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Motile *Aeromonas* Septicemia (MAS) in Fish

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Aeromonas is a genus of bacteria that is widespread in the aquatic environment. These bacteria, referred to as aeromonads, can be free living in water, they may be non-pathogenic bacteria living in the digestive tract in a variety of aquatic animals and they can be important pathogens. The group of these pathogens that are most often seen in warm water environment are called motile aeromonads because they actively swim using flagella (another species of aeromonad pathogen seen mostly in cold water fish is *Aeromonas salmonicida* and it is non-motile). The disease caused by these bacteria in fish is called Motile *Aeromonas* Septicemia (MAS). These pathogens are also an important cause of disease in amphibians (red leg in frogs), reptiles, birds, and mammals including humans. Motile aeromonads infect all freshwater and brackish water fish species and cause substantial economic damage to fish culture operations. Many cases of MAS are associated with a predisposing environmental condition or injury, but there are certain strains that are very virulent that can cause rapid mortality and substantial cumulative losses without an obvious predisposing condition.

Cause

The most common species of motile aeromonads that cause disease in fish are *A. hydrophila, A. sobria, A. caviae,* and *A. veroniae*. The traditional designations of species have recently been in flux as the genomes of these pathogens have been sequenced. These bacteria are small gram negative rods that are motile, cytochrome oxidase positive, ferment glucose, and are resistant to the vibriostatic agent 0/129 and novobiocin. These bacteria survive and grow in a variety of temperatures from near freezing to well above human body temperature (98.6°F or 37 °C). Most are also naturally resistant to penicillin and related antibiotics. In addition to the multiple species making up this group, each species has several strains. This has made the development of a broadly applicable vaccine to MAS for finfish aquaculture very difficult. These bacteria can be isolated from the water, from biofilm, mud, and non-diseased animals in most fresh and brackish water bodies worldwide. Motile aeromonads have intricate chemical communication methods that allow them to interact cooperatively in the environment and in an infected host. They can also sense changes in the environment and react to those changes. Aeromonads often form a biofilm on vegetation and other natural and man-made surfaces. These biofilms consist of bacteria embedded in a matrix that makes them resistant to environmental insults and mild disinfectants. This provides a natural reservoir for the pathogen and if this surface is rubbed against by a fish, the abrasion provides a break in the fish's surface defense while the biofilm provides the inoculum. In the host, when the bacterium is acting as a pathogen, it can express a variety virulence factors that help it evade the hosts immune system. Also, they release enzymes including proteases that break down the fish's tissues and provide nutrients to the bacterium. If a fish succumbs to infection, the bacterium continues to proliferate in the carcass, increasing its environmental numbers. The carcass can also function as vehicle to infect scavenging fish, birds, reptiles, and mammals that aid in transmission. The numbers of aeromonads present in a water body can generally be correlated to temperature and the organic content of the water.

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Common MAS

Most outbreaks of MAS are associated with an environmental condition that has weakened the fish's ability to fight infections. Because these bacteria are ubiquitous in the aquatic environment as free swimming bacteria, in biofilms, and often inhabit the gastrointestinal (GI) tract of the animal, they can readily take advantage of an immune compromised fish. The factors that allow aeromonad infections include temperature stress, stressful water quality conditions, poor nutritional factors, injury, and infection with another bacteria, virus, parasites, or water mold. Thus, combining the associated conditions, aeromonads are among the most common pathogens contributing to disease outbreaks in finfish aquaculture. Often primary infections are initiated when the bacterium enters the site of an injury or break the mucus barrier that covers the fish. The bacterium can then establish a localized infection that is characterized by breakdown of the skin, muscle, and connective tissue due to release of proteolytic enzymes. These are often manifested as deep ulcers, scale loss, fin erosion, or abscesses (Fig. 1). The bacteria can then spread from these localized infections to cause systemic infections (this is known as septicemia or bacteremia). The aeromonads also can invade the fish through the gut or gills causing a systemic infection. Fish with septicemia often have hemorrhages (red areas or spots) on the skin, eyes, and fins, a distended abdomen, flared scales due to edema in the scale pockets (dropsy), and/or a red, inflamed anus. Internally, the muscle and visceral tissue will be red and the body cavity often contains much bloody fluid (Fig. 2). Typical MAS can be attributed to a predisposing factor such as a handling event, temperature shock, water quality stressor, spawning, or aggression. Depending on the severity of the predisposing factor, rapidly increasing mortality can occur but the losses usually subside relatively quickly if the predisposing factor can be corrected. *Aeromonas* sp. infections can also be secondary to columnaris disease, a viral infection, *Saprolegnia* (water mold) infection, or *Epistylis* infections. The level of the *Aeromonas* infection's contribution to the morbidity and mortality in these disease conditions is hard to assess but generally the losses subside after the other agent is cleared.

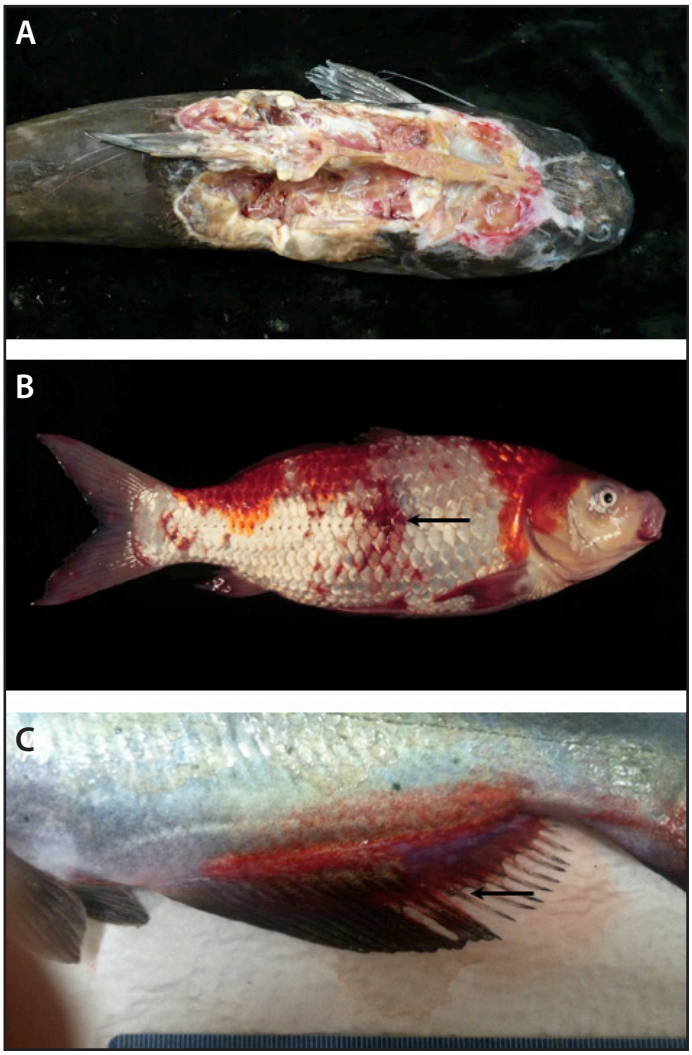

Figure 1. External lesions caused by *Aeromonas* infections. A deep ulcer on the head of a channel catfish (A). Scale loss and hemorrhage (arrow) on a Koi (B). Fin erosion and hemorrhage (arrow) on a channel catfish (C) *(Images A, B by John Hawke, C by Larry Hanson)*

MAS caused by a more virulent strain of *Aeromonas hydrophila* (VAH)

In 2009, a more virulent strain of *Aeromonas hydrophila* (VAh) began causing widespread and severe MAS in commercial catfish operations in West Alabama and East Mississippi. The disease was characterized by rapidly increasing mortality with the fish displaying profound MAS as lesions described above. These outbreaks were predominantly in the summer and early fall (June through October). From 2009 through 2016, MAS was the most diagnosed infectious disease in this region. No obvious predisposing factor was associated with them. The only consistent finding was that the pathogen was a strain of *Aeromonas hydrophila* with a unique biochemical profile and distinct genetic profile. The bacteria was unusual for this species in being able to utilize inositol (a carbohydrate) and degrade lysine (an amino acid). Genome sequencing showed that the bacterium is related to a strain of *A. hydrophila* that was found causing high losses of carp in China. The biochemical and unique genetic sequences

associated with this bacterium allowed diagnostic assays to be developed and environmental studies to be performed. In early outbreaks of the disease, the transmission was shown to be correlated with the movement of seines and hauling equipment between operations. Also, predatory and scavenging birds that have consumed infected fish are capable of shedding the bacterium in their feces, suggesting that fish eating birds may be an important vector for the spread of this pathogen. Outbreaks of this pathogen have been detected in the Mississippi Delta region of Mississippi and Arkansas, but the disease has not become as wide spread as in West Alabama and East Mississippi. This pathogen is clearly associated with high losses and appears to be relatively geographically restricted. Therefore, biosecurity measures between and within an operation are highly encouraged. To be effective, a proper diagnosis is needed to determine if a MAS outbreak is caused by VAh and if it is, aggressive treatment may be warranted. The documented outbreaks have been in catfish operations, but the mortality events also include centrarchids (the sunfish family) and other fish species in the ponds, and they have cultured positive for the pathogen during outbreaks. This suggests that many species of fish may serve as a reservoir for the pathogen.

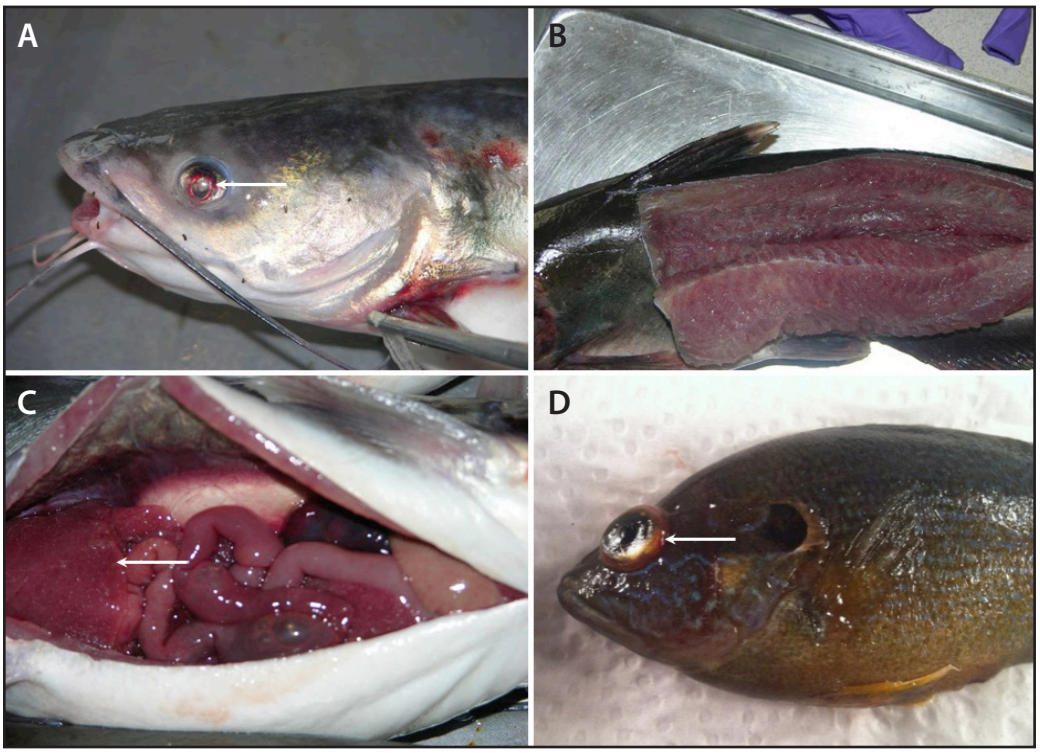

Figure 2. External (A&D) and internal (B&C) images of fish with Motile *Aeromonas* Septicemia. Note the hemorrhage in the eye (arrow) of the channel catfish in A and the bulging eye of the green sunfish in D. The channel catfish displays the diffuse hemorrhages in the muscle in B and throughout the viscera including the adipose tissue (arrow) in C. These fish had the VAh strain of *Aeromonas hydrophila.* *(Images A, B &C by William Hemstreet, D by Larry Hanson).*

Importance of a good diagnosis

It is critical to have a complete diagnosis done when an aquaculture operation begins to take losses from infectious diseases. In order to get the best diagnosis a complete background of events leading to the losses, a water quality sample, and diseased fish should be examined by a fish health professional. Many bacterial infections can show similar clinical signs, so to clearly identify the cause of the disease, the bacteria associated with the disease should be cultured and identified. With MAS it is useful to determine if the pathogen is the more virulent form of *A. hydrophila* to help determine how to manage the disease. Also, the sensitivity of the bacterium to antibiotics should be tested to determine effectiveness of the available antibiotics. Water quality and background information allows the fish health professional to identify potential stressors that may have induced the disease and may reveal important management options. The best sample is a sick fish that is captured alive. It is important to take the time to get a good sample. When one is captured, it should be placed in a plastic bag with no water in the bag, and refrigerated or packed in ice or surrounded by icepacks in a cooler. Do not freeze the fish (see SRAC Publication No. 472, *Submitting a Sample for Fish Kill Investigation*). Often the best time to capture a sick fish is early in the morning before scavengers catch the fish or drive them off the banks. Hook and line caught fish and floating, decomposing fish are usually of no use.

Prevention and treatment

Preventing MAS requires optimizing aquaculture management to minimize stress and injury and preventive management of parasites and other infectious diseases. In addition, aquaculturists should make sure they are using properly formulated feeds that have been stored properly and conservative management of water quality.

Stocking rates of tanks and cages must be optimized to prevent aggression for fish species that are territorial. In intensive systems and tanks, removal of dead and dying fish is an important measure to break the infectious cycle.

When handling fish, schedule crowding and transfer activities for cool periods, using properly sized nets to minimize abrasions and entanglement, not overloading transfer nets and adding 1 to 5 g/L (ppt) salt to hauling tanks to minimize the effects of stress (this is approximately 1 to 4 pounds of salt per 100 gallons of water). Also, the use of sedation with ample aeration is beneficial, if possible, whenever fish are handled. Refer to SRAC Publication No. 474, *The Role of Stress in Fish Disease* for more information on methods to minimize stress.

Effort should be made to avoid introducing VAh to facilities that have not experienced outbreaks by avoiding the use of nets, equipment, or water from VAh endemic facilities. If this is not possible, all nets and equipment should be thoroughly cleaned and then disinfected. Any mud, aquatic weeds, and trash fish must be removed from the net. Then the net can be dried and exposed to sunlight or immersed in a disinfectant such as 1 percent formalin. Refer to SRAC Publications No. 4707, *Biosecurity in Aquaculture, Part 1: An Overview,* and No. 4712, *Biosecurity in Aquaculture, Part 3: Ponds,* for more information on biosecurity measures. Although it is nearly impossible to keep up with mortality in large operations during severe outbreaks, removal and disposal of carcasses can help prevent spread within the pond and between ponds. Catfish readily cannibalize carcasses and the tissues of fish that have died from MAS have huge numbers of the pathogen. Also, scavenging birds have been shown to spread the pathogen in their feces. If an outbreak of VAh associated MAS does occur on a facility that was previously VAh negative, rapid deployment of an antibiotic medicated feed treatment would be warranted to minimize the opportunity of spread. This should be followed with biosecurity measures to reduce the spread and the affected pond should be completely harvested at the end of the production period to break the infectious cycle.

There are no commercially available vaccines for MAS. Research has been done to develop vaccines for *A. hydrophila* infections, but bacterial strain diversity has hampered the development of a broadly effective vaccine. However, recent studies with VAh suggest less diversity and several researchers have developed experimental vaccines that show promise.

MAS caused by motile aeromonads, other than VAh, can usually be managed by treating or managing the predisposing condition. However, if the predisposing factor is not obvious and the fish are feeding well, the use of medicated feed is an option. In VAh endemic areas, the use of medicated feeds should be evaluated by the producer based on previous experience. Three feed-based medications are available for fish, Terramycin® (oxytetracycline), Romet 30® (sulfadimethoxine plus ormetoprim), and Aquaflor® (florfenicol). Only Terramycin® is U.S. Food and Drug Administration approved for use on *Aeromonas* in catfish and salmonids (it is also approved for use on *Pseudomonas* infections in catfish and salmonids, furunculosis in salmonids, cold water disease in freshwater salmonids and columnaris disease in rainbow trout). Romet® is approved for use on enteric septicemia of catfish (ESC) and furunculosis in salmonids, and Aquaflor® is approved for use on columnaris disease and streptococcus infections in warm water fin fish, furunculosis and coldwater disease in salmonids, and ESC in catfish. The use of any of these antibiotics requires a prescription from a licensed veterinarian in the form of a veterinary feed directive (VFD). The options for medicated feed depend on what is available in your area. In areas that have a well-established aquaculture infrastructure, it is often possible to obtain medicated feed from a feed producer within a day or two of receiving a VFD. In other areas the producer must mix their own medicated feed by top coating. The disease should be diagnosed by a trained professional and the VFD can be provided based on clinical signs, but it is important for the diagnostician to culture the bacteria and perform antibiotic sensitivity testing. Sensitivity testing provides a prediction of the effectiveness of the available antibiotics and helps monitor the buildup of antibiotic resistance of bacterial pathogens in the system. Many *Aeromonas* isolates are resistant to one or more of the aquaculture antibiotics. Refer to SRAC Publication No. 473, *Medicated Feeds for Use in Food Fish,* for detailed information on the formulation and use of medicated feeds for food fish.

It is important for the aquaculturist to have an action plan for dealing with diseases caused by bacteria. This should include identifying trained fish health professionals, including a veterinarian, and making sure they are familiar with your operation before a disease problem occurs so they can assist with disease management plans. This also establishes the veterinary-client relationship that is required to allow the veterinarian to write a VFD quickly if needed. The producer will need to know what medication options (sources) are available so that if medicated feed is needed, it can be applied as soon as possible.

Appropriate and judicious use of antibiotics is critical for aquaculture. This is important to minimize the development of antibiotic resistance in the fish pathogens and to assure a safe and wholesome food product for the

consumer. Antibiotics should only be used to treat an active infectious disease caused by bacteria. They should never be used to prevent disease or to treat virus or parasite caused diseases. Anytime an antibiotic is used, it selects for bacteria that are resistant to that antibiotic. The overuse of antibiotics and inappropriate use increases the antibiotic resistance at a much higher rate than appropriate judicious use. An antibiotic should only be used when its use will be effective and it is the only practical option. The fish must be feeding well enough to get a therapeutic dose, and the feed must be consistently applied at the therapeutic dose for the indicated treatment period. Furthermore, the fish must be off the antibiotic for the prescribed period before they can be used for human consumption. This withdrawal period is critical so that the antibiotic can be metabolized and removed from the fish flesh before they are processed for consumption. Not only is this important for human safety, but also low dose consumption of antibiotics will cause the buildup of antibiotic resistance in human pathogens. Another component of appropriate use, is the safe use of these drugs. Every effort must be made to minimize the exposure of aquaculture personnel to the antibiotics. Any person handling the antibiotics should have protective clothing and eye and respiratory protection. When using the medicated feed, avoid direct skin contact and breathing the dust from the feed. Likewise, keep children, pets, and livestock away from the medicated feed.

Conclusions

MAS is common problem in freshwater and brackish water fin fish culture. Unhealthy or stressful conditions are often the underlying cause for this bacterial disease. Although widespread and problematic, this disease can usually be prevented by optimizing the environment, reducing other infectious agents, and minimizing physical injury. MAS caused by the more virulent strain of *A. hydrophila* often requires the use of medicated feed to control losses. It is important for aquaculturists to work with fish health professionals to get a diagnosis, to evaluate antibiotic sensitivity, and to obtain a prescription for medicated feed if it is needed.

Suggested readings

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USDA United States Department of Agriculture National Institute of Food and Agriculture

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