Neonatal Isoerythrolysis

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Neonatal Isoerythrolysis (NI) or hemolytic anemia of the newborn, although not commonly encountered in equine practice, is an important immunologic disease of foals that often results in a fatal hemolytic crisis. Erythrocytes of affected foals are destroyed by alloantibodies produced by the mare as a result of alloimmunization by erythrocyte antigens foreign to her but possessed by the stallion and inherited by the foal. Alloantibodies are concentrated in the colustrum and initiate intra- and extra vascular hemolysis when absorbed into the foal's circulation following successful passage of colostral antibodies.

Foals that develop NI are normal at birth, ingest colustrum uneventfully, and exhibit normal behavior until erythrolysis precipitates anemia and resulting clinical signs. Since colostral proteins, including antibodies, can be demonstrated in the foal's serum as early as six hours after first suckling, signs of NI in severely affected foals may occur shortly after this time. More commonly, affected foals begin to exhibit signs between 12 hours and 5 days of age. Generally, the younger the foal when signs are observed, the poorer the prognosis for life, and the greater the need for therapy and supportive management.

DIAGNOSIS

Two of the most important clinical signs of NI are icterus and hemoglobinuria. Although not pathognomonic for NI, these signs suggest NI in a weak, depressed foal born to a multiparous mare. Icterus rarely occurs before the second day of the disease, and increases in intensity during the following days. Thus, a foal can die of NI before developing obvious icterus. Icterus seen in foals with NI is associated with an increase in predominantly unconjugated bilirubin, which may reach levels of 20 to 40 mg per dl. The presence of icterus can best be determined by examination of the foal's sclerae in natural sunlight. It is noteworthy to remember that neonatal foals with septicemia or meconium impactions frequently exhibit varying degrees of icterus.

Hemoglobinuria, although not commonly present, may be seen in peracute cases and may be the first clinical sign observed. Its presence suggests profound intravascular hemolysis with release of hemoglobin into the bloodstream. This free hemoglobin overwhelms the binding capacity of plasma haptoglobin and is passed in the urine.

Progressive weakness is commonly observed in foals with NI. Foals may be found in lateral recumbency or in sternal recumbency, resting the chin on the ground. A mare with an udder full of milk should alert the practitioner that the foal may be spending less time than normal nursing. Rectal temperature is usually within normal limits or slightly elevated owing to the accompanying hemolysis. The pulse and respiratory rates are usually elevated because of the hemolytic anemia and the foal's tissue oxygen debt. With progression of the anemia, respirations become rapid and shallow, and shortly before death they become labored as the foal exhibits periods of gasping. During terminal stages, the foal may convulse and become comatose. Postmortem findings suggestive of NI include splenomegaly, pale and/or icteric body tissues, and hemoglobinuria.

Laboratory support greatly facilitates the clinical diagnosis of NI. It provides valuable information regarding the severity of the disease and the approach to therapy indicated. The blood erythrocyte count and packed cell volume (PCV) should be assessed to determine the severity of the anemia. Anemia is present in a neonatal foal when the erythrocyte count is less than 6×10^6 per cu mm

or the PCV is less than 25 per cent. Foals with severe NI usually have a rapidly progressive anemia with a PCV of 6 to 10 per cent and erythrocyte count of less than 3 X 10^6 per cu mm. In most clinical cases, the severity of clinical signs is directly related to the degree of the anemia. Generally, foals with NI that have an erythrocyte count of 4 X 10^6 per cu mm nurse frequently for short periods, whereas foals with less than 3 X 10^6 per cu mm rarely nurse.

Despite a history compatible with NI, laboratory confirmation of anemia, and clinical signs of weakness, icterus, hemoglobinuria, and increased vital parameters, a definitive diagnosis of NI can be made only when alloantibodies are demonstrate on the foal's erythrocytes. Basically, serodiagnostic testing for NI demonstrates either agglutination or hemolysis of sensitized foal erythrocytes. Since alloantibodies responsible for NI act stronger as hemolysis that cause hemolysis than as agglutinins that cause agglutination, preferred tests should detect hemolysis of the foal's erythrocytes. Even though the hemolytic test has been established as a superior test when compared with tests detecting agglutination such as the conventional cross-match and the antiglobulin or Coombs' test, it is rather impractical for use in the field. The test requires an exogenous complement source that is furnished in commercially available pooled rabbit sera.[1] Since Rabbits have naturally occurring antiequine antibodies, rabbit sera must be processed with erythrocytes of healthy horses to remove these antibodies. The rabbit sera is best preserved by freezing at extremely low temperatures, which adds to its impracticality for the equine practitioner. A major advantage of the hemolytic test when compared with tests that demonstrate agglutination is the ease of test interpretation. Hemolysis is quickly identified grossly as a wine like discoloration of the supernatant in a tube containing sensitized erythrocytes whereas rouleaux formation of equine erythrocytes often interferes with microscopic assessment of agglutination.

Several laboratories presently perform the hemolytic test for NI in foals. Even though with this approach the diagnosis is delayed by mailing of samples to the laboratory, a confirmation of the disease can be obtained. Two laboratories that provide excellent testing services are the Serology Laboratory at the University of California and Stormont Laboratories at Woodland, California. Addresses are given in the discussion of blood transfusions (see p. 320_.

Test that demonstrates agglutination of sensitized erythrocytes are more available to the practicing veterinarian and can be helpful in the diagnosis of NI. Agglutination of washed foal erythrocytes re-suspended in saline or following the addition of Coombs' sera containing antiequine IgG confirm the diagnosis of NI. Before use, the Coombs' serum must also be processed to remove antiequine antibodies possessed by rabbits used to prepare the reagent. Generally a foal with NI will not exhibit auto agglutination-- that is; erythrocytes will not agglutinate in the saline following washing, until the disease is severe and life-threatening. Even though these procedures may confirm the diagnosis, it should be remembered that alloantibodies react primarily as hemolysins, and may therefore not be detected by tests that detect agglutination.

In summary, in vitro agglutination and hemolysis are dependent upon the relative amounts of alloantigen or erythrocytes and alloantibody or serum. Therefore, simple tests in which blood of the foal is mixed with serum or colustrum from the mare are inaccurate and often misleading. Furthermore, serodiagnostic tests for NI are rather laborious and difficult to perform in the practice setting for several reasons. First, erythrocytes must be washed free of serum proteins, other blood cell types, and platelets, which may interfere with results. Second incubation for at least 30 minutes is usually required before any meaningful results can be obtained. Third, special reagents are required, which include pooled rabbit sera for the hemolytic test and antiglobulin serum for the Coombs' test. And finally these

[1] Pel-Freez Biologicals, P.O. Box 68, Rogers, AK

reagents require special storage and processing before use in the diagnostic tests. The tests required for accurate NI diagnosis are not too sophisticated for the practicing veterinarian to perform. However, if the tests are to be worthwhile and the results reliable, dedication to the steps involved and a sound basic understanding of the testing procedures are mandatory.

THERAPY AND MANAGEMENT

General approaches to management of foals with NI include prevention of further ingestion of alloantibodies, adequate nutrition, consideration of corticosteroid therapy, protection against opportunistic infections, and replacement of plasma volume and erythrocytes until the bone marrow responds to the anemia. If the foal is less than 24 hours old when NI is diagnosed, it should be muzzled and fed supplemental milk or a commercially available milk preparation.[2] Since the foal's intestine becomes impermeable to colostral antibodies by 24 hours of age, prevention of nursing until the foal is 30 hours old should be adequate. During this period, the mare should be milked at approximately two-hour intervals to ensure continued milk production. Separation of the foal and mare is not recommended, as it tends to unnecessarily stress the already compromised foal. Testing the mare's milk for alloantibody levels and not allowing the foal to return to nursing until alloantibodies are no longer detectable is probably not worth while. Even if the antibody levels are detectable, the foal's intestine should be unable to absorb them into circulation after 24 hours of age.

The question of corticosteroid therapy in foals with NI has not been adequately addressed. Although infections by opportunistic organisms are of concern in foals with NI, one administration of corticosteroids may be helpful in diminishing the hemolytic process. An intravenous injection of dexamethasone (5 to 20 mg) appears to have some clinical benefit in severe NI cases. As mentioned previously, antibiotic coverage with such preparations as trimethoprim-sulfa or Ampicillin also aids in prevention of secondary bacterial infections.

Erythrocyte supplementation is an important adjunct to the successful management of foals with NI. However, it is important to remember that a decrease in circulating blood volume also occurs and should be addressed while supplemental blood is being obtained. Administration of plasma from an acceptable donor or sodium-containing isotonic fluid replacement is helpful in the immediate management of a foal with NI. The PCV should be monitored during such therapy because further hemo-dilution will occur.

The need for supplemental erythrocytes should be based on the erythrocyte count or the PCV or both. Not only is the absolute value important, but also important is the rapidity with which these parameters are declining. Foals with NI warrant PCV determinations at least twice daily until the progression of anemia can be characterized. Irrespective of the rapidity of the declining PCV, a transfusion is indicated when the PCV and erythrocyte count fall to less than 12 per cent and 3×10^6 per cu mm, respectively. Clinical appearance of the affected foal should also be taken into consideration when the need for supplemental erythrocytes is considered, as several foals have spontaneously recovered from NI following anemia with a PCV as low as 12 per cent.

The best donor of erythrocytes for a foal with NI is the mare, but this means that the serum containing the offending alloantibodies must be removed. Allowing whole anticoagulated blood collected from the mare to settle followed by siphoning of the serum is acceptable, but the settled erythrocyte preparation contains alloantibodies. Washing mare erythrocytes by mixing with saline and repeated centrifugation is the preferred method, but rather impractical unless the practitioner has access

[2] Foal Lac. Borden, Inc. Pet-Ag Division, Pet/Vet Products, Hampshire, IL

to a large refrigerated centrifuge.

Administration of the sire's erythrocytes will result in destruction of his erythrocytes until the alloantibodies are consumed. This adds further insult to the foal's reticuloendothelial system and is not advisable. If washed erythrocytes of the mare are not available, erythrocytes from an "acceptable blood-typed donor horse" are recommended. An acceptable blood-typed donor horse is one whose blood has been characterized by one of the two serology laboratories already mentioned, and found to be free of significant alloantibodies in the serum and Aa and Qa surface antigens on the erythrocytes (see p. 317). Alloantibodies against these antigens are more often responsible for field cases of NI. Transfusion of 1 to 2 liters of whole blood usually has a dramatically favorable effect on the foal's clinical appearance. Since transfused equine erythrocytes have a short half-life, the transfusion may need to be repeated if the anemia is progressive. The PCV should be determined after each transfusion to serve as a baseline, which will enable detection of the need for repeat transfusions. Exchange transfusion whereby blood is withdrawn from one vein while it is supplemented through another vein may benefit severely affected foals. However, the need for increased manipulation of the foal and possible sedation make this procedure less attractive as a useful adjunct to therapy.

PREVENTION

Neonatal isoerythrolysis can be effectively prevented by withholding the offending colustrum and providing colustrum from a nonsensitized mare. It is important to test supplemental colustrum, as NI can be induced with supplemental colustrum that contains alloantibodies. An aliquot of stored colustrum should be analyzed by one of the laboratories mentioned previously to ensure it is free of alloantibodies, especially anti-Aa and anti-Qa.

A common question by concerned horse owners regarding NI is: "I'm afraid that my mare is carrying a foal that might develop NI. What should I do?" The best approach to this question is to acquire serum from the mare during late pregnancy, preferably during the last two to three weeks, and mail the sample to one of the serologic laboratories to determine if the mare has a significant titer to erythrocyte alloantigens. If a high titer to one or more alloantibodies is detected, recommendations should include withholding the mare's colustrum from the foal and provision for supplemental colustrum. Such results do not mean that the foal will definitely develop NI, because the foal may not inherit the offending alloantigen types from the stallion, and therefore the foal's erythrocytes would not be affected by alloantibodies in the colustrum.

Supplemental Readings

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