This period of time should be sufficient in most instances to support the foal prior to a bone marrow response [13]. The most recent advancement in treatment of foals whose clinical signs and anemia necessitate a blood transfusion is the use of polymerized bovine hemoglobin. Polymerized hemoglobin, an ultra-pure bovine origin hemoglobin solution, contains 13 g/dl of hemoglobin in a modified lactated Ringer’s solution [14]. This product has been used successfully in the management of a foal with neonatal isoerythrolysis where a blood donor was not immediately available. The recommended dose in dogs is 10-30 ml/kg intravenously at a rate not to exceed 10 ml/kg/h. Foals with NI have been anecdotally treated with as little as 5 ml/kg with good result (F. Bain, personal communication). Polymerized hemoglobin is readily available and requires no special storage conditions with a shelf life of 36-months. However, in vivo, its half-life is very short (30-40 hrs) and therefore should not be used as the sole means of oxygen carrying supplementation. A transfusion with washed Qa/Aa red blood cells or red cells from a cross-matched donor should ideally follow [14]. Supplemental oxygen is often a useful adjunct therapy. Foals with mild anemia, who continue to have a good appetite and do not have signs of cardiovascular compromise may often be managed with close monitoring and placement in a low stress environment.

**Prevention**

Neonatal isoerythrolysis is a highly preventable disease if several pre-foaling precautions are taken. The best means of
disease prevention is to identify mares that are at risk of producing NI causing antibodies. Mares may be blood typed prior to breeding. Mares who lack the Qa or Aa blood group factor should be identified as mares at risk. The presence of the Ca antigen in the mare should also be evaluated. Mares who lack the Ca antigen often produce antibodies to that antigen, foals ingesting that antibody have not been shown to have clinical illness. However, Ca antibodies may produce false-positive reactions in cross matching tests. One should be aware that a weak background reaction may occur in these tests in mares producing the Ca antibody. Primiparous mares are much less likely to produce an NI foal, regardless of the stallion they are bred to. Primiparous mares lacking the Qa or Aa blood group factor that may have been exposed to that factor are more at risk of producing an NI foal. Mares who have produced an NI foal in a previous pregnancy are considered at high risk for producing another NI foal, especially if bred back to the same stallion. It has been estimated that mares having an NI affected foal at one pregnancy are likely to produce another NI foal in 70% of their other foalings [11]. Stallion blood types are often readily available from the breeder. Ideally stallions possessing the Qa or Aa blood group factor should not be bred to mares lacking either of those factors. From a practical standpoint it is often extremely difficult to base breeding selection upon blood type, most often potential stallions are chosen based upon athletic abilities, temperament, and conformation. If a blood factor positive stallion must be bred to a high NI risk mare, there are post-breeding means of preventing NI in the high risk foal.

Anti-RBC antibody in the mare’s serum, especially if the antibodies are for Qa or Aa with a rise in titer, is suggestive of potential NI. Testing for these antibodies should be done as close to the end of gestation as possible, the last month of gestation is preferred. Antibody levels should be increasing as parturition nears, however, it has been reported that peak level for anti-RBC antibody is approximately nine days after foaling [15]. Antibody testing may be performed at a blood typing laboratory. The stallion's RBC are ideally used to test against as his blood represents all possible factors inherited by the foal. Often the stallion’s blood is not available for this procedure and a representative sample of horse blood is obtained with all likely factors being present in that sample. If antibody testing procedures demonstrate the presence of antibody, the foal must be prevented from ingesting any colostrum from the mare. There has been a reported incident of NI occurring in a mare where anti-erythrocytes antibodies were not detected prior to foaling but detected nine days after foaling. The foal developed mild clinical signs of NI as it had been permitted access to the mare’s colostrum. It is much more likely for false positive antibody detection to be reported [8]. NI only occurs after the ingestion of colostrum as transplacental transfer of maternal immunoglobulin does not occur in horses.

The foalings of mares at risk for producing NI foals should always be attended so that the foal may be prevented from ingesting colostrum. The foal need not be separated from the mare as this may be stressful and impractical. A muzzle may be placed on the foal, or an udder guard placed on the mare, and the mare and foal monitored closely. Detection of antibody may be demonstrated in the colostrum using a relatively simple stall side test, the jaundiced foal agglutination test. A pre-suckle blood sample is obtained from the foal. In the jaundiced foal agglutination test (JFA) an agglutination response is detected between the foal’s red blood cells and the colostrum. For this reason it is essential that a pre-suckle blood sample be obtained. Red blood cells obtained after ingestion of colostrum are likely antibody coated and may auto-agglutinate in the JFA. In horses if the JFA titer is 1:16 or greater, colostrum should be withheld. In mule foals colostrum should be withheld if the titer is 1:64 or greater. Alternative sources of passive immunity such a plasma or antibody negative colostrum should be given to foals unable to consume their own mare’s colostrum. The mare may be milked out and serial JFA samples collected; when the colostrum titer decreases to less than 1:16 in horses, and less than 1:64 in mules, the colostrum may be considered safe for consumption. Often this occurs in less than eight hours from birth. Many farms opt to keep the foal muzzled, give alternative passive immunity, and strip out the mare’s udder for a 24 to 72 hour period before allowing the foal to nurse. This practice is extremely laborious and in many instances unnecessary if the JFA is used.

**Neonatal Isoerythrolysis in Mule Foals**

All mare-donkey breedings have the potential to result in a NI mule foal. All donkeys possess the RBC antigen known as the donkey factor. Thus, previous donkey/mare breedings may allow a mare to become sensitized to the factor. NI may result as previously described in horses. All mule foals of multiparous mares should be monitored closely for signs of NI. There is an estimated 10% incidence of NI in mule foals [16]. There has also been a suggestion that mule foals are less sensitive to moderate anemia and the clinical signs seen in mule foals may be less severe than those seen in horse foals. If a blood donor is necessary any horse may be used, as they will not likely possess the anti-donkey factor [12].

**Conclusions**

Neonatal isoerythrolysis occurs in a small percentage of foalings. The condition may be life-threatening to the foal in severe cases and may require intensive management of the foal, including blood transfusion. The most unique feature of this disease is that it may be effectively prevented by means of careful pre-foaling screening practices. If pre-foaling measures have not been taken to determine the likelihood of the mare producing an NI foal, evaluation of colostral antibody is sufficient to prevent the foal from ingesting potentially harmful anti-erythrocyte antibody. The pathogenesis of this disease has been well
established, however, future research will be used to determine effective means of immune regulation in order to prevent anti-erythrocyte antibody formation, potentially eliminating this disease.

References


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