

NOTE

Preliminary description of lesions in juvenile largemouth bass injected with largemouth bass virus

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ABSTRACT: Juvenile largemouth bass *Micropterus salmoides* were intraperitoneally injected with largemouth bass virus (LMBV), a member of the genus *Ranavirus*, family *Iridoviridae*. Moribund fish which had been injected with $10^{6.2}$ tissue culture infectious doses, 50% endpoint (TCID₅₀), were sampled 4 d after injection; other largemouth bass injected with this dose died between 3 and 5 d after injection. Fish injected with $10^{2.8}$ TCID₅₀ of LMBV were also examined after 4 d and had lesions similar to those of fish injected with the high dose. Clinical signs included darker pigmentation, inflammation and necrosis at the site of injection, distended abdomen, corkscrew swimming, and lateral recumbency. Internally, fish had focally pale livers, bright red spleens and reddened intestinal ceca. Histologically acute fibrinous peritonitis affected the surface of all organs in the peritoneal cavity, but deeper portions of organs appeared normal. There was also necrosis of the gastrointestinal mucosa. Except for the injection site, lesions were confined to the peritoneal cavity.

KEY WORDS: Fish virus · Histopathology · *Iridoviridae* · *Ranavirus*

Largemouth bass virus (LMBV) was initially isolated from adult largemouth bass *Micropterus salmoides* from the Santee-Cooper Reservoir in South Carolina (Plumb et al. 1996) and has since been found in several other locations in the southeastern United States (Plumb et al. 1999). Fish from which virus was isolated floated at the surface, but gross lesions were absent except for an excessively red gas gland on the swimbladders. In experimental transmission studies, LMBV was injected intraperitoneally (IP) into 0.5 to 1 kg adult largemouth bass, and virus was reisolated from several organs at concentrations greater than 10^6 tissue culture infectious doses, 50% endpoint (TCID₅₀), per gram of tissue for at least 26 d after infection. These fish did not

die and showed no signs of disease except inflammation at the site of injection. In a more recent study, 60% of 4 g largemouth bass injected with LMBV died within 4 d (Plumb & Zilberg 1999a). It was also shown that juvenile largemouth bass were more susceptible to injected LMBV than juvenile striped bass *Morone saxatilis*, while both species were less susceptible to virus exposure by immersion (Plumb & Zilberg 1999b).

Largemouth bass virus was tentatively classified as a member of the family *Iridoviridae* (Plumb et al. 1996), a classification further confirmed by Mao et al. (1999) and Piaskoski et al. (1999). Using polymerase chain reaction, Mao et al. (1999) also determined that LMBV was nearly identical to the doctor fish virus (DFV) and guppy virus (GV6) isolated from ornamental fish imported into the United States from southeast Asia (Hedrick & McDowell 1995). Mao et al. (1999) proposed that LMBV, DFV and GV6 constitute a new species of *Ranavirus* in the *Iridoviridae* and that it be named Santee-Cooper ranavirus, reflecting the origin of the original LMBV isolate.

Histopathology of natural LMBV infections is not available; therefore, the objective of the current study was to preliminarily describe gross and microscopic lesions in juvenile largemouth bass experimentally infected with LMBV. Four moribund largemouth bass (~4.7 g) from the LMBV infectivity study of Plumb & Zilberg (1999b) were necropsied 4 d after intraperitoneal injection with 0.1 ml of LMBV containing $10^{6.2}$ TCID₅₀. Two additional fish were injected with approximately $10^{2.8}$ TCID₅₀ of LMBV, held at 27°C, and killed 4 d after injection. Control fish were injected with 0.1 ml of Hanks' balanced salt solution (HBSS). Live fish were euthanized (200 mg l⁻¹ of tricaine) and preserved in Bouin's fixative after the peritoneal cavity was opened. Whole fish were decalcified for 1 h (RDO, Apex Engineering Products Corporation, Plainfield, Illinois), cut into 3 mm thick transverse blocks and embedded in paraffin. Sections of embedded fish were

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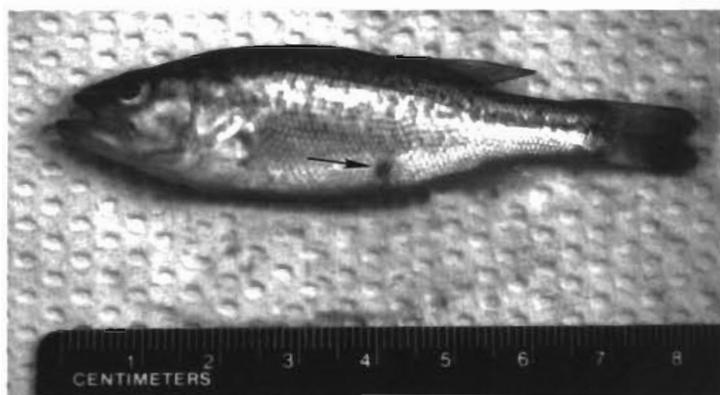


Fig. 1. A juvenile largemouth bass injected intraperitoneally with largemouth bass virus showing the 2 to 3 mm lesion (arrow) at the injection site

cut 6 μm thick, stained with hematoxylin and eosin, and examined by light microscopy.

The LMBV-infected largemouth bass from which fish for the current study were taken began to die 3 d after infection and all had died after 5 d (excluding 6 live fish sampled) (Plumb & Zilberg 1999b). Virus concentrations in moribund fish ranged from $10^{7.8}$ to $10^{9.2}$ $\text{TCID}_{50} \text{g}^{-1}$ 4 d after injection. Death was preceded by spiral swimming on Day 3 followed shortly thereafter by dark pigmentation, lethargy and a slightly distended abdomen on Day 4. Each LMBV-infected fish developed an inflamed lesion measuring 2 to 3 mm in diameter at the site of injection 3 or 4 d after infection (Fig. 1). Internally, infected fish had focally pale areas on the surface of livers, and bright red spleens compared to dark red spleens in control fish. Slight redness was present in mesenteries and in abdominal fat near the intestinal ceca. Control fish showed no signs of disease; no virus was isolated from 2 control fish or 3 fish assayed before injection.

Acute peritonitis was severe in all LMBV-injected fish examined histologically. A fibrinous exudate containing numerous leukocytes and abundant cellular debris was present throughout the peritoneal cavity (Fig. 2a, b). Eosinophilic granular cells were present in the exudate but were also seen in the peritoneum of control fish. Superficial portions of the liver, stomach, intestine, and spleen were necrotic and severely inflamed, but deeper areas of these organs appeared normal. Exudate was present on the ventral surface of the swim bladder contacting the peritoneal cavity. The exocrine pancreas, which is widely disseminated throughout mesenteries of the anterior peritoneal cavity, was typically infiltrated by leukocytes or was necrotic (Fig. 2c), but was unaffected in some areas. Focal necrosis was present in the mucosal epithelium of the stomach, intestine, and occasional intestinal ceca (Fig. 2d). Except for necrotic and inflamed muscle

near the injection site, organs not in the peritoneal cavity appeared normal. Even though a high dose of virus ($10^{6.2}$ $\text{TCID}_{50} \text{fish}^{-1}$) was injected in most fish, the same lesions developed in fish injected with only $10^{2.8}$ TCID_{50} . Microscopic lesions were not found in the control fish.

The small, inflamed, necrotic lesion in the skin and muscle at the site of injection of infected largemouth bass was similar to the transient lesions that developed in injected adult fish described by Plumb et al. (1996). Similar, but less obvious, skin lesions also occurred in IP-injected striped bass reported by Plumb & Zilberg (1999b). Because of the consistent isolation of high virus titers in assayed live, moribund and dead fish, lack of significant bacterial pathogens in the frozen carcasses (Plumb & Zilberg 1999a), and absence of lesions in control fish, it was concluded that LMBV was the cause of death and associated lesions.

Molecular characterization (Mao et al. 1999) indicates that LMBV is nearly identical to DFV and GV6 of ornamental fish and closely related to viruses isolated from sheatfish *Silurus glanis* (Ogawa et al. 1990), black bullheads *Ameiurus (Ictalurus) melas* (Pozet et al. 1992), and Eurasian perch *Perca fluviatilis* (Reddacliff & Whittington 1996). Except for LMBV, necrosis of hematopoietic tissue in kidney and spleen is the most consistent lesion reported for fish infected with these viruses, and lesions also occur in several other organs including gill, liver, intestine, and heart. Histological lesions in largemouth bass injected with LMBV were dramatically different, consisting principally of peritonitis.

The restricted distribution of necrotizing inflammation seen in largemouth bass injected with LMBV is reminiscent of the toxic hepatitis in rodents injected with frog virus 3 (FV3), which is also in the genus *Ranavirus* (Mao et al. 1999). Frog virus 3 injected into mice and rats initially kills Kupffer cells and endothelial cells in hepatic sinusoids, but does not replicate because of the high temperature (Gut et al. 1981, Kirn et al. 1983). Injury to hepatocytes follows the damage to sinusoidal cells, even though FV3 does not enter these cells. Toxic proteins of the virions apparently injure hepatic sinusoidal cells and cause nuclear changes in hepatocytes, and these early lesions are followed by hepatocytolysis caused by endogenous endotoxins (Kirn et al. 1983, Gut et al. 1984). Unlike the toxic reaction in rodents injected with FV3, lesions in largemouth bass injected with LMBV were not confined to the liver; however, surfaces of visceral organs directly in contact with the injected virions were the predominant sites of lesions in largemouth bass. This

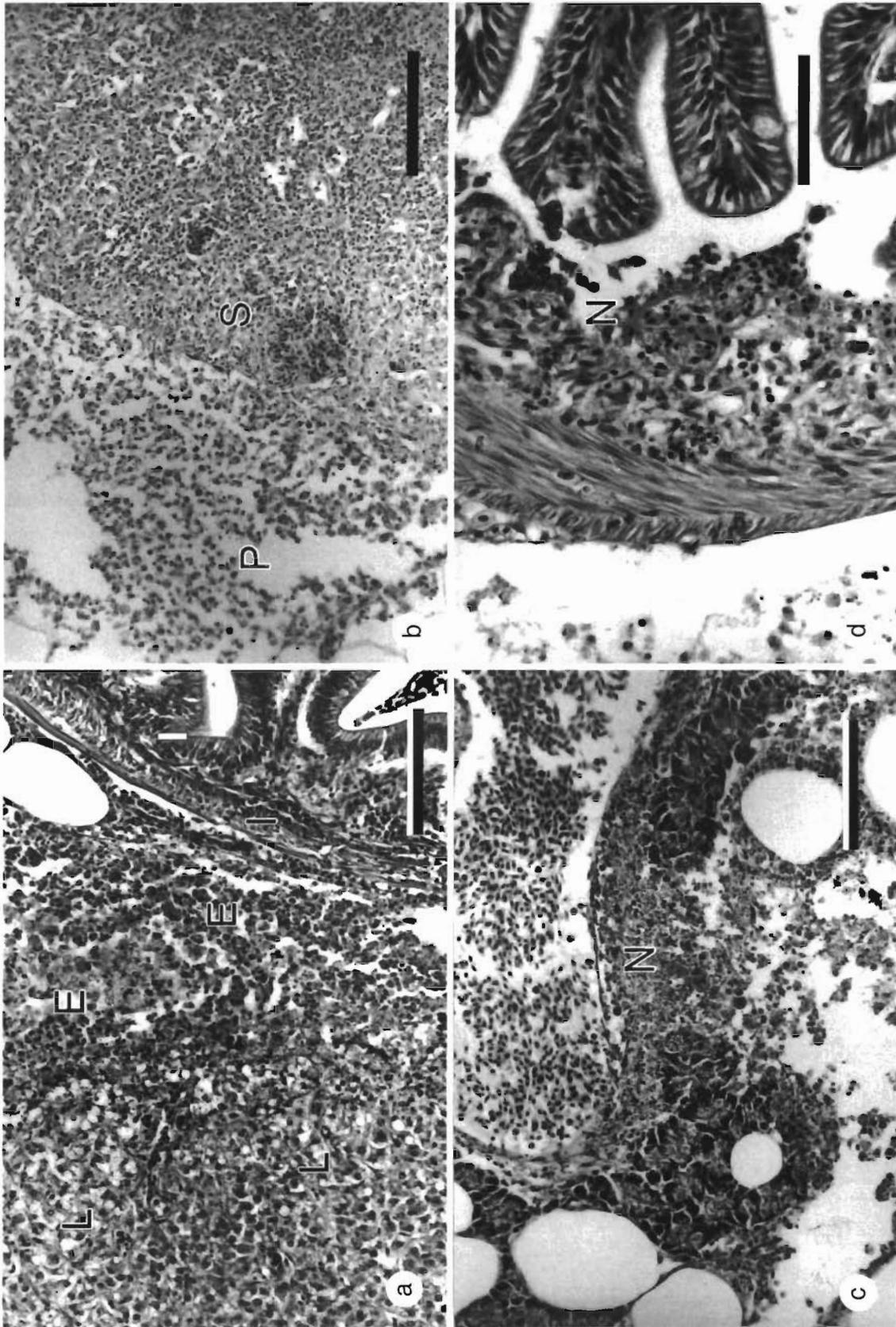


Fig 2. Paraffin sections of largemouth bass injected with largemouth bass virus. (a) Cell-rich exudate (E) between the intestine (I) and liver (L) (scale bar = 100 μ m). (b) Peritonitis (P) on surface of the spleen (S) (scale bar = 100 μ m). (c) Necrosis (N) of exocrine pancreas (scale bar = 100 μ m). (d) Necrosis (N) of mucosa in an intestinal cecum (scale bar = 50 μ m)

distribution of lesions indicates either a greater susceptibility of this site to infection by LMBV, that the peritoneum served as a barrier to transmission of LMBV from the peritoneum to other sites, or that virion toxicity primarily affected only those sites in direct contact with the injected virus. The high dose of virus ($10^{6.2}$ TCID₅₀) injected into most fish in our study does not appear to be essential in producing disease. Similar lesions occurred in fish injected with only $10^{2.8}$ TCID₅₀, indicating that results were not dose dependent; therefore, a mechanism other than simple direct toxicity of injected virions is probably involved. Further study is needed to determine the reasons for localization of lesions in largemouth bass injected with LMBV.

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