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Objective—To assess data regarding clinical features, clinicoopathologic and blood gas variables, and outcome from horse and mule foals with confirmed neonatal isoerythrolysis (NI).

Design—Retrospective case series.

Animals—17 horse and 1 mule foals.

Procedure—Medical records of foals (<14 days old) with NI were reviewed. Information collected included signalment; clinical examination findings; results of hematologic, serum and plasma biochemical, and venous blood gas analyses and urinalysis; treatments; and outcome.

Results—Data from 17 horse foals and 1 mule foal with NI (mean age, 71 hours) were evaluated. Many foals had high serum indirect and direct bilirubin concentrations and sorbitol dehydrogenase activity. Whole blood immunoglobulin concentrations were >400 mg/dL in 4 of 15 foals. Fresh whole blood transfusions were administered to 10 of 18 foals. Among the blood factors implicated in 11 foals, one (Dg) had not previously been associated with NI. Of 10 foals that received blood transfusions, 7 had significant improvements in central venous oxygen tension. Fifteen foals survived to discharge.

Conclusions and Clinical Relevance—Data suggest that blood factor Dg may be associated with NI in foals. Liver disease may be concurrent with NI in foals, and NI can develop in foals with inadequate passive transfer of colostral antibodies. Whole blood transfusions were successful at increasing oxygen-carrying capacity and improving peripheral tissue oxygenation in NI-affected foals. With appropriate treatment, the prognosis for foals with NI is good. (J Am Vet Med Assoc 2005;227:1276–1283)

Neonatal isoerythrolysis (NI) is the most common cause of clinical icterus in neonatal foals. It is the most common alloimmune disease in foals that is characterized by hemolytic anemia. Other causes of hemolytic disease in neonatal foals include disseminated intravascular coagulation, bacteria-induced hemolysis, and iatrogenic causes associated with an incompatible blood or plasma transfusion. To the authors’ knowledge, there are no published retrospective evaluations of a clinical case series of NI in foals. The purpose of the study reported here was to assess data regarding clinical features, clinicoopathologic and blood gas variables, and outcome collected from horse and mule foals with confirmed NI.

Criteria for Selection of Cases

Medical records of all neonatal foals (age range, 0 to 14 days) with a confirmed diagnosis of NI that were admitted to the in-house or field services of the University of California-Davis Veterinary Medical Teaching Hospital (VMTH) from January 1988 to December 2003 were selected for review. For each foal, confirmation of NI was defined as detection of hemolysis and positive results of a direct antiglobulin test (Coombs’ test) or identification of anti-erythrocyte antibody in the dam’s serum or colostrum. The identified blood factor for the anti-erythrocyte antibody in a dam’s serum or colostrum identifies the blood factor antigen on a foal’s RBCs, and these terms are used interchangeably throughout this report. Foals with anemia were excluded if results of a direct antiglobulin test or anti-erythrocyte antibody testing were not available.

Procedures

Medical records were reviewed; information obtained included signalment; clinical examination findings; results of hematologic, serum and plasma biochemical, and venous blood gas analyses and urinalysis; treatments; and outcome. All laboratory tests were performed at the VMTH. Hematologic variables were measured by use of an automated CBC analyzer. All serum and plasma biochemical data were obtained by use of an analyzer. Venous blood gas analyses were performed with a blood gas analyzer. Urinalysis was performed by use of urinalysis strips; the presence of bilirubin in urine was detected by use of reagent tablets. Plasma fibrinogen concentration was assessed via the heat precipitation method. Passive transfer of immunoglobulin was determined by use of 1 of 2 semiquantitative commercial immunoglobulin immunoassays; adequate passive transfer was defined as whole blood immunoglobulin concentration > 800 mg/dL. The reference ranges for all these variables in neonatal horses were not created specifically; published reference ranges were used.

Additional information collected included details of administration of blood products and corticosteroid treatment; heart rate, respiratory rate, and rectal temperature before and after the first blood or plasma transfusion; hemoglobin concentration, Hct, RBC count, icterus index (ie, the value that indicates the relative amount of bilirubin in serum or plasma by assessment of the intensity of color of the sample), and plasma protein and fibrinogen concentrations before and after the first blood or plasma transfusion; serum anion gap; serum

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creatinine, direct bilirubin, indirect bilirubin, total bilirubin, BUN, total CO₂, and total protein concentrations before and after the first blood or plasma transfusion; and venous blood pH, venous base excess, venous bicarbonate concentration, and central venous oxygen tension (PcvO₂) before and after the first blood or plasma transfusion. Posttransfusion data were collected within 4 hours of and 24 hours after the first blood or plasma transfusion for heart rate, respiratory rate, rectal temperature, and venous blood gas values; 0.5 to 4 days after the first blood or plasma transfusion for hematologic variables; and 0.5 to 5 days after the first blood or plasma transfusion for serum, plasma, and urine biochemical variables.

The NI-associated blood factor antigens on a foal's RBCs were indirectly determined via identification of the blood factor alloantibodies in the dam. Three dilutions of the mare's serum or colostrum were evaluated for anti-erythrocyte hemolysin and agglutinin activities against a panel of washed RBCs from 11 horses and 1 donkey of known blood types (performed at the Veterinary Genetics Laboratory, School of Veterinary Medicine, University of California, Davis, as previously described). From the recorded data, blood volume administered per kilogram was calculated for foals with known body weights and estimated for foals with unknown body weights (body weight assumed to be 50 kg [110 lb] on the basis of breed). Hospital visit data were computer generated for all foals 0 to 14 days of age that were treated at the VMTH from 1988 to 2003.

Survival-to-discharge status was determined for hospitalized cases as well as the field service cases for which follow-up history (minimum duration, 3 weeks) was available. Long-term outcome data were obtained from medical records and via telephone conversations with owners and farm managers. Long-term outcome was considered successful if the horse was alive ≥1 year after discharge.

Statistical analyses—A nonparametric Mann-Whitney test was used to compare Hct values at initial evaluation between foals that were euthanatized and foals that survived to discharge. This test was also used to evaluate the differences between values before and after the first blood transfusion for the following variables: heart rate; respiratory rate; rectal temperature; hemoglobin concentration; Hct; RBC count; icterus index; plasma protein concentration; plasma fibrinogen concentration; venous blood pH; venous base excess; venous bicarbonate concentration; PcvO₂; anion gap; and serum direct bilirubin, indirect bilirubin, total bilirubin, BUN, creatinine, total CO₂, and total protein concentrations. Statistical comparisons, a value of P < 0.05 was considered significant.

Results—Signalment—Eighteen foals fulfilled the criteria for inclusion in the study. An additional foal that was dead on arrival at the VMTH was suspected to have NI but was not included in the study because the selection criteria could not be met. Among the 18 foals, there was 1 mule, 2 American Paints, 1 warmblood cross, 1 Standardbred, 8 Thoroughbreds, and 5 Quarter Horses; the percentage of foals with NI by breed was 5.5%, 11%, 5.5%, 5.5%, 44.4%, and 27.8%, respectively. Among the general population of neonatal equids evaluated at the VMTH during the study period, the percentages of foals of these breeds were 1.1%, 3.6%, 5.8%, 3.2%, 31%, and 20.3%, respectively. In the study group, mules, American Paints, Standardbreds, and Thoroughbreds were overrepresented. There were no Arabians, American Miniature Horses, or draft breeds with NI despite these breeds representing 10.8%, 4.2%, and 2% of the general neonatal equid population at the VMTH. The 18 foals with NI represented 1% of the total population of equids (n = 1,988) aged 0 to 14 days evaluated at the VMTH during the study period. There were 12 females among the 18 foals with NI; among the total VMTH population of 1,988 neonatal equids, there were 913 (45.9%) females and 994 (50%) males (sex was not recorded for 81 [4.1%] foals). Mean age at initial evaluation was 71 hours (age range, 7.5 to 288 hours; median age, 60 hours).

Clinical examination findings—Clinical examination findings were available for 17 of the 18 foals. Each foal's attitude at initial evaluation was classified. Of the 17 foals, 10 were quiet, alert, and responsive; 4 were weak; and 3 were obtunded. Initial rectal temperature ranged from 35.9°C to 39.3°C (96°F to 102.8°F; mean, 38.1°C ± 1.1°C [100.6°F ± 2.0°F]; median, 38.4°C [101.2°F]). Five of the 17 foals were considered febrile (rectal temperature > 38.8°C [101.8°F]). Respiratory rate ranged from 20 to 140 breaths/min (mean, 46 ± 31 breaths/min; median, 40 breaths/min). Six foals were considered tachypneic (respiratory rate > 40 breaths/min). Heart rate ranged from 75 to 144 beats/min (mean, 108 ± 19 beats/min; median, 108 beats/min). Four foals were considered tachycardic (heart rate > 120 beats/min). Additional clinical abnormalities detected in the 17 foals at initial evaluation included icterus (n = 11), cardiac murmurs (4), enlarged umbilicus (1), petechiae (2 [1 of which was the mule foal]), echymoses (1 [the mule foal]), hematochezia (1 [the mule foal]), diarrhea (1), contracted tendons (1), seizures (1), and red urine (3 of the 5 foals that urinated during the initial evaluation).

Hematologic findings—Results of a CBC performed at the initial evaluation were available for 16 foals. Hematocrit ranged from 9% to 38.4% (mean,
17.7 ± 9.6%; median, 13.7%; reference range, 33.4% to 46.6%). At the initial evaluation of these 16 foals, 6 had Hct ≤ 11% and 9 had Hct ≤ 15%. Although the Hct was lower in foals that were eventually euthanized, there was no significant difference in initial Hct between the 3 foals that were euthanized (mean, 12.1 ± 2.4%; median, 11%) and the 13 foals that survived (mean, 19 ± 10.2%; median, 16%). At the initial evaluation, hemoglobin concentration ranged from 3.4 to 13.4 g/dL (mean, 6.4 ± 3.0 g/dL; median, 5.3 g/dL; reference range, 12.2 to 16.6 g/dL) and RBC count ranged from 8.5 to 12.5 X 10⁶ cells/µL; median, 142,000 platelets/µL; reference range, 10 to 70 X 10⁶ cells/µL; median, 9,600 cells/µL; reference range, 3,492 to 13,772 cells/µL), and band neutrophil count ranged from 0 to 2,340 bands/µL (mean, 561 ± 839 bands/µL; median, 16 bands/µL; reference range, < 50 bands/µL). Leukocyte index ranged from 15 to 100 units (mean, 78 ± 27 units; median, 88 units; reference range, 10 to 70 units). Platelet counts ranged from 1,100 to 278,000 platelets/µL (mean, 145,174 ± 80,078 platelets/µL; median, 142,000 platelets/µL; reference range, 100,000 to 225,000 platelets/µL). Plasma fibrinogen concentrations were available for 15 of 19 foals; these values ranged from 300 to 900 mg/dL (mean, 387 ± 192 mg/dL; median, 300 mg/dL; reference range, 95 to 391 mg/dL). Serum total protein concentrations were available for 12 of 19 foals; these values ranged from 3.9 to 7.1 g/dL (mean, 5.5 ± 1.0 g/dL; median, 5.6 g/dL; reference range, 4.5 to 7.7 g/dL).

**Hematocrit (Hct)**

- **Median Hct:**
  - Initial evaluation: 12.2%
  - Survival: 16%
- **Range:**
  - Initial evaluation: 4.3% to 16.6%
  - Survival: 9.6% to 13.7%
- **Reference range:**
  - Initial evaluation: 33.4% to 46.6%
  - Survival: 11% to 15%

**Hemoglobin concentration**

- **Median Hgb:**
  - Initial evaluation: 6.4 g/dL
  - Survival: 5.3 g/dL
- **Range:**
  - Initial evaluation: 3.4 to 13.4 g/dL
  - Survival: 5.3 to 13.3 g/dL
- **Reference range:**
  - Initial evaluation: 12.2 to 16.6 g/dL
  - Survival: 12.2 to 16.6 g/dL

**RBC count**

- **Median RBC:**
  - Initial evaluation: 9.6 X 10⁶ cells/µL
  - Survival: 142,000 platelets/µL
- **Range:**
  - Initial evaluation: 8.5 to 12.5 X 10⁶ cells/µL
  - Survival: 5.3 X 10⁶ to 142,000 platelets/µL
- **Reference range:**
  - Initial evaluation: 12.2 to 16.6 X 10⁶ cells/µL
  - Survival: 12.2 to 16.6 X 10⁶ cells/µL

**WBC count**

- **Median WBC:**
  - Initial evaluation: 4.3 X 10⁶ cells/µL
  - Survival: 561 ± 839 bands/µL
- **Range:**
  - Initial evaluation: 0 to 2,340 bands/µL
  - Survival: 0 to 2,340 bands/µL
- **Reference range:**
  - Initial evaluation: 10 to 70 X 10⁶ cells/µL
  - Survival: 10 to 70 X 10⁶ cells/µL

**Platelet count**

- **Median Platelet:**
  - Initial evaluation: 145,174 ± 80,078 platelets/µL
  - Survival: 142,000 platelets/µL
- **Range:**
  - Initial evaluation: 1,100 to 278,000 platelets/µL
  - Survival: 100,000 to 225,000 platelets/µL
- **Reference range:**
  - Initial evaluation: 100,000 to 225,000 platelets/µL
  - Survival: 100,000 to 225,000 platelets/µL

**Blood glucose concentration**

- **Median Glucose:**
  - Initial evaluation: 5.3 g/dL
  - Survival: 5.6 g/dL
- **Range:**
  - Initial evaluation: 2.2 to 13.1 g/dL
  - Survival: 4.3 to 7.1 g/dL
- **Reference range:**
  - Initial evaluation: 4.5 to 7.7 g/dL
  - Survival: 4.5 to 7.7 g/dL

**Serum creatinine concentration**

- **Median Creatinine:**
  - Initial evaluation: 0.9 mg/dL
  - Survival: 1.3 mg/dL
- **Range:**
  - Initial evaluation: 0.8 to 3.4 mg/dL
  - Survival: 0.7 to 1.9 mg/dL
- **Reference range:**
  - Initial evaluation: 0.7 to 1.9 mg/dL
  - Survival: 0.7 to 1.9 mg/dL

**Serum alkaline phosphatase concentration**

- **Median ALP:**
  - Initial evaluation: 33.9 U/L
  - Survival: 189 U/L
- **Range:**
  - Initial evaluation: 10 to 70 U/L
  - Survival: 0 to 104 U/L
- **Reference range:**
  - Initial evaluation: 10 to 70 U/L
  - Survival: 0 to 104 U/L

**Serum γ-glutamyl transferase concentration**

- **Median γ-GT:**
  - Initial evaluation: 29.5 U/L
  - Survival: 7.5 U/L
- **Range:**
  - Initial evaluation: 29.5 to 6.5 U/L
  - Survival: 29.5 to 5.5 U/L
- **Reference range:**
  - Initial evaluation: 8.5 to 6.5 U/L
  - Survival: 8.5 to 6.5 U/L

**Urinalysis findings**

- **Bilirubinuria:**
  - Present in all 4 foals
- **Hemoglobinuria:**
  - Present in all 4 foals

**Histologic examinations**

- **Liver tissue specimens:**
  - Focal hemorrhagic necrosis
  - Massive hemosiderosis
  - Multifocal hemolysis
  - Liver cirrhosis
  - Liver fibrosis

**Venous blood gas analysis findings**

- **Initial venous bicarbonate concentration:**
  - Range: 18.2 to 45.5 mm Hg
- **Initial venous pH:**
  - Range: 7.0 to 7.4
- **Initial venous PCO₂:**
  - Range: 24.4 to 43.8 mEq/L

**NI diagnosis**

- Nine of the 18 foals were assessed via a direct antiglobulin test (Coombs’ test); results of
the tests were positive in 8 of the 9 foals. Sera from 12 of the 18 dams were tested for anti-erythrocyte antibody. Anti-erythrocyte antibody was detected in sera obtained from 10 of the 12 dams; in 1 of the 2 dams with no detectable serum anti-erythrocyte antibody, anti-erythrocyte antibody was identified in colostrum. Three dam-foal pairs were assessed via the direct antiglobulin test (foal) and the serum anti-erythrocyte antibody test (dam). One of the 3 foals had negative results via the direct antiglobulin test, but the dam's serum was positive for anti-erythrocyte antibody. The other 2 foals had positive results via the direct antiglobulin test, and their dams' sera were positive for anti-erythrocyte antibody. The mule foal had positive results via the direct antiglobulin test, but the anti-erythrocyte testing of the dam's serum was not performed.

Among the 10 foals in which the NI-associated antigen was indirectly identified via testing of their dams for anti-erythrocyte alloantibody, 4 were positive for Aa; 1 was positive for Qa; 2 were positive for Pa; 1 was positive for Qb; 1 was positive for Dg; and 1 was positive for Qa, Qb, and Qc. In the foal that was positive for Qa, Qb, and Qc, the strongest reaction was associated with Qb, but all 3 antigens were considered important. The antigen was not identified for 2 foals; donkey key factor was assumed to be the NI-associated antigen in the mule foal (Table 1). Four of the 6 foals with the lowest initial Hct values (ie, the foals with Hct ≤ 11%) had anti-erythrocyte alloantibodies against the following blood factors identified in the mare's serum or colostrum: Dg (n = 1), Aa (1), Qa (1), and the combination of Qa, Qb, and Qc (1).

Treatments—Polymerized bovine hemoglobin solution was administered to 2 of the 18 foals as initial treatment while each dam's erythrocytes were washed in preparation for blood transfusions. The mule foal received 250 mL (5 mL/kg [2.23 mL/lb]) and a Thoroughbred foal received 1,250 mL (25 mL/kg [11.36 mL/lb]) of polymerized bovine hemoglobin solution. Administration of oxygen supplementation via nasal insufflation was recorded for 6 of the 18 foals.

Whole blood transfusions were administered to 10 of the 18 foals; 6 foals received 1 transfusion, 1 foal received 2 transfusions, and 2 foals (including the mule foal) received 3 transfusions. Number and volume of blood transfusions were unknown for 1 foal. Of the 9 foals for which the volume of blood administered during the first transfusion was recorded, the volume ranged from 750 to 4,000 mL (mean, 2,333 ± 887 mL; median, 2,200 mL). This volume range was estimated on a body weight basis to be 17 to 60 mL/kg (7.7 to 27.3 mL/lb; mean, 41 ± 12 mL/kg [18.6 ± 5.45 mL/lb]; median, 40 mL/kg [18.2 mL/lb]). Time to complete the transfusions ranged from 2 to 11 hours (mean, 5 ± 3 hours; median, 3 hours). Of these first whole blood transfusions, 5 were comprised of the dam's washed RBCs, 1 was a combination of unwashed and washed mare's RBCs, and 1 was whole blood from a crossmatched blood donor; the blood transfusion type was not recorded for 3 transfusions. Information regarding the resuspensions of the washed RBCs and the resulting PCVs of the transfusion preparations was not available from the medical records. The mule foal received 1 transfusion (750 mL [17 mL/kg]) of washed RBCs from the jenny followed by 2 smaller transfusions of washed RBCs from that dam (250 mL each [5.7 mL/kg (2.99 mL/lb)]). The mule foal also received a platelet-rich plasma transfusion from a crossmatched blood donor after the first washed RBC transfusion because of severe thrombocytopenia and on-going hemorrhage. Only plasma transfusions were administered to 6 of the 18 foals. The volume of the plasma transfusions ranged from 900 to 2,000 mL (mean, 1,142 ± 172 mL; medi-

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<th>Direct antiglobulin test performed</th>
<th>Results of direct antiglobulin test</th>
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NA = Not applicable.
Eight of 18 foals received 1 treatment with a corticosteroid (mean dosage, 0.19 mg/kg [0.09 mg/lb], IV). Five of these 8 foals received the cortico-steroid treatment before the first transfusion, and 7 of the 8 foals received the treatment on the day of initial evaluation. The mule foal received treatment with a corticosteroid (0.11 mg/kg [0.05 mg/lb], IV) between the treatment with the polymerized bovine hemoglobin solution and the transfusion of washed RBCs. One of the 8 foals that received corticosteroid treatment did not survive, and 2 of the 10 foals that did not receive corticosteroid treatment did not survive.

Heart and respiratory rates; rectal temperature; and hematologic, biochemical, and blood gas findings—For the following variables, no significant difference was detected between the values before and after the first blood transfusion: heart rate; respiratory rate; rectal temperature (within 4 and at 24 hours after the transfusion); RBC count; icterus index; plasma protein concentration; venous blood pH; venous base excess; anion gap; venous bicarbonate concentration; and serum creatinine, direct bilirubin, indirect bilirubin, total bilirubin, BUN, total CO₂, and total protein concentrations. By use of a nonparametric Wilcoxon matched-pairs signed rank test, a significant (P = 0.047) difference in hemoglobin concentration between values before and after the first blood transfusion was detected in 7 foals. Before the transfusion, mean hemoglobin concentration was 4.5 ± 0.7 g/dL, and after the transfusion, the value was 8.0 ± 3.2 g/dL. By use of a nonparametric Wilcoxon matched-pairs signed rank test, a significant (P = 0.047) difference in Hct between values before and after the first blood transfusion was also detected in 7 foals. Before the transfusion, mean Hct was 11.8 ± 2.2%, and after the transfusion, the value was 21.6 ± 9.3%. Compared with findings before the first blood transfusion, there was a large increase in PcvO₂ after the transfusion; before the transfusion, PcvO₂ was 21.7 ± 44.5 mmHg, and after the transfusion, the value was 34.2 ± 37.4 mmHg. However, this finding was based on samples from only 2 foals and therefore must be interpreted with caution. For the following variables, no significant difference was detected between the values before and after the first plasma transfusion: heart rate; respiratory rate; rectal temperature (at 4 or 24 hours after the transfusion); hemoglobin concentration; Hct; RBC count; icterus index; venous blood pH; venous base excess; venous bicarbonate concentration; PcvO₂; anion gap; and serum creatinine, direct bilirubin, indirect bilirubin, total bilirubin, BUN, total CO₂, and total protein concentrations. When data regarding heart rate, rectal temperature, and respiratory rate for the 2 transfusion groups (plasma and blood) were combined for analyses within 4 and at 24 hours after transfusion, significant differences between values before and after transfusion were still not detected.

Outcome—Two foals were evaluated by veterinarians on field service only. Fifteen of the 18 foals survived to discharge (13 hospitalized foals with a mean time to discharge of 6 ± 5 days); 3 foals were euthanatized as a result of a combination of sepsis, hepatic disease, or neurologic disease while hospitalized. Bacterial or fungal culture of blood from all 3 foals yielded positive results. In 1 foal, hemolysis of erythrocytes continued after a third blood transfusion. The specificity of the anti-erythrocyte antibodies was never identified in the serum of this foal’s dam. Results of the necropsy of this foal indicated chronic hemolysis, diffuse pigment hepatopathy with multifocal hepatocellular necrosis, acute bile stasis, pigmented nephrosis, and severe hemosiderosis of the spleen. Necropsy of the second foal that was euthanatized revealed chronic cholangitis, biliary obstruction, bile stasis, giant cell hepatopathy, and hepatocyte syncytia formation; there was bone marrow hypoplasia, degenerative changes of the spleen, thymic atrophy, and Alzheimer type II gliosis of the frontal and parietal lobes of the brain. A necropsy of the third euthanatized foal was not performed at the owner’s request. The 2 euthanatized foals for which necropsies were performed had also undergone liver biopsy procedures. Among the 3 euthanatized foals, 2 had initial Hct ≤ 11% and all 3 had initial Hct ≤ 15%. One of the 3 foals that was euthanatized had high serum SDH (300 U/L), alkaline phosphatase (4,379 U/L), and aspartate aminotransferase (998 U/L) activities and high serum total bilirubin concentration. All 3 foals that were euthanatized had high serum SDH activity and high serum total bilirubin concentration. The only anti-erythrocyte alloantibody identified in the dam’s serum of the 3 foals that were euthanatized was Dg. Long-term follow-up data were available for 5 foals. One foal was euthanatized within the first year of life for reasons unrelated to NI (ie, physitis, Salmonella group B enteritis, and a patent urachus). Via telephone conversations with the owners (a minimum of 1 year after discharge following the initial evaluations), the other 4 foals were reported to be alive, 1 of which was the mule foal. Because many of the foals had been hospitalized several years prior to commencement of this study, many of the owners could not be contacted because of change of ownership of the foal and lack of current contact information.

Comparison of survival-to-discharge rates with treatments revealed that 8 of 10 foals that received whole blood transfusions survived to be discharged from the hospital. Five of 6 foals that received only a plasma transfusion survived to discharge, and 2 of 2 foals that received no transfusions survived to discharge. The whole blood transfusions were then divided by type. Five of 5 foals that received washed RBCs from their respective dams survived to discharge. The 1 foal that received a combination of unwashed and washed RBCs from its dam was euthanatized. The foal that received a crossmatched blood transfusion and 2 of 3 foals that received a whole blood transfusion of unknown type survived to discharge.

Discussion

To the authors’ knowledge, there are no reports of immune-mediated hemolytic anemia in neonatal foals without a prior history of incompatible blood or plasma transfusions.† A positive result of a direct antiglob-
Proportions of affected Standardbred and Thoroughbred foals were also consistent with those previously reported.2 Interestingly, in the present study, only 6 of 11 foals in which the antigen was identified had Qa or Aa antigen; 3 foals were Quarter Horses, 1 foal was a Paint, and 2 foals were Thoroughbreds. An increased percentage of alloantigens other than those historically associated with NI could be a result of an enhanced ability to detect these blood factors or the relative percentage of these breeds in breeding herds resident near our hospital. Three factors are involved with the development of an alloantigen as the cause of NI in a foal: lack of the antigen on the dam's RBCs, development of antibodies against the antigen by the dam, and the probability that foal will inherit the antigen from the sire. Therefore, the frequency of occurrence of any of these 3 events could change with time for a particular breed. The increased percentage of alloantigen other than Qa and Aa detected in our study should be interpreted with caution because of the small sample size. The alloantigen Dg has not been previously implicated in NI.9.9 In the Standardbred foal in which this alloantigen was detected, the agglutination behavior of Dg was quite strong, resulting in clinical signs in the foal at 18 hours of age. This foal did not survive but also had the confounding factor of concurrent sepsis. The combination of Qa, Qh, and Qc antigens has also not been previously reported in foals with NI.

In the present study, there was a slight overrepresentation of fillies among the foals with NI relative to the hospital's neonatal equid population. This finding may reflect the small number of foals in our study. Because there was only 1 mule foal in our study, it is difficult to know the importance of the apparent overrepresentation of this breed, compared with the general population. It is possible that offspring of all horse-dam and donkey-sire breedings are at risk for NI because of the presence of donkey factor antigen on the sire's erythrocytes.9 Proportions of affected Standardbred and Thoroughbred foals were also consistent with those previously reported.7.20 Interestingly, Arabs, American Miniature Horses, and draft breeds were underrepresented in the study population, compared with the hospital's neonatal equid population. The reasons for these under- and overrepresentations are unclear but may reflect the small number of foals in our study or the relative frequencies of erythrocyte antigens in the horse population served by the VMTH.

It has been previously reported11 that foals with NI develop clinical signs within 5 days of birth. Our data suggest that this interval may be as long as 12 days (median time to development of signs after birth, 2.5 days). This extended age range for development of clinical signs of NI could represent subclinical or mild NI in foals that subsequently became clinically apparent. For example, the 12-day-old foal in the study of this report was evaluated as a field service case; signs of NI were mild, and the foal did not require hospitalization but did receive a blood transfusion. If the hemolytic process was slow or mild, foals could have compensated for the low Hct and RBC mass. Iatrogenic immune-mediated hemolytic crises, as might develop after administration of incompatible blood transfusions, were ruled out in the foals of the present study on the basis of anamnesis; none of the foals in our study had previously received plasma or blood product transfusions, and, in most instances, hemolysin or agglutinin alloantibody was detected in the mares' sera.

Tachypnea and icterus were the most notable abnormal physical examination findings among the foals with NI.7,8,10 Anemia or low oxygen content would likely stimulate the respiratory center response, resulting in tachypnea. Reduced mentation was also common. All 3 foals that were described as obtunded at the initial evaluation were discharged from the hospital alive, but 1 was later euthanatized as a result of sepsis and salmonellosis.

Hematologic findings at the initial evaluation were consistent those of previous reports7,9 of NI in foals, which included various degrees of anemia associated with clinical disease. At the initial evaluation, hemocrit concentration can falsely increase Hct. In the present study, 2 foals had Hct within the reference range and 2 foals had Hct close to the reference range at the initial evaluation; following rehydration with plasma transfusions or administration of crystalloids, these foals became anemic. Interestingly, the former 2 foals both had failure of passive transfer, which may have resulted in poor absorption of the alloantigen from colostrum. The severity of the hemolytic crisis (determined by the antigenicity of the alloantigen) can also affect the rate at which Hct decreases. Some foals had WBC counts near the upper reference limit with high band neutrophil concentrations. Similar findings are associated with systemic inflammatory response syndromes (SIRS), such as sepsis. Neutrophilia develops as a result of sympathoadrenal and neurohormonal responses to anemia. Thrombocytopenia has been identified concurrently with NI, most commonly in mule foals.12,13 In our study, 3 of 18 foals had concurrent thrombocytopenia and anemia, which highlights the fact that thrombocytopenia may be present in some foals in NI. Whether this abnormality is a result of alloimmune thrombocytopenia, coagulopathies, or other causes is unknown and requires further investigation. All 3 foals were confirmed to be thrombocytopenic via platelet counts performed by an automated analyzer on blood samples collected in tubes contain-
ing sodium citrate. Platelet-associated alloantibody testing was performed on a whole blood sample from the mule foal, but results were negative. Examination of the bone marrow of this foal was consistent with a thrombolytic process via maternal antibodies. The reliability of platelet-associated alloantibody testing for alloimmune thrombocytopenia in horses and mules has been questioned. Coagulopathies with activation or consumption of platelets as a result of SIRS or hypoxic-ischemic injury may also explain the thrombocytopenia detected in these NI-affected foals. The thrombocytopenia (48,000 platelets/µL) in 1 foal could have developed secondary to disseminated intravascular coagulation associated with sepsis. The mule foal required treatment with platelet-rich plasma from a VMTH donor horse because of active hemorrhage. The other 2 foals with thrombocytopenia responded to whole blood transfusions, and all 3 were discharged from the hospital alive.

At the initial evaluations of the NI-affected foals, serum and plasma biochemical variables were consistent with previously reported values except for the additional abnormality of high serum direct bilirubin concentration. To our knowledge, direct hyperbilirubinemia in association with NI has never been described in the peer-reviewed literature and likely reflects hepatic disease in our population of foals. Hepatocellular damage leading to direct hyperbilirubinemia is suspected to develop secondary to excessive bilirubin accumulation in hepatocytes and iron toxicity as a result of massive hemosiderosis as well as anemic hypoxia. Serum SDH activity correlated with these findings. Urine abnormalities were consistent with hyperbilirubinemia and hemolytic disease.

Neonatal isoerythrolysis has historically been associated with adequate passive transfer of IgG because a foal absorbs the dam’s colostrum that contains the anti-erythrocyte antibodies. Interestingly, 4 of 15 foals in our study had failure of passive transfer (defined as whole blood immunoglobulin concentration < 400 mg/dL). Three of these 4 foals did not require blood transfusions, possibly indicating more mild disease associated with poor passive transfer of colostral antibodies. One of these 4 foals with failure of passive transfer did not survive to discharge because of sepsis-associated complications. There are several theories regarding development of NI in the face of failure of passive transfer. It is possible that the anti-erythrocyte antibodies may have different antigenic reactivities or sensitivities because certain blood groups are more antigenic than others; to cause erythrocyte destruction, lower concentrations of some antibodies may be required, compared with concentrations of other anti-erythrocyte antibodies. Other theories include consumption of IgG secondary to concurrent sepsis (sepsis was identified in 3 of the 4 foals with failure of passive transfer) or antibody binding to RBCs that prevents measurement of free IgG in whole blood via the immunoassay.

At initial evaluation of the NI-affected foals, results of venous blood gas analyses were consistent with metabolic acidemia and high anion gap metabolic acidosis. Anemia causes peripheral tissue hypoxia and increased lactic acid production; in the foals of our study, low PcvO2 and high blood lactate concentrations were evidence of this. Oxygen extraction increases in tissues with low oxygen delivery, resulting in levels of PcvO2 that are below the lower reference limit. Unfortunately, results of arterial blood gas analyses were unavailable to estimate oxygen extraction ratios. As indicated by the oxyhemoglobin dissociation curve, a decrease in blood pH shifts the curve to the right, and unloading of oxygen from hemoglobin to the peripheral tissues is facilitated. High blood lactate concentrations are also consistent with increased production of lactate as a result of inadequate peripheral oxygen delivery, although increased production due to catecholamine or inflammatory mediators may have also contributed to the hyperlactatemia. Compared with initial values, significant differences in hemoglobin concentration and Hct were detected after the administration of whole blood transfusions, suggesting an increase in oxygen-carrying capacity provided by the transfusions. In 2 foals in which PcvO2 was measured before and after the first blood transfusion, there was a large posttransfusion increase in that value, reflecting improved peripheral tissue oxygenation. The use of PcvO2 as a transfusion trigger in foals with NI warrants further study. Based on the results of the present study, it appears that not all foals with clinical signs of NI need to be treated with whole blood transfusions. Affected foals with high Hct values, slower rates of RBC hemolysis, more normalized clinical variables such as heart and respiratory rates and mentation, and mild abnormalities in PcvO2 may require only supportive care including colloid administration via plasma transfusions. Foals with hypoproteinemia may be best treated with colloids to avoid further dilutional decreases in serum total protein concentration by the crystalloids. Evidence also suggests that many cases of NI are subclinical.

It would be expected that the clinical variables (heart rate, temperature, and respiratory rate) measured in the NI-affected foals within 4 hours of completion of plasma and blood transfusions would be decreased, compared with initial values, because of an increase in the oxygen-carrying capacity of the blood. In the present study, there were no significant differences in those clinical variables between values before and after transfusions. Following both types of transfusion, respiratory rate and rectal temperature actually increased, which could be explained by concurrent SIRS or mild transfusion reactions. At 24 hours after transfusions, reevaluation of all clinical variables still revealed no significant differences, compared with values before transfusions. Because clinical data before and after the first transfusion were available for only 3 foals that were administered plasma and 5 foals that were administered blood, the sample was too small to detect significant differences. The clinical data for the 2 transfusion groups were then combined (plasma and blood) for analyses at 4 and 24 hours after transfusion, but significant differences between values before and after transfusion were still not detected.

The present retrospective study included too few foals to examine risk factors for death associated with
NI. However, microbial culture of blood yielded positive results for all 3 foals that were euthanatized. At the initial evaluation, Hct values were lower in the 3 foals that were euthanatized than values in the foals that were not euthanatized, but the difference was not significant, which may be a consequence of the low number of foals that were euthanatized. Of all foals in the study, the foals with Hct values < 11% did have significantly higher serum SDH activities, compared with the foals with Hct values ≥ 11%, and both foals in which liver biopsies were performed were euthanatized, which may suggest that another possible risk factor associated with outcome of NI-affected foals could include the degree of concurrent liver disease. Two of the 3 nonsurviving foals had high serum liver enzyme activities, which may also reflect an association between liver disease and outcome. However, because there were so few nonsurviving foals in our study, it is difficult to draw strong conclusions. The number of transfusions required to stabilize the foal and the particular anti-erythrocyte antibody may also be possible risk factors associated with outcome of NI-affected foals.

In the study of this report, the alloantigen Dg and the combination of Qa, Qb, and Qc antigens were associated with the development of NI in foals. Our data indicated that NI-affected foals may have concurrent liver disease as a result of low oxygen content in blood associated with anemia. Blood transfusions administered to affected foals can improve tissue oxygenation, as evidenced by increases in hemoglobin concentration, Hct, and PCvO2. Although blood transfusions are not necessary for every foal with NI, treatment or support of tissue oxygenation by means of crystalloid, colloid, or blood transfusion therapy can result in a favorable prognosis, as indicated by survival of NI-affected foals that had not received blood transfusions.

References

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