Pathology in Practice

In March 2012, 2 stocker size (approx 50 g) channel catfish (*Ictalurus punctatus*), approximately 9 months of age, from a commercial catfish farm were brought to the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Miss, for health assessment and necropsy following euthanasia. Several hundred fish had previously died on the farm.

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In cooperation with

**Clinical and Gross Findings**

The fish were euthanized by an immersion method with water containing tricaine methanesulfonate (MS-222), and necropsies were performed. During necropsy of one of the fish, an approximately 3-cm-diameter, spherical, firm, smooth, pale pink, posterior kidney mass was noted (Figure 1). No other gross abnormalities were found in this fish, and no gross abnormalities were evident in the other fish. Fresh impression smears were prepared from the posterior kidney mass for cytologic evaluation. Fresh wet mounts were also prepared from gill tissue of the affected fish for microscopic evaluation. Some of the kidney mass and brain tissue was submitted for bacteriologic culture. A sample of the kidney mass was then fixed in neutral-buffered 10% formalin and routinely processed for histologic evaluation.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

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**Figure 1**—Photograph of a posterior kidney mass (arrow) in an approximately 9-month-old stocker catfish from a fish farm in Mississippi. This and another fish underwent health assessment, euthanasia, and necropsy after several hundred fish on the farm had died. The other fish had no gross abnormalities.
Cytologic and Histopathologic Findings

Impression smears prepared from the fish’s kidney mass were stained with Wright-Giemsa stain. The smears revealed abundant microorganisms in various stages of development with few inflammatory cells (Figure 2). The developmental stages consisted of many immature trophozoites along with numerous mature spores. The trophozoites were round to oval in shape and approximately 4 to 7 µm in diameter. They were found both singly and in aggregates, and each contained a round, purple nucleus with a small amount of deeply basophilic and occasionally vacuolated cytoplasm. The mature spores were found singly as well as frequently in pairs. They had elongated bodies (approx 20 X 5 µm) that contained 2 anterior, magenta, piriform, polar capsules and a single posterior, clear, round vacuole. The polar capsules were characterized by approximately 6 to 10 internal polar filament coils. The organisms also had long, tapering tails with indistinct endings that were approximately 20 to 40 µm in length. Few oval structures were noted that contained both trophozoites and developing spores with developing polar capsules. The inflammatory cells consisted of mostly macrophages with few lymphocytes. The background of the smears contained abundant amorphous, basophilic material (necrotic debris) with many variably distinct clear vacuoles, many bare nuclei of lysed trophozoites, and many erythrocytes. Few necrotic, renal epithelial cells were also noted in thick clusters.

Formalin-fixed sections of the kidney were stained with H&E or Giemsa stain. These sections revealed a large, well-demarcated, nonencapsulated mass that compressed the neighboring renal parenchyma. The mass was composed of densely packed plasmodia, which are pseudocysts containing myxozoans of various stages of development. The pseudocysts were round to oval and highly variable in size (approx 30 to 500 µm). They had a thin outer eosinophilic hyaline membrane and contained abundant, eosinophilic, fibrillar to granular, necrotic debris with developing trophozoites and spores similar in morphology to those in the impression smears of the mass (Figure 3). There were moderate numbers of macrophages and lymphocytes in the surrounding renal tissue along with rare myxozoans. Microscopic examination of gill wet-mount preparations also revealed few plasmodia along with few *Trichodina* organisms. Results of bacterial cultures of brain tissue and kidney mass were negative.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis and case summary: moderate, interstitial, granulomatous nephritis with abundant myxozoans consistent with *Henneguya* organisms in a catfish.

Comments

The genus *Henneguya* belongs to the phylum Myxozoa and the class Myxosporea. Myxosporeans are common parasites of a wide range of teleost fish and invertebrates worldwide, with a few species found in elasmobranchs, amphibians, and reptiles. There are at least 204 species in the genus *Henneguya*, with 43 new species described between 2002 and 2012. These parasites, in addition to having a...
wide range of potential hosts, also have highly variable host specificity. Their life cycle is complex and involves development of an actinospore in an intermediate host, such as an oligochaete. The infective actinospore, after being discharged from the intermediate host, then penetrates the piscine host via the skin or gill epithelia (commonly via mucous cells). It has also been reported that the buccal cavity and the stomach were important sites of entry after experimental infection of channel catfish with *Henneguya ictaluri* actinospores.

Infections with various *Henneguya* spp in channel catfish (*I. punctatus*) have been reported. These parasitic organisms can be devastating to the catfish farming industry; mortality rates as high as 100% have been reported, with losses occurring among both fingerlings and market-size catfish. Clinical signs in channel catfish infected with *Henneguya* spp vary depending on the infective species. Catfish infected with *H. ictaluri*—the causative agent of proliferative gill disease—develop gill thickening, necrosis, and hemorrhage secondary to intense granulomatous bronchitis, which results in reduced oxygen exchange. Infected fish are lethargic and swim near the water surface, presumably indicating that the parasite was unable to complete its life cycle. Small-subunit rDNA sequences link the cause of proliferative gill disease in channel catfish and channel × blue catfish hybrids in 1 study. In fact, plasmodia were not detected in blue catfish, indicating that the parasite was unable to complete its life cycle in those fish. Other *Henneguya* spp have been reported to cause cutaneous masses in channel catfish, with *Henneguya diversis* infection also resulting in cyst formation in the liver and kidneys.

Definitive diagnosis of *Henneguya* infection relies on identification of the typical spores (although the spores are not always readily observed) and secondary inflammatory response in affected tissue. The spores can be visualized via microscopic evaluation of unstained wet-mount preparations or impression smears stained with Wright-Giemsa stain or histologic examination of formalin-fixed tissue sections stained with H&E or Giemsa stain. Determining the species of these organisms has traditionally been accomplished via the combination of detailed measurements of the spores in wet-mount preparations and the tissue and host predilection of the organisms. The species can also be determined by sequencing the 18S small subunit rDNA after isolation and PCR amplification and comparing that sequence against the several known 18S small-subunit rDNA sequences of *Henneguya* spp.

With regard to the case described in the present report, differential diagnoses for the kidney mass included a neoplasm or a granuloma caused by certain fungi or parasites. The diagnosis of granulomatous nephritis caused by *Henneguya* infection was made by identifying the characteristic organisms surrounded by macrophages and lymphocytes in kidney mass impression smears and by histologic evaluation of the mass tissue. The *Henneguya* spores were readily identified microscopically owing to their distinctive elongated spore body, long tail, and paired polar capsules. However, these organisms could only be definitively identified to the genus level, given that spore measurements were not made from wet mounts and fresh tissue was unavailable for DNA isolation. The spore measurements from the cytologic smears should not be compared against those reported for fresh wet mounts because of the effect of the alcohol-based fixation on spore morphology.

To the authors’ knowledge, there have been no reports of *Henneguya* spp only infecting the kidney and gills in channel catfish. It is possible that the species described in this report is a novel species. However, the case described in the present report could also represent an unusual progression of disease for one of the known species of *Henneguya*. In this case, the only gross abnormality was the renal mass. Furthermore, only the gills and the renal mass were evaluated microscopically. Therefore, it is also possible that the fish may have had microscopic evidence of *Henneguya* infection in other tissues that was not yet detectable grossly. Currently, there is no effective treatment for *Henneguya* infection; however, supportive care to increase the oxygenation of the water can be implemented. Furthermore, it has been shown that channel catfish with lesions due to experimentally induced proliferative gill disease can heal with minimal residual damage after they have been removed from infested ponds. On the basis of the findings for case described here, water oxygenation in the fish farm tanks was enhanced by increased aerator use and decreased feeding to decrease oxygen consumption. Unfortunately, the farm owners could not be reached for follow-up. *Henneguyosis* should be considered as a differential diagnosis for a kidney mass in channel catfish.

### References


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a. Western Chemical, Ferndale, Wash.