Veterinary Microbiology 148 (2011) 425–430

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Importance of skin abrasion as a primary site of adhesion for *Edwardsiella ictaluri* and impact on invasion and systematic infection in channel catfish *Ictalurus punctatus*

Simon Menanteau-Ledouble, Attila Karsi, Mark L. Lawrence *

College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762-6100, USA

ARTICLE INFO

Article history:
Received 27 March 2010
Received in revised form 16 August 2010
Accepted 23 August 2010

Keywords:
Edwardsiella ictaluri
Enteric septicemia of catfish
Ictalurus punctatus
Channel catfish
Virulence
Skin
Invasion
Abrasion
Bioluminescence

ABSTRACT

The route of entry of *Edwardsiella ictaluri* into its catfish host has been a subject of investigation since the pathogen was first discovered. There is evidence to support entry through the intestinal tract, the nares, and the gills. Here, we evaluated the role of skin abrasion through a series of experimental challenges using bioluminescent *E. ictaluri* carrying the plasmid pAKLux1. Our results show that *E. ictaluri* is able to colonize abrasion sites on catfish skin and that catfish with abrasions developed systematic infection faster. We also found that abrasions are associated with significantly increased mortalities following experimental immersion exposure. Finally, a protocol was developed during this study that allowed for immunohistochemical examination of the tissue layers underneath the abrasion sites, confirming the presence of *E. ictaluri* in subdermal tissues from abrasion sites. This study constitutes the first report on the role of channel catfish skin as a portal of entry for *E. ictaluri* and further illustrates how versatile this pathogen can be in its mechanisms of entry.

1. Introduction

The mucus and skin barriers of fish constitute the first line of defense against infection. Not only do they form a physical barrier against pathogens impeding their attachment (Aranishi and Nakane, 1997; Magnadottir, 2006), but they also harbor a variety of protective antimicrobial compounds (Aranishi and Nakane, 1997; Ellis, 2001; Fast et al., 2002; Shepard, 1994). It is therefore not surprising that many fish pathogens take advantage of skin abrasion to establish infection (Bader et al., 2003; Plumb, 1999).

Edwardsiella ictaluri causes enteric septicemia of catfish (ESC), one of the most important pathogens affecting channel catfish aquaculture. Classically, the intestine has been considered the primary route of entry for this pathogen into the channel catfish host (Baldwin and Newton, 1993; Newton et al., 1989), but the olfactory sinus (Morrison and Plumb, 1994) and gills have also been implicated as potential sites of entry (Nusbaum and Morrison, 1996a,b).

We previously reported a method for expressing bioluminescence in *E. ictaluri*, and we showed that bioluminescence has good correlation with bacterial CFUs (Karsi et al., 2006) allowing for the quantification of the bacteria *in vivo* in catfish during infection. Furthermore, evidence collected during this study suggested that *E. ictaluri* has a predilection for sites of skin damage (Karsi et al., 2006). However, to the best of our knowledge, the effect of skin abrasions on the pathogenesis of *E. ictaluri* infection has not yet been investigated. Therefore, we designed a set of experiments to determine the plausibility of skin abrasion as an initial site of entry for *E. ictaluri* and subsequent development of systemic infection.

* Corresponding author. Tel.: +1 662 325 1205; fax: +1 662 325 1031.
E-mail address: lawrence@cvm.msstate.edu (M.L. Lawrence).

0378-1135/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2010.08.022

2. Methods

2.1. Bacterial strains

E. ictaluri strain 93–146 is a clinical isolate originally isolated from an outbreak of ESC in a commercial aquaculture pond. This strain was used to determine the *E. ictaluri* genome sequence and has been used to standardize experimental infection protocols in our laboratory. As a species, *E. ictaluri* isolates are relatively homogenous (Roberts, 2001). Plasmid pAK*lux*1 was transferred into this strain from *Escherichia coli* strain SM10 by conjugation as described (Karsi and Lawrence, 2007), which resulted in constitutive expression of the bacterial luciferase operon in 93–146. The resulting constitutive luminescence allows for detection and quantification of bacteria using the IVIS Imaging System (Xenogen, San Francisco, CA).

2.2. Fish origin

Juvenile channel catfish were obtained from the specific pathogen free (SPF) hatchery at the College of Veterinary Medicine, Mississippi State University and had no prior *E. ictaluri* exposure. Experimental infections were conducted in 40-L flow-through tanks supplied with dechlorinated municipal water. Water temperature was maintained at approximately 25 °C (±2 °C) throughout experimental infections. All fish were used according to the university policy for animal care, and protocols were approved by the Institutional Animal Care and Use Committee.

2.3. First immersion challenge

In the first experiment, 30 SPF channel catfish (approximately 4 months and 10 ± 2 cm long) were randomly allocated between two treatment groups: abraded and non-abraded. Fish were sedated using Finquel-MS222 (Argent Chemical Laboratories, Redmond, Washington), and fish from the abraded group received three ~4 cm abrasions on the right lateral abdomen (penetrating to the subdermis) using a 20-gauge syringe needle. Abraded and non-abraded fish were experimentally infected with bioluminescent *E. ictaluri* by bath immersion in water containing approximately 6.0×10^6 colony forming units (CFU) per liter for 1 h.

At predetermined time points, disease progression was quantified in each of the 15 fish for each treatment by measuring bioluminescence with an IVIS Living Image system as described (Karsi et al., 2006). Quantitative luminescence measurements were collected from the abdominal area at the abrasion sites to determine number of attached bacteria, and measurements were also collected from the whole body of each fish to determine the amount of systemic infection. A standard area was applied to all the fish at each time point to extract luminescence data from both whole body and abdomen. Individual fish were identified using fin clips to allow monitoring of disease progression for each individual.

To determine the effect of treatment on the bacterial loads at each time point, statistical analysis was performed on the mean bioluminescence from each treatment by performing an analysis of variance (ANOVA) using the Proc GLM procedure of version 9.1 of SAS (SAS Institute, Cary, NC).

2.4. Second immersion challenge

To investigate more specifically how skin abrasion affects mortality, 300 fish of approximately 7 months of age (10–15 cm long) were evenly allocated between three treatments: abraded, non-abraded, and non-challenged control. Four tanks were allocated to each treatment, for a total of 12 tanks. Immersion challenge in water containing 9.0×10^6 CFU/ml of bioluminescent *E. ictaluri* was conducted using the same method described for the first challenge experiment.

Mortalities were recorded daily to calculate the mean percent survival per tank. Dead fish were necropsied, and spleen and head kidney were sampled and homogenized. One hundred microliters of the homogenate was spread on Brain Heart Infusion (BHI) agar to confirm the cause of death as bioluminescent *E. ictaluri*. At the end of the study, the risk ratio correlated to abrasion was calculated using Fisher's exact test.

2.5. Skin challenge

Six tanks were used in this third challenge. For each tank, one fish was abraded on the abdomen using the same method described for the immersion challenge. A second fish in each tank served as a non-abraded control. A bacterial suspension containing approximately 6.0×10^8 CFU *E. ictaluri* pAKlux1 was applied directly to the lateral abdomen of abraded and non-abraded fish using a cotton swab. In addition, one non-abraded fish was also added to each tank that was not directly challenged. The objective was to determine the potential for these fish to develop ESC through tank water during co-habitation with infected fish. Luminescence was quantified from the whole body of each fish, and statistical analysis was performed on the mean bioluminescence for each treatment as during the first challenge.

2.6. Histological analysis of abrasion sites

In this final challenge, 22 abraded fish were topically inoculated with 6.0×10^6 CFU of bioluminescent *E. ictaluri* (quantified by plate counts) as described for the skin challenge, and three fish were sampled at 1, 6, 12, 24, 48 and 96 h. At each time point, bioluminescence was recorded using the IVIS system, and tissue samples of a homogenous 3 cm × 1.5 cm size were collected from the zones of abrasion and processed histologically. In addition to standard H&E staining, serial sections underwent indirect immunohistochemistry using monoclonal antibody Ed9 (Ainsworth et al., 1986) and FITC labeled goat anti-mouse immunoglobulin (Southern Biotech, Birmingham, AL) as described below. Sections were observed using an Olympus BX51 microscope and Picture Frame software (MicroFire, Goleta, CA). For each slide, the total number of bacteria present on the slide was recorded along with their distance from the epithelial surface. Because some slides