Southern Regional Aquaculture Center

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Revision

Enteric Septicemia of Catfish

John P. Hawke[1]

Enteric septicemia of catfish (ESC), caused by the gram-negative bacterium *Edwardsiella ictaluri,* is one of the most important diseases of farm-raised channel catfish (*Ictalurus punctatus*). ESC accounts for 20 to 30 percent of all disease cases submitted to fish diagnostic laboratories in the southeastern United States. In Mississippi, where catfish make up the majority of case submissions, ESC has accounted for as much as 47 percent of the yearly total. Other members of the genus, *Edwardsiella tarda* and the newly discovered *E. piscicida,* make up a much smaller portion of the total bacterial cases (about 2 percent). Economic losses resulting from ESC are in the millions of dollars yearly but have leveled off in recent years with the downsizing of the catfish industry. The causative bacterium of ESC, *Edwardsiella ictaluri,* is strictly a pathogen of fish and does not infect mammals or humans so there is no concern of zoonotic infection.

ESC was first recognized as a new infectious disease of pond-raised channel catfish in 1976 from the examination of specimens from Alabama and Georgia submitted to the Southeastern Cooperative Fish Disease Laboratory (SECFDL) at Auburn University. The disease was similar to another catfish disease caused by the bacterium *Edwardsiella tarda,* but differed in several key characteristics used for identification. ESC was first described in a published journal in 1979 and the causative bacterium, *Edwardsiella ictaluri,* was identified as a new species in 1981.

Although evidence from archived specimens indicates that ESC was present in Arkansas as early as 1969, records from fish diagnostic laboratories show that it was not prevalent in the catfish industry immediately following its discovery. Only 26 cases of ESC were diagnosed by the SECFDL between January 1976 and October 1979 and ESC occurred in only 8 percent of the total cases received by the Mississippi Cooperative Extension Service in 1980 and 1981. Between 1982 and 1986, the incidence of ESC increased dramatically and the impact on the catfish industry was significant. ESC is now known to occur throughout the geographic range of the catfish industry. ESC and columnaris disease are now the top two bacterial diseases in the industry.

Hosts and geographic range

In the U.S., the channel catfish is the fish most susceptible to infection by *Edwardsiella ictaluri,* but other species of catfish such as the white catfish and the brown bullhead are also susceptible. Blue catfish are somewhat resistant to infection and blue × channel catfish hybrids are moderately susceptible to infection. The geographic range in the U.S. now extends to all states where catfish farming is practiced. Early on, ESC was believed to affect only ictalurid catfishes, but in recent years the hosts and geographic range of *Edwardsiella ictaluri* have expanded. The disease has been reported in cultured *Pangasius* "basa" from Vietnam, *Clarias* "walking catfish" from Thailand, and yellow catfish from China. New strains of *Edwardsiella ictaluri* have been isolated from farm-raised tilapia in the tropical Western Hemisphere that differ slightly from the U.S. catfish strains. The Asian strains also vary from the U.S. catfish isolates. The bacterium has been isolated from non-catfish species such as the green knife fish, devario, rosy barb, and Ayu. Recently, new strains of *Edwardsiella ictaluri* have caused high mortality rates in laboratory and pond populations of zebrafish. What was once thought to be a homogeneous bacterial species is now known to be more diverse. Natural fish kills due to ESC are rare; only two cases are on record.

[1]Louisiana Aquatic Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University

Clinical signs and diagnosis

Behavior

Catfish affected with ESC often are seen swimming in tight circles, spiraling, spinning, and tail chasing. This erratic behavior is the result of nervous system impairment resulting from inflammation of the brain (meningitis, meningoencephalitis). Affected fish may become lethargic and swim slowly near the edge of the pond, or they may hang in the water column with the head up and tail down. Catfish with ESC tend to stop eating shortly after becoming infected.

External clinical signs (symptoms)

Affected catfish may have internal fluid accumulation (ascites) that can lead to a swollen abdomen and exophthalmia (popeye) (Fig. 1); small red and white ulcers (ranging from pinhead size to about ¼ inch in diameter) covering the skin (Fig. 2); pinpoint red spots (petechial hemorrhage) appearing under the lower jaw or belly region (Fig. 3); and a raised or eroded red ulcer with inflammatory exudate protruding through the cranial foramen at the top of the skull (Fig. 4). The clinical signs seen in Figs. 1, 2, and 3 are more typical of acute infection, whereas the clinical signs in Fig. 4 are more representative of chronic disease.

Figure 1. Channel catfish fingerling showing signs of ESC such as distended abdomen and exophthalmia (popeye) due to ascites.

Figure 2. Channel catfish with acute ESC showing small red and white ulcers on the skin.

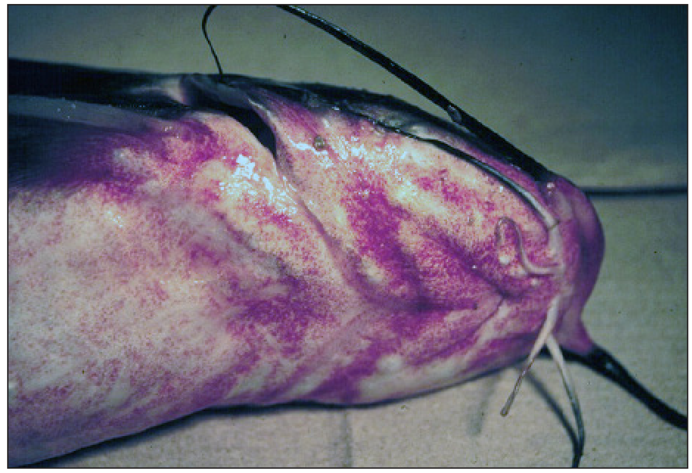

Figure 3. Channel catfish with acute ESC showing petechial hemorrhaging on the ventral surface.

Figure 4. Channel catfish fingerling with chronic ESC showing ulceration of the cranial foramen.

Internal signs

Clear, straw-colored, or bloody fluid (ascites) is often present in the body cavity of the diseased catfish (Fig 5.) The liver typically has pale areas of tissue destruction (necrosis) or a general mottled red and white appearance (Fig. 6). The posterior kidney and spleen may also appear swollen. Petechial hemorrhages can be found in the muscle, intestine, and fat of the fish. The intestine is often filled with bloody fluid.

Diagnosis

ESC is typically diagnosed by observing clinical signs and isolating the bacterium from the internal organs or brain tissue on tryptic soy agar (TSA) with 5 percent sheep blood or brain heart infusion (BHI) agar. Tissue

Figure 5. Channel catfish fingerling with acute ESC showing straw-colored fluid (ascites) in the body cavity.

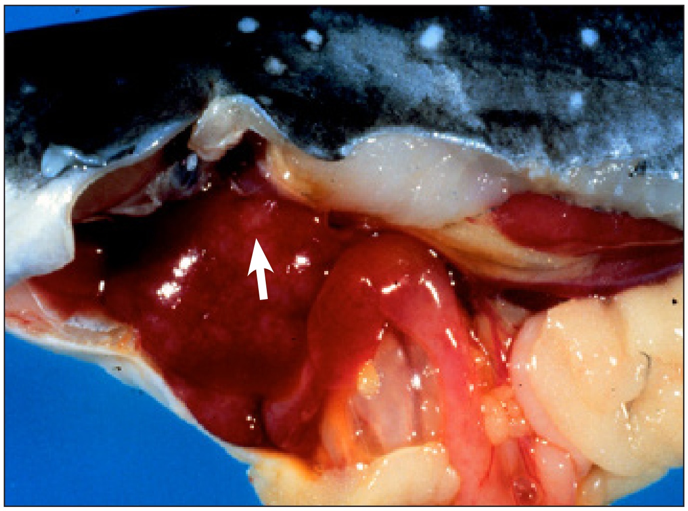

Figure 6. Channel catfish with ESC showing mottled liver; arrow indicates necrotic foci.

samples from the internal organs and brain are streaked on agar plates and small white colonies become visible in about 2 days at 77 to 82 °F (25 to 28 °C). Briefly, for presumptive identification, the bacterium should be gram negative, weakly motile, rod shaped, oxidase negative, fermentative in O/F glucose or glucose motility deeps, triple sugar iron (TSI) slant reaction K/A with no H2S, and negative for indole production in tryptone broth. Confirmatory identification is typically done in miniaturized biochemical test systems such as the Crystal™ (BBL Microbiology Systems) or the API 20E (bioMerieux Vitek, Inc.). Strains isolated from channel catfish in the U.S. typically give a code number of 4004000 in the API 20E strip. Amplification and sequencing of the 16S rRNA gene and amplification and sequencing of the *gyrB* gene product have been valuable in confirmatory diagnosis. A real-time PCR assay developed for *E. ictaluri* shows promise as a highly sensitive diagnostic test that can detect and quantify the pathogen in the host or in water and/or pond mud.

Cause of ESC

Predisposing factors

Enteric Septicemia of Catfish (ESC) can occur when a susceptible host (the channel catfish) encounters a virulent pathogen (*Edwardsiella ictaluri*) under environmental conditions that are conducive to proliferation of the pathogen and stressful for the host. Stress can be induced by a number of factors such as netting, handling, close confinement (overstocking), improper diet, low chloride levels in the pond water, and poor water quality (low oxygen, high levels of ammonia and nitrite). Mortality rates during ESC outbreaks in ponds can vary from less than 10 percent to more than 50 percent. Fingerlings are primarily affected but adults may also succumb to infection and disease. Although stress may increase susceptibility to infection and losses, it is not a prerequisite for this highly virulent pathogen. The immune status of individuals in the population may also determine the outcome.

Sources of infection

Edwardsiella ictaluri is not an organism that lives naturally in the environment. It must have a fish host to survive and must be introduced into a pond fish population in order for disease to develop. Newly constructed ponds filled with well water should be free of ESC. The bacterium may be introduced into the pond environment via contaminated nets, seines, or equipment or by fish that carry the disease. Birds can introduce the pathogen by transferring infected carcasses from pond to pond, but it is unlikely that it is transmitted through their feces. *Edwardsiella ictaluri* was originally thought to be an obligate pathogen because it survives for only a short time in water; however, it was later demonstrated to survive for up to 95 days in sterile pond mud at 77 °F (25 °C).

Transmission

The primary mode of transmission of *E. ictaluri* in ponds occurs when sick fish shed the pathogen in their feces and other fish ingest pond water containing the pathogen during feeding. The bacterium also can be transmitted by cannibalism of infected fish or from feeding on dead, infected carcasses. The *Edwardsiella* bacterium is "enteric" (referring to its preference for the intestine as a primary site for infection). Survivors of outbreaks can become latent carriers of the organism, showing no overt signs of disease. These survivors develop specific immunity that protects them from subsequent infection and disease. The introduction of ESC-infected fish (fingerlings) or carriers into ponds containing healthy fish that have no prior exposure to ESC, or the stocking of healthy (naive) fingerlings into ponds containing older catfish that are carrying *E. ictaluri* are examples of how the multiple-batch cropping system can contribute to the perpetuation and spread of ESC.

Pathogenesis

Pathogenesis studies in channel catfish have shown that the gut is a primary portal of entry for *E. ictaluri.* After ingestion and passage through the stomach to the intestine, the bacterium can cross the intestinal epithelium, enter the bloodstream, and migrate to the kidneys as soon as 15 minutes after experimental infection. The gills, skin, and nares (nasal openings) are also sites where the bacterium can enter the host. The chronic form of the disease can result when the bacterium enters the nares, travels up the olfactory tract to the brain, and causes meningitis or meningoencephalitis.

ESC occurs within a specific temperature range sometimes referred to as the "ESC window." Outbreaks typically occur in the spring and fall when water temperatures are 68 to 82 °F (20 to 28 °C). Fingerlings spawned in the summer are at greatest risk when they are exposed to ESC for the first time in the fall and subsequent spring. As fish increase in age and survive prior exposure, they become less susceptible to infection.

Prevention

Strategies

The old saying, "an ounce of prevention is worth a pound of cure," certainly applies when talking about fish disease in general and ESC in particular. Prevention of ESC has become difficult, however, because of its widespread distribution throughout the catfish industry. Various management practices can reduce the incidence and severity of ESC outbreaks. These include reducing stress, managing water quality, practicing biosecurity in the hatchery, using proper nutrition and feeding practices, and administering drugs and chemicals appropriately. Hybrid crosses of catfish such as the blue x channel catfish cross are more resistant to infection and underscore the importance of genetic improvement of fish stocks in the future. Vaccination has become a common strategy in aquaculture and the cost can be low because of the use of immersion vaccines (see section on Vaccination).

Stress

The most common advice given for the prevention of bacterial disease in fish is to avoid stress. This is a difficult goal to accomplish because commercial aquaculture is stressful by nature. Stocking density is an important factor because higher stocking densities facilitate disease transmission throughout a population. Although reducing stress is helpful for reducing disease losses, it is not always effective in prevention because *E. ictaluri* can cause disease even in the absence of apparent stress.

Nutrition

Improved nutrition through vitamin and mineral supplements may not increase the resistance of catfish to *E. ictaluri* infection. Research indicates that increasing the amounts of various individual vitamins and minerals such as vitamin E 60 to 2500 ppm (60 to 2500 iv mg/kg), iron 60 to 180 ppm (60 to 180 mg/kg), vitamin C 50 to 2071 ppm (50 to 2071 mg/kg), folic acid 0.4 to 4 ppm (0.4 to 4 mg/kg), and zinc 5 to 30 ppm (5 to 30 mg/kg) in the feed does not affect disease resistance. Menhaden oil increased susceptibility to ESC infection compared to corn oil or beef tallow as a lipid source. Winter feeding programs were found to affect susceptibility to ESC infection the following year. Year 1 fish that were fed in December, January and February were more resistant to *E. ictaluri* infection the following spring. Immunostimulants and/or immunomodulators such as beta-glucans, extracts of the blue green algae Spirulina, or extracts of the sea squirt *Ecteinascidia turbinata,* added to feed were found to enhance non-specific immunity in channel catfish but did not improve resistance to infection by *E. ictaluri.*

Genetic improvement

Various strains, crosses between strains, and hybrid crosses between species have been examined for resistance to infection by *E. ictaluri*. Higher resistance to infection was noted in the Red River strain as compared to Mississippi-select and Mississippi normal strains. The cross between Norris strain females and Marion × Kansas males showed improved resistance to ESC. Resistance to infection has also been documented experimentally in blue catfish and intermediate resistance is achieved with blue/channel crosses. A word of caution: Genetic selection for resistance to one pathogen may increase susceptibility to another; for example, increased resistance to ESC may result in increased susceptibility to ictalurid herpesvirus (CCV) in strains of channel catfish.

Specific pathogen-free (SPF) fish

The production and stocking of specific pathogen-free (SPF) fingerlings, while a possibility, has not been widely accepted by the industry. Stocking fish that have never been exposed to ESC into ponds containing fish that are carriers can lead to extremely high mortality rates. The opposite approach is often practiced, where fingerlings that are known survivors of an outbreak are actually preferred because of their acquired immunity to subsequent infection.

Vaccination

Vaccination has become a common strategy in aquaculture and a commercial vaccine for ESC, AQUAVAC-ESC™ (Merck), is available. The cost is relatively low because large numbers of advanced fry can be vaccinated by immersion. Because catfish are vaccinated at a very young age and may not be fully immunocompetent, the outcome of vaccination has been variable. The AQUAVAC-ESC™ vaccine is a modified live vaccine that has infectivity but low virulence in the catfish. Research on live attenuated vaccines with defined mutations in virulence genes is ongoing. Some pharmaceutical companies offer custom autogenous vaccines made from the parent strain from that particular facility.

Treatment

Strategies

Treatment of ESC can be approached in a variety of ways. A good pond manager makes daily observations on feeding response, behavior, and mortality. When an abnormal trend develops, specimens of sick fish should be transported or shipped to the nearest aquatic diagnostic laboratory where a complete diagnosis can be made. The diagnostic laboratory should identify the pathogens involved and if *E. ictaluri* is isolated, antibiotic susceptibility tests should be performed. Once an effective approved antibiotic is identified, fish should be treated as soon as possible with medicated feeds since fish progressively reduce feed intake during an infection. Ultimately, this makes medicated feed treatments less effective. Antibiotics should be administered under the guidance of a fish health professional or veterinarian.

Antibiotic medicated feed

Relatively few antibiotics are approved for warm water fish. Currently only Romet 30®, Romet TC®, Terramycin®, and Aquaflor® (florfenicol) are approved by the U.S. Food and Drug Administration (FDA) to treat food fish. Only Romet® and Aquaflor® are specifically labeled to treat ESC in catfish. Romet TC® is purchased as a powder that can be mixed with water or oil and used to top coat the feed pellets. Romet 30® must be incorporated into the feed at the feed mill. Usually a minimum purchase is required for the feed mill to do this. Aquaflor® medicated feeds must be administered by Veterinary Feed Directive (VFD). This process requires the participation of a veterinarian to issue a directive to enable producers to acquire VFD medicated feeds. This means that Aquaflor® can be used only under the supervision of a licensed veterinarian in the context of a valid veterinarian-client relationship. When using medicated feeds, withdrawal times must be observed prior to slaughter. For Romet®, the treatment is 5 days and withdrawal time is 3 days. For Aquaflor®, the treatment is for 10 days and the withdrawal time is 15 days. Dissolving antibiotics in the water is not recommended for treating fish in troughs or closed recirculating systems. Refer to SRAC publication No. 473, *Medicated Feeds for Use in Foodfish,* for detailed information on the formulation and use of medicated feeds for food fish.

Antibiotic resistance

Growth of *Edwardsiella ictaluri* is usually inhibited by both drugs (Romet® and Aquaflor®) in vitro. Treating fish in ponds with these drugs is effective if the disease is detected early and fish are still eating well. However, clinical efficacy has not been established to define sensitive and resistant strains. Research is currently in progress, in cooperation with the Clinical Laboratory Standards Institute (CLSI) Aquaculture Working Group, to determine threshold values for *E. ictaluri* isolates to be considered sensitive or resistant. Strains of *E.ictaluri* have been isolated that are considered resistant to Romet® and Terramycin® in vitro. Resistance to Aquaflor® (florfenicol) has recently been reported. In all cases the causative bacterium should be isolated and antibiotic susceptibility determined before a decision to treat is made. There is evidence that improper use or overuse of antibiotics increases the chance for resistant strains to develop. Medicated feeds should always be used as labeled when a proper diagnosis has been made and a disease condition exists. Medicated feeds should never be used as a preventive measure. Medicated feed should be administered for the total number of days recommended even if mortalities no longer occur. A mixture of medicated and non-medicated feed should never be used. The total weight of fish in the pond should be estimated, and fish should be fed at the recommended per cent body weight per day so all fish in the pond receive a therapeutic amount of the drug.

Economic considerations of medicated feed treatment

Economics must be considered when determining the best treatment procedure. Medicated feeds are considerably more expensive than regular feeds. Does the cost of treatment exceed the value of the fish? Will the number of fish dying (or likely to die) justify the cost of the treatment? Has there been an early diagnosis and are most of the fish still on feed? These questions should be answered and the current pond side value of fish and medicated feed costs should be factored into the equation before a decision to treat is made.

Chemical treatments

The use of chemical treatments such as copper sulfate to control algal blooms and parasites should be avoided during the ESC temperature window. The increased stress due to degraded water quality and the possible immunosuppressive effect of copper sulfate can result in a severe outbreak of ESC with high mortality rates. The addition of salt to ponds to achieve levels of 1,000 to 3,000 ppm NaCl (1 to 3 g/L) has been suggested to reduce mortality from ESC; however, the greatest benefit may be in ponds extremely low in chloride and subject to chronic nitrite toxicity.

Restrictive feeding

Research has shown that, in some cases, mortality rates can be reduced by withholding feed, restricting the amount of feed per day, or feeding every third day. There is merit to this practice and it has become widespread in the industry. However, there are risks involved and fish can, in many instances, continue to die at a high rate. The success of this method can be explained based on research that has been done on the pathogenesis of ESC. The bacterium is transmitted very efficiently by the fecal-oral route during feeding, as fish ingest water containing the bacterium along with the feed. Some investigators have noted increased rates of infection by feeding fish during an experimental water-borne challenge. Therefore, by withholding feed from a population that is in the early stages of infection, the transmission efficiency of the disease is greatly reduced and losses may be diminished. A drawback to this approach is the reduced growth of fish during this period due to restricted feeding. To assist in the decision to use restrictive feeding or antibiotics as a management strategy, it is important to carefully monitor changes in water temperatures during ESC outbreaks. If a diagnosis is made when water temperatures are rising rapidly in the spring and approaching 82 °F (28 °C), it may be wise to restrict feed and not use medicated feed. Fish will stop dying when water temperatures climb above this level. Likewise, if a diagnosis is made when temperatures are dropping in the fall and will soon drop below 68 °F (20 °C), it would also be best to withhold feed and not use medicated feeds because losses will be short-lived and minimal. However, if a diagnosis is made in the middle of the “ESC window” and temperatures will remain stable for several weeks, medicated feeds may be the proper strategy.

Management of ESC in the future

ESC will probably continue to be a problem for the catfish industry, but perhaps it will not have the negative economic impact it has had in the past. Since its discovery more than 35 years ago, hundreds of scientific articles have been published on various aspects of its pathobiology. Major advances have been made in our understanding of the disease, its causative agent, and the immune response of the channel catfish. In the future, a combination of good management techniques, vaccination, and new antibiotics such as Aquaflor® will enable the catfish producer to better cope with this disease problem. Genetically improved stocks of fish with improved resistance to ESC are being developed. With the application of modern molecular techniques, such as next generation sequencing and RNAseq, the secrets to virulence mechanisms of the pathogen and the host response to infection will be unlocked.

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

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