DOI: 10.7589/2018-03-082

Journal of Wildlife Diseases, 55(2), 2019, pp. 516–517

A Preliminary Study of Largemouth Bass Virus in Mexico

Isidro O. Montelongo-Alfaro,[1] Gerardo Amador-Cano,[1] Jaime L. Rábago-Castro,[2,4] Jesús Genaro Sánchez-Martínez,[2] Flaviano Benavides-González,[2] and Héctor H. Gojon-Báez[3] [1]Universidad Tecnológica del Mar de Tamaulipas Bicentenario, Carretera Estatal N° 52 Soto La Marina - La Pesca, Km. 46+400, S/N, La Pesca, 87678, Soto La Marina, Tamaulipas, Mexico; [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Km 5 Carretera Victoria-Mante, 87000, Cd. Victoria, Tamaulipas, Mexico; [3]Dirección de Desarrollo Sustentable y Proyectos Estratégicos, Secretaría de Pesca y Acuacultura, Gobierno del Estado de Tamaulipas, Centro Esmeralda 3er piso, Av. Hidalgo #3309, Col. Guadalupe C.P. 89120, Tampico, Tamaulipas, Mexico; [4]Corresponding author (email: jrabago@uat.edu.mx)

ABSTRACT: Disease outbreaks and mortalities caused by largemouth bass virus (LMBV) in largemouth bass (*Micropterus salmoides*) have been reported in the US. Blood and mucus samples tested by PCR to assess the presence of LMBV in largemouth bass in northeastern Mexico were negative, and further monitoring is needed.

Largemouth bass virus (LMBV) was first isolated from sporadic epizootics involving largemouth bass (*Micropterus salmoides*) in Lake Weir, Florida, US in 1991 (Grizzle 2007). Since then, disease outbreaks caused by LMBV have been reported in 17 states in the US (Goldberg 2002), including Texas (Southhard et al. 2009). Largemouth bass virus can be difficult to diagnose based on clinical signs alone because there are no pathognomonic signs associated to this disease. This virus primarily affects fish larger than 30 cm, especially trophy-sized fish (Maceina and Grizzle 2006), causing prolonged mortality over several weeks (Zilberg et al. 2000; Woodland et al. 2002). The Vicente Guerrero reservoir (23°57′34′′N, 98°39′57′′W), located in Tamaulipas, Mexico, is one of the best sites in the world for the sport fishing of largemouth bass. Because this fish species was introduced from the US, and Tamaulipas shares its border with Texas, it is possible for largemouth bass in Vicente Guerrero Reservoir to be exposed to LMBV. Given the economic importance of largemouth bass to Mexican recreational fishing, it is essential to implement monitoring programs to establish its sanitary status. The PCR has been used for the detection of LMBV from internal organs (Grizzle et al. 2003); however, the virus can also be isolated from skin mucus (Woodland et al. 2002), allowing for nonlethal sampling. The aim of this study was to assess the presence of LMVB in nonlethal samples from largemouth bass in a reservoir in northeastern Mexico by real-time PCR (qPCR).

A total of 360 fish were sampled, 270 through the summers of 2014 and 2015 and 90 during the winter season of 2014–15 (Table 1). Fish were caught by angling and released after blood (0.3 mL blood from the branchial venous sinus) and skin mucus samples (scraped off a 1-cm^2 area from the lateral line with a sterile blade) were nonlethally collected. Seventy-two pooled samples (including mucus and blood) were prepared; each pool consisted of samples from five animals. The pooled samples were stored at 4 C in sterile, 15-mL tubes containing 8 mL of 96% ethanol until shipped to a laboratory (Centro de Investigación y Desarrollo Biotecnológico y Diagnóstico S.A. de C.V. Escobedo, N.L., México).

Samples were homogenized and DNA was extracted using the Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Promega Corporation, Madison, Wisconsin, USA). Samples were quantified with a NanoVue™ spectrophotometer (General Electric, Healthcare Bio-Sciences Corp., Piscataway, New Jersey, USA), adjusting the sample concentration to 20 ng/μL; a concentration of 40 ng was used in 20 μL of reaction mix. In order to amplify the gene fragment of LMBV, the smallmouth bass (*Micropterus dolomieu*) virus major capsid protein gene was used to design a primer set to complete coding sequence of the major capsid protein gene of LMBV (GenBank no. KY825782.1; F:TCAAAGAGCATTATCC-CGTGG, R:AGAGTTGAGCACATAGTCGC)

TABLE 1. Date, season, and number of largemouth bass (*Micropterus salmoides*) collected in 2014–15 at Vicente Guerrero Dam, Tamaulipas, México, for sampling of blood and mucus for detection of largemouth bass virus.

Date	Season	No. fish analyzed
July 2014	Warm	155
December 2014	Cold	30
January 2015	Cold	30
February 2015	Cold	30
July 2015	Warm	55
August 2015	Warm	60

and probe ACT TCT GGT ACG CCT GCT TTC GGA CA, and a qPCR was carried out under the following conditions: 50 C for 2 min, 95 C for 20 s, 40 cycles of 95 C for 1 s, and 60 C for 20 s. The enzyme used was TaqMan™ Fast Advance Master Mix (Applied Biosystems, Life Technologies Corporation, Austin, Texas, USA); the extracted DNA was amplified with StepOne™ (Applied Biosystems). Fluorescence was measured at the end of each qPCR cycle to make the amplification plot.

We did not detect LMBV in any of the samples. The absence of the virus in our study may have two explanations: first, that the fish introduced from the US came from LMBV-free areas or, second, that the fluids sampled for LMBV detection were not adequate for detection of the virus. This study suggested that this virus is absent in *Micropterus salmoides* from the Vicente Guerrero Reservoir, Tamaulipas, and to our knowledge this is the first study on the LMBV in Latin America.

The authors thank participating sports anglers as well the Asociación Estatal de Pesca Deportiva de Tamaulipas A.C. and the organizers of the fishing tournaments in the Vicente Guerrero reservoir for facilitating the fish sampling.

LITERATURE CITED

Goldberg TL 2002. Largemouth bass virus: An emerging problem for warmwater fisheries? In: *Black bass: Ecology, conservation, and management*, Philipp DP, Ridgway MS, editors. American Fisheries Society, Symposium 31, Bethesda, Maryland, pp. 411–416.

Grizzle JM. 2007. 2.2.12 Largemouth bass virus disease. In: *Southeastern Cooperative Fish Disease Project, Department of Fisheries and Allied Aquacultures*. Auburn University, Auburn, Alabama, pp. 1–7. https://units.fisheries.org/fhs/wp-content/uploads/sites/30/2017/08/2.2.12-LMBV-2014.pdf. Accessed December 2016.

Grizzle JM, Altinok I, Noyes AD. 2003. PCR method for detection of largemouth bass virus. *Dis Aquat Organ* 54:29–33.

Maceina MJ, Grizzle JM. 2006. The relation of largemouth bass virus to largemouth bass population metrics in five Alabama reservoirs. *Trans Am Fish Soc* 135:545–555.

Southard GM, Fries LT, Terre DR. 2009. Largemouth bass virus in Texas: Distribution and management issues. *J Aquat Anim Health* 21:36–42.

Woodland JE, Brunner CJ, Noyes AD, Grizzle JM. 2002. Experimental oral transmission of largemouth bass virus. *J Fish Dis* 25:669–672.

Zilberg D, Grizzle JM, Plumb JA. 2000. Preliminary description of lesions in juvenile largemouth bass injected with largemouth bass virus. *Dis Aquat Organ* 39:143–146.

Submitted for publication 24 March 2018.
Accepted 4 September 2018.