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Isolation of *Aeromonas salmonicida* from Paddlefish, *Polyodon spathula*

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ABSTRACT: *Aeromonas salmonicida* was isolated from paddlefish (*Polyodon spathula*) mortalities collected during an epizootic of furunculosis at the Spring River State Hatchery, Arkansas (USA), in 1992. Isolates of the bacterium were obtained from culture of gill and kidney tissue. This is the first epizootic of bacterial etiology to be reported in paddlefish.

Key words: Paddlefish, *Polyodon spathula*, furunculosis, *Aeromonas salmonicida*.

Paddlefish (*Polyodon spathula*) are endemic to river systems of the Mississippi River Basin of the United States; over the last century both the range and the numbers of the species has diminished (Russell, 1986). We document the first known epizootic and isolation of *Aeromonas salmonicida* from paddlefish being reared for stocking Arkansas (USA) rivers.

Aeromonas salmonicida, the causative agent of furunculosis of fish, is a Gram-negative, non-motile bacterium; most isolates of *A. salmonicida* are homogeneous both in their biochemical and serological reactions (Griffen, 1954). Although this bacterium is considered to be distributed throughout the world's marine and freshwater environments, most epizootics are reported in salmonid fish (Bullock et al., 1983).

We observed acute mortality among paddlefish fingerlings (12 to 13 cm) reared during spring and summer of 1992 at the Spring River State Fish Hatchery, Mammoth Springs (36°28′N, 91°32′W), Arkansas. Spring River, the main water supply for the hatchery, flooded several times during April, May and June, resulting in increased water turbidity. By June, fish had fungal growth on their rostrums that was attributed to overcrowding. Fish were divided to reduce stocking densities and treated with formalin (250 ppm for 1 hr). Although the fungal problems were controlled, mortality continued to rise. Two fish were necropsied on 12 June 1992 and kidney tissue was cultured on tryptic soy agar plates (TSA) (Difco, Detroit, Michigan, USA) for bacteria. Bacterial cultures isolated from kidney samples plus additional dead fish were shipped to the National Fish Health Research Laboratory (NFHRL), Leetown, West Virginia (USA) for confirmation. Gill tissue, kidney, spleen and liver were sampled from four fish and plated onto Coomassie Brilliant Blue (CBB) agar (Cipriano and Bertolini, 1988) according to the methods of Cipriano et al. (1992). *Aeromonas salmonicida* was isolated from gill tissue or kidneys of three of the four paddlefish evaluated. Identification was based on the methods of Amos (1985) and MacFaddin (1981). Isolates were identified as *A. salmonicida* if they were Gram-negative, non-motile rods that formed dark blue colonies on CBB, produced brown pigment on TSA, and an alkaline over acid reaction on triple sugar iron agar (Difco, Detroit, Michigan, USA), were positive for cytochrome oxidase, esculin hydrolysis, nitrate reduction and gelatinase, were negative for indole production, urease, phenylalanine deaminase, ornithine decarboxylase, and did not use citrate or malonate as a source of carbon. The isolates also were sensitive to oxytetracycline (30 μg) as determined by disc-diffusion tests (Bauer et al., 1966).

Upon confirmation of *A. salmonicida*, the paddlefish were treated with 6 g active ingredient terramycin (Pfizer, Inc., New York, New York, USA) per 45.4 kg of fish for 10 days under an emergency Investigational New Animal Drug permit issued

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by the Food and Drug Administration. Treatment reduced mortality (approximately 90% of 7,000 paddlefish already had died); after a 50-day withdrawal period, most of the approximately 550 remaining fish were stocked.

A subsample of about 150 paddlefish was transported from the Spring River State Fish Hatchery to the Andrew Hulsey State Fish Hatchery, Hot Springs (34°32'N, 93°6'W), Arkansas. These fish were maintained on well water chilled to 18 to 20 C to imitate the rearing conditions at Spring River as closely as possible, and were used to conduct an infectivity trial. Pathogen-free stocks of juvenile paddlefish were not available, therefore survivors of the suspected furunculosis epizootic were used. A 24-hr culture of *A. salmonicida* isolated during the epizootic and frozen (−70 C) was grown on TSA and the cells were washed with sterile phosphate buffered saline (PBS; pH 7.0). Cell suspensions were standardized with sterile PBS to 30% and 95% transmission (T) using a spectrophotometer (Milton Roy, Rochester, New York, USA). An inoculum (0.1 ml) from each standardized suspension was injected intraperitoneally into a group of 20 paddlefish. Another group of 20 paddlefish was injected with PBS (0.1 ml) and served as controls. Paddlefish were fed daily and water temperature remained between 18 and 20 C throughout the trial. Only three fish died due to probable furunculosis during the 17-day period of the trial. Two died from the group of fish that received the highest concentration of bacteria (30% T) and one died from the control tank.

These three mortalities could be attributed to natural exposure to *A. salmonicida* during the epizootic. Isolation, purification and transport of the bacterial culture between facilities, on the other hand, could have affected the virulence of the isolate and resulted in low level of mortality reported for the infectivity trial. At the NFHRL, a duplicate sample of the *A. salmonicida* isolate (standardized to 30% T), was used to inject brook trout (*Salvelinus fontinalis*) using the same methods. Sixteen of 20 trout injected died within 10 days and *A. salmonicida* was re-isolated from the kidney of each dead fish. Perhaps, most paddlefish that received an injection of *A. salmonicida* had developed some level of immunity or clearance ability that enabled them to resist infection from a second exposure to the bacterium. Although information on responses of paddlefish to *A. salmonicida* or other bacterial pathogens of fish has not been reported, paddlefish can mount a humoral immune response against such antigens as *Salmonella* spp. whole cells, sheep erythrocytes and human erythrocytes (Legler et al., 1971).

In July 1993, paddlefish became available from a new year-class of juveniles. The infectivity trial, as described previously, was repeated using groups of only five fish. Suspensions of *A. salmonicida* cells were prepared from a frozen aliquot of the original isolate. Within five days, all five paddlefish injected with 30% T suspension of cells died. One fish injected with the 95% T suspension of cells died during this period and fish injected with PBS did not die. *Aeromonas salmonicida* was re-isolated from each mortality during this trial. Clearly, susceptibility of paddlefish to *A. salmonicida* now must be considered in the management of wild and hatchery-reared stocks, and the potential for paddlefish to become a carrier of the pathogen should be examined.

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