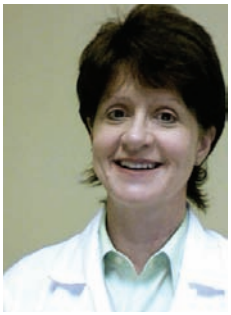
AQUA FOCUS



Catfish immunity and the effect of antibacterial treatment

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KEY POINTS

- **ESC is endemic in most areas where catfish are commercially produced and is a leading cause of economic loss in the catfish industry.**
- **ESC outbreaks are best controlled by a combination of preventive measures and the judicious use of therapeutic antibiotic treatments.**
- **Therapeutic antibiotics do not interfere with developing immunity to ESC.**
- **To preserve their effectiveness, in-feed antibiotics must be used according to label directions.**

Introduction

Enteric septicemia of catfish, or ESC, is an important contributor to economic loss in the catfish industry. The cause of ESC is a gram-negative bacterium known as *Edwardsiella ictaluri.*[1]

Transmission of ESC occurs when infected fish — whether they're sick or simply carriers — shed the ESC bacterium. The disease can also be spread when fish eat diseased fish or dead, infected fish.

Environmental factors such as poor water quality or low dissolved oxygen water levels can predispose fish to ESC. The widespread occurrence of ESC can be attributed in part to industry practices such as repeated partial harvest and re-stocking or under-stocking of fingerlings into older populations.

The severity of an ESC outbreak depends on several factors including water temperature, the stocking rate and feeding frequency.

Mortality among fish is greatest when water temperatures range from 22°C to 28°C (71.6° F to 82.4°F), the optimal growth temperature for *E. ictaluri.*[2] In the southern United States, these temperatures occur in the spring and autumn months, resulting in the typical spring and fall ESC outbreaks experienced by most catfish producers. In carrier populations, low-level mortality due to ESC can occur outside of this temperature range.

Birds cannot carry or spread *E. ictaluri* themselves, but they can move bacteria by surface contamination or carry sick or dead fish from one pond to a non-infected pond, thereby spreading the disease. Interestingly, high losses from *E. ictaluri* have only been observed in channel catfish.

Some producers have expressed concern that the use of antibiotics will clear an infection too fast and interfere with developing immunity to ESC. However, this is a misconception and does not occur when antibiotics are used according to label directions.

Clinical ESC

There are two clinical forms of ESC in channel catfish. One is a ***chronic*** encephalitis (or brain infection) and the other is an ***acute***

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septicemia or disseminated infection that involves most vital organs.[3, 4, 5]

In its chronic form, *E. ictaluri* infects the olfactory sacs in the nose and migrates along the olfactory nerves to the brain. Nasal pit (nose) infections and resulting brain infections are usually associated with immersion[4] and natural, waterborne infections.[5, 6, 7] Brain infection results in abnormal behavior and swimming patterns. In late stages of the disease, the tissue above this part of the brain erodes and exposes the brain, hence the term "hole in the head disease."

The type of ESC most commonly seen in the spring and fall is acute ESC, which leads to infection throughout the intestines[8] after the fish have eaten. The total number of deaths correlates with the dose of bacteria ingested.[4, 5, 9, 10] Affected fish have small hemorrhages around the mouth, on the throat and abdomen and at the base of the fins. Multiple small, raised hemorrhagic lesions can occur on the skin and turn into shallow ulcers. Anemia, moderate gill inflammation and bulging eyes commonly occur at the same time. Inside the fish, fluid accumulates in the body cavity and hemorrhage appears throughout vital organs. The kidney and spleen are the organs where *E. ictaluri* reaches the highest numbers and causes the most damage.[7]

When fish are suffering from ESC, they lose their appetite. Once a large portion of fish has reached this stage of disease, oral medication is unlikely to be effective. Dying fish are often found lethargic on the pond bank or they are disoriented and swimming at the surface.

Carriers

Fish that survive an ESC outbreak are considered carriers. *E. ictaluri* has been detected in fish kidney tissue 4 months after exposure.[11, 12] Survivors have protective immunity, but occasionally, fish deaths due to recurrent ESC occur, especially if the fish are stressed by low dissolved oxygen, poor water quality or high stocking densities.

Many fish populations experience low-level or subclinical ESC, develop protective immunity and become carriers of the disease without the producer realizing there was an outbreak. It is believed that infected fish shed the bacterium in their feces. The contaminated pond water then transmits the bacterium to other fish. *E. ictaluri* can survive in pond sediments for an extended period of time; in mud incubated at 25°C (77°F), *E. ictaluri* survived for 95 days.[13]

Immunity to *E. ictaluri*

There are two basic types of immunity and each functions a bit differently:

• **Innate immunity (natural immunity).** With innate immunity, the animal does not develop protection against a specific pathogen or disease. Protection does not get stronger with repeated exposure. When fish are exposed to *E. ictaluri* for the first time, innate immune defenses determine if they will become infected.

• **Acquired immunity.** The animal develops protection against a specific pathogen or disease. Protection gets stronger with repeated exposure. Acquired immunity plays a critical role in protecting fish when they are exposed the second time to *E. ictaluri.*

Once fish become infected with ESC, the severity of the disease depends on the ability of the innate immune system to suppress the pathogen until acquired immunity can resolve the infection. The severity of disease also depends on the ability of *E. ictaluri* to overcome the defenses of the fish.

Aquaculture practices tend to favor a rapid spread of high doses of bacteria within a fish population, resulting in severe ESC outbreaks that occur due to one of two scenarios:

• **SCENARIO 1:**
A large portion of the population has never been exposed before to *E. ictaluri* and then comes in contact with a high dose of the bacterium for the first time. This usually occurs in fingerling ponds during the fall, or in newly stocked fingerlings in production or stocker ponds in the spring. Fish that survive this outbreak will usually establish immunity to ESC.

• **SCENARIO 2:**
A population of fish carries ESC and then becomes immunosuppressed. In other words, the immune system is depressed due to an environmental stress such as nitrite toxicity, chemical over-treatment or oxygen depletion. Another disease can also depress the immune system, predisposing fish to ESC. When the secondary disease clears, immunosuppression is resolved and immunity to ESC returns.

Whether ESC develops from new exposure or immunosuppression, rapid diagnosis of the disease should be made and application of antibiotic-medicated feed is usually warranted.

A common misconception

Some producers have expressed concern that antibiotics clear an infection too fast, thereby interfering with developing immunity to ESC. However, in a production pond, this is impossible.

To interfere with developing immunity, the antibiotic would need to prevent infection, but antibiotic-medicated feed is used to treat infection in accordance with the label. In other words, antibiotics are used after ESC has been diagnosed. This generally means that fish have been dying from ESC for at least 2 days before the antibiotic is used.

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Although antibody levels of E. ictaluri *can predict exposure to* E. ictaluri, *they alone are not a good measure of protective immunity to ESC.*

FIGURE ONE

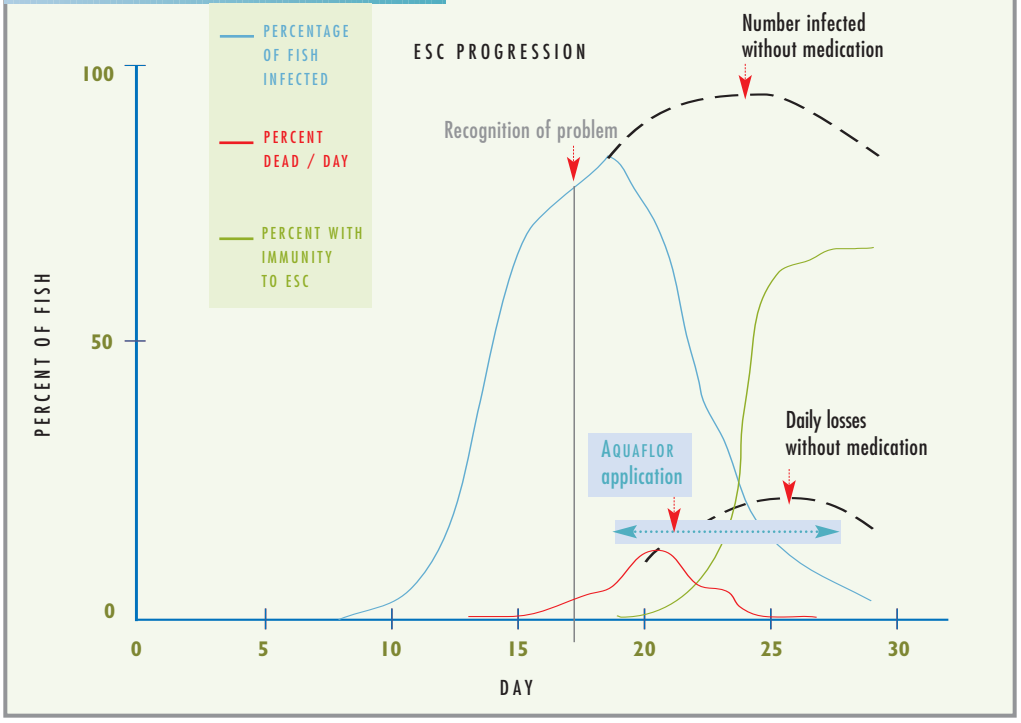

FIGURE 1: This schematic illustrates the typical progression of ESC in a naïve fingerling production pond.

During the natural course of ESC, the incubation period from first exposure to the first deaths is about 8 days.[14, 15] Intestinal levels of *E. ictaluri* increase by the fourth day after infection, which suggests active shedding.[8] The remaining 4 days of life in the first diseased fish, plus the 2 days it takes for the producer to detect and treat the problem, provides a minimum of a 6-day period for bacterial dissemination within the population. As soon as the fish are infected, the immune system is triggered (Figure 1).

During subclinical infections, when mortality is low and ESC is not diagnosed, fish undergo exposure that results in increased protection against ESC. Subclinical exposure undoubtedly occurs repeatedly in a production pond and the fish population develops immunity to ESC over time.

The misconception that antibiotics compromise immunity may arise from studies suggesting that fish given feed medicated with Romet® (sulfadimethoxine and ormetoprim) have decreased antibody levels to *E. ictaluri* when compared to non-medicated groups of fish exposed to ESC.[15] However, although antibody levels of *E. ictaluri* can predict exposure to *E. ictaluri*, they alone are not a good measure of protective immunity to ESC.

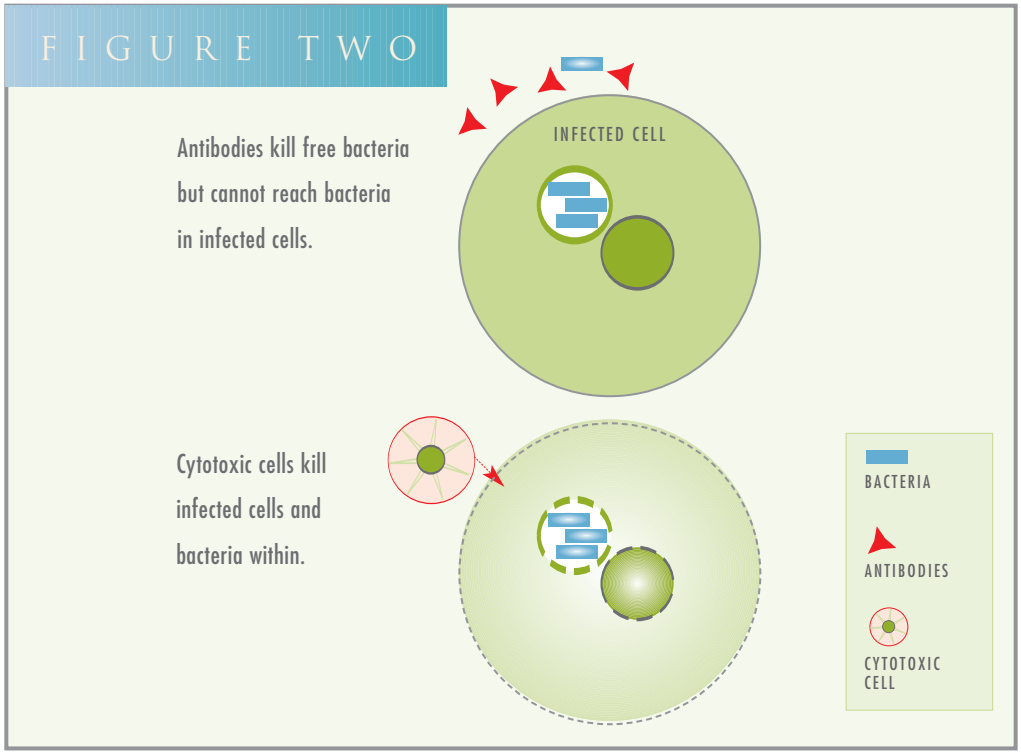

FIGURE 2: Some pathogens cause an antibody-mediated immune response, but *E. ictaluri* is an intracellular pathogen that results in another type of immune response known as cell-mediated immunity. Protection is accomplished with the help of cytotoxic T-lymphocytes.

Antibodies are measured in body fluids such as serum and reflect a type of immunity known as antibody-mediated immunity. In contrast, *E. ictaluri* works within the cells and causes a type of immune response known as cell-mediated immunity. With cell-mediated immunity, protection is carried out by killer T cells known as cytotoxic T-lymphocytes that recognize infected cells and kill them. (Figure 2).

Research performed in our lab suggests that the protection seen after catfish fry are vaccinated against ESC at 7 to 10 days of age is due to stimulation of innate defenses and long-term specific immunity may not be induced.[16] However, in a pond, repeated natural exposure to ESC following initial vaccination would ensure and strengthen long-term specific immunity. The immaturity of the acquired immune system in fry (in many species of fish) has been published.

Management of ESC disease

Avoiding *E. ictaluri* is a useful way to prevent ESC in facilities that are isolated from ESC-endemic populations. This is best done by maintaining a separate breeding population and/or by only bringing disinfected eggs onto the facility.

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Another preventive measure is vaccination against ESC. Live vaccines with reduced virulence are available.

TABLE ONE

Tool	Vaccination	Management	Feed restriction	ROMET medicated feed	AQUAFLOR medicated feed
APPLICATION	PREVENTIVE: Immersion of fry	PREVENTIVE: Avoid stress especially during ESC window	PREVENTIVE or THERAPEUTIC during ESC window	THERAPEUTIC	**THERAPEUTIC**
NOTES AND LIMITATIONS	Immunity limited — effectiveness shown only during 1st year	High-density aquaculture results in stress	Loss of weight gain	Effective but poor palatability; Fed for 5 days, 3-day withdrawal	**Effective, palatable. VFD required; fed for 10 days, 12-day withdrawal**
STAGE	Fingerlings	All stages	All stages	All stages	**All stages**

TABLE 1: ESC-mangement tools available to catfish producers.

In ESC-endemic regions, outbreaks are best controlled by a combination of preventive measures and the judicious use of therapeutic antibiotic treatments as outlined in Table 1.

Preventive measures include management aimed at reducing stressful conditions and the transmission of *E. ictaluri* during the ESC temperature window. Management options include avoidance of overcrowding, unnecessary chemical treatments and conservative oxygen management, as well as high chloride levels (at least 60 ppm) to reduce the risk of nitrite toxicity.

Once an outbreak has occurred, diligent removal and disposal of dead fish will reduce transmission within the pond and the potential of scavengers spreading the disease to nearby ponds.

Another preventive measure is vaccination against ESC. Live vaccines with reduced virulence are available. They do not cause disease but will induce protection against disease. One of these, Aquavac-ESC®, has been licensed for use and shown to provide protection when given at 7 days of age.[17] Vaccination has been shown to be helpful for fingerling production to reduce but not eliminate ESC.

Until 2006, the most common antimicrobial treatment for ESC in channel catfish was oral administration of Romet. However, as with all antimicrobials and antibiotics, resistant bacteria have developed. Furthermore, the drug was not highly palatable, which hampered effective use of the drug. Therefore, many producers focused on alternative methods of reducing losses such as reducing stress in fish, the use of alternate feeding days during the ESC window and the cessation of feeding when ESC-induced losses were detected.[15] This approach may help control ESC losses, but results in a significant loss in growth. If the ESC outbreak worsens, diseased fish lose their appetite, which reduces the producer's therapeutic options, such as the use of medicated feed.

In late 2005, AQUAFLOR® (florfenicol) was made available to producers as a Veterinary Feed Directive (VFD) drug, a relatively new category established by the FDA to more closely control new therapeutic products, primarily antimicrobials, and their use in food animals. AQUAFLOR has proven to be highly efficacious and palatable and an analysis of ESC-management methods indicates that it could provide greater receipts and net returns compared to using non-medicated feed or not feeding at all.[18] The product complements preventive strategies without interfering with appetite and associated growth (Table 1).

Conclusion

ESC is endemic in most areas that commercially produce channel catfish. Minimizing ESC losses requires diligent management in the temperature window of 22°C to 28°C (71.6°F to 82.4°F) to reduce stress on fish.

Ponds should be observed daily so that increased losses are recognized and ESC can be identified during the early stages. Determining if ESC is occurring requires collecting diseased specimens. This is best accomplished in the early morning, before scavengers and pond-side activity drive affected fish off the bank.

Fresh, diseased fish should be taken to a trained fish health professional for diagnosis. If ESC is the diagnosis, the producer must choose a therapeutic regimen. If producers use an antibiotic-medicated feed, they must adhere to the application times, methods and withdrawal periods stated on the label. Improper use of antibiotics may lead to antibiotic-resistant bacteria and make ESC less manageable. A licensed veterinarian can issue the VFD for AQUAFLOR if ESC is diagnosed.

Once ESC has resolved, the affected population will have established immunity to the

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disease and should be relatively resistant to future ESC outbreaks unless an event occurs that suppresses the immune system. In this case, repeat antibiotic treatment may be warranted.

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CAUTION: Federal law limits this drug to use under the professional supervision of a licensed veterinarian. Animal feed bearing or containing this Veterinary Feed Directive (VFD) drug shall be fed to animals only by or upon a lawful Veterinary Feed Directive issued by a licensed veterinarian in the course of the veterinarian's professional practice.

AQUAFLOR (florfenicol) is indicated for the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*. Feeds containing florfenicol must be withdrawn 12 days prior to slaughter. The effects of florfenicol on reproductive performance have not been determined. A dose-related decrease in hematopoietic/lymphopoietic tissue may occur. The time required for the hematopoietic/lymphopoietic tissues to regenerate was not evaluated.

Schering-Plough