Heterobilharzia americana infection as a cause of hepatic parasitic granulomas in a horse

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Case Description—A 22-year-old American Paint Horse gelding from the Gulf Coast region of Texas was examined at the Texas A&M University Veterinary Medical Teaching Hospital because of regrowth of a perirectal squamous cell carcinoma that had been surgically removed 11 months previously.

Clinical Findings—A necrotic and ulcerated mass was present below the anus. The horse had paraphimosis and was having difficulty with urination. Histologic examination revealed that it was squamous cell carcinoma, and the horse was euthanized because of the unlikelihood that the mass could be adequately resected and its close proximity to the urethra.

Outcome—At necropsy, in addition to the squamous cell carcinoma, hundreds of round, white to pale yellow nodules were disseminated throughout the liver, resulting in a so-called starry-sky appearance. Similar granulomas were seen in the right caudal lung lobe and small intestinal serosa. A single granuloma in the liver, which differed from the others by its larger size, contained a pair of adult schistosomes. Several hepatic granuloma specimens were used for PCR amplification and sequencing. Use of primers specific for a portion of the Heterobilharzia americana small subunit rRNA gene resulted in amplification of a 487-base pair product that had 100% sequence identity with H. americana.

Clinical Relevance—Severe cases of disseminated granulomas in the liver of horses may result in a liver with a grossly abnormal starry-sky pattern. To our knowledge, this is the first report documenting the association of granulomas with H. americana infection along with adult schistosomes in the liver of a horse. (J Am Vet Med Assoc 2011;239:1117–1122)

A 22-year-old American Paint Horse gelding from the Gulf Coast region of Texas was examined at the Texas A&M University Veterinary Medical Teaching Hospital because of regrowth of a perirectal squamous cell carcinoma that had been surgically removed 11 months previously.

Scrapings of tissue were also processed for histologic examination and were found to contain islands and trabeculae of neoplastic epithelial cells with central areas of acantholysis consistent with an acantholytic squamous cell carcinoma. Passage of a stallion catheter revealed an intact urethra, which could be digitally palpated through the soft tissue hole created by the tumor. Because of the invasive and malignant nature of the tumor, its close proximity to the urethra, and the considerable amount of tumor-related tissue necrosis, the horse was anesthetized with xylazine (1 mg/kg [0.45 mg/lb], IV) followed by diazepam (0.1 mg/kg [0.045 mg/lb], IV) and ketamine hydrochloride (2 mg/kg [0.91 mg/lb], IV); once under anesthesia, the horse was euthanized with potassium chloride (2 mEq/kg, IV). A complete necropsy was performed.

At postmortem examination, the most striking feature noted upon opening the abdominal cavity was the presence of hundreds of small (1- to 2-mm-diameter), round, pale yellow to white, slightly raised, firm, granulomas disseminated uniformly over the capsular surface of all liver lobes (Figure 1). The granulomas extended throughout the parenchyma of the liver on all cut sections. Small clusters of up to 10 to 20 firm, slightly raised, 1- to 2-mm granulomas were also found on the serosal surface of the duodenum and jejunum (Figure 2). Bilat-
Generally, the cranioventral aspect of the caudal lung lobes was firm and contained multiple white, slightly raised, 1- to 1.5-mm-diameter granulomas on cut section. The perirectal squamous cell carcinoma, which was the primary and expected lesion in this horse, surrounded the base of the penis and compressed the bulbospongious muscle but did not invade the urethra.

Samples of all tissues were fixed in neutral-buffered 10% formalin at the time of necropsy. Selected tissues were later trimmed, dehydrated, embedded in paraffin, and stained with H&E by use of routine histologic procedures. Histologic examination of the perirectal mass revealed the presence of an acantholytic squamous cell carcinoma. Additional lesions included a gastric leiomyoblastoma and metastatic acantholytic squamous cell carcinoma in the lungs, mediastinum, and pericardial sac that were unrelated to the pulmonary granulomas.

Histologic examination of the liver revealed marked, subacute to chronic inflammatory changes that are typically associated with parasite migration. These included numerous well-circumscribed granulomas containing a central eosinophilic core of necrotic cells surrounded by a peripheral rim of large numbers of palisading epithelioid macrophages and variable numbers of multinucleated giant cells (Figure 3). These areas were further surrounded by scattered eosinophils and moderate numbers of lymphocytes and plasma cells, with circumferential areas of fibrosis. Some of the granulomas that were considered more chronic in nature contained variable amounts of mineralization, while many others consisted primarily of lamellated layers of collagen with only small areas of inflammation.

Figure 1—Photographs of the liver from a horse with disseminated parasitic granulomas due to *Heterobilharzia americana* infection. A—Large numbers of small, disseminated granulomas cover the capsular surface of the liver, giving it a diffuse pattern of round, white to pale yellow lesions on a dark background (ie, starry-sky appearance). B—Multiple discrete, white to pale yellow nodules bulge from the cut surface of the liver.

Figure 2—Photographs of parasitic granulomas due to *H. americana* infection of the same horse as in Figure 1. A—Multiple raised, white nodules form small clusters in the wall of the small intestine that bulge from the serosal surface. B—Multiple discrete, white nodules are visible throughout the cut section of the right caudal lung lobe.

Figure 3—Photomicrograph of a section of a typical eosinophilic granuloma in the liver of the same horse as in Figure 1. The granuloma contains a central coagulum of degenerating eosinophils surrounded by a palisading layer of epithelioid macrophages and multinucleated giant cells. H&E stain; bar = 50 µm.
One small area of the liver, however, was substantially different from all other areas. On gross examination, it contained a single 1-cm-diameter granulomatous-appearing lesion that was up to 10 times as large as all of the other granulomas. Histologically, this area contained a thick fibrous capsule that surrounded a well-circumscribed area of coagulation necrosis. Within the center of the lesion was a cyst-like space that was presumed to be a blood vessel and contained a pair of coiled adult male and female trematodes, whose internal structure was morphologically compatible with classic descriptions of members of the family Schistosomatidae. Each of the schistosomes lacked a body cavity and had a syncytial tegument (cuticle) that surrounded a parenchyma filled with loosely arranged mesoderm cells (Figure 4). The larger and more flattened of the 2 worms, which was presumed to be a male cut in transverse section, was 800 $\times$ 300 $\mu$m wide and had incurved lateral edges, which if fully extended would make it up to 1.7 mm wide. The smaller and more rounded worm was likely a female that was held within the male’s gynecophoric canal, the central groove formed by the curved lateral margins where the female lives in permanent copula with the male. The positioning of the female adjacent to the male is believed to be the result of tissue processing or sectioning artifact may have artificially separated the 2. Numerous small, spine-studded elevations lined the concave outer surface of the tegument of the larger schistosome, adding further support to the view that this area represents part of the gynecophoric canal, where the pointed spines are used to hold the female in place.

Additional evidence for hepatic schistosome infection in this horse included the presence of fragments of thin yellow-brown membranes resembling trematode eggshells within the center of several of the granulomas (Figure 4). One of the granulomas contained a single schistosome egg with an outer eggshell and internal miracidium. Although it was somewhat collapsed and degenerate, the morphology of the egg, which had a diameter of 35 $\mu$m, lacked a spine or hook on its thin eggshell, and contained a single multicellular mira-
ciddium, was compatible with eggs of the schistosome *Heterobilharzia americana*.

5, 6 In almost all instances, egg and eggshell fragments were found within the center of granulomas and were surrounded by an abundance of fibrous tissue with little remaining inflammation. A similar pattern of multiple chronic granulomas with rare eggshell fragments was found in the affected areas of the lungs and wall of the small intestine (Figure 5). The marked hepatic, pulmonary, and small intestinal granuloma formation in this horse was similar to what has been observed following infections with *H. americana* or *Schistosoma* spp in other mammals.6, 7

To test the theory that the hepatic granulomas were the result of infection with *H. americana*, fresh liver tissue from this horse was used to harvest DNA from 6 granulomas for PCR amplification. The primers used were designed to amplify DNA from a portion of the *H. americana* 18S SSU rRNA gene. The DNA from a previously sequenced fragment of *H. americana* recovered from an infected dog was used as a positive control. Polymerase chain reaction amplification of the harvested DNA resulted in amplification of identical 487-base pair fragments from 2 of the 6 granulomas. Amplified DNA was not obtained from the remaining 4 granulomas. The 2 amplified products were sequenced directly (GenBank accession No. HM363369). Each of the products had 100% sequence identity with a previously reported partial sequence for the *H. americana* SSU rRNA gene, which was obtained following experimental infection of a golden hamster (*Mesocricetus auratus*) with *H. americana* (GenBank accession No. AY157220).9

The SSU rRNA gene is 1 of 3 genes that has been used extensively to phylogenetically classify members of the Schistosomatidae family.9 The *Heterobilharzia* SSU rRNA sequence from the horse of the present study had close sequence identity with several other schistosome species. Their life cycle, biologic characteristics, host specificity, and geographic restriction, however, make their presence unlikely in this horse. The SSU rRNA sequence (GenBank accession No. AY157221) of *Schistosomatium douthitti*, the only other species of schistosome besides *H. americana* that is known to infect mammals in the United States, had 98% sequence identity (481/487 bp), but that species is restricted to rodent hosts (muskrats and mice) in the northern United States and southern Canada.10 The SSU rRNA sequence (GenBank accession No. AY157233) of *Schistosoma margrebowiei* had 97% sequence identity (477/487 bp), but that species is found in antelope in Africa.11 The SSU rRNA sequence (GenBank accession No. AY157231) of *Schistosoma indicum* had 97% sequence identity (477/487 bp), and that species infects horses and ruminants but is found in South Asia.12 Lastly, the SSU rRNA sequence (GenBank accession No. AY157229) of *Schistosoma incognitum* had 97% sequence identity (477/487 bp), but that species is found in pigs, dogs, and rodents in South Asia, Indonesia, and Thailand.13–15 The sequence of the *Heterobilharzia* SSU rRNA gene fragment did not have close sequence identity to the avian schistosomes (genus *Trichobilharzia*), which are typically associated with schistosomiasis caused by cercarial penetration of human skin.

**Discussion**

*Heterobilharzia americana*, a trematode in the family Schistosomatidae, is an important cause of chronic diarrhea, weight loss, gastrointestinal and liver disease, and hypercalcemia in dogs and is predominantly found in the Gulf Coast and south Atlantic region of the United States,9 where infections have been reported in Texas,17-20 a Louisiana,21 Florida22,23 and, more recently, North Carolina.24 The geographic range of the trematode and its intermediate hosts, the lymnaeid freshwater snails *Fossaria* (*Lamnacea*) *cabensis* and *Pseudosuccinea columella*, includes Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and, perhaps most surprisingly, Kansas, following importation of infected raccoons from Texas and Florida.25 The natural definitive host is the raccoon, although a number of other wild and captive mammals, including the bobcat, armadillo, Brazilian tapir, beaver, coyote, mountain lion, mink, nutria, opossum, red wolf, swamp rabbit, and white-tailed deer, have been naturally infected.25 The importance of schistosomiasis in dogs as an emerging disease in the United States is underscored by recent studies of *H. americana* infection in North America.
and migrate to the intestinal tract. Following penetration, cercariae that allow them to break through the vessel walls release proteolytic enzymes through pores in the eggshell that allow them to break through the vessel walls and migrate to the intestinal tract. Following penetration of the intestinal wall, they enter the intestinal lumen and are shed in the feces. The eggs, which may also circulate as emboli in the vessels, induce a severe granulomatous response when they become lodged in visceral organs, and although the immune response destroys the eggs, it also results in most of the pathologic findings associated with this disease.

Small but consistent numbers of horses with disseminated hepatic granulomas similar to those of the horse of the present study are seen in the necropsy service at Texas A&M University each year. Of 1,672 horses that were necropsied at our institution from January 2004 through July 2010, 38 (2.3%) had a morphologic diagnosis of hepatic parasitic granulomas included in the necropsy report, which represents between 5 and 6 affected horses/y. Although H. americana has been implicated as a possible cause of disseminated hepatic granulomas in horses, a definitive cause has never been established.

Our previous attempts to amplify H. americana–specific DNA from formalin-fixed liver granulomas of other horses have all been unsuccessful. We believe this was attributable to a combination of the negative effect of formalin fixation on the yield of extracted schistosome DNA and the small amount of DNA remaining in the liver because of the chronic nature of the disease. In the horse of the present study, fresh tissue was used and positive PCR products were obtained from 2 of 6 granulomas that were carefully dissected from the surrounding liver tissue. In a single previous attempt at our institution to amplify H. americana DNA, in which fresh liver from another affected horse and a different set of primers were used, a 1,187-bp fragment of the H. americana SSU rRNA gene was obtained (GenBank accession No. DQ230918). We conclude, therefore, that the use of fresh tissue may be critical for obtaining positive PCR amplification and that isolation of the granulomas by careful dissection prior to DNA extraction may also be necessary.

There has been only 1 previous study on fibrosing granulomas in an equine liver that were postulated to be the result of chronic schistosomiasis of undetermined origin. The gross and histopathologic changes were nearly identical to those observed in the horse of the present study, and the authors suggested that H. americana or another species of schistosome may be involved. Of 11 horses examined in that study, only 1 had hepatic granulomas containing broken or empty eggshells consistent with schistosome eggs, supporting our contention that in most instances, by the time a diagnosis of hepatic schistosome infection in horses is made, the changes are long-standing and the trematode eggs that incited the lesion are no longer present. We have also found that in many instances, the hepatic granulomas are an incidental finding unrelated to some primary disease as it was in this horse, which had a metastatic squamous cell carcinoma.

On first observation of an affected liver, via histologic examination of hepatic biopsy specimens, imaging studies, surgical intervention, or postmortem examination of the abdominal cavity, the changes associated with this disease can be quite impressive; however, they can also be misleading because only the most severely affected horses appear to develop clinically relevant hepatic dysfunction. Hepatic dysfunction may in fact be more severe during the time of initial infection when the liver is being seeded with schistosome eggs and a marked inflammatory response to their presence ensues. It may also be important to note that the most severely affected horses that we have observed at necropsy have been those with some other chronic disease, either infectious, inflammatory, or neoplastic, and less frequently horses euthanized because of colic, trauma, or other acute conditions.

We believe that schistosomiasis in horses due to H. americana is an undiagnosed disease that is likely to continue to spread to new areas as the geographic range of infected raccoons and dogs as well as the snail intermediate hosts continues to expand. Although the formation of small numbers of randomly scattered eosinophilic and fibrosing granulomas in the liver may be a common response to localized injury due to other parasitic insults, such as ascarid34 and strongyle35 migration and perhaps tissue-invading ciliated protozoa,36 the presence of large numbers of disseminated granulomas arranged in the distinctive shotgun pattern of small, round, white to pale yellow nodules on a dark background (ie, starry-sky appearance) observed in this horse, particularly when found in horses raised in the southeastern United States, is something we propose is more likely associated with H. americana infection.

References


