Journal of Fish Diseases 2003, **26,** 433–437

Short communication

Pathogen loads, clearance and plasma cortisol response in channel catfish, *Ictalurus punctatus* (Rafinesque), following challenge with *Edwardsiella ictaluri*

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Keywords: channel catfish, *Edwardsiella ictaluri*, kinetics, plasma cortisol.

Enteric septicaemia of catfish (ESC), caused by the Gram-negative bacterium *Edwardsiella ictaluri* (Hawke 1979), is the most prevalent disease affecting farm-raised catfish in the southeastern United States. The disease occurs primarily in the autumn and spring when water temperatures are between 18 and 28 °C (Francis-Floyd, Beleau, Waterstrat & Bowser 1987). The number of ESC cases investigated by fish diagnostic laboratories can be as high as 40% of total cases per year (USDA 1997). The pathology of ESC is well defined (Shotts, Blazer & Waltman 1986; Newton, Wolfe, Grizzle & Plumb 1989; Baldwin & Newton 1993); however, the exact mechanisms of pathogenesis and disease kinetics within populations and between individual fish are not well understood.

Management strategies for combating ESC vary in both application and degree of success and include vaccination (Shoemaker & Klesius 1997; Wise, Klesius, Shoemaker & Wolters 2000), withdrawing feed at appropriate times, and treatment of ponds once an ESC outbreak has become evident (Hawke, Durborow, Thune & Camus 1998). Effectiveness of these strategies depends upon timing of treatment and the level of infection in the ponds. An additional approach is to utilize applied breeding strategies to select for disease resistance (Wolters & Johnson 1994). Particular spawns (or families) of channel catfish are consistent in their susceptibility to *E. ictaluri* challenge (i.e. they demonstrate resistance or susceptibility) (Wolters & Johnson 1994). Stress is known to lower disease resistance in fish (see Schreck, Maule & Kaattari 1993; Iwama, Pickering, Sumpter & Schreck 1997); however, differences in disease kinetics and plasma cortisol concentrations in response to disease progression between resistant and susceptible fish are not well understood. The purpose of this study was to investigate differences in *E. ictaluri*-resistant and -susceptible families by examining pathogen loads, pathogen clearance and the cortisol response during an ESC challenge.

Two families of NWAC103 strain channel catfish, *Ictalurus punctatus* (Rafinesque), from the USDA-ARS Catfish Genetics Research Unit in Stoneville, MS, USA, were selected based on mortality rates from three prior experimental *E. ictaluri* immersion challenges; family 81 (susceptible) mean percentage of mortality = 86.67 ± 13.06 (SE) and family 110 (resistant) mean percentage of mortality = 13.33 ± 11.55 (SE). A total of 85 juvenile fish from each family were acclimatized for 7 days in 30 120-L aquaria (17 aquaria per family) supplied with 26 °C well water. Of these, 75 fish were challenged with virulent *E. ictaluri* with a 30-min static challenge (Wolters & Johnson 1994) on day 7 of acclimatization and sampled four times during the 12-day trial. All fish were harvested from a tank on each sample day. The 10 remaining fish (five per family) were

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harvested and tested for the presence of *E. ictaluri* (as below). Head kidney, spleen and whole blood (100 μL treated with K_2-EDTA and 100 μL treated with sodium heparin) were collected from all fish in three replicate aquaria per family at the following times: 2 h, 1, 2 and 5 days post-exposure. Fish were anaesthetized with 0.6 mg L^{-1} metomidate hydrochloride (Small 2003) prior to blood collection, then overdosed in a solution of MS-222 (tricaine methanesulphonate) before collection of head kidney and spleen. Plasma was collected from the heparinized blood following centrifugation and stored at −80 °C until cortisol analyses were conducted. Metomidate hydrochloride blocks the handling-related release of cortisol into circulation, thus decreasing plasma cortisol variability due to sampling (Small 2003). Plasma cortisol concentrations were determined by a time-resolved fluoroimmunoassay (Small & Davis 2002). Plasma cortisol concentrations for each family were subjected to analysis of variance to determine differences between families (GLM procedure; SAS Institute, Cary, NC, USA, 1999) using individual fish as replicates. Tank effect was non-significant ($P = 0.5455$).

Genomic DNA was extracted from all EDTA-treated blood samples using a modified erythrocyte lysis protocol (Bilodeau, Terhune, Waldbieser, Wolters & Wise 2003). Genomic DNA was extracted from kidney and spleen samples by overnight digestion with 50 μg mL^{-1} proteinase K at 55 °C followed by protein precipitation with 7.5 M NH_4OAc and DNA precipitation with isopropanol. An *E. ictaluri*-specific target sequence was then amplified using a validated real-time PCR assay that enabled direct quantification of bacterial DNA/cell-equivalents (Bilodeau *et al.* 2003). Although this assay has been validated against standard plate counts of viable *E. ictaluri*, target DNA fragments from dead bacteria may also be included in the quantification. Hence the data is expressed in cell-equivalents. Bacterial levels (cell-equivalents) for each family were subjected to analysis of variance to determine differences between families (GLM procedure, SAS). A total of five blood samples were removed from analysis because they had values of more than five SDs from the mean (Sokal & Rohlf 1995) and do not represent realistic values of bacterial infection. Bacterial clearance was measured as a change in cell-equivalents over time and was assessed by performing a linear regression and comparison of slopes for days 1–5 post-exposure for each family (GLM procedure, SAS; Armitage 1980).

All fish tested with the real-time PCR assay (Bilodeau *et al.* 2003) prior to immersion challenge were negative for the presence of *E. ictaluri* DNA. Pathogen uptake was evident as early as 2 h post-challenge in blood and kidney tissues as positive real-time PCR results. There were no family differences in bacterial levels for both kidney and spleen tissue samples ($P > 0.5$ and $P > 0.1$, respectively). However, there were differences between families for blood pathogen levels both across the entire challenge and specifically for days 1–5 post-exposure ($P < 0.01$ and $P < 0.01$, respectively) (Fig. 1). Fish from the susceptible family had higher levels of bacterial cell-equivalents throughout the trial and may represent a difference in disease progression/kinetics based on susceptibility to ESC. There was no difference in pathogen levels between families upon initial uptake, as measured at the 2 h timepoint ($P > 0.05$) (Fig. 1).

Cortisol concentrations in the plasma were significantly ($P < 0.05$) different between the two

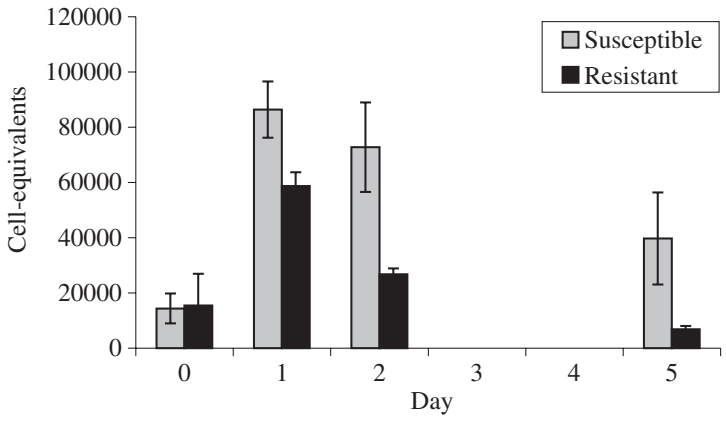

Figure 1 Concentration of *Edwardsiella ictaluri* cell-equivalents in whole blood samples from channel catfish and frequency of PCR-positive blood samples. Quantities are per 100 μL whole blood.

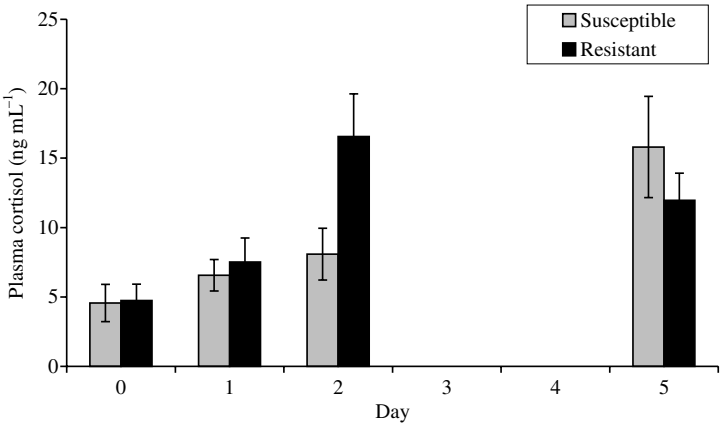

Figure 2 Plasma cortisol response of susceptible and resistant channel catfish subjected to an immersion challenge with *Edwardsiella ictaluri*.

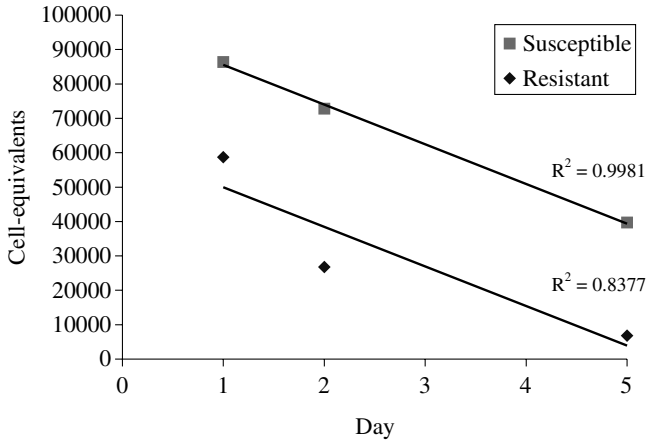

Figure 3 Clearance of *Edwardsiella ictaluri* in susceptible and resistant families of channel catfish. Quantities are per 100 μL whole blood. R^2 values are calculated from linear regression.

families only 2 days after *E. ictaluri* challenge (Fig. 2). Among resistant fish, cortisol levels peaked on day 2 within 24 h of detecting the highest bacterial levels on day 1. This was concurrent with a decrease in *E. ictaluri* concentration on day 2. Peak cortisol levels in susceptible fish were not observed until 5 days post-pathogen exposure, again corresponding to a decline in bacterial levels (Fig. 1).

Real-time PCR results from blood samples indicated a relationship between susceptibility and infection levels. At 5 days post-exposure, bacterial levels were significantly lower for both families ($P < 0.05$) than on day 1 which indicates that pathogen clearance was occurring (Figs 1 & 3). However, fish from the susceptible family carried higher levels of bacterial DNA in their blood throughout days 1–5. Cumulative mortality rates through day 12 post-exposure were 13% for the resistant family and 40% for the susceptible family. Combining information on pathogen levels and cumulative mortality rates indicates differences in host response to *E. ictaluri* infection. Differences in response can result in low mortality or chronic infection at sub-acute and perhaps sub-lethal levels in fish from the resistant family, or acute infection resulting in high mortalities in fish from the susceptible family.

Fish from the resistant family (i.e. lower challenge mortality) had decreased levels of the pathogen in the blood when compared with susceptible fish, although both families showed a pattern of clearance over time. Pathogen loads peaked at day 1 for both families, but remained significantly lower for fish in the resistant family. An increase in plasma cortisol 48 h after the peak in pathogen load for resistant fish indicates that cortisol may play an adaptive role in pathogen resistance. Chronic elevations in circulating cortisol levels are considered to have negative effects on disease resistance (Maule, Tripp, Kaattari & Schreck 1989) and to interfere with lymphocyte

function (Tripp, Maule, Schreck & Kaattari 1987; Espelid, Lokken, Steiro & Bogwald 1996). However, more recent data suggest that an acute cortisol response may have immunologically protective effects. Weyts, Flik & Berburg-van Kemenade (1998) demonstrated a reduction in neutrophil apoptosis, suggesting that an acute cortisol response may be beneficial to innate immune function by enhancing the phagocytic defences.

The acute cortisol response observed in channel catfish following an *E. ictaluri* challenge is similar to that observed in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following *Vibrio anguillarum* infection (Ackerman & Iwama 2001). Further evidence supporting the role of acute stress in enhancing both cellular and humoral components of innate defences are provided in a study by Demers & Bayne (1997). Their results with rainbow trout demonstrate that acute stress responses are generally adaptive and will probably enhance survival. It is logical to assume that, only later, as a result of chronic stress, does immunosuppression occur. Investigations are currently underway in our laboratory to further examine the relationships between an acute cortisol response and the innate immune system.

Acknowledgements

We thank Debra Harris for her work in DNA sample preparation and Jimmy Warren and Bridgette Esters-Fields for sample collection. We also thank Dr Brian Bosworth and Dr Brian Peterson for their helpful comments on earlier versions of this manuscript. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply approval to the exclusion of other products that may be suitable.

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Received: 15 January 2003
Accepted: 4 June 2003