Hepatoblastoma with erythrocytosis in a young female horse

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EQUINE

- Hepatoblastoma is a rare hepatic tumor that develops most commonly in juvenile domestic animals and humans.
- Hepatoblastoma can be associated with paraneoplastic syndromes such as erythrocytosis.
- Histologic examination of affected hepatic tissue provides the only definitive method for diagnosis of hepatoblastoma.
- Concentrations of α-fetoprotein in serum or affected hepatic tissue may serve as a tumor marker but cannot be used alone to confirm a diagnosis of hepatoblastoma.

A 2.5-year-old 457-kg (1,005-lb) female Thoroughbred was referred to our veterinary medical teaching hospital for assessment of lethargy, anorexia, and weight loss. The horse did not have a history of illness and had raced successfully 6 weeks before admission. After returning from the racetrack, the horse became lethargic, anorectic, and lost weight.

Physical examination on the day of admission (day 1) revealed a rectal temperature of 37.5 C (99.5 F), heart rate of 52 beats/min, and respiratory rate of 12 breaths/min. The horse had mild signs of depression and had a body condition score of 3 (scale of 1 to 9[1]). Oral, conjunctival, and vulvar mucous membranes were moist but dark red, and capillary refill time was 2 seconds. The sclera were icteric. Auscultation of the thorax and abdomen as well as per rectal palpation did not reveal abnormal findings. Feces were normal in appearance. Analysis of results of a CBC on day 1 revealed erythrocytosis (16.14×10^6 cells/µl; reference range, 4.6 to 11.6×10^6 cells/µl) with an increase in total hemoglobin concentration (23.2 g/dl; reference range, 9.7 to 15.7 g/dl) and an increase in PCV (63%; reference range, 27 to 43%). Plasma total protein concentration was within the reference range (6.7 g/dl; reference range, 6.1 to 7.9 g/dl); leukocyte and platelet counts also were within reference ranges. Serum biochemical analyses revealed mild increases in activity of alkaline phosphatase (273 U/L; reference range, 45 to 246 U/L), aspartate transaminase (479 U/L; reference range, 166 to 386 U/L), creatinine kinase (451 U/L; reference range, 57 to 404 U/L), and L-iditol dehydrogenase (10 U/L; reference range, 1 to 7 U/L). Activity of γ-glutamyltransferase was profoundly increased (207 U/L; reference range, 4 to 48 U/L). Blood urea nitrogen concentration was slightly less than the reference range (12 mg/dl; reference range, 13 to 25 mg/dl). Results of a urinalysis were unremarkable. Evaluation of all these results indicated hepatic dysfunction and an absolute or relative erythrocytosis.

Abdominal ultrasonography on day 1 revealed severe hepatomegaly. The liver had profoundly rounded and bulging margins and extended 5 to 6 cm ventral to the costochondral junction. The liver could be identified on the right side extending from the paralumbar fossa to the seventh intercostal space and on the left side in the sixth to ninth intercostal spaces. Right and left lobes of the liver had a heterogeneous appearance throughout, which was most prominent on the ventral aspect. In the ventral aspect of the seventh to eleventh intercostal spaces, the hepatic parenchyma had a loss of architecture. The hepatic parenchyma appeared anechoic with multiple bright hyperechoic areas, which cast shadows compatible with areas of necrosis and mineralization **(Fig 1)**. The spleen and kidneys were considered to be of normal size and architecture. Ultrasonographic examination of the thorax revealed a small number of comet-tail artifacts on the pleural surface of the lungs, but we did not detect other evidence of pulmonary or cardiac disease. On the basis of clinical findings, a preliminary diagnosis of hepatic neoplasia was made. Other differential diagnoses included amyloidosis or storage disease, chronic active cholangiohepatitis, hepatic abscesses, and septic cholangiohepatitis with a secondary polycythemia.

A specimen of the liver was obtained by use of percutaneous needle biopsy, using a 14-gauge, 16-cm automated biopsy instrument.[a] The specimen was submitted for histologic examination.

Initial treatment included IV administration of fluids (lactated Ringer's solution, 100 ml/kg of body weight/d [45.4 mg/lb/d]), metronidazole (25 mg/kg [11.4 mg/lb], PO, q 12 h), and flunixin meglumine (0.25 mg/kg [0.11 mg/lb], IV, q 8 h). The PCV and plasma total protein concentrations were monitored at 8-hour intervals; PCV ranged from 53% (day 1) to 68% (day 2), and plasma total protein concentration varied from 6.4 g/dl (day 1) to 8.0 g/dl (day 2).

Supportive treatment for erythrocytosis included administration of heparin sodium (40 U/kg [18.2 U/lb], IV, q 8 h) and hypertonic saline (7.5% NaCl) solution (2 L, IV) as well as phlebotomy (removal of 2 L of blood).

Examinations on day 2 revealed a grade II/V late systolic heart murmur, polyuria, and anorexia. On day 3, severe watery diarrhea developed, which resolved without treatment within 12 hours after onset. The horse was given bismuth subsalicylate suspension (10

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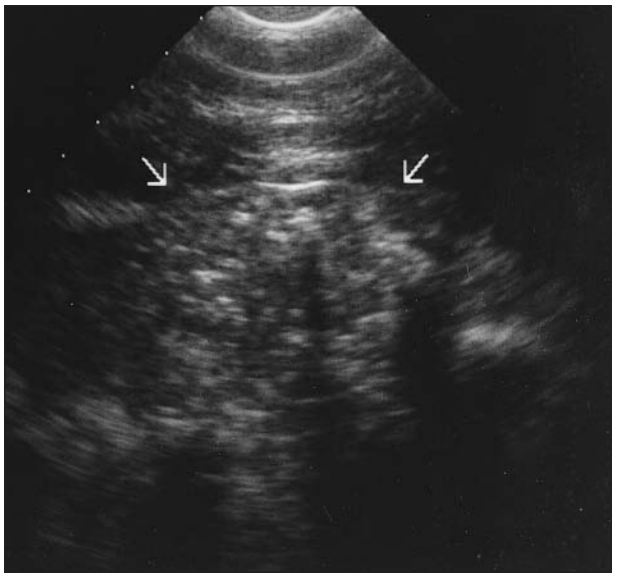

Figure 1—Ultrasonographic view of a hepatoblastoma in the right lobe of the liver in a 2.5-year-old female Thoroughbred. The sonogram was obtained by use of a 5-MHz sector scanner. Notice that the typical architecture of the hepatic parenchyma is replaced by a mixed heterogeneous pattern (white arrows) of the neoplasm. Hepatomegaly is evident. The right side represents the dorsum of the horse. The sonogram was obtained via the right eleventh intercostal space, ventral to the lung.

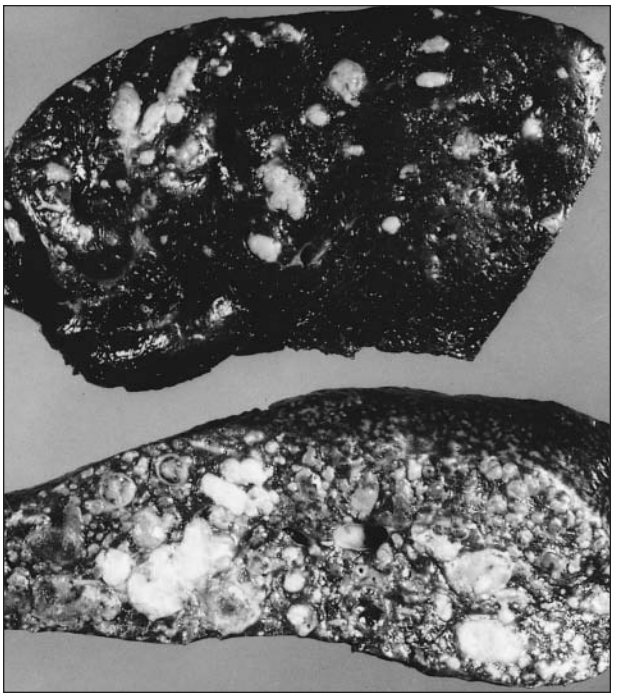

Figure 2—Photograph of sections of liver containing the hepatoblastoma from the horse in Figure 1. Notice the variegated separate and coalescing tumor nodules.

mg/kg [4.5 mg/lb], PO, q 6 h), and a solution of electrolytes was available for consumption ad libitum. On day 4, the horse did not have evidence of improvement, and the PCV was still abnormally high (median 58.1%), whereas the plasma total protein concentration (7.7 g/dl) was within the reference range.

Results of histologic examination of the hepatic biopsy specimen revealed mild portal hepatitis with bile duct proliferation and focal lobular atrophy. On the basis of the strong ultrasonographic evidence of hepatic neoplasia and associated poor prognosis, the horse was euthanized on day 6.

Necropsy revealed profound hepatomegaly. The liver weighed 22.7 kg [49.9 lb]). The left lobe of the liver contained numerous, raised, white foci (1 to 15 mm in diameter), with central areas of necrosis **(Fig 2)**. The right lobe of the liver was more extensively involved and had multiple, firm, coalescing, yellowish nodules (3 to 10 cm in diameter). Sectioning exposed firm, necrotic masses (22 × 18 cm and 20 × 18 cm in the right and middle lobes, respectively). A thick mineralized capsule surrounded both masses. The left lobe of the lungs contained numerous gray nodules (1 to 5 mm in diameter).

Histologically, a thick fibrous capsule delineated neoplastic foci in the liver. Neoplastic cells resembled fetal hepatocytes, embryonal-type cells, and cells with features intermediate between these 2 cell types **(Fig 3)**. Fetal cells were arranged in sheets, nests, and cords; these arrangements were composed of cells smaller than typical hepatocytes with round to oval vesicular nuclei, vacuolated eosinophilic cytoplasm, and indistinct cell borders. In contrast, the embryonal component was more organ-like, forming acini, tubules, and rosettes lined by hyperchromatic cuboidal to columnar epithelial cells. Groups of tumor cells contained variable amounts of mucin that stained with Alcian blue. Mitotic activity was, on average, 3 to 4 mitotic figures/HPF. Foci of hemorrhage and necrosis, accompanied by a mixed population of inflammatory cells, were detected in tumor nodules. Metastatic lesions in the lungs appeared similar to the primary tumor. Immunohistochemically, fetal hepatocytes in liver and lung tissues stained strongly with anti-α-fetoprotein[b,c] (Fig 3). Embryonal cells and hepatocytes in the adjacent nonneoplastic tissue stained weakly or did not stain. Normal bile ducts had negative results when stained for **α-fetoprotein (AFP)**. Positive-control (testicular yolk sac tumor) and negative-control (primary antibody substituted by nonimmune mouse ascites) samples stained as expected. A diagnosis of hepatoblastoma, pure epithelial type, with embryonal and fetal components and pulmonary metastases was made. After the type of tumor was identified, frozen serum was submitted for determination of erythropoietin concentration by use of an ELISA[d]; results were within the reference range (5 mU/ml; reference range, 2 to 20 mU/ml).

Primary hepatic neoplasms are rare in horses, accounting for only 0.82% of all neoplasms in 1 study[2] of 1,222 horses with cancer. Of these primary neoplasms (cholangiocarcinoma, hepatocellular carcinoma, mixed hamartoma, and hepatoblastoma), cholangiocarcinoma is the most common, accounting for 9 of 10 such tumors, and appears to be a condition of older horses.[3,4] Conversely, hepatocellular carcinomas have been reported[5-8,e,f] in 5 horses, 4 of which were ≤ 3 years old. A mixed hamartoma was described in the liver of an equine fetus aborted late in gestation.[9] To our knowledge, there have been 2 other reports of hepatoblastoma in horses, 1 in a stillborn Thoroughbred fetus[10] and 1 in a 3-year-old Appaloosa gelding.[11] In

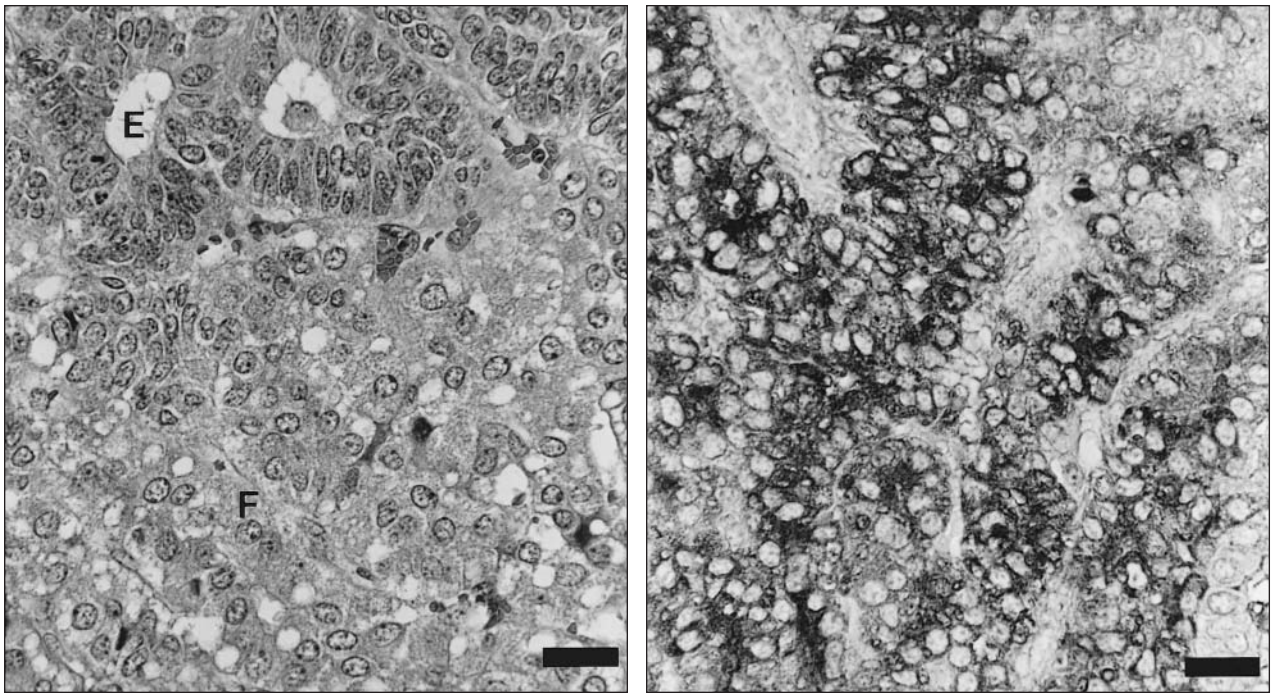

Figure 3—Photomicrographs of sections of the liver of the horse in Figure 1. Left—Notice the fairly well-defined neoplastic, embryonal, and fetal-type epithelial cells. Embryonal cells (E) are elongated and darkly stained and form acini, whereas fetal cells (F) are polyhedral, less intensely stained and vacuolated and grow in cords. H&E stain; bar = 30 μm. Right—Photomicrograph of a section of the liver containing hepatoblastoma. Notice the results after tissues were reacted with anti-α-fetoprotein. Cords of darkly stained fetal-type cells are strongly immunoreactive for α-fetoprotein. Anti-α-fetoprotein (1:300 dilution) with Harris' hematoxylin counterstain; bar = 21 μm.

both of those horses, the tumor metastasized to the thoracic cavity.

Hepatoblastoma is the most common primary hepatic tumor of children,[12] accounting for 25 to 45% of all primary hepatic tumors in children < 5 years old.[13] The cause of hepatoblastoma in humans is unknown, but it has been indicated in one report[12] that maternal exposure to several environmental agents may be important. In children, the tumor has been reported in association with several congenital abnormalities (hemihypertrophy, Wilm's tumor of the kidney, glycogen storage disease, and familial colonic polyposis) and chromosomal mutations (trisomy 20 and abnormalities of chromosome 1).[13] Clinical features include an enlarging abdominal mass, anorexia, and weight loss. Paraneoplastic syndromes may also result from tumor production of various protein hormones such as human chorionic gonadotrophin or testosterone, causing precocious puberty in boys.[14] In 80 to 90% of affected humans, a dramatic increase in serum α–fetoprotein concentration serves as a tumor marker, aiding in the initial diagnosis as well as in monitoring disease progression and recurrence.[14]

As an embryonic tumor of the liver, hepatoblastoma has a wide range of histologic patterns that include epithelial and mesenchymal elements. The epithelial type is the most common in humans, constituting 50 to 60% of all hepatoblastomas. The epithelial type is subdivided into fetal-type tumors and tumors with fetal- and embryonal-type cells. Other less common types of hepatoblastomas include the mixed epithelial-mesenchymal tumors, which can have teratoid features such as trabecular bone, cartilage, striated muscle, or intestinal epithelium, and the anaplastic tumor, which is a small-cell, undifferentiated neoplasm.[15]

Clinical diagnosis of an invasive hepatic tumor in a young animal should raise suspicion of a developmental pediatric-type tumor such as hepatoblastoma. Diagnostic tests should include serum AFP analysis and examination of a hepatic biopsy specimen. An increase in serum AFP concentrations may support an antemortem diagnosis of hepatoblastoma, but it is not pathognomonic, because **hepatocellular carcinoma (HCC)** also can produce AFP.[16] Similarly, both tumors will immunoreact with AFP. Definitive diagnosis of hepatoblastoma requires histologic evaluation of tumor architecture and classification of the tumor type to allow differentiation from HCC. Ultrastructurally, epithelial tumor cells of hepatoblastomas can have features of immature hepatocytes that histologically resemble HCC.[17] Tumors with this cellular morphologic characteristic must be carefully examined for histologic patterns or features that allow differentiation between hepatoblastoma and HCC. Hepatocellular carcinoma cells are usually larger than nonneoplastic hepatocytes and commonly have cellular pleomorphism, multinucleated giant cells, and intranuclear inclusions, which are not detected or extremely rare in hepatoblastomas. Hepatoblastoma tumor cells are usually smaller than nonneoplastic hepatocytes and may contain embryonal cells forming acini or primative tubules.[17] Ultrasound-guided biopsy of the liver is preferable to unaided techniques and may have prevented the error in sample collection that was evident in the horse reported here. Had a sample of the tumor been obtained during percutaneous biopsy, then ante-

mortem diagnosis of hepatoblastoma potentially could have been made on the basis of histologic examination.

Several paraneoplastic syndromes are associated with hepatic neoplasia, with 1 of the most common being an inappropriate secondary erythrocytosis.[16] Erythrocytosis is defined as an absolute or relative increase in RBC mass characterized by an increase in PCV, RBC count, and hemoglobin concentration. Relative erythrocytosis is an apparent increase in RBC mass caused by a decrease in plasma volume (dehydration) or splenic contraction. Dehydration was eliminated as the cause of erythrocytosis in the horse of our report on the basis of results of physical examination, plasma total protein concentration within the reference range, and lack of response to administration of fluids and induced diuresis. Likewise, a persistently high PCV without evidence of stress or exertion eliminated splenic contraction as the cause of polycythemia.

Absolute erythrocytosis is an actual increase in the total circulating RBC mass, which may be primary or secondary. Primary erythrocytosis is a myeloproliferative disorder in which erythropoiesis is independent of erythropoietin concentrations. Secondary erythrocytosis develops because of appropriate (chronic hypoxia) or inappropriate excess production of erythropoietin. A diagnosis of inappropriate erythrocytosis would seem most likely in the horse reported here, because it did not have physical evidence of pulmonary or cardiac disease that would lead to a state of hypoxia and because of the fact that erythrocytosis is a paraneoplastic syndrome in humans and domestic animals.[6,16,18,19] By definition, however, it is difficult to make a diagnosis of secondary inappropriate erythrocytosis when the concentration of serum erythropoietin is within the reference range, as was found in this horse. In humans with paraneoplastic erythrocytosis and serum erythropoietin concentrations within the reference range, tumor production of androgenic hormones can cause erythrocytosis.[19] Additionally, prostaglandin production from the tumor may enhance the effect of erythropoietin, leading to erythrocytosis.[19] Another possibility includes synthesis by tumor cells of a protein with erythropoietin-like action. In animals with erythrocytosis as a paraneoplastic syndrome, the definition of inappropriate secondary erythrocytosis must be expanded to include the possible production of other protein hormones that may cause increased production of RBC. To our knowledge, the horse reported here represents the first documented case of erythrocytosis as a paraneoplastic syndrome in a horse with a hepatoblastoma.

It is difficult to identify animals with hepatoblastoma; thus, this diagnosis may have been overlooked previously. Hepatoblastoma and HCC have similar clinical characteristics and a similar ultrasonographic appearance. Of the 5 reported cases of HCC in horses,[5-8,e,f] 4 were confirmed to have developed in juvenile horses, which is typical of a pediatric tumor such as hepatoblastoma. Furthermore, 2 of the 5 horses had concurrent erythrocytosis, which was similar to the horse with hepatoblastoma reported here. Histologic evaluation and tumor classification must be performed to achieve a definitive diagnosis. Submission of tissue samples and awareness of the similar histologic appearance of HCC and hepatoblastoma, along with educated assessment of signalment and results of clinical examination, may allow diagnosis of more hepatoblastomas in the future. On the basis of the 2 confirmed horses with hepatoblastoma that were born alive, as well as literature on affected children, we believe that horses with hepatoblastoma have a grave prognosis.

[a]Bard Monopty biopsy instrument, CR Bard Inc, Covington, Ga.
[b]Polyclonal anti-α-fetoprotein, Dako Corp, Carpinteria, Calif.
[c]DAB detection kit, Ventana, Tucson, Ariz.
[d]ETO Kit, Medac Diagnostics, Hamburg, Germany.
[e]Beech J, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Pa: Personal communication, 1998.
[f]Valberg S, Large animal internal medicine, University of Minnesota Veterinary Teaching Hospital, St Paul, Minn: Personal communication, 1998.

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