2.2.12 Largemouth Bass Virus Disease

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A. Name of Disease and Etiological Agent

Largemouth bass virus disease (LMBVD) is caused by an iridovirus known as largemouth bass virus (LMBV; Figure 1). In the Universal Virus Database (ICTVdB Management 2006), this virus along with guppy virus 6 (GV) and doctor fish virus (DFV) is listed in the species Santee-Cooper ranavirus. Additional names for LMBV are largemouth bass ranavirus and largemouth bass iridovirus. A *Megalocytivirus* isolated from largemouth bass in Taiwan has also been called largemouth bass iridovirus (Chao et al. 2004). For reviews, see Goldberg (2002) and Grizzle and Brunner (2003).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Largemouth bass virus has been found only in the central and eastern United States. The closely related GV and DFV were isolated from ornamental fish being imported into the U.S. from southeast Asia (Hedrick and McDowell 1995).

2. Host Species

The most commonly reported species with LMBVD or subclinically infected with LMBV is largemouth bass *Micropterus salmoides*. Smallmouth bass *Micropterus dolomieu* are also affected by LMBVD. Subclinical infections occur in other members of the Centrarchidae family, but LMBV in fish of other families appears to be rare.

C. Epizootiology

The first known isolation of LMBV was from largemouth bass collected from Lake Weir, Florida, in 1991 (Grizzle et al. 2002). The virus is highly communicable by waterborne transmission (Grant et al. 2005), but most infected fish do not show signs of disease. Causes of subclinical infections converting to overt disease are unknown. There is no evidence that stress is a trigger for outbreaks of LMBVD in wild populations; water temperature and other variables are usually suitable for largemouth bass during LMBVD outbreaks. Increased mortality can occur in confined largemouth bass captured from populations infected with LMBV, probably because of virus transfer among fish

and increased occurrence of bacterial diseases in the LMBV-infected fish (Schramm et al. 2006). The genetics of the virus (Goldberg et al. 2003) and the fish (Goldberg et al. 2005) may be important factors affecting the severity of disease outbreaks. Fish that have been exposed to LMBV develop antibodies, but the role of antibodies in disease resistance is unknown.

This disease occurs during summer in adult fish, and subclinical infections occur during all seasons. Occasionally, LMBVD causes a noticeable fish kill in wild populations, but there can be a decreased abundance of older age classes even when a fish kill is not observed (Maceina and Grizzle 2006). Young-of-year largemouth bass can be infected experimentally, but spontaneous outbreaks of LMBVD have not been reported in age 0 fish. Most hatcheries with LMBV-infected fish have not observed an increased mortality rate.

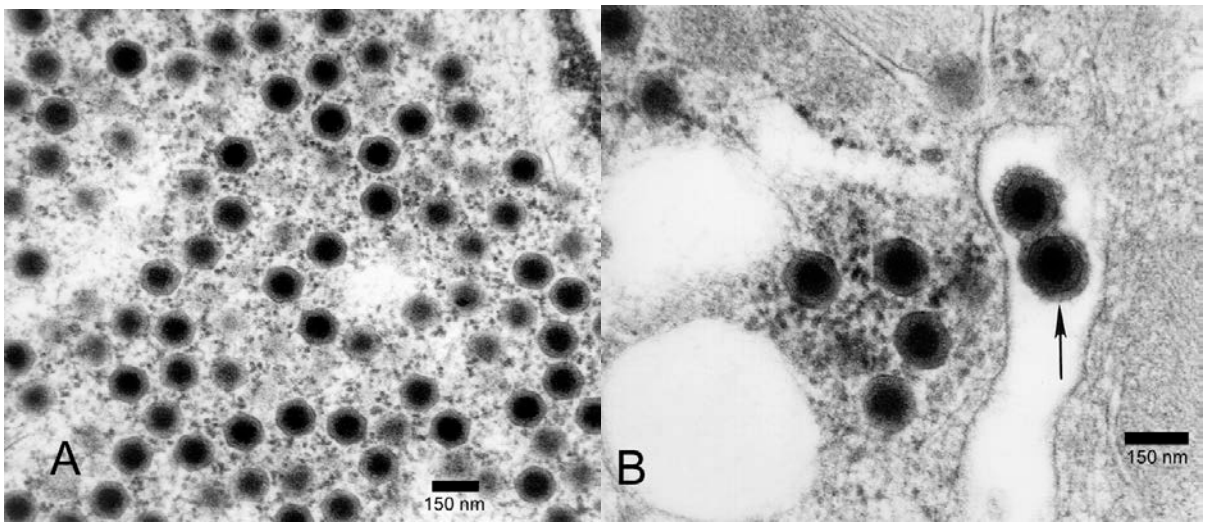

Figure 1. Largemouth bass virus. (A) Transmission electron microscope image of LMBV in cell culture. (B) Enveloped virus (arrow) after passing through the cell membrane.

D. Disease Signs

Diseased fish swim lethargically near the surface of the water and then lose equilibrium. Some moribund fish have an overinflated swim bladder (Figure 2) and less commonly have exudate in the swim bladder (Figure 3). There are no other gross lesions characteristic of LMBVD. If other gross lesions are noted, an additional disease should be suspected. Fish injected intraperitoneally with LMBV have acute peritonitis; however, histological lesions have not been adequately documented in fish with spontaneous LMBVD.

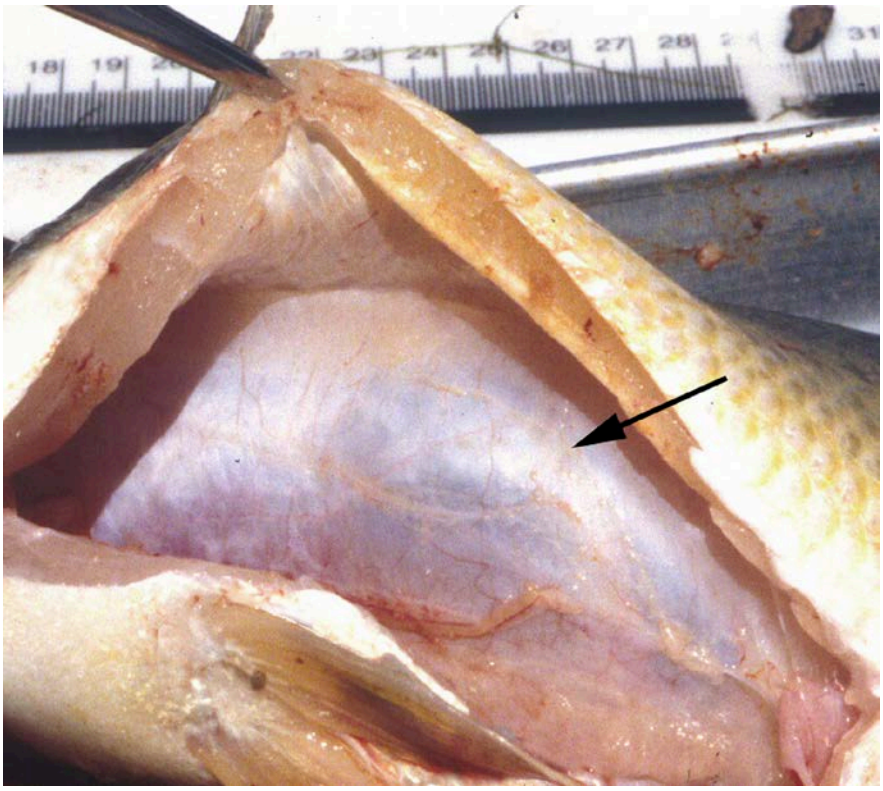

Figure 2. Largemouth bass with largemouth bass virus disease. The swim bladder is overinflated (arrow). Scale for the ruler in this photograph is centimeters.

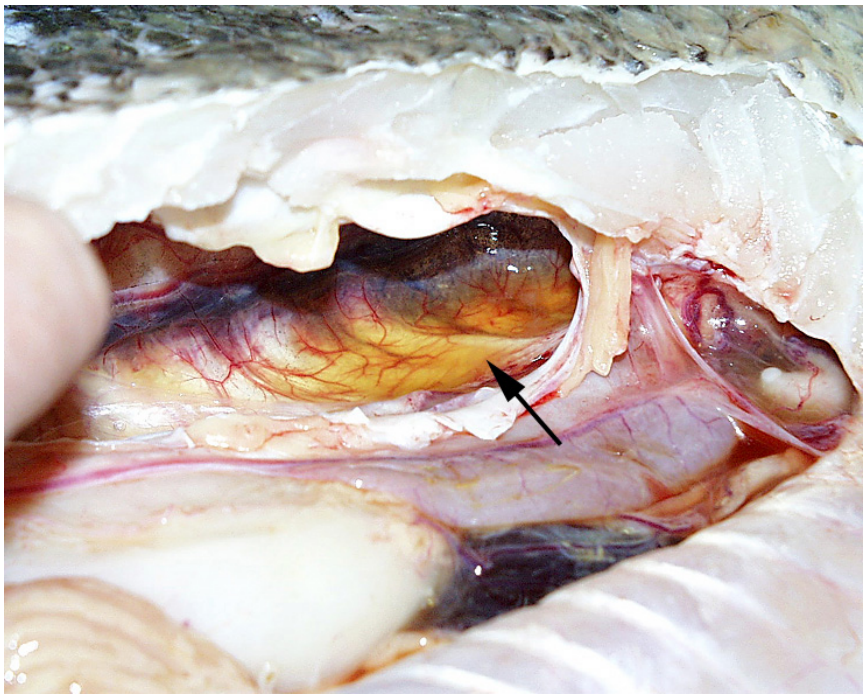

Figure 3. Yellow exudate (arrow) in the swim bladder of a largemouth bass with LMBVD. Photo by Andrew Goodwin, University of Arkansas at Pine Bluff.

E. Disease Diagnostic Procedures

Diagnosis of LMBVD requires the detection of LMBV in fish that have signs typical of LMBVD. The mere presence of LMBV does not mean that a fish is diseased, and the behavior and swim bladder lesions that have been observed in fish with LMBVD are not pathognomonic. Fish infected with LMBV can also have bacterial infections, most often *Flavobacterium columnare* or motile *Aeromonas* spp. If an LMBV-infected fish with signs of LMBVD also has additional diseases, a decision about the relative importance of LMBV as a cause of morbidity depends in part on the presence and severity of lesions not attributed LMBVD.

The organs most commonly used for LMBV testing are swim bladder, trunk kidney, and spleen. Gill may also be a suitable organ for sampling, but additional field validation is needed. No single organ always has a detectable amount of LMBV in infected fish so it is important to sample multiple organs (Beck et al. 2006).

1. Presumptive Diagnosis

a. Behavior and Gross Lesions

Lethargy and swimming near the surface, followed by a loss of equilibrium and floating in a laterally recumbent position, occur in some fish dying from LMBVD. Internally, the swim bladder may be reddened or overinflated.

b. Virus Isolation

The cell lines most commonly used are bluegill fry (BF-2) cells and fathead minnow (FHM) cells, but several other fish cell lines will support replication (Piaskoski et al. 1999; McClenahan et al. 2005a). Incubation of the inoculated cells at 25°C to 30°C provides more rapid detection of CPE than at lower temperatures, but LMBV will replicate at least as low as 15°C. Cytopathic effect (CPE) is indicated by cell rounding followed by lysis [see Section 2, 4.6.D. "Largemouth Bass Virus (LMBV)"]; cytoplasmic inclusion bodies are not visible without staining. At 30°C, CPE can be noticeable in less than one day if the sample has a high titer of LMBV, but 7 days may be necessary to detect low titers.

2. Confirmatory Diagnosis

The virus is identified by PCR [see Section 2, 4.6.D.2 "Confirmation Method for LMVD - Polymerase Chain Reaction (PCR) (Modified from Plumb et al. 1999)"]. The PCR method can be simplified by adding the culture fluid from a presumptively positive cell culture to the PCR mixture, rather than using extracted DNA for the PCR template (McClenahan et al., 2005b). Before adding the cell culture fluid to the PCR mixture, the cell culture fluid should be centrifuged for 5 min at 10,000 X gravity and then diluted 1:500 in buffer.

F. Procedures for Detecting Subclinical Infections

The virus can be isolated from subclinically infected fish at any time of the year; however, low titers of virus are often present so the use of a highly sensitive assay is important to avoid false negative results. Optimization of cell culture isolation procedures (e.g., 30°C for cell culture incubation and blind passage of initially negative samples) can increase sensitivity (McClenahan et al. 2005a) or even greater sensitivity is possible by using PCR to detect viral DNA that has been extracted from homogenized organs (Grizzle et al. 2003). Testing organs, such as gill, head kidney, and liver, in addition to swim bladder, trunk kidney, and spleen, can also increase the probability of LMBV detection (Beck et al. 2006).

G. Procedures for Determining Prior Exposure to the Etiological Agent

An enzyme-linked immunosorbant assay (ELISA) has been developed by John Hawke (Louisiana State University) for the detection of serum antibodies against LMBV, but this test has not been widely field-tested. Agar gel diffusion assay can also be used to detect antibodies against LMBV (Fraser et al. 2002).

H. Procedures for Transportation and Storage of Samples

Organs or whole fish can be frozen for shipment. Little or no loss of infectious LMBV occurs during short-term storage of frozen samples (Plumb and Zilberg 1999a). However, samples should not frozen and thawed repeatedly during storage, and long-term storage should be at -80°C.

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