EVALUATION OF AN AQUAVAC-ESC® BOOSTER ON PRODUCTION OF

FOOD-SIZE CHANNEL CATFISH *Ictalurus punctatus*

IN EARTHERN PONDS

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EVALUATION OF AN AQUAVAC-ESC® BOOSTER ON PRODUCTION OF FOOD-SIZE CHANNEL CATFISH *Ictalurus punctatus* IN EARTHERN PONDS

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FOOD-SIZE CHANNEL CATFISH *Ictalurus punctatus*

IN EARTHERN PONDS

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VITA

Joshua Paul McNeely, son of Paul and Kathy McNeely, was born in Houston, Texas on June 4, 1980. He attended Splendora High School in Splendora, Texas, and graduated in 1998. In August 2002, he earned a Bachelor of Science degree in Wildlife and Fisheries Sciences from Texas A&M University. He married Elizabeth Grace Adams, daughter of Michael and Ingrid Adams, on July 12, 2003. After teaching 6th grade science at Splendora Middle School for two years, he entered graduate school in August 2004 to pursue a Master of Science degree in Aquaculture at Auburn University.

THESIS ABSTRACT

EVALUATION OF AN AQUAVAC-ESC® BOOSTER ON PRODUCTION OF FOOD-SIZE CHANNEL CATFISH *Ictalurus punctatus* IN EARTHERN PONDS

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The effect of administering a more economically feasible dose of AQUAVAC-ESC® to channel catfish (*Ictalurus punctatus*) fingerlings for protection against enteric septicemia of catfish (ESC) was evaluated. The study consisted of three laboratory components and a field study.

In the first laboratory study, fish were vaccinated by immersion at 24 C at 2.44×10^4, 3.48×10^5, or 3.16×10^6 CFU/mL for 4 or 8 hrs. Serum samples were later collected and evaluated for agglutinating antibody titers against *Edwardsiella ictaluri*, the causative agent of ESC. Mean antibody titers were significantly higher in fish vaccinated at 3.48×10^5 CFU/mL than in fish vaccinated at 2.44×10^4 CFU/mL. In addition, antibody titers were significantly higher in fish vaccinated at 3.16×10^6 CFU/mL than in

fish vaccinated at the lower doses. The main effect of time, however, was not found to be significant, nor was a dose*time interaction observed.

In the second laboratory study, fingerlings were stocked into aquaria, vaccinated by immersion at 18 C at 1.0 x 10^4, 1.0 x 10^5, or 1.0 x 10^6 CFU/mL for 4 h, and later challenged with *E. ictaluri*. No significant differences in antibody titers were observed among treatments.

In the third laboratory study, fingerlings were stocked into aquaria, vaccinated by immersion at 25 C at 0, 1.95 x 10^4, 3.80 x 10^5, or 1.95 x 10^6 CFU/mL for 4 h, and later challenged with *E. ictaluri*. Significant differences were observed between control fish and fish vaccinated at the highest dose and between fish vaccinated at the highest dose and fish vaccinated at the lowest dose.

In the field study, treatment groups consisted of fish (1) non-vaccinated as fry and non-vaccinated as fingerlings, (2) vaccinated as fry at 10-d post-hatch and non-vaccinated as fingerlings, (3) non-vaccinated as fry and vaccinated as fingerlings, and (4) vaccinated as fry at 10-d post-hatch and vaccinated as fingerlings. Fish were vaccinated by immersion at 18 C for 4 h at a concentration 1:10 dilution rate of the manufacturer's recommended dose rate. The main effect of vaccination at the fry stage on survival was found to be significant. However, the main effect of vaccination at the fingerling stage on survival was not found to be significant, nor was a fry*fingerling interaction observed. The main effects of vaccination at the fry stage and vaccination at the fingerling stage on FCR values, average weights, or net production figures were not found to be significant, nor were any fry*fingerling interactions observed.

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INTRODUCTION

Enteric Septicemia of Catfish (ESC) is the most prevalent disease affecting the commercial catfish industry (USDA 2003a,b). In past years, ESC has been reported to cause annual direct losses to the industry totaling $20-30 million (Plumb and Vinitnantharat 1993). In 1981, Hawke et al. characterized and named the causative agent of ESC *Edwardsiella ictaluri*. The disease primarily affects young fingerlings in the fall. However, fingerlings that have not survived severe outbreaks in their first fall may be highly susceptible once they are stocked into food-fish ponds.

Fish farmers have attempted to control ESC outbreaks by offering medicated feed or by restricting the amount of feed offered (Wise and Johnson 1998). Romet® (a 5:1 mixture of sulfadimethoxine and ormetoprim) and Aquaflor® (florfenicol) are the only antibiotics approved by the Food and Drug Administration (FDA) for treating ESC (Gaunt et al. 2003). However, antibiotic treatment is expensive and fish affected with ESC typically reduce their feeding activity, leading to antibiotic delivery problems (Klesius and Shoemaker 1998). In addition, plasmid-mediated resistance of several strains of *E. ictaluri* to Romet has been reported (Starliper et al.1993). Restricting feed may control ESC, but may lead to a loss of production.

Due to lack of efficient control measures, interest in developing a vaccine for ESC began growing in the late 1980s. Bacterins were developed (Shoemaker and Klesius

1997), but they failed to consistently provide protection (Thune et al. 1994; Plumb et al. 1994; Shoemaker and Klesius 1997). Lack of exposure to or uptake of bacterins may have contributed to their inconsistent success (Ellis 1988; Thune et al. 1994; Shoemaker and Klesius 1997; Nusbaum and Morrison 1996). However, the most significant contributing factor was probably their limitation in producing only a humoral immune response, which may not necessarily provide protection (Klesius and Sealy 1995; Plumb et al. 1986, 1994; Wolters et al. 1996; Thune et al. 1997).

Due to the failure of bacterins to provide consistent protection, the search for an attenuated, live vaccine was begun. Unlike bacterins, live vaccines are capable of inducing cell-mediated immunity (Klesius 1992a). In time, Klesius and Shoemaker (1998) produced a live, attenuated *E. ictaluri* vaccine (*E. ictaluri* RE-33) that, when administered by immersion, could protect juvenile channel catfish 3-9 mos of age for at least 4 mos. Intervet Inc.® licensed and produced the vaccine under the name AQUAVAC-ESC® (Shoemaker et al. 2002). The AQUAVAC-ESC vaccine has been shown to provide protection when administered to fry as young as 7 d (Shoemaker et al. 1999).

Evaluation of the AQUAVAC-ESC vaccine under experimental pond conditions has shown mixed results. Wise et al. (2000) vaccinated channel catfish fingerlings 72 d post-hatch at 1.0 x 10^7 colony-forming units CFU/mL, and later challenged them in the laboratory and in the field in floating net pens. In both the field and laboratory challenges, survival was significantly higher in vaccinated fish than in control fish. However, data from a study by Carrias (2005), which evaluated under normal pond conditions the effect of vaccinating fry with AQUAVAC-ESC on survival, was

inconclusive. Carrias (2005) vaccinated channel catfish fry at 10 d post-hatch or 32 d post-hatch and stocked them into primary nursery tanks for 1 mo prior to being stocked into ponds. The fish were then grown to the fingerling stage. Survival of 32-d vaccinates was significantly higher than controls, but survival of 10-d vaccinates was not significantly higher. Because the study was conducted under pond conditions, unknown factors that also contribute to fish mortalities may have obscured a statistically significant difference.

To date, no studies have evaluated the relative survival of channel catfish immunized with AQUAVAC-ESC as fingerlings under pond conditions. The volume of vaccine required to administer it to fingerlings at the dose currently recommended by the manufacturer is cost prohibitive. However, it may be possible to substitute a longer immersion time (4 to 8 h as compared to the standard protocol of 17 min) for a lower dose. Administering the vaccine to fingerlings would be beneficial in that it may allow farmers the option of vaccinating small fingerlings while moving them to food-fish ponds or stocker-phase ponds. It may also allow the administration of a booster vaccine to fingerlings previously vaccinated as fry. The objectives of this study were to (1) evaluate the ability of lower vaccine doses administered at longer exposure times to confer protection in channel catfish fingerlings against ESC, (2) evaluate under normal pond conditions the relative survival of fish immunized as fry with AQUAVAC-ESC and given a booster of the vaccine as fingerlings, and (3) begin evaluating the long-term benefits of vaccination to determine if a booster is necessary for life-long protection.

LITERATURE REVIEW

Enteric Septicemia of Catfish (ESC) is the most prevalent disease affecting the commercial catfish industry and causes the most economic loss to the industry. In 2002, ESC was reported to have caused losses on 53% of fry/fingerling operations and 61% of food-size fish operations (USDA 2003a,b). In past years ESC has been reported to cause annual direct losses to the industry totaling $20-30 million (Plumb and Vinitnantharat 1993).

The Disease

The first cases of ESC were documented at the Southeastern Cooperative Fish Disease Laboratory of Auburn University in 1976 in channel catfish (*Ictalurus punctatus*). Hawke (1979) first described clinical signs associated with ESC, including: ascites; necrosis of the liver; petechial hemorrhaging; exophthalmia; small cutaneous lesions; and a large open lesion between the eyes, which gives ESC its common name, "hole-in-the head disease". Behavioral signs of ESC include: anorexia, tail-chasing, hanging at the surface tail-down, and spiraling.

ESC can present itself in either an acute or chronic form. However, the acute form is more common (Klesius 1992a; Newton et al. 1989). High mortalities and general septicemia typically characterize the acute form, whereas low mortalities and "hole-in-the-head" typically characterize the chronic form (Newton et al. 1989). Fingerlings

generally suffer from the acute form, whereas food-fish generally suffer from the chronic form (Wise et al. 2004).

The Pathogen

When the first cases of ESC were investigated, a bacterium was suspected to be the causative agent. Koch's postulates were used to confirm that the suspect bacterium was indeed causing the disease (Hawke 1979). Microscopy and biochemical characterization of the bacterium by Hawke (1979) indicated that the causative agent of ESC belonged to family Enterobacteriacae. Microscopy revealed that the bacterium was Gram negative and rod-shaped. Biochemical characterization revealed that isolates were cytochrome oxidase negative, fermentive in glucose O/F medium, and reduced nitrate to nitrite. With a few exceptions, all members of Enterobacteriacae share these characteristics (Holt 1994).

Use of DNA:DNA hybridization technique indicated that the causative agent of ESC was more closely related to *Edwardsiella tarda* than any other member of Enterobacteriacae (Hawke 1979). Later work by Zhang and Arias (2006), in which a bootstrap analysis was used to produce a phylogenetic tree based on 23S rRNA sequences, confirmed the close relationship. The organism differed from *E. tarda* in that it was negative for hydrogen sulfide production and the indole biochemical test and was nonmotile at 37 C. Therefore, the Center for Disease Control in Atlanta, Georgia considered the organism a new species in the genus *Edwardsiella* (Hawke 1979). In 1981, Hawke et al. fully characterized, classified, and named the bacterium *Edwardsiella ictaluri*.

Epidemiology

In the Southeastern United States, where the channel catfish industry is concentrated, epizootics of ESC commonly occur during late spring and early fall due to favorable temperatures for bacterial growth and reproduction. The acute form of ESC tends to occur when temperatures are between 22 C and 28 C, while the chronic form tends to occur when temperatures are between 18 C and 22 C or higher than 28 C. Below 18 C or above 30 C, ESC outbreaks are rare (Tucker and Robinson 1990). Temperature ranges associated with both the acute and chronic forms correlate well with the optimum growth temperature for *E. ictaluri*, which is between 25 C and 30 C (Hawke et al. 1981), but these temperature ranges are mere generalizations. Wise et al. (1997) exposed channel catfish fingerlings to *E. ictaluri* in aquaria, and, after holding the fish at a constant temperature (26-28 C) for 216 d, necropsied fish surviving acute infection. The researchers were able to isolate *E. ictaluri* from the posterior kidney or brain of a small percentage of fish having cleared the infection from the blood and may have been able to isolate the bacterium from a larger percentage of fish had they used enrichment techniques. The study demonstrated that chronic infections can develop in fish surviving acute infections, even if the temperature is held constant within the range typically associated with acute infections.

E. ictaluri primarily infects channel catfish and especially causes severe economic losses in naïve, young of the year fry and fingerlings in the fall of the year. However, the bacterium has been shown to naturally infect walking catfish *Clarias batrachus*, blue catfish *Ictalurus furcatus*, white catfish *Ameiurus catus*, and brown bullhead *Ameiurus nebulosus* as well (Tucker and Robinson 1990). An aquatic assessment of the Cohansey

River in New Jersey revealed that tadpole madtoms *Noturus gyrinus* are also susceptible to natural infection (Klesius et al. 2003). Although no reports have been made of *E. ictaluri* causing natural infections in blue tilapia *Oreochromis aureus*, chinook salmon *Oncorhynchus tshawytscha*, or rainbow trout *Oncorhynchus mykiss*, these species have been experimentally infected with the bacterium (Plumb and Sanchez 1983; Baxa and Hedrick 1989).

E. ictaluri has been shown to survive in pond water for short periods of time and pond mud for longer periods of time. Plumb and Quinlan (1986) inoculated sterile pond water samples with *E. ictaluri* and incubated them at 25 C. Samples of pond mud were prepared in the same way, but were incubated at 18 C as well. After incubation, samples were assayed for the presence of *E. ictaluri* on designated days. The bacterium survived for only 15 d in the water sample, but survived 40 d in mud incubated at 18 C and 95 d in mud incubated at 25 C. The researchers concluded that *E. ictaluri* is probably not a strict obligate pathogen, but that only certain environments will support its survival outside channel catfish.

Channel catfish have been shown to serve as carriers of *E. ictaluri* after surviving infection by *E. ictaluri*, even if treated with antibiotics. Mqolomba and Plumb (1992) experimentally infected channel catfish and later attempted to recover the bacterium from surviving fish. *E. ictaluri* was present in tissues of asymptomatic channel catfish 81 d post-infection. Klesius (1992b) offered fingerlings known to have suffered from clinical ESC feed containing Romet-30 or non-medicated feed. Periodically over a 270 d period, the trunk kidney was tested for the presence of *E. ictaluri* and antibody titers. In fish given medicated feed, the bacterium was detected at 90 d and 270 d and serum antibodies

to *E. ictaluri* were detected at 30 d and 270 d. A greater percentage of fish contained *E. ictaluri* at 270 d than at 90 d, and a greater percentage of fish tested positive for antibodies at 270 d than at 30 d. Klesius (1992b) hypothesized Romet-30 failed to kill *E. ictaluri* within macrophages, leading to establishment of a carrier state.

E. ictaluri can be transmitted in a variety of ways. Shotts et al. (1986) placed channel catfish fingerlings exposed to *E. ictaluri* in tanks with unexposed fish. Some of the fish unexposed to *E. ictaluri* by the researchers developed systemic infections, thus demonstrating that horizontal transmission can occur. Horizontal transmission may also occur when fish eat infected carcasses (Tucker and Robinson 1990) or by the shedding of viable *E. ictalu*ri into the water by moribund fish just prior to death or during decomposition of moribund fish (Wise et al. 1997). Wise et al. (1997) exposed channel catfish fingerlings to *E. ictaluri* in aquaria and monitored the number of *E. ictaluri* cells in water over a 216-h period. The concentration of *E. ictaluri* in the water significantly increased after 48 h and this increase coincided with the onset of mortalities. Widespread use of the multiple batch system by channel catfish producers may be contributing to the continued spread of ESC (Tucker and Robinson 1990). In multiple batch systems, naïve fish are often mixed with carrier fish. In addition, fish farmers may unintentionally facilitate transmission by passing effluent from one pond to another or using seines that had previously been used in ponds with infected animals without disinfection or drying.

Other animals may facilitate transmission by serving as vectors (Tucker and Robinson 1990). Taylor (1992) performed intestinal and rectal smears of snowy egrets *Egretta thula*, great egrets *Casmerodius albus*, great blue herons *Ardea herodias*, and double-crested cormorants *Phalacrocorax auritus* and tested for the presence of *E.*

ictaluri using an indirect fluorescent antibody (IFA) test. The bacterium was detected in 53% of sampled birds. Results of a study conducted by Waterstrat et al. (1999), however, suggested that birds play a limited role in *E. ictaluri* transmission. Over a 4-d period, great blue herons *A. herodias* were fed channel catfish obtained from a pond experiencing an outbreak of ESC and injected intraperitoneally with live *E. ictaluri*. During the feeding period, fecal samples, throat and rectal swabs, and feather samples were collected. At the end of the 4-d feeding period, birds were euthenized and gastrointestinal tracts were sampled. Selective media was used to attempt to recover the bacterium from the samples, and an IFA test was used to attempt to detect the bacterium. The bacterium was detected using IFA, but the researchers failed to recover viable *E. ictaluri*. In the same study, Waterstrat et al. (1999) determined the body temperature of great blue herons to be 40.2 C, and then attempted to grow *E. ictaluri* at 24 C after incubating the cultures at 40 C for either 5 h or 8 h. No growth was observed in the cultures. The failure to recover viable *E. ictaluri* from samples and the failure to grow the bacterium at 40 C led the authors to conclude that great blue herons probably play a limited role in *E. ictaluri* transmission. In support of their findings, Waterstrat et al. (1999) pointed out that, although Taylor (1992) detected *E. ictaluri* in 53% of sampled birds, viable *E. ictaluri* was recovered from only two of the samples taken over the three-year study period.

E. ictaluri can enter channel catfish via the olfactory mucosa, intestine, or gills. Miyazaki and Plumb (1985) observed inflammation in olfactory organs of channel catfish naturally infected with *E. ictaluri*. Newton et al. (1989) observed cutaneous hemorrhaging and enteritis 4 d after experimentally inducing *E. ictaluri* infection by

immersion. Meningoencephalitis involving the olfactory bulbs and dorsocranial ulceration were observed 3-4 weeks post-exposure. Morrison and Plumb (1994) observed damage to cilia on olfactory mucosal surfaces of channel catfish 1 h post-exposure to *E. ictaluri*. Baldwin and Newton (1993) showed that *E. ictaluri* can cross intestinal mucosa 0.25 h post-injection of the gut. Nusbaum and Morrison (1996) immersed channel catfish in heat-killed radiolabeled *E. ictaluri* and were able to detect the bacterium in or on the gills within 2 h of exposure.

Like some other members of Enterobacteriacae, such as *Salmonella sp.* and *Yersinia sp*., *E. ictaluri* has been shown to be an intracellular pathogen, surviving and reproducing within neutrophils and macrophages of channel catfish. Miyazaki and Plumb (1985) examined moribund fish with natural infections of *E. ictaluri*. The researchers observed *E. ictaluri* cells in the process of dividing within neutrophils, as well as neutrophils having died from extensive multiplication of the bacterium. Morrison and Plumb (1994) observed phagocytic cells migrating through olfactory epithelium to phagocytize *E. ictaluri*, but failing to destroy the bacterium. Baldwin and Newton (1993) experimentally infected channel catfish intragastrically and later examined tissues by electron microscopy. Cytoplasmic vacuoles of macrophages were found to contain *E. ictaluri*, with the number of cells present increasing over time. The researchers also observed *E. ictaluri* cells in the process of dividing within cytoplasmic vacuoles of macrophages. Shotts et al. (1986) observed lesions in channel catfish resulting from exposure to *E. ictuluri* and noted macrophages containing bacteria in every tissue examined. The authors suggested that macrophages might play an important role in spreading the bacterium throughout the host.

Extracellular products may contribute to the ability of *E. ictaluri* to cause disease in channel catfish. Stanley et al. (1994) found clear differences between extracellular products produced by virulent strains of *E. ictaluri* and those produced by avirulent (attenuated) strains. Scanning electron microscopy and transmission electron microscopy both showed virulent strains to have a greater amount of capsular material. Congo red, a dye that binds to proteins, was used to show that virulent strains possess more surface proteins than avirulent strains. A plate assay used to evaluate chondrotinase activity revealed virulent strains to have greater ability to degrade chondrotin sulfate than avirulent strains. Stanley et al. (1994) suggested that the relatively greater amount of capsular material and surface proteins possessed by virulent strains may contribute to their ability to attach to macrophages and to survive in the acidic environment within macrophages. The authors also suggested that the greater ability of virulent strains to degrade chondroitin sulfate, an abundant compound in cartilage, may allow them to escape host localization and facilitate their spread throughout the host. Shotts et al. (1986) suggested that chondroitinase activity may be responsible for the hole-in-the-head lesion characteristic of ESC.

Immune System of Channel Catfish

All multicellular organisms possess some form of innate immunity, and adaptive immune mechanisms first evolved in jawed vertebrates (Abbas and Lichtman 2003d). Therefore, channel catfish possess an innate and an adaptive immune system. Innate immune mechanisms are nonspecific, meaning they recognize structures shared by groups of organisms, but cannot distinguish small differences among microbes. Adaptive immune mechanisms are specific, meaning they can distinguish among different

microbes by recognizing antigens specific to those microbes. Adaptive immune mechanisms also show memory, meaning that subsequent exposures to a particular antigen elicit faster and stronger responses than the primary exposure, whereas innate immune mechanisms do not have this capability. After a host encounters an antigen for the first time, it passes from a naïve status to an immune status, with regard to that particular antigen (Abbas and Lichtman 2003d).

The innate immune system of channel catfish consists of physical barriers, phagocytic cells, nonspecific cytoxic cells, and the complement system. Physical barriers include mucous and skin. Goblet cells in the skin, gills, and gastrointestinal tract secrete mucous, which prevents colonization of these organs by microbes. The skin prevents microbes from directly entering the blood stream. Phagocytic cells are cable of adhering to, engulfing, and destroying microbes (Moore and Hawke 2004). Macrophages and neutrophils are the primary phagocytic cells in channel catfish (Ainsworth 1992; Finco-Kent and Thune 1987). Nonspecific cytoxic cells are similar to mammalian natural killer (NK) cells in that they are nonspecific, are not capable of phagocytosis, participate in mediating the lysis of virus infected cells, protozoan parasites and tumor target cells, and require cell contact with the target cell to mediate lysis (Evans et al. 1984).

In mammalian systems, complement is a system composed of serum and mucous proteins, designated C1 through C9, that when activated interact in a sequential cascade of protelytic steps. Activation of complement can result in formation of the membrane attack complex, which lyses cell membranes; complement-mediated opsonization; or complement-mediated inflammation. Complement-mediated opsonization is the process in which complement fragments coat microbes, thereby facilitating phagocytosis of

microbes. Complement-mediated inflammation describes the process in which complement fragments trigger an inflammatory response—vasodilation, increased vascular permeability, and migration of white blood cells to the site of infection. Complement can be activated by either the alternative or classical pathway. Activation of the alternative pathway occurs nonspecifically, whereas activation of the classical pathway requires specific antibodies (Abbas and Lichtman 2003c).

Complement activation pathways in fish appear to be similar to those in mammals (Holland and Lambris 2002). The alternative and classical pathways can activate complement in channel catfish (Ourth and Wilson 1982). The effector mechanisms of complement in fish also appear to be similar to those in mammals. Fish complement has been shown to function in lysis of foreign cells, opsonization, and inflammation. Furthermore, complement proteins in fish appear to be similar to those in mammals, but tend to be more diverse. For example, C3 has been shown to occur in five different forms (Holland and Lambris 2002). In channel catfish a C1q-type protein (Dodds and Petry 1993) and proteins analogous to mammalian C3 and C4 have been isolated (Jenkins and Ourth 1991; Holland and Lambris 2002).

As in mammals, two types of adaptive immunity exist in fish: humoral and cell-mediated (Klesius 1992b). Humoral immunity consists of B cells—defined as those lymphocytes (antigen specific leukocytes) that express immunoglobulin (antibodies) on their surface and secrete specific antibody in response to antigenic stimulation (Kaattari 1992). Cell-mediated immunity consists of T-like cells, which, despite lacking cell surface markers—as mammalian T cells possess—have functions similar to mammalian T-helper and cytolytic T cells (CTLs) (Chilmonczyk 1992). B cells are formed in the

anterior kidneys of fish (Kaattari 1992). Upon activation, B cells either differentiate into memory cells or plasma cells. Plasma cells no longer express membrane-bound immunoglobulin, but instead secrete antibodies (Abbas and Lichtman 2003c). Activation of B cells occurs when they specifically recognize one of two types of antigens: T-independent or T-dependent. T-independent antigens, which are generally polysaccharides, do not require the assistance of T-helper cells to activate B cells, but T-dependent antigens, which tend to be proteins, do require the assistance of T-helper cells (Kaattari and Piganelli 1996).

Immunization of fish triggers B cells to produce specific antibodies that appear in blood, bile, and mucous (Wilson and Warr 1992). Studies have shown that *E. ictaluri*-specific serum antibodies are indicative of prior exposure to *E. ictaluri* (Klesius 1992b; Vinitnantharat and Plumb 1993; Antonio and Hedrick 1994). Serological detection of *E. ictaluri*-specific antibodies have been used in diagnosing *E. ictaluri* infections (Klesius et al. 1991). The presence of serum antibodies after immunization has been confirmed in numerous studies. Plumb and Vinitnantharat (1993) vaccinated fry against ESC by immersion in bacterin (killed bacteria), stocked the fish into ponds, and offered an oral bacterin booster to a portion of the previously vaccinated fry. Antibody titers were significantly higher in vaccinated fish than in control fish. Thune et al. (1997) vaccinated channel catfish against ESC by immersion bacterin or immersion bacterin followed by an oral bacterin booster and later assayed serum antibodies. Both vaccination regimes resulted in antibody titers significantly higher than those observed in control fish. Shoemaker and Klesius (1997) vaccinated juvenile channel catfish by immersion bacterin

or oral bacterin and later assayed serum antibodies. Antibody titers were significantly higher in fish vaccinated by immersion bacterin than in control fish.

Increases in antigen concentration, water temperature or antigen exposure time have been shown to increase the magnitude of the primary immune response. Vinitnantharat and Plumb (1992) injected channel catfish intraperitoneally with killed *E. ictaluri* at varying doses. Fish exposed to 2.0×10^4 cells/fish had lower antibody titers than fish exposed to 2.0×10^6 or 2.0×10^8 cells/fish. The researchers injected a second group of channel catfish intraperitoneally with an extract of *E. ictaluri* at varying temperatures. Fish immunized at 20°C had lower antibody titers than fish immunized at 25 C or 30 C. The researchers immersed a third group of channel catfish in an extract of *E. ictaluri* for varying exposure times. Fish exposed to antigen for 30 min or 8 h had higher antibody titers than fish exposed for 2 min or 5 min.

As in mammals, fish antibodies function by neutralization, agglutination, opsonization, and complement activation (Wilson and Warr 1992). Neutralization is the process in which specific antibodies bind to antigen receptors, thereby preventing attachment of antigens to cell targets. Agglutination is the process in which antibodies cross-link antigens on multiple cell surfaces—causing cells to clump together—and thereby facilitating removal of bacteria by macrophages. As with complement, opsonization is the process in which antibodies coat the surface of antigens, facilitating phagocytosis of antigens. Antibodies can activate complement via the classical pathway (Abbas and Lichtman 2003c).

Unlike mammalian antibodies, which occur in five different isotypes, only one isotype is present in fish. Because of its resemblance in size, structure, gene organization

and physiochemical properties to mammalian IgM, the isotype present in fish is referred to as IgM-like. As in mammals, the monomeric form of the molecule has two binding sites. However, the monomers tend to associate to form tetramers, so that each tetrameric association has eight binding sites (Wilson and Warr 1992).

In mammalian systems, a secondary exposure to an antigen results in faster and greater increases in specific antibodies, isotype switching (generation of isotypes other than IgM), and affinity maturation (generation of antibodies with increasingly higher affinities over time) (Abbas and Lichtman 2003a). The secondary response of fish is similar to that of mammals, in that faster and greater increases in specific antibodies are seen, but is dissimilar to that of mammals, in that isotype switching and affinity maturation are not seen (Wilson and Warr 1992). Studies have shown channel catfish mount a secondary humoral immune response to *E. ictaluri* greater than the primary response. Vinitnantharat and Plumb (1992) injected channel catfish intraperitoneally with extracts of *E. ictaluri* and thereafter monitored serum antibody titers on a weekly basis. Antibody titers rose for 4 weeks and then declined. Following a booster injection administered 6 weeks post-primary vaccination, antibody titers reached levels nearly twice those reached during the primary response. Petrie-Hanson and Ainsworth (1999) vaccinated channel catfish fry 4 weeks post-hatch with live *E. ictaluri* and reexposed them to the bacterium at 8 weeks post-hatch. Antibodies were assayed post-primary and post-secondary exposure. Antibody titers were significantly higher post-secondary exposure than post-primary exposure.

Although Vinitnantharat and Plumb (1992) and Petrie-Hanson and Ainsworth (1999) observed greater secondary responses, it should be noted that Klesius and Sealy

(1995) observed a lesser secondary response. However, the authors did not attribute the lesser secondary response to lack of immunological memory. Klesius and Sealy (1995) immersed channel catfish in virulent *E. ictaluri* to achieve primary or secondary responses and thereafter monitored serum antibody titers on a weekly basis. During both the primary and secondary exposures, significant specific antibody titers were observed, although titers were higher during the primary response. The researchers offered four explanations for why they may have observed lower antibody production during the secondary response than in the primary response: antibodies may have functioned in opsonization; antibodies may have been trapped in immune complexes; fish may have down-regulated a strong immune response; or immersion may simply have not resulted in a strong memory response.

In most cases, a specific immune response involves both humoral and cell-mediated immune mechanisms (Moore and Hawke 2004). B cells can only become activated in the absence of a cell-mediated response if the antigen is T-independent; additionally, T-independent antigens do not lead to immunological memory in B-cells. Immunological memory develops only when B cells are activated by T-dependent antigens in conjunction with T-helper cells (Arkoosh and Kaattari 1991). T cells, both T-helper and CTLs, arise in the thymus (Chilmonczyk 1992), and when activated—by specifically recognizing a particular antigen—they either differentiate into effector T cells or memory cells (Abbas and Lichtman 2003a). Effector T helper cells secrete cytokines—soluble messenger proteins—that are involved in activating B cells, macrophages, and neutrophils. Effector CTLs are involved in destroying altered host cells (Manning and Nakanishi 1996).

For T cells to recognize antigens in mammalian systems, antigens must be displayed by major histocompatability (MHC) molecules. MHC molecules are present on host cells—referred to as target cells—and on B cells, macrophages and neutrophils—referred to as antigen presenting cells (APCs). MHC class I molecules are present on target cells and display antigens to CTLs. MHC class II molecules are present only on APCs and display antigens to T-helper cells. APCs must process, or break antigens up into short peptides, before displaying them on MHC molecules (Abbas and Lichtman 2003b). Antigen processing and presentation in teleosts have been shown to be similar to that of mammals (Vallejo et al. 1992).

Factors Affecting the Immune System

Nutrition significantly influences the immune system of channel catfish. Micronutrients known to improve resistance to disease include vitamins C, B_6, E, and A and the minerals iron and fluoride (Blazer 1992). Wise et al. (1993) fed channel catfish fingerlings diets containing 0, 60, or 2500 mg vitamin E/kg, vaccinated them by immersion in a bacterin followed by an oral booster, and later analyzed for macrophage activity. The ability of macrophages to phagocytize virulent *E. ictaluri* was enhanced in both vaccinates and non-vaccinates fed either 60 or 2500 mg vitamin E/kg. Sealey et al. (1997) found that channel catfish deficient in dietary iron were more susceptible to infection by *E. ictaluri*. Paripatananont and Lovell (1995) fed channel catfish diets supplemented with varying amounts of zinc and later challenged them with *E. ictaluri*. Fish receiving no zinc in their diets showed 100% mortality, but those fish receiving an adequate amount of zinc showed 25-30% mortality. Duncan and Lovell (1994) fed channel catfish diets containing 0, 0.4, or 4.0 mg folic acid/kg with 0, 20, or 200 mg

vitamin C/kg in a factorial design in aquaria and later challenged them with *E. ictaluri*. Maximum survival was observed in fish fed diets containing 0.4 or 4.0 mg folic acid/kg in combination with the high level of vitamin C. However, improved survival was not observed in fish fed diets supplemented with vitamin C or folic acid alone.

Certain fatty acids may play a role in disease resistance (Blazer 1992). Li et al. (1994) fed channel catfish diets supplemented with 2% catfish offal oil, beef tallow, or menhaden oil and challenged them with *E. ictaluri*. Fish fed the diet supplemented with menhaden oil showed increased susceptibility to *E. ictaluri*, but those fed the other diets did not show increased susceptibility. Sheldon and Blazer (1991) vaccinated channel catfish by immersion in killed *E. ictaluri*, offered vaccinated and non-vaccinated fish diets of varying lipid content, and later isolated macrophages from both vaccinated and non-vaccinated fish. Isolated macrophages were exposed to *E. ictaluri* to assay bactericidal activity. Bactericidal killing of macrophages increased as the level of n-3 fatty acids in the diet increased in both non-vaccinated and vaccinated fish.

An indirect relationship has been shown to exist between the longevity of circulating antibodies and water temperature. Plumb et al. (1986) assayed antibodies 60 d post-vaccination in channel catfish vaccinated by injection with killed *E. ictaluri* and held at different temperature regimes. Higher antibody titers were observed in vaccinated fish held at 25 C for 30 d and 12 C for an additional 30 d than vaccinated fish held at 25 C for 60 d. Higher antibody titers were also observed in vaccinated fish held at 25 C for 5 or 10 d and then held at 12 C until 60 d post-vaccination than in vaccinated fish held at 25 C.

A rapid drop in water temperature from 23 C to 11 C has been shown to result in suppression of T-helper cells and B-cells. Bly and Clem (1991) decreased the water temperature in which channel catfish were immersed from 23 C to 11 C over a 24 h period. Subsequently, T and B cells responses to mitogen and antibody production following stimulation by T-dependent or T-independent antigens were assayed. The researchers observed a reduction in T and B cell proliferation in response to mitogen and suppressed antibody production in response to either T-dependent or T-independent antigen.

A rapid drop in water temperature from 22 C to 17 C has also been shown to result in suppression of T-helper cells. Clem et al. (1984) cultured B-cells and T-helper cells isolated from channel catfish acclimated to 22 C in the presence of LPS (a B-cell mitogen) or Con A (a T-cell mitogen) at 32, 27, 22, or 17 C. B-cells showed a significant response to LPS, regardless of culture temperature. T-helper cells showed significant responses to Con A at 32 and 27 C, a diminished response at 22 C, and no response at 17 C. Miller and Clem (1984) cultured B-cells isolated from channel catfish acclimated to 22 C in the presence of T-dependent and T-independent antigens at 32, 27, 22, or 17 C. B-cells showed significant responses to T-independent antigens, regardless of culture temperature. However, B-cells only showed significant responses to T-dependent antigens at 32 and 27 C. Diminished B-cell responses were observed at 22 and 17 C, with the response at 17 C much lower than that at 22 C.

Although a rapid drop in temperature from 22 C to 17 C has been shown to result in suppression of T-helper cells, acclimation of fish to 17 C has been shown to result in recovery of T-helper cell function. Clem et al. (1984) cultured T-helper cells isolated

from channel catfish acclimated for at least 2 weeks to 17 C in the presence of Con A at 32, 27, 22, or 17 C. T-helper cells showed significant responses to Con A at 32, 27, and 22 C. Miller and Clem (1984) cultured B-cells isolated from channel catfish acclimated to 17 C in the presence of T-dependent and T-independent antigens at 27, 22, or 17 C. B-cells showed significant responses to both T-independent and T-dependent antigens, regardless of culture temperature.

Stress has been defined as the response of animals to changes or challenges in their environments by a variety of interlocking anatomical, physiological, biochemical, immunological, and behavioral adaptation mechanisms (Ewbank 1989). Events triggering a stress response include: osmotic and ionic changes, pollutants, crowding, handling, grading, hauling, and other aquaculture practices (Eddy 1981). The physiological changes associated with stress can be divided into three response phases: primary, secondary and tertiary. During the primary response phase, plasma concentrations of corticosteriods and catecholamines rise, leading to the secondary phase which affects metabolic and osmoregulatory functions. Changes in plasma glucose levels and fatty acids also accompany these disturbances (Mazeaud et al. 1977). During the tertiary phases, behavioral changes, decreased growth and immunosuppression can be observed (Wedemeyer and McLeay 1981). The tertiary effect of immunosuppression results from the ability of corticosteriods, such as cortisol, to inhibit leukocyte migration, lymphocyte effector mechanisms, inflammation, and cytokine production (Kennedy-Stoskopf 1993).

Stress caused by transport and handling has been shown to result in immunosuppression in channel catfish. In a study by Klesius (1992b) channel catfish

fingerlings known to have suffered from clinical ESC were to be used as control fish, but approximately one-third of the fish developed clinical signs associated with ESC and died 3 d after moving them into the laboratory. Klesius determined that a combination of ESC and transport-induced stress caused the mortalities. Wise et al. (1993) subjected channel catfish to a 30-min standardized confinement treatment—intended to simulate current handling practices used in channel catfish culture—and 60 min later to static immersion in *E. ictaluri* for 24 or 48 h. Unstressed fish were also subjected to static immersion for 24 or 48 h. Mortalities were significantly higher in stressed fish and those exposed to *E. ictaluri* for 48 h. Ciembor et al. (1995) exposed channel catfish sac fry to *E. ictaluri* by bath immersion with stress (induced by handling and netting) or bath immersion without stress. Three weeks after exposure, 52.7% of fish from the stressed group tested positive for *E. ictaluri*, while only 15.7% of fish from the unstressed group tested positive.

Stress caused by low dissolved oxygen or rapid changes in water temperature has also been shown to result in immunosuppression in channel catfish. Mqolomba and Plumb (1992) experimentally infected channel catfish with *E. ictaluri* and subjected them to one of three treatments: 25 C with aeration, 25 C with no aeration, and variable temperature (18-23°C) with no aeration. Concentrations of *E. ictaluri* within tissues were significantly higher in fish held in 25 C water with no aeration and in variable temperature water with no aeration than in fish held in 25 C water with aeration.

Immunosuppression associated with temperature fluctuations may contribute to the seasonality of ESC outbreaks. Outbreaks of ESC tend to occur in the late spring and early fall, when temperature fluctuations most often occur. Temperature fluctuations might compromise the immune system of carrier fish, leading to development of ESC in

these fish and transmission to susceptible fish (Klesius 1992a). Mention has already been made of the fact that spring and fall temperatures in the southeastern United States correspond to the optimal temperatures for ESC outbreaks (Tucker and Robinson 1990). Another possible reason for the seasonality of ESC may be that high feed allotments offered during the fall can lead to low oxygen and poor water quality, which in turn lead to stress and immunosuppression.

Immune Response of Channel Catfish to *E. ictaluri*

A study by Ainsworth and Dexiang (1990) suggested that complement may play a role in protecting against ESC by opsonizing *E. ictaluri*. The researchers performed phagocytic assays by mixing neutrophils, *E. ictaluri*, and normal or inactivated serum. The authors calculated percent phagocytosis by counting the number of neutrophils containing at least one bacterium out of 100 neutrophils counted. Percent phagocytosis of the normal serum treatment (63.31%) was significantly higher than that of the inactivated serum treatment (8.63%). The authors suggested that opsonization by specific antibodies or complement may have accounted for the difference, but pointed out that only 10% of fish in the pond in which experimental fish were sampled had detectable titers of antibodies specific to *E. ictaluri*.

While complement may play a role in protecting against ESC by opsonizing *E. ictaluri*, a study by Ourth and Bachinski (1987) indicated that it is unlikely that complement protects against ESC by lysing *E. ictaluri*. Knowing that sialic acid hindered the binding of complement factor B of the alternative complement pathway (ACP), the researchers determined sialic acid content and examined bactericidal activity by channel catfish serum incubated at 30 C for 1 h on fifteen Gram-negative fish

pathogens, including *E. ictaluri*, and non-pathogens. Ourth and Bachinski (1987) found that non-pathogens contained 90 μg or less per 100 mg of dried bacteria and had 100% bactericidal activity produced against them. Pathogens contained greater than 90 μg per 100 mg of dried bacteria had 0-13% bactericidal activity produced against them. The sialic acid content of *E. ictaluri* was 123 μg per 100 mg of dried bacteria and had 0% bactericidal activity produced against it. Thus, sialic acid may be a mechanism of resistance of *E. ictaluri* against the ACP.

A few studies have shown a positive correlation between *E. ictaluri*-specific antibody titers and protection against ESC. Vinitnantharat and Plumb (1993) took serum samples from channel catfish that had survived a natural *E. ictaluri* infection and fin-clipped each fish to allow for later identification. After assaying serum antibodies, fish were divided into four groups based on antibody titers: negative (0), low (<128), medium (256-512), and high (>1024). Treatment groups were challenged by intraperitoneal injection and mortality rates were compared. A strong positive correlation (regression coefficient = 0.95) was observed between mortality rate and antibody titer. The researchers determined that antibody titers greater than 1:256 were capable of providing protection. However, it should be noted that challenge was performed by intraperitoneal injection, which does not offer the best simulation of natural exposure. Plumb and Vinitnantharat (1993) vaccinated fry by immersion bacterin, stocked them into ponds, and offered an oral bacterin booster to a portion of the previously vaccinated fry. Some fish were removed before harvest and subjected to a laboratory challenge. Survival and antibody titers were significantly higher in fish vaccinated by either regime than in control fish.

Humoral immunity may play a role in protecting against ESC by opsonizing *E. ictaluri*. Waterstrat et al. (1991) performed phagocytic, bactericidal, and chemiluminescent assays to determine the *in vitro* responses of channel catfish neutrophils to *E. ictaluri*. Although phagocytosis of *E. ictaluri* by neutrophils was made evident by light and electron microscopy, the number of intracellular bacteria remained either constant or increased over the time period. However, a control assay—containing a mixture of cells, bacteria, and immune catfish serum—exhibited a decline in viable *E. ictaluri* cells, indicating that serum components or serum components working in conjunction with neutrophils had bactericidal activity. Using an oxidative-microbiocidal activity as an indicator of the chemiluminescent response of neutrophils to *E. ictaluri* opsonized with immune serum was twice the response of neutrophils to *E. ictaluri* opsonized with non-immune serum. The authors suggested that, although intracellular killing was not observed, the extracellular killing of *E. ictaluri* observed in the control assay may have been due to serum components, extracellular products liberated from the neutrophil, or by a combination of both cellular and serum factors. The increased chemiluminescent response observed with immune serum may have been due to increased extracellular killing.

Although a few studies have showed a positive correlation between *E. ictaluri*-specific antibody titers and protection against ESC, a majority have showed a lack of or negative correlation between antibody titer and survival, indicating that humoral immunity is probably not the only arm of adaptive immunity acting to protect against ESC. Klesius and Sealy (1995) passively immunized naïve channel catfish with serum from immune fish and challenged them. Although antibody titers were confirmed to be

significantly higher in passively immunized fish than in control fish, protection was not observed. The researchers suggested that antibody titers may not have been as high as those reached in the study by Vinitnantharat and Plumb (1993), which might account for the disparity between the two studies. Alternatively, they point out that antibodies may be more important in providing protection when fish are challenged intraperitoneally, as in Vinitnantharat and Plumb's study (1993), than when fish are challenged by immersion.

In a laboratory experiment, Plumb et al. (1986) found that vaccination by immersion in sonicated bacteria offered greater protection than that found in groups vaccinated by other methods, even though it resulted in the lowest antibody titers. In a field experiment, Plumb et al. (1986) vaccinated fish by immersion or by intraperitoneal injection, with either sonicated or whole cell *E. ictaluri*. Vaccinated fish were grown out in ponds and challenged under laboratory conditions at harvest. Vaccination by immersion in sonicated bacteria offered the greatest protection, even though it resulted in the lowest antibody titers. Plumb et al. (1994) stocked fry into earthen ponds at low, intermediate, or high densities, and later vaccinated them orally. The researchers found that survival within a vaccinated or non-vaccinated regime increased as stocking density decreased. However, antibody titers were low in all treatments, regardless of stocking density or vaccination regime.

In a laboratory study, Wolters et al. (1996) vaccinated channel, blue, and hybrid catfish by immersion or injection with live *E. ictaluri* and challenged them. When serum antibodies were later assayed, antibody titers were negatively correlated with survival, regardless of the challenge method. Thune et al. (1997) performed a laboratory study to evaluate the ability of vaccination by immersion in bacterin, immersion in bacterin

followed by an oral booster, or injection to induce a humoral immune response. Antibody titers were monitored throughout the study by periodically taking serum samples. All vaccination regimes resulted in measurable antibody titers; however, a large percentage of seropositive fish in a group did not correlate with increased survival in that group.

The intracellular nature of *E. ictaluri* suggests that cell-mediated immunity, working in conjunction with innate and humoral immunity, would be important in providing protection. Cell-mediated immunity is the major protective immune response against most intracellular pathogens (Abbas and Lichtman 2003e). Humoral immunity acting alone was shown to fail in protecting mice and cattle against the intracellular pathogen, *Brucella abortus* (Montaraz and Winter 1986). Klesius (1992a) pointed out that, just as both the humoral and cell-mediated components of the adaptive immune system cooperate to provide protection in mammals, the same is probably true in catfish.

Several studies have shown that cell-mediated immunity is important in providing protection against ESC. Shoemaker and Klesius (1997) vaccinated channel catfish fingerlings with live *E. ictaluri* and later challenged them. Antibody titers and bactericidal activity by peritoneal macrophages were assayed following challenge. Survival, antibody titers, and macrophage bactericidal activity were significantly higher in vaccinated fish than in non-vaccinated fish. Shoemaker et al. (1997) isolated macrophages from naïve channel catfish and channel catfish surviving exposure to live *E. ictaluri*. Isolated macrophages were incubated with live *E. ictaluri*, and macrophage bactericidal activity was assayed. Macrophage bactericidal activity was significantly

higher in immune fish than in naive fish, indicating that macrophages isolated from immune fish had been activated by T-helper cells.

In a separate study, Shoemaker et al. (1997) incubated macrophages isolated from naïve or immune channel catfish in opsonized or unopsonized *E. ictaluri*. The bactericidal activity of macrophages isolated from immune fish incubated in opsonized bacteria was higher at 2.5 h post-exposure than that of macrophages incubated in unopsonized bacteria. However, the bactericidal activity of macrophages isolated from naïve fish incubated in opsonized bacteria was suppressed at 1.5, 3, and 5 h post-exposure, compared to that of macrophages incubated in unopsonized bacteria. Shoemaker et al. (1997) suggested that activation of macrophages occurred in immune fish, allowing the macrophages to engulf opsonized bacteria. Klesius and Shoemaker (1997) suggested that, upon a secondary exposure to *E. ictaluri*, antibodies generated by immune fish may opsonize the bacterium, facilitating its engulfment by activated macrophages, where the bacterium is then killed.

Antonio and Hedrick (1994) treated carrier fish with Kenalog, an immunosuppressive drug, and reexposed them to *E. ictaluri*. Two weeks after challenge, the researchers attempted to recover the bacterium and assayed serum antibodies. Some fish died from the reexposure, despite having high antibody titers. In addition, the researchers were able to recover the bacterium from some surviving fish, regardless of their antibody titers. Antonio and Hedrick (1994) concluded Kenalog may have suppressed one of the components of cell-mediated immunity important in providing protection against *E. ictaluri*.

Sheldon and Blazer (1991) vaccinated channel catfish by intraperitoneal injection of killed *E. ictaluri*, offered vaccinated and non-vaccinated fish diets of varying lipid content, and later isolated macrophages from both vaccinated and non-vaccinated fish. Isolated macrophages were exposed to *E. ictaluri* at optimal and suboptimal temperatures to assay bactericidal activity. Immunization generally resulted in substantial enhancement of bactericidal activity, regardless of diet and temperature. Phagocytic assays were also performed with isolated macrophages at optimal and suboptimal temperatures. Neither diet nor immunization increased phagocytosis at the suboptimal temperature, even though serum antibodies were high. The authors suggested that macrophages from immune fish showed increased bactericidal activity, not because of opsonization by antibody, but because macrophages in immune fish had undergone activation.

Control Methods

Fish farmers have attempted to control ESC outbreaks by offering medicated feed (Tucker and Robinson 1990). Romet® (a 5:1 mixture of sulfadimethoxine and ormetoprim) and Aquaflor® (florfenicol) are the only antibiotics approved by the FDA for treating ESC (Gaunt et al. 2003). Various problems are inherent in treating with antibiotics. Antibiotic treatment is expensive and fish affected with ESC typically reduce their feeding activity, leading to antibiotic delivery problems (Klesius and Shoemaker 1998). In addition, there is the potential for antibiotic resistance to develop. Plasmid-mediated resistance of several strains of *E. ictaluri* to Romet has been reported (Starliper et al.1993). At one time, the effectiveness of Romet was reduced due to palatability problems, but that problem has since been corrected. Poe and Wilson (1989) found that

feed consumption decreased as the amount of Romet incorporated into feed increased, due to palatability problems caused by ormetroprim. A study by Robinson et al. (1990) showed that the problem could be corrected by increasing the amount of fish meal in the feed from 4% to 16%.

Florfenicol has been shown to be effective in treating ESC and palatable when incorporated into feed in two separate studies conducted by Gaunt et al. (2003). In the palatability study, the researchers placed channel catfish into tanks, and over a 10-d period, fed them feed containing different amounts of florfenicol, such that fish were offered 0, 10, 20, 40, or 100 mg of florfenicol per kg of body weight. Each day tanks were given a numerical score based upon feeding activity, and at the end of the 10-d feeding period, these scores were used to compute palatability scores for each treatment group. At the end of the 10-d feeding period, fish were removed from the tanks and weighed. No significant differences were observed in palatability scores or weight gain among the treatment groups. In the efficacy study, Gaunt et al. (2003) stocked channel catfish into tanks and compared mortalities among five treatment groups: (1) not challenged with *E. ictaluri* and fed unmedicated feed, (2) challenged with *E. ictaluri* and fed unmedicated feed, (3) challenged and fed florfenicol at the rate of 10 mg per kg of body weight, (4) challenged and fed florfenicol at the rate of 20 mg per kg of body weight, and (5) challenged and fed florfenicol at the rate of 40 mg per kg of body weight. The cumulative mortality rate for each treatment group was 2.5, 57.5, 0, 1.25, and 1.25%, respectively, such that the mortality rate of each treated group was significantly lower than the untreated, challenged group.

Fish farmers have attempted to control ESC by restricting the amount of feed offered. Wise and Johnson (1998) confirmed the effectiveness of practicing restricted feeding in controlling ESC. In their study, fish were completely withheld from feed; offered medicated feed for five consecutive days and non-medicated feed thereafter; or offered medicated or non-medicated feed every day, every other day, or every third day. Fish completely withheld from feed or offered Romet-medicated feed every other day or every third day had the highest survival. Wise and Johnson (1998) hypothesized that a reduction in the amount of feed offered limits exposure to *E. ictaluri* through ingestion. Although restricted feeding can mitigate the effects of ESC, it results in a loss of production (Wise et al. 2000, 2004).

Fish farmers have also attempted to control ESC by practicing good management (Tucker and Robinson 1990). Lack of adequate nutrients and stress can result in immunosuppression. Therefore, providing food with adequate nutrients, acclimating fish to water properly, and maintaining good water quality reduce incidences and severity of ESC.

Due to the lack of efficient control measures, interest in developing a vaccine for ESC began growing in the late 1980s. Vaccines had been in use in the salmonid and trout industry for years, beginning in the early 1960s with the development of the first practical, commercial vaccine for enteric redmouth (Ross and Klontz 1965). However, a serious obstacle to developing a vaccine was the heterogeneous serology of most pathogens affecting fish (Thune et al. 1994). Fortunately, early studies indicated that isolates of *E. ictaluri* were biochemically and serologically homogenous. Biochemical characterization of 119 isolates of *E. ictaluri* revealed very few differences among them

(Waltman et al. 1985). Plumb and Vinitnantharat (1989) examined 40 isolates and found very few biochemical or serological differences. A later study by Panangala et al. (2005) showed *E. ictaluri* isolates to be genetically homogenous, with respect to the 16S-23S rRNA intergenic spacer region (ISR). The researchers amplified the 16S-23S rRNA ISR sequences of 19 *E. ictaluri* isolates by polymerase chain reaction, and after performing sequence analyses, found no differences among the ISR sequences.

Another challenge facing researchers was that it would be necessary to administer any vaccine developed for channel catfish orally or by immersion. Vaccination in salmonid and trout culture had typically been performed by intraperitoneal injection of bacterins (Klesius and Shoemaker 1998). Intraperitoneal injection generates large immune responses, because it allows for the use of adjuvants (Ellis 1988) and guarantees that each fish in a group is exposed to and receives approximately the same number of bacteria (Ciembor et al. 1995). The high individual worth of salmonids and trout had allowed for high labor costs associated with injecting each fish. On the other hand, the low individual worth of channel catfish would prohibit development of vaccines administered by injection.

Development of a Vaccine

Vaccines are preparations of nonpathogenic microbial antigen, either dead or live, that are administered by injection, orally, or by immersion. Dead antigens may consist of microbial extract, inactivated organisms, or bacterins that are prepared by heat or formalin treatment. Live bacteria must be attenuated to render them nonpathogenic. Vaccines protect against disease by inducing the adaptive immune system to develop long-lived effector and memory cells. Although vaccines protect against disease, they do

not necessarily protect against establishment of an asymptomatic carrier state (Ellis 1988).

Oral and Immersion Bacterin Vaccine Trials

Following the success of bacterin vaccines developed for coldwater species, immersion and oral bacterins were developed for vaccinating channel catfish against ESC. An immersion bacterin was developed by BIOMED Inc. (Bellevue, WA, USA) (Shoemaker and Klesius 1997). An oral bacterin was developed by Escogen, Aqua Health Ltd. (Charlestown, PEI, Canada) (Shoemaker and Klesius 1997).

Oral and immersion bacterins failed to consistently provide protection. An early study by Plumb and Vinitnantharat (1993) found bacterin vaccines to provide protection. The researchers stocked channel catfish fry vaccinated by immersion or immersion followed by an oral booster into ponds. Some fish were removed before harvest and subjected to a laboratory challenge. The relative percent survival (RPS) in fish vaccinated by immersion or immersion followed by an oral booster was 93.1 and 96.6, respectively. At harvest survival of groups vaccinated by immersion followed by an oral booster was higher than in groups vaccinated by immersion alone and in control groups.

Thune et al. (1994) had mixed successes with bacterin vaccine trials. In a preliminary pond study, the authors vaccinated channel catfish fry by immersion or orally, stocked them into ponds, and gave an oral booster to a portion of the fry vaccinated by immersion. Some fish were removed before harvest and subjected to a laboratory challenge. Only vaccination by immersion resulted in an RPS value above 50. At harvest no significant difference in survival was observed among treatment groups.

In a pond study conducted in 1987-1988, Thune et al. (1994) vaccinated channel catfish fry by immersion or orally, stocked them into ponds, and offered an oral booster to a portion of the fry vaccinated by immersion. At harvest, RPS values were calculated to be 57.4, 50.3, and 53.5, respectively, for fish vaccinated by immersion, orally or by immersion followed by an oral booster. In two separate pond studies conducted in 1989-1990 and 1990-1991, Thune et al. (1994) vaccinated fry by immersion, stocked them into ponds, and offered them an oral booster. In the pond study conducted in 1989-1990, no significant difference in survival was observed between vaccinated and non-vaccinated fish. In the pond study in 1990-1991, survival was significantly higher in vaccinated fish, but the overall RPS value was only 35.1.

In later unpublished laboratory studies, Thune et al. was unable to reproduce any of the successes in their earlier pond studies (Thune et al. 1997). Thune et al. (1994) suggested the apparent success of the vaccine in the 1987-1988 field trial might have been due to the ability of naturally occurring *E. ictaluri* to stimulate primary or secondary immune responses. This suggestion may also serve to explain the successes observed in the field study of Plumb and Vinitnantharat (1993), who isolated *E. ictaluri* from two apparently healthy fish from their study indicating that *E. ictaluri* was present in ponds used for their study.

Other researchers also had mixed success with bacterin vaccine trials. Plumb et al. (1994) stocked fry into earthen ponds at low, intermediate, or high densities, and later vaccinated them orally. At harvest, survival was not significantly higher in vaccinated fish at any of the stocking densities. Some fish from harvest were subjected to a

laboratory challenge. An RPS value greater than 50 was observed only in vaccinated fish stocked at the highest density.

Shoemaker and Klesius (1997) stocked juvenile channel catfish into aquaria, vaccinated them by immersion or oral bacterins, and later challenged them. No significant difference in survival was observed between fish vaccinated by either regime and non-vaccinated fish. In a second experiment, Shoemaker and Klesius (1997) challenged fish vaccinated by immersion bacterin. No significant difference in survival was observed between vaccinated fish and non-vaccinated fish. In a third experiment Shoemaker and Klesius (1997) challenged fish vaccinated by immersion bacterin followed by an oral bacterin booster. Again, no significant difference in survival was observed between vaccinated fish and non-vaccinated fish.

Inconsistent success with immersion and oral bacterin trials may have been observed for a variety of reasons. The heat inactivation needed to prepare both immersion and oral bacterins often severely denatures surface proteins (Nusbaum and Morrison 1996). Oral bacterins tend to expose fish to different doses of antigen, due to fish consuming different amounts of feed (Ellis 1988); are often destroyed within the digestive system before the immune response has time to respond (Ellis 1988); and may leach from feed into surrounding water (Thune et al. 1994). Even if antigen does make it through the stomach without being destroyed, it may not be absorbed in the intestines. Shoemaker and Klesius (1997) suggested that immersion bacterins may fail to expose fish to a sufficient amount of antigen and macrophages may be unable to process bacterin and present it to B cells. Results from a study conducted by Nusbaum and Morrison (1996) supported the former suggestion of Shoemaker and Klesius (1997). Nusbaum and

Morrison (1996) immersed channel catfish in water containing heat-killed radiolabeled *E. ictaluri* and attempted to detect the presence of radiolabeled bacteria in various organs where *E. ictaluri* typically enters (i.e. nares, gills, and gut). No levels of radiolabeled bacteria above background levels could be detected, leading the authors to suggest the need to reassess the effectiveness of oral and immersion bacterins. Probably the most significant factor contributing to the inconsistent success of oral and immersion bacterins was their inability to induce a cell-mediated immune response (Klesius 1992a), which plays a major role in protecting against intracellular pathogens (Abbas and Lichtman 2003e).

Virulent, Live Vaccine Trials

Due to the failure of bacterins to provide consistent protection, the search for an effective live vaccine was begun. Unlike bacterins, live vaccines are capable of inducing both the humoral and cell-mediated components of immunity (Klesius 1992a). Vaccination of channel catfish by immersion in virulent, live *E. ictaluri* was found to result in protection against later exposures. Shoemaker and Klesius (1997) stocked juvenile channel catfish into aquaria, vaccinated them with virulent, live *E. ictaluri*, and later challenged them. Survival in vaccinated fish was significantly higher than in non-vaccinated fish. Klesius and Shoemaker (1997) immunized channel catfish with a single isolate of virulent, live *E. ictaluri* and later challenged them with the same isolate. An RPS value of 88.3 was calculated.

Although virulent, live vaccines were found to be protective against ESC, isolates were shown to vary in their ability to provide protection. Klesius and Shoemaker (1997) immunized channel catfish with a single isolate of virulent, live *E. ictaluri* and later

challenged them with heterologous isolates. Protection was not observed against challenge with one of the isolates. In a separate experiment, channel catfish were immunized with heterologous isolates of virulent, live *E. ictaluri*, and later challenged with a single isolate. Not all isolates used for immunization resulted in protection against the single isolate. The results of both experiments led the authors to suggest that oral vaccination trials of the past (Thune et al. 1994; Thune et al. 1997; Plumb et al. 1994; Shoemaker and Klesius 1997) may have failed due to oral bacterins consisting of only one isolate.

The results of Klesius and Shoemaker (1997) seemed to be inconsistent with studies indicating that isolates of *E. ictaluri* were homogenous isolates (Waltman et al. 1986; Plumb and Vinitnantharat 1989). However, Klesius and Shoemaker (1997) suggested that, although isolates may be homogenous according to established techniques designed for assessing the homogeneity of mammalian pathogens, fish may not necessarily recognize them as being homogenous (Mutheria et al. 1993; Hasting and Ellis 1988).

Attenuated, Live Vaccine Trials

Although exposure to virulent *E. ictaluri* was shown to induce a cell-mediated response and provide protection against subsequent exposures, using virulent, live *E. ictaluri* as a vaccine obviously would have failed to fulfill the "safe" criterion of an effective vaccine. Therefore, the search for an attenuated, live vaccine that would not result in significant mortalities upon administration was begun. In time Klesius and Shoemaker (1998) produced a live, attenuated *E. ictaluri* vaccine (*E. ictaluri* RE-33).

Intervet, Inc.® licensed and produced the vaccine under the name AQUAVAC-ESC® (Shoemaker et al. 2002).

In considering how to attenuate *E. ictaluri*, Klesius and Shoemaker (1998) took into account that other researchers were able to produce an effective vaccine against furunculosis in rainbow trout by removing the O-side chain of LPS in *Aeromonas salmonicida* (Thornton et al. 1994). The researchers also took into account the findings of Klesius and Shoemaker (1997), which indicated that a successful vaccine would be composed of heterologous isolates. Therefore, Klesius and Shoemaker (1998) hypothesized that an O-antigen deficient *E. ictaluri* vaccine would protect channel catfish against ESC. To remove the O-side chain, a weakly virulent isolate of *E. ictaluri* (EILO) originally isolated from walking catfish in Thailand, was passed through increasing concentrations of rifampicin 33 times. The O-antigen deficient EILO isolate was designated RE-33.

Klesius and Shoemaker (1998) demonstrated the safety of the RE-33 vaccine. Channel catfish (3-9 mos of age) were vaccinated by immersion at 200 times the vaccine dose (2.0×10^7 CFU/mL) for 30 times longer than the vaccine exposure time (60 min). No mortalities or clinical signs arose following immersion. Procedures were performed to determine if reversion to virulence occurred. No reversion was observed.

Attenuated, live vaccines induce low-level infections that persist in the host for a somewhat lengthy period of time, and then are cleared by the host. Persistence in the host provides continual antigenic stimulation, and therefore, greater immunity than if the vaccine did not persist for longer periods of time. Clearance is obviously necessary for safety purposes (Wise and Terhune 2001). Therefore, Klesius and Shoemaker (1998)

evaluated survival and clearance of *E. ictaluri* after administration of the vaccine. Their evaluation revealed that *E. ictaluri* survived in vaccinates only 14 d before being cleared. They attributed early clearance to their unpublished findings that complement was more effective in killing *E. ictaluri* RE-33 than virulent *E. ictaluri*.

The ability of the RE-33 vaccine to induce long-lasting protection under laboratory conditions was shown (Klesius and Shoemaker 1998). Channel catfish 3-9 mos of age were vaccinated by immersion in RE-33 at 1.0×10^5 CFU/mL for 2 min and challenged with 13 isolates of virulent *E. ictaluri* 14 d post-vaccination. The RE-33 vaccine was successful in providing protection to 8 of 13 isolates. When challenge occurred at a later time, protection was demonstrated in two additional isolates. Protection lasted at least 4 mos, as demonstrated by challenge. A significant point is that *E. ictaluri* RE-33 was not found to induce large antibody responses, which supports earlier studies showing a lack of or negative correlation between antibody titers and survival.

The AQUAVAC-ESC vaccine has also been shown to provide protection under laboratory conditions when administered to young fry. The ability to vaccinate fry before moving them into production ponds is desirable, because it is difficult to recover fry once they have been released (Petrie-Hanson and Ainsworth 1999). Wise and Terhune (2001) vaccinated channel catfish fry at 12 d post-hatch and later challenged them. Survival was significantly higher in vaccinated fry than in control fry. Shoemaker et al. (1999) vaccinated channel catfish fry by immersion at 7 d post-hatch and later challenged them. Relative percent survival ranged from 58.4 to 77.5.

The AQUAVAC-ESC has even been shown to provide protection when administered in ovo. Shoemaker et al. (2002) vaccinated eyed channel catfish eggs and challenged hatch fry 60 d post-vaccination. Relative percent survival was 59.7%.

Prior to studies indicating that *E. ictaluri* RE-33 could induce protection in young fry, questions had arisen as to whether the immune system of young fry was developed enough to respond to a vaccine. Petrie-Hanson and Ainsworth (1999) vaccinated channel catfish fry at 1, 2, 3 or 4 weeks of age or 2, 3, 4, 5, or 6 mos of age with live *E. ictaluri* and assayed antibodies 2 weeks post-vaccination. A humoral immune response greater than that of controls was not observed in fish younger than 4 weeks of age. However, studies have shown that cell-mediated immunity, not humoral immunity, is important in providing protection against ESC. Wise and Terhune (2001) suggested that *E. ictaluri* RE-33 may persist in tissues until the immune system fully develops. As support, they pointed out the positive correlation between exposure dose, which relates to the establishment of low-level infections, and survival to challenge with a virulent strain in their study.

The success of the AQUAVAC-ESC vaccine has been shown to depend on exposure dose. Wise et al. (2000) vaccinated channel catfish fingerlings 72 d post-hatch by a 30-min bath immersion at concentrations of 1.0×10^6, 1.0×10^7, or 2.0×10^7 CFU/mL and later challenged them in the laboratory and in the field in floating net pens. Survival was significantly higher in fish vaccinated at the high and intermediate doses as compared to the low dose in both the laboratory and field challenges. No significant difference was observed in survival between fish vaccinated at the intermediate and high doses. Wise and Terhune (2001) vaccinated channel catfish fry at 12 d post-hatch by a

2-min bath immersion at doses of 2.5 x 10^5, 2.5 x 10^6, or 2.4 x 10^7 CFU/mL and later challenged them. The researchers were able to recover *E. ictaluri* RE-33 from a greater percentage of fry vaccinated at the high and intermediate doses than fry vaccinated at the low dose. Regardless of dose, the researchers were able to recover the bacterium from the greatest percentage of fry between 1 and 6 d post-vaccination. After challenge, survival was significantly higher in fry vaccinated at the high and intermediate doses than in fry vaccinated at the low dose and control fish. No significant difference in survival was observed between fry vaccinated at the low dose and control fish.

Evaluation of the AQUAVAC-ESC vaccine under pond conditions has shown mixed results. Wise et al. (2000) vaccinated channel catfish fingerlings 72 d post-hatch at 1.0 x 10^7 CFU/mL, and later challenged them in the laboratory and in the field in floating net pens. In both the field and laboratory challenges, survival was significantly higher in vaccinated fish than that in control fish. However, data from a study by Carrias (2005), in which the effect of vaccinating fry with AQUAVAC-ESC on survival was evaluated under normal pond conditions, was inconclusive. Carrias (2005) vaccinated channel catfish fry at 10 d post-hatch or 32 d post-hatch, stocked them into primary nursery tanks, and grew them out to fingerling stage. Mean survival of 32-d vaccinates was significantly higher than controls, but mean survival of 10-d vaccinates was not significantly higher. The author suggested that a significant difference in survival of the 10-d vaccinates may not have been observed, due to columnaris infections mixed with ESC.

For a fish vaccine to be economically beneficial, savings associated with the vaccine must outweigh costs of administering the vaccine. Lillehaug (1989) developed a

formula to calculate the break-even point between costs of vaccination and economic losses due to disease. Factors used to calculate costs associated with administering the vaccine include: the man-hours required for the vaccination method, workers' wages, the volume of vaccine consumed, the price of the vaccine per liter, and additional costs (necessary equipment, anesthetics, and the value of fish lost due to side effects). Factors used to calculate savings associated with vaccine include: the expected mortality, the RPS of the vaccine, the mean weight of fish at slaughter, the price obtained for the fish per kg, the FCR, and the price of feed per kg. The formula does not include the possible savings in reduced drug costs associated with treating an outbreak of the disease.

To date no studies have evaluated under pond conditions the relative survival of channel catfish immunized with AQUAVAC-ESC as fingerlings. Administration of the vaccine to fingerlings at the dose recommended by Intervet Inc. currently is not economically feasible, because the weight of the fish and the volume of water required to hold the fish requires a considerable volume of vaccine. However, fingerlings can be immersed in vaccine for longer periods of time than fry, because fry are more susceptible to crowding stress than fingerlings (Tucker and Robinson 1990). Therefore, increasing vaccine immersion time from the 17-min period currently recommended to a 4- or 8-h period may allow a lower dose of vaccine to provide protection against ESC. Administering the vaccine to fingerlings would be beneficial in that it would allow farmers the option of (1) vaccinating naïve fingerlings while moving them to food-fish ponds and (2) giving a booster of the vaccine to fingerlings previously vaccinated as fry.

Low costs would be associated with administering the vaccine to fingerlings. Fingerlings are often transported from one farm to another for periods of approximately

4 h, leaving open the possibility of administering the vaccine in route. In route vaccination would require no additional man-hours, no anesthetics, and no additional equipment. Vaccination of fingerlings can easily be integrated into the production cycle, as they are already being handled and gathered up for transfer from fingerling ponds to food-fish ponds. Administering the vaccine to fingerlings may provide savings in that, although food-fish typically suffer low mortalities from the chronic form of ESC, larger fish are worth more per individual than smaller fish (Wise et al. 2004). The objectives of this study were to (1) evaluate a more economically feasible dose of AQUAVAC-ESC to administer to channel catfish fingerlings for protection against ESC, (2) evaluate under normal pond conditions the relative survival of fish immunized as fry with AQUAVAC-ESC and given a booster of the vaccine as fingerlings, and (3) evaluate the long-term benefits of vaccination to determine if a booster is necessary for life-long protection.

MATERIALS AND METHODS

Laboratory Experiment 1

Channel catfish fingerlings (NWAC 103 strain, ~ 34 g) were stocked into 18, 32-L aquaria at a density of ten fish/tank and water temperature maintained at 24 C. Fish used for the experiments had been maintained in flow-through tanks, with water derived from a well, at the North Auburn S6 laboratory. No previous outbreaks had occurred among the fish, nor had any samples tested positive for *E. ictaluri*-specific antibodies. Fish were fed to satiation daily throughout the duration of the experiment. Water flow to each tank was shut off 1 h prior to vaccination, and salt was added to reach a chloride concentration of ~ 2 ppt. According to manufacturer's directions, one vial of vaccine reconstituted in 50 mL of water and added to 3.78 L of water creates an approximate concentration of 1.0×10^7 CFU/mL. Fish were vaccinated at target concentrations of 1.0×10^4, 1.0×10^5, or 1.0×10^6 CFU/mL by adding 0.43, 4.3, or 43 mL of reconstituted vaccine, respectively, to tanks. Water flow to each tank was resumed either 4 or 8 h post-vaccination. Six treatment groups (three replicate tanks each) were created in a 3x2 factorial design, with the main factors being dose and time. Reconstituted vaccine was enumerated by standard plate count methods on brain heart infusion (BHI) agar incubated at 27 C for 48 h. Water samples (1.0 mL) from each replicate aquaria within each

treatment were pooled and vaccine isolate enumerated as above. Final vaccine dose concentrations were 2.44 x 10^4, 3.48 x 10^5, or 3.16 x 10^6 CFU/mL.

Nineteen days post-vaccination serum was collected from five fish per tank. Fish were anaesthetized with Finquel (Argent Chemical Laboratories, Redmond, Washington, USA) and blood was collected from the caudal sinuses using Vaccutainer® tubes (Becton Dickinson and Co., Franklin Lakes, New Jersey, USA). Blood samples were held at room temperature for 1 h and thereafter refrigerated for 24 h. Serum from samples was decanted into microfuge tubes and centrifuged at 1000 rpm for 5 min to remove any remaining cellular debris. Serum from centrifuged tubes was decanted into a second set of microfuge tubes and frozen at -80°C for later analysis.

Antibody titers of collected serum samples were determined by an agglutination assay described by Contrath (1972). A two-fold dilution series, using PBS with 0.05% tween 80, was prepared for each sample in a 96-well plate. Two positive and two negative control wells, using known positive and negative sera, were included. A virulent strain (S97-773) of *E. ictaluri* was grown to stationary phase and killed with 2% formalin to serve as antigen. Equal amounts of antigen were added to each well in the dilution series. Plates were allowed to sit at room temperature overnight. The reciprocal of the highest serum dilution at which agglutination occurred was considered to be the antibody titer of the sample.

Laboratory Experiment 2

Channel catfish fingerlings (NWAC 103 strain, ~34 g) were stocked into nine, 32-L aquaria at a density of 15 fish/tank. Fish were acclimated at 18 C for two weeks and maintained at this temperature for the duration of the experiment. Fish were fed to

satiation daily. Water flow to each tank was shut off 1 h prior to vaccination, and salt was added to reach a chloride concentration of ~ 2 ppt. Fish were vaccinated at target concentrations of 1.0×10^4, 1.0×10^5, or 1.0×10^6 CFU/mL according to the procedures described previously. Water flow to each tank was resumed 4 h post-vaccination. Enumeration procedures for the vaccine were followed as previously described. Reconstituted vaccine concentration was determined to be 1.5×10^9 CFU/mL. Twenty-one days post-vaccination serum was collected from ten fish per tank and agglutinating-antibody titers were determined according to the procedures described above.

Laboratory Experiment 3

Channel catfish fingerlings (NWAC 103 strain, ~ 15 g) were stocked into 20, 32-L tanks at a density of 12 fish/tank. Fish were fed to satiation daily throughout the duration of the experiment. Water level in each tank was reduced to 15 L, flow was shut off 1 h prior to vaccination, and salt was added to reach a chloride concentration of ~ 2 ppt. According to manufacturer's directions, one vial of frozen vaccine added to 19 L of water creates an approximate concentration of 1.0×10^7 CFU/mL. A stock of vaccine was made by adding three vials to 5.7 L of water to create an approximate concentration of 1.0×10^8 CFU/mL. Four treatment groups (five replicate aquaria each) were created by vaccinating fish at target concentrations of 1.0×10^4, 1.0×10^5, or 1.0×10^6 CFU/mL by adding 0.15, 15, or 150 mL of vaccine stock, respectively, to replicate tanks. Control tanks were also created in which no vaccine was added. Water flow to each tank was resumed after 4 h post-vaccination. Water temperature at time of vaccination was 25 C. Final vaccine concentrations in tanks were determined to be 1.95×10^4, 3.80×10^5, or 1.95×10^6 CFU/mL.

Sixty days post-vaccination, fish were challenged with a virulent strain of *E. ictaluri* (S97-775) for 1 h. A culture of *E. ictaluri* was grown in BHI broth at 25 C for 24 h. Subsequent plating of serial dilutions of the challenge culture revealed that fish were challenged at a concentration of 1.2×10^5 CFU/mL. Dead fish were removed daily, and necropsies were performed on at least five suitable mortalities from each tank to verify *E. ictaluri* infection. Bacterial pathogens were isolated from the liver on BHI and Hsu-Shotts agar plates to test for the presence of *E. ictaluri* or *Flavobacterium columnare*. Negative results for indole production and cytochrome oxidase biochemical tests were used to confirm the presence of *E. ictaluri* (Hawke et al. 1981). The challenge was stopped after 16 d.

Pond Study

Twenty 0.04 ha ponds at the Auburn University Fisheries Experiment Station were randomly assigned to one of the four treatments. One week prior to stocking, powdered agricultural limestone was added to each pond at a rate of 2,260 kg/ha, and then ponds were filled with water from a reservoir, with screens placed on inlet pipes to prevent entry of wild fish.

Channel catfish fingerlings (NWAC 103 strain) used in a previous study (Carrias 2005) were harvested from ponds located at the North Auburn Fisheries Research Station for use in the present study. Fingerlings had been divided among individual ponds, according to two treatment groups, and over-wintered since December 2004. Four ponds contained fingerlings vaccinated with AQUAVAC-ESC® as fry at 10 d of age and four ponds contained fingerlings non-vaccinated as fry. Fingerlings from all four ponds in each treatment were pooled together in holding tanks and passed through a series of

bar-graders to sort the fish according to size. Retained fingerlings (~ 39 g) were transferred from the holding tanks to experimental ponds via hauling tanks. Within hauling tanks, vaccine was administered to half of the fingerlings that were non-vaccinated as fry in the previous study and half of the fingerlings vaccinated as fry at 10 d post-hatch. Fingerling fish were vaccinated for 4 h in 250 L of water inoculated at a concentration 1:10 dilution rate of the manufacturer's recommended dose rate. Water temperature at time of vaccination was 18 C. Salt was added to the hauling tanks to raise chloride levels in the tanks to 2 ppt. Vaccine was withheld from the other half of fingerlings either vaccinated or non-vaccinated as fry. Thus, the experimental design consisted of four treatment groups (five replicate ponds; 800 fish/replicate; 20,000 fish/ha) in a 2x2 factorial design:

(1) non-vaccinated as fry and non-vaccinated as fingerlings, (2) vaccinated as fry at 10 d post-hatch and non-vaccinated as fingerlings, (3) non-vaccinated as fry and vaccinated as fingerlings, and (4) vaccinated as fry at 10 d post-hatch and vaccinated as fingerlings.

Fingerlings were grown from early April to mid-November 2005 (225 d). Ponds were managed using a single-batch cropping system and best management practices as described by Tucker and Robinson (1990). Mortalities were recorded throughout the experiment. If mortalities were observed, the pond number, number of mortalities, date of occurrence, and clinical signs were documented. Fish suitable for necropsy were sent to the Southeastern Cooperative Fish Disease Laboratory, Auburn University, Auburn AL, for diagnosis. Fish were fed a 32% commercial catfish diet as a percentage of their body weight once a day early- to mid-morning six days a week. The percentage body weight fed was adjusted periodically to compensate for weight gain, as described by

Robinson et al. (1998). Daily feed allotments for each pond were recalculated weekly, based upon standing crop estimates. Fish were fed to satiation, rather than as a percentage of their body weight, when temperatures began to drop in the fall. A maximum daily feed allotment of 141 kg/ha was established to prevent deterioration of water quality.

To ensure that all treatments had a minimal exposure to *E. ictaluri*, naïve fingerlings exposed to *E. ictaluri* were stocked into cages in the experimental ponds in early June 2005. A 1.0-L BHI culture of virulent *E. ictaluri* was grown for 24 h and added to a hauling tank with 160 L of water to expose fingerlings via immersion for 1 h. The concentration of the culture innoculum was 8.7×10^8 CFU/mL. One mesh cage (0.61 m diameter, 1.07 m deep, 0.31 m^3) attached to a stake was placed in each pond. Exposed fish were stocked into each cage at a density of 20 fish/cage for three weeks and survivors then removed.

Dissolved oxygen (DO), temperature, total ammonia nitrogen (TAN), pH, unionized ammonia, nitrite, chloride, total hardness (TH; as $CaCO_3$) and total alkalinity (TA; as $CaCO_3$) levels were monitored throughout the course of the experiment. Temperatures and DO levels were checked using a YSI-550A® oxygen meter (Yellow Springs Instruments, Yellowsprings, Ohio, USA) in early morning and mid- to late-afternoon seven days a week. Measurements of pH were performed using a Hach Sension1® portable pH meter (Hach Chemical Company, Loveland, Colorado, USA) on a weekly basis during the early evening. A Hach® water quality kit (model FF-1A) was used to measure TAN and nitrite levels on a weekly basis, chloride levels on a bi-weekly basis, and TH and TA levels on a monthly basis. Unionized ammonia levels were

calculated weekly, using pH levels and the highest temperature reached in ponds throughout the week.

Water quality parameters were modified as needed to maintain ranges conducive to good fish health. Dissolved oxygen levels were maintained above 4.0 ppm (parts per million) by equipping each pond with a 0.5-hp pump-spray aerator (Aerolator Systems, Inc., Monroe, North Carolina, USA). Aerators were set on a timer to run daily from late evening to early morning (7 h). Salt was added to ponds if chloride levels fell below 50 ppm or ten times the level of nitrites. Powdered agricultural limestone was added if TA levels fell below 35 ppm.

At harvest, fish were counted and weighed to determine the final standing crop of each pond. Survival, net production, feed conversion ratios (FCRs), and average fish weight were calculated. During the study, standing crops estimates were calculated using initial standing crops and estimates of FCR, based upon average fish size, provided by Robinson et al. (1998).

Statistical Analyses

All statistical analyses were performed using Statistical Analysis System version 9.1.3 software (SAS Institute, Inc., North Carolina, USA). For the first laboratory study, mean antibody titers ($\log_2$) of each treatment were subjected to a 2-way ANOVA (MIXED procedure), with LS means compared by t-tests, to determine if significant statistical differences existed among them. The second laboratory experiment posed a challenge because of the presence of zeros in many cells, leading to a highly skewed distribution. Based on the plots of studentized residuals (histogram and quantile-quantile plot) the data were analyzed with PROC GLIMMIX using lognormal as the distribution

function. For the third laboratory study, percent survival for each dose treatment was subjected to a 1-way ANOVA (MIXED procedure), with LS means compared by t-tests. For the pond study, mean survival rates, FCRs, net production, and water quality parameters for each treatment were subjected to a 2-way ANOVA (MIXED procedure), with LS means compared by t-tests. For all analyses, $P \leq 0.05$ was considered significant.

RESULTS

Laboratory Experiment 1

In the first experiment, with the water temperature maintained at 24 C, antibody titers (log_2) against *E. ictaluri* ranged from 0.80± 0.46 in fish vaccinated at 2.44 x 10^4 CFU/mL for 4 h to 4.40± 0.36 in fish vaccinated at 3.16 x 10^6 CFU/mL for 8 h (Table 1). The main effect of dose was found to be significant. Mean antibody titers were significantly higher in fish vaccinated at 3.48 x 10^5 CFU/mL than in fish vaccinated at 2.44 x 10^4 CFU/mL. In addition, antibody titers were significantly higher in fish vaccinated at 3.16 x 10^6 CFU/mL than in fish vaccinated at the lower doses. The main effect of time, however, was not found to be significant. No significant difference was observed between the mean antibody titer for fish vaccinated at 4 h (2.11) and the mean antibody titer for fish vaccinated at 8 h (2.61), nor was a dose*time interaction observed.

Laboratory Experiment 2

In the second experiment, mean antibody titers for fish vaccinated at 3 doses and maintained at 18 C (log_2; 95% confidence intervals) were 0.09 (0.0-0.42), 0.35 (0.05-0.82), and 1.05 (0.50-1.88) for the low, intermediate, and high doses, respectively (Fig. 1). No significant differences in antibody titers were observed between treatments.

Laboratory Experiment 3

Channel catfish fingerlings were vaccinated by immersion at 1.95 x 10^4, 3.80 x 10^5, or 1.95 x 10^6 CFU/mL, along with a control, for 4 h, and later challenged with *E. ictaluri*. Mean mortality rates (± SE) ranged from 78.3± 2.04% in control fish to 33.9± 12.28% in fish vaccinated at the highest dose (Table 2). Significant differences were observed between control fish and fish vaccinated at the highest dose and between fish vaccinated at the lowest dose and fish vaccinated at the highest dose. The relative percent survival (RPS) for fish (Amend 1981) vaccinated at the highest dose was 56.7 compared to the control treatment.

Pond Study

Channel catfish fingerlings that had been vaccinated with AQUAVAC-ESC were grown-out in ponds for 8 mos. Mean survival rates (±SE) ranged from 85.3± 1.71% in the control fry-control fingerling treatment to 91.3± 0.71% in the vaccinated fry-vaccinated fingerling treatment. Mean FCR values ranged from 1.38± 0.02 in the vaccinated fry-vaccinated fingerling treatment to 1.42± 0.02 in the vaccinated fry-control fingerling treatment. Mean final average weights ranged from 0.51± 0.02 kg in the vaccinated fry-control fingerling treatment to 0.56± 0.01 kg in the vaccinated fry-vaccinated fingerling treatments. Mean net production ranged from 8,356± 380 kg/ha in the vaccinated fry-control fingerling treatment to 9,511± 163 kg/ha in the vaccinated fry-vaccinated fingerling treatment (Table 3). The main effect of vaccination at the fry stage on survival was found to be significant. Survival in food-fish vaccinated as fry (90.18%) was 3.62% higher than survival in food-fish non-vaccinated as fry (86.56%). However, the main effect of vaccination at the fingerling stage on survival was not found

to be significant, nor was a fry-fingerling interaction observed. The main effects of vaccination at the fry stage and vaccination at the fingerling stage on FCR values, average weights, or net production figures were not found to be significant, nor were any fry*fingerling interactions observed.

Water quality parameters were monitored throughout the course of the field study. Nitrite, TAN, pH, and chloride levels are reported for each treatment (Table 4), however, no significant differences were observed for either of the vaccination effects, nor were there any interaction effects. Likewise, no significant effects were observed in morning or afternoon DO, total alkalinity, or total hardness levels for any of the main factors, nor were there any interaction effects (Table 5). The mean (± SE) number of times morning or afternoon DO levels dropped below 2.5 ranged from 0.4 ± 0.24 in the non-vaccinated fry/vaccinated fingerling treatment to 1.0 ± 0.55 in the non-vaccinated fry/non-vaccinated fingerling treatment (Table 6). The mean (± SE) number of times pH levels rose above 9.5 ranged from 1.2 ± 0.10 in the vaccinated fry/non-vaccinated fingerling treatment to 1.8 ± 0.73 in the non-vaccinated fry/vaccinated fingerling treatment. The overall change of average morning and afternoon water temperatures in the catfish ponds is displayed in Figure 2.

Table 1. Mean antibody titers ($log_2 \pm$ SE) to *E. ictaluri* of variable vaccine (AQUAVAC-ESC) dose and immersion-time trial conducted at 24 C. Values within the same row or column with different letter designations are significantly different ($P \leq 0.05$).

	Dose (CFU/mL)				
Time (h)	10^4	10^5	10^6	Mean	Pooled SE
4	0.80±0.46	2.00±0.54	3.53±0.46	2.11[a]	0.28
8	0.93±0.46	2.50±0.64	4.40±0.36	2.61[a]	0.28
Mean	0.87[a]	2.25[b]	3.97[c]		
Pooled SE	0.35	0.35	0.35		
P-value dose	<0.0001				
P-value time	0.2170				
P-value dose*time	0.756				

Figure 1. Mean antibody titers (log_2) to *E. ictaluri* of fingerlings vaccinated with AQUAVAC-ESC at three different doses for 4 h at 18 C. Different letters next to value points indicate significant differences.

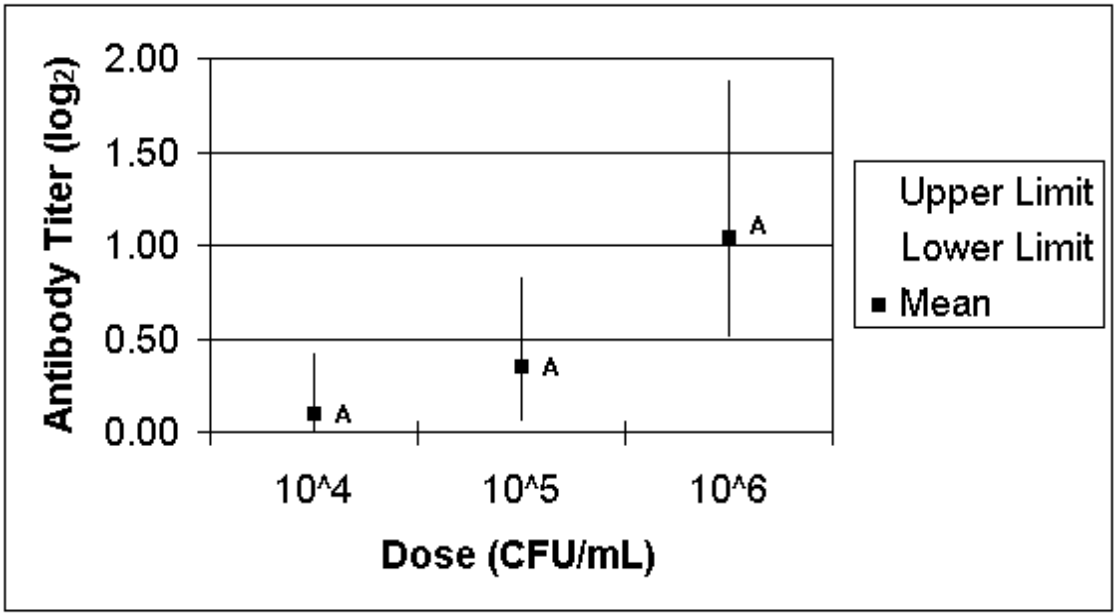


Table 2. Mean survival rates (±SE) of fingerlings vaccinated with AQUAVAC-ESC for 4 h at variable doses. Values within the same row or column with different letter designations are significantly different (P≤0.05).

Vaccine dose	Mortality (%)	RPS[1]
Control	78.3±2.04[a]	–
10^4	65.0±8.90[a]	NS[2]
10^5	55.3±10.62[a,b]	NS
10^6	33.9±12.28[b]	56.4
Pooled SE	9.31	

[1] Relative Percent Survival

[2] Not Significant

Table 3. Mean survival rates, FCR, average final weights, and net production (±SE) of vaccinated food-fish trial. An asterisk indicates the P-value is significant at P≤0.05. A "V" indicates vaccination occurred, while a "NV" indicates no vaccination.

	Survival Rate (%)	FCR	Average Final Weights (kg)	Net Production (kg/ha)
Treatments				
Fry Fingerling				
NV NV	85.3±1.71	1.41±0.02	0.54±0.03	8,540±632
V NV	88.9±1.91	1.42±0.02	0.51±0.02	8,356±380
NV V	87.7±1.44	1.41±0.03	0.56±0.02	9,004±316
V V	91.3±0.71	1.38±0.02	0.56±0.01	9,511±163
Pooled SE	1.51	0.02	0.02	409
Fry effect				
NV	86.5	1.41	0.55	8,772
V	90.1	1.40	0.54	8,933
Pooled SE	1.07	0.02	0.02	290
P-value	0.03*	0.76	0.62	0.70
Fingerling effect				
NV	87.1	1.42	0.53	8,448
V	89.5	1.39	0.56	9,257
Pooled SE	1.07	0.02	0.02	290
P-value	0.13	0.37	0.15	0.07

Table 4. Mean values (ppm except pH ± SE) of TAN, pH, nitrite, and chloride during vaccinated food-fish trial. An asterisk indicates the P-value is significant at P≤0.05. A "V" indicates vaccination occurred, while a "NV" indicates no vaccination.

		TAN[1]	pH	Nitrite	Chloride
Treatment					
Fry	Fingerling				
NV	NV	1.32±0.08	8.06±0.11	0.40±0.06	57.70±3.45
V	NV	1.54±0.31	8.16±0.09	0.44±0.04	58.70±1.38
NV	V	1.34±0.17	8.06±0.15	0.48±0.14	55.00±2.16
V	V	1.62±0.23	7.96±0.15	0.41±0.07	55.14±1.41
Pooled SE		0.22	0.09	0.06	2.26
Fry effect					
NV		1.33	8.06	0.45	56.35
V		1.58	8.06	0.43	56.92
Pooled SE		0.15	0.09	0.06	1.60
P-value		0.26	1.00	0.82	0.80
Fingerling effect					
NV		1.43	8.11	0.42	58.20
V		1.48	8.01	0.45	55.07
Pooled SE		0.15	0.09	0.06	1.60
P-value		0.82	0.46	0.73	0.18

[1]Total Ammonia Nitrogen

Table 5. Mean values (ppm ± SE) of morning and evening DO, total hardness, and total alkalinity. A "V" indicates vaccination occurred, while a "NV" indicates no vaccination.

	DO_a[1]	DO_p[2]	TH[3]	TA[4]
Treatment				
Fry Fingerling				
NV NV	6.57±0.17	7.30±0.50	72.80±6.44	43.90±2.92
V NV	6.82±0.15	7.94±0.53	66.94±4.27	44.50±3.49
NV V	6.66±0.12	7.44±0.56	65.48±5.54	41.20±3.89
V V	6.62±0.15	6.81±0.62	60.10±6.44	34.98±1.27
Pooled SE	0.14	0.55	5.75	3.05
Fry effect				
NV	6.61	7.37	69.14	42.55
V	6.72	7.38	63.52	39.74
Pooled SE	0.10	0.39	4.06	2.16
P-value	0.47	0.99	0.34	0.37
Fingerling effect				
NV	6.69	7.62	69.87	44.20
V	6.64	7.13	62.79	38.09
Pooled SE	0.10	0.39	4.06	2.16
P-value	0.72	0.39	0.24	0.06

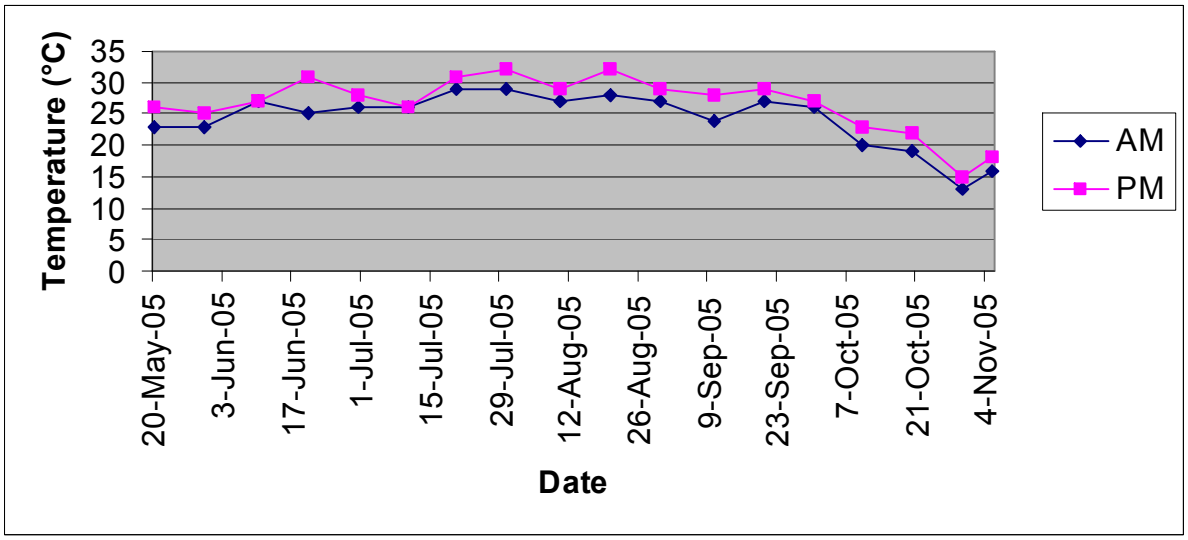
[1] Dissolved Oxygen A.M. [3] Total Hardness as $CaCO_3$

[2] Dissolved Oxygen P.M. [4] Total Alkalinity as $CaCO_3$

Table 6. Mean number of times (± SE) dissolved oxygen fell below 2.5 ppm and pH rose above 9.5 in channel catfish ponds stocked at 20,000 fish/ha.

Treatment		n	Times of dissolved oxygen	Times of pH above
Fry	Fingerling		below 2.5 mg/L	9.5
NV	NV	5	1.0 ± 0.55	1.6 ± 0.81
V	NV	5	0.6 ± 0.24	1.2 ± 0.10
NV	V	5	0.4 ± 0.24	1.8 ± 0.73
V	V	5	1.0 ± 0.45	1.6 ± 1.60

Figure 2. Overall change of average morning and afternoon water temperature (°C) in the catfish ponds stocked at 20,000 fish/ha.



DISCUSSION

In the first laboratory experiment, mean antibody titers were measured after administering lower doses of AQUAVAC-ESC® for longer immersion times at 24 C. Following administration of the vaccine, antibodies specific for *E. ictaluri* were produced, which agrees with previous studies. Serum antibodies have been shown to be present after immunization with bacterin (Vinitnantharat and Plumb 1992, 1993; Plumb and Vinitnantharat 1993; Klesius and Sealy 1995; Thune et al. 1997; Shoemaker and Klesius 1997) and have been shown to be indicative of prior exposure (Klesius et al. 1991; Klesius 1992b; Vinitnantharat and Plumb 1993; Antonio and Hedrick 1994).

A direct relationship was observed between mean antibody titers and vaccine dose. Other researchers have observed a direct relationship between dose and vaccine uptake utilizing this vaccine. Wise and Terhune (2001) were able to recover *E. ictaluri* RE-33 from a greater percentage of fry vaccinated at 2.4×10^7 CFU/mL and 2.5×10^6 CFU/mL than fry vaccinated at 2.5×10^5 CFU/mL. The level of a primary humoral immune response is usually correlated with vaccine or antigen dose (Ellis 1988). Vinitnantharat and Plumb (1992) injected channel catfish intraperitoneally with killed *E. ictaluri* at varying doses and found fish exposed to 2.0×10^4 cells/fish had lower antibody titers than fish exposed to 2.0×10^6or 2.0×10^8 cells/fish. Marsden et al. (1996) measured antibody responses in rainbow trout vaccinated with a mutant strain of

Aeromonas salmonicida at doses ranging from 2.0×10^6 to 2.0×10^9 and found a clear vaccine dose effect. Moore et al. (1998) immersed 1.5-3.0-g rainbow trout, *Onchorhynchus mykiss*, for 24 h in suspensions of 1.0×10^5, 1.0×10^6, or 1.0×10^7 Bovine Serum Albumin (BSA)-conjugated fluorescent latex microspheres per mL and found that uptake was proportional to suspension microsphere concentration.

The main effect of time on antibody response was not found to be significant in the first laboratory experiment. To our knowledge, this is the first report of time being investigated as a main effect with a live vaccine in channel catfish. Other researchers, however, have observed time to have a significant effect on antigen uptake in studies with rainbow trout (Tatner 1987; Moore et al. 1998). The absence of a significant effect of time on antibody titers in the present study may have been due to the inability of extending time from 4 h to 8 h to compensate for the dilutions used. Tatner (1987) suggested that, when antigen concentration is not limiting in direct immersion vaccinations, only a certain amount of antigen will enter a fish at a given antigen concentration, regardless of immersion time. Conversely, if a vaccine is diluted too greatly, then fish will quickly uptake all available antigens, and a time extension will not result in further uptake.

In the second laboratory experiment conducted at 18 C, overgrowth of contaminants from the pooled water samples prevented the final vaccine doses in the aquaria from being determined. Because similar procedures for diluting were followed in the first laboratory experiment, it is assumed that final doses were in the range of 1×10^4, 1×10^5, or 1×10^6 CFU/mL. We refer to the doses here as low, intermediate, and high. Mean antibody responses of fingerlings vaccinated at 18 C appeared to be lower than

those generated when vaccination occurred at 24 C. However, no direct comparisons can be made between the two experiments.

One explanation for the lack of a significant titer response lies in the fact that the immune system of warm-water fish is generally depressed at sub-optimal temperatures (Rijkers 1982). A rapid drop in water temperature from 22 C to 17 C has been shown to result in suppression of T-helper cells and lower B-cell responses to T-dependent antigens (Clem et al. 1984; Miller and Clem 1984). However, acclimation of fish to 17 C for longer than 2 weeks has been shown to result in recovery of T-helper cell function *in vitro* (Clem et al. 1984). B-cells isolated from channel catfish acclimated for at least two weeks at 17 C showed significant responses to both T-independent and T-dependent antigens, regardless of culture temperature (Miller and Clem 1984). In the present study fish were acclimated to 18 C for two weeks before administering the vaccine. The experiments of Clem et al. (1984) and Miller and Clem (1984) were conducted *in vitro*, whereas the present study was a reflection of the immune response *in vivo*. Components of the adaptive immune system of channel catfish may respond to sub-optimal temperatures differently *in vivo* than *in vitro*.

A second explanation for the low antibody response observed in fish vaccinated at 18 C might be that fish were sampled too early. Rijkers et al. (1980) showed that the magnitude of the humoral immune response in carp *in vivo* remained the same at low temperatures, but the amount of time required to generate the response was increased. If the kinetics of the immune response in channel catfish are comparable to that of carp, then sampling fish later than 21 d post-vaccination may have shown fish vaccinated at

18 C to generate higher antibody titers and may also have resulted in a positive correlation between antibody titers and dose, as we observed in fish vaccinated at 24 C. In addition, future studies will need to investigate whether fingerlings vaccinated at 18 C are indeed protected against *E. ictaluri*. Rijkers et al. (1980) did not observe fish vaccinated at 18 C to generate significantly greater secondary immune responses. This observation suggests that vaccinating fingerlings at 18 C fails to stimulate development of long-lived memory cells. The delayed nature of the primary immune response at 18 C would obviate even the short-term protection afforded by higher levels of antibodies and activated T-cells in the Southeastern U.S.A., where pond temperatures can rapidly rise from low temperatures to those conducive to *E. ictaluri* infection.

A third explanation for the observance of low antibody titers in fish vaccinated at 18 C may be due to the slow growth rate of *E. ictaluri* following vaccination at this temperature. Plumb and Vinitnantharat (1989) demonstrated that optimum growth of *E. ictaluri* occurs between 25 and 30 C and that little or no growth occurs at 15 C. Francis-Floyd et al. (1987) found that percent mortality in channel catfish challenged intraperitoneally with *E. ictaluri* at concentrations of 1×10^4, 1×10^5, or 1×10^6 bacteria/fish was significantly lower in fish challenged at 17 and 21 C than in fish challenged at 23, 25, and 28 C. The researchers also observed that fish mortality was correlated with inoculation dose at 23, 25, and 28 C, but not at 17 or 21 C.

In the third laboratory experiment, channel catfish fingerlings vaccinated at the highest dose had significantly greater survival than control fish and fish vaccinated at the lowest dose. Other researchers have found a positive correlation between RE-33 vaccine dose and protection (Wise et al. 2000; Wise and Terhune 2001). Significantly greater

survival in fish vaccinated at the highest dose was probably observed due to generation of a larger specific immune response, as demonstrated by the first laboratory study. Antibody titers were used in the first laboratory study, not as a measure of the protection that would be conferred following challenge, but as a measure of the overall specific immune response occurring in vaccinated fish (Klesius and Sealy 1995; Plumb et al. 1986; Plumb et al. 1994; Wolters et al. 1996; Thune et al. 1997). The intracellular nature of *E. ictaluri* suggests that cell-mediated immunity, working in conjunction with humoral immunity, would be important in providing protection (Abbas and Lichtman 2003e), and several studies have shown this to be the case (Shoemaker and Klesius 1997; Antonio and Hedrick 1994; Sheldon and Blazer 1991).

The highest dose used in the challenge corresponds to a 1:10 dilution of the AQUAVAC-ESC vaccine. Protection against ESC may have been observed with the highest dose, even though it represents a dilution of the vaccine, due to the ability of time to compensate for a lower dose. Standard protocol for administration of AQUAVAC-ESC calls for fish to be vaccinated for a total of 17 min, but fish were vaccinated for 4 h in the present study. Other studies have shown the ability of time to compensate for lower doses. Tatner and Horne (1985) observed that brown trout, *Salmo trutta*, vaccinated and then challenged with *Yersinia ruckeri* had significantly higher survival in treatment groups with lower vaccine doses and longer exposure times than those treatments with higher vaccine doses and shorter exposure times. Tatner (1987) immersed 3-mo-old rainbow trout fry in a radiolabelled *Aeromonas salmonicida* for various dilution*time combinations, and found that certain combinations of time and dose were "equivalent", with respect to the amount of antigen taken up by the fish.

Confounding factors may be responsible for the failure to observe significantly greater survival in fish vaccinated at the low and intermediate doses. The experiment included only a small number of replicates per treatment. Wise et al. (1993) found that a high degree of innate variability is associated with the immersion challenge system, limiting its ability to detect only large differences in treatment effects when smaller numbers of replicates within treatments are used. In addition, many of the replicate tanks contained anorexic fish and *F. columnare* was isolated from 27% of fish throughout the first week following challenge, possibly having an impact on overall results.

The efficacy of AQUAVAC-ESC in fry has been evaluated in several studies utilizing both laboratory and field investigations (Klesius and Shoemaker 1998; Petrie-Hanson and Ainsworth 1999; Wise and Terhune 2001; Shoemaker et al. 1999; Wise et al. 2000; Wise et al. 2000; Carrias 2005). Unpublished data (D. Wise, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS) confers that commercial fingerling operations can improve economic profitability through the use of this vaccine. However, the effects of this vaccine on fingerlings grown to the food-fish stage have not been investigated. Klesius and Shoemaker (1998) suggested that long-term immunity is possible, but field production and economic data are lacking. In the field experiment of this study the main effect of vaccination at the fry stage on survival of fingerlings to the food-fish stage was found to be significant. However, other production parameters were not influenced by vaccination at the fry stage, possibly due to the relatively low number of mortalities that occurred during the experiment.

The main effect of vaccination at the fingerling stage on survival was not found to be significant. However, fish were vaccinated at 18 C in the field study and as previously

demonstrated and discussed, low temperatures may negatively influence the immune response of channel catfish to *E. ictaluri*, possibly due to suppression of immune system components, low growth of the bacterium within the fish, and less uptake of the vaccine (Rijkers 1982; Clem et al. 1984; Miller and Clem 1984; Plumb and Vinitnantharat 1989; Francis-Floyd et al. 1987; Tatner 1987). Channel catfish producers typically transfer fingerlings into food-fish ponds during the cooler months, before temperatures rise above 20 C, to avoid stressing the fish. In addition, they desire to begin feeding fish as soon as possible to maximize pond production capabilities. To try and mimic these production practices, we also chose to vaccinate the fingerlings and transfer them to food-fish ponds in April just before the start of the new production season.

Another explanation for the failure to observe significantly greater survival in vaccinated fingerlings and possibly even greater differences in survival rates in the fry effect may be that fingerlings used for the field study were obtained from ponds where ESC epizootics had occurred (Carrias 2005). Studies have shown that a primary exposure to *E. ictaluri* results in protection against secondary exposures (Shoemaker and Klesius 1997; Klesius and Shoemaker 1997). In the channel catfish industry, ESC tends to affect food-fish to a much lesser extent than it does fingerlings, primarily because food-fish are naturally exposed to the pathogen in fingerling production phase (Wise et al. 2004). Additionally, a significant ESC outbreak may not have occurred in the study ponds. Although cages stocked with naïve fish challenged with *E. ictaluri* were placed in each pond to introduce fish actively undergoing an acute infection, there is no way of knowing if all the experimental fish were exposed to a high enough pathogen dose to affect disease development. Mortalities from the experimental ponds exhibited clinical

signs associated with ESC, but no fish collected were suitable for necropsy. Some mortalities also exhibited clinical signs associated with *F. columnare* infections, a confounding effect that other researchers have noted in field experiments (Wise et al. 2000; Carrias 2005). Furthermore, ESC tends to affect fish in ponds managed using a single-batch system, as they were in the present study, to an even lesser extent because of improved water quality and environmental conditions (Tucker et al. 1990).

Summary

The results of the laboratory studies are relevant to fish farmers in that it might be possible to increase survival in food-fish by administering AQUAVAC-ESC to fingerlings at more economically feasible doses for longer periods of time. Fingerlings are often transported from one farm to another over long periods of time, leaving open the possibility of administering the vaccine in route. The ability to obtain the same results in exposing fish for 4 h as in exposing fish for 8 h is desirable, because a greater likelihood exists that fish will become stressed due to remaining in hauling tanks for additional time. In addition, administering the vaccine for 8 h may not be practical on commercial fish farm operations for logistical and economic reasons, especially on farms where fingerlings and food-fish are produced on or near the same facility and the number of hauling tanks and trucks are limited.

Based on these findings, the vaccinations described here in older/larger fingerlings may have to be restricted to fish being transported in warmer temperatures. Additionally, the vaccine may have to be restricted to a fish size less than 5 cm, due to the cost of the vaccine and the total volume of water used in the vaccination process. Some food-fish producers target this size of fish for stocking directly into food-fish

production ponds or "stocker" ponds. The inclusion of a stocker phase in single-batch production systems is becoming more common in the catfish industry (Pomerleau and Engle 2003). This would afford the opportunity to integrate the vaccine as either a booster or primary vaccination if the fish had not previously survived an epizootic.

The results of the field study are relevant to producers in that purchasing fingerlings that have been previously vaccinated may have economic benefits. In our study, food-fish vaccinated as fry were shown to have 3.6% greater survival than food-fish non-vaccinated as fry. In the U.S. catfish industry, fingerling producers typically charge as much as $0.004/fish for vaccinated fingerlings, compared to non-vaccinated fingerlings, although costs vary less than this depending on the fingerling operation (K. Shuster, Intervet, Inc.). In this study, fish were stocked at 20,000 fish/ha, which would translate into an extra $80/ha for vaccinated fingerlings compared to non-vaccinated fingerlings. If an average harvest weight of 0.54 kg and an average market value of $1.54/kg are assumed, as observed in this study, then a 3.6% increase in survival translates into an increase in net revenue of $519/ha.

To properly assess the relevance of the field study to U.S. channel catfish farmers, however, it will be necessary to conduct the study over several years in ponds managed using a multiple-batch system. Currently, most channel catfish farmers use multiple-batch systems, rather than single-batch systems, because large standing crops are always present and more ponds contain harvest-sized fish throughout the year. However, multiple-batch systems typically have poorer water quality and are more conducive to infectious diseases. A study conducted over several years using a multiple-batch system

would be able to take these factors into account and, thus, more accurately evaluate vaccination at the fry and fingerling stage under pond conditions.

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