International Journal of Veterinary Science and Medicine 5 (2017) 187–195

HOSTED BY

Contents lists available at ScienceDirect

International Journal of Veterinary Science and Medicine

journal homepage: www.elsevier.com/locate/ijvsm
www.vet.cu.edu.eg

Full Length Article

Non-invasive *in vivo* imaging of fluorescence-labeled bacterial distributions in aquatic species

S. Ramachandran [a], S. Thiyagarajan [a], G. Dhinakar Raj [a,b,*], A. Uma [c]

[a] Translational Research Platform for Veterinary Biologicals, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai 51, India
[b] Department of Animal Biotechnology, Madras Veterinary College, Chennai 07, India
[c] State Referral Laboratory for Fish Disease Diagnosis and Aquatic Animal Health, Tamilnadu Fisheries University, Chennai 51, India

ARTICLE INFO

Article history:
Received 30 June 2017
Revised 11 September 2017
Accepted 17 September 2017
Available online 10 November 2017

Keywords:
Preclinical
Pathogen distribution
Catfish
Shrimp
IVIS optical imaging

ABSTRACT

In vivo imaging is becoming an advanced tool for noninvasive distribution of longitudinal small animals. However, the aquatic species have been limited to the optical imaging of noninvasively tracking on pathogen distribution. The purpose of this study was to develop shell-less fish and shrimp models of non-invasive *in vivo* imaging technique for visualization of pathogens. This experiment was utilized *Escherichia coli, Edwardsiella tarda*, *Vibrio alginolyticus and Vibrio harveyi* labeled with fluorescence probes to imaging bacterial distributions by IVIS Lumina LT system. The study was traced the internal distribution of fluorescence probes labeled bacteria in systemic organs by quantified their fluorescence intensities. The *ex vivo* organ images were showed more obvious fluorescent signal in catfish intestine, liver, heart, kidney and the shrimp showed heart, hepatopancreas, and colon. Hence, the *in vivo* imaging methods using fluorescent labeled bacterial distribution were suggested to quantify by fluorescence intensity in whole pre-infected subjects. Therefore, it can offer the information about the localization and distribution of pathogens in the preclinical research, after immersion and injections.

1. Introduction

An aquaculture is the fastest growing food-production industry in the world sector, however it is overwhelmed by infectious diseases due to various biological and non-biological agents [1]. The annual economic loss in the aquaculture industry through diseases was estimated to be billions of US dollars in worldwide [2]. Among the groups of microorganisms that cause serious losses in aquaculture, the best known are bacterial strains because of the devastating economic effects they have on affected farms [3]. Predisposition to such outbreaks is often associated with poor water quality, organic loading of the aquatic environment, handling, and transport of fish, marked temperature changes, hypoxia, or other stressful conditions [4,5]. In addition, several potential biological contaminations of aquaculture products can occur from bacteria, viruses, parasites and biotoxin causing zoonotic infection every single year [6]. These pathogens cause transmitted diseases as the result of a massive progeny amplification that follows exposure to various forms of the environment or physiological stress. The diagnosis of these pathogens is carried out by isolation from the infected tissues in pure culture and identification of the bacterial strains [7].

However, the pathogen is often present within the infected site; it is occasionally undetectable, despite the persistence of immunological symptoms [8]. To address the complexity of the chronic infections of bacterial pathogens, a number of mammalian infection models have been developed [9]. Most of them rely on the injection of *E. coli*, or other bacteria, directly into the tissues. Here, we confirm that the injection of pathogenic strain Gram-negative bacteria *E. coli* (B2) tracing *in vivo* imaging in catfish. Further, *Edwardsiella tarda (E. tarda)* is one of major fish pathogen producing the disease known as edwardsiellosis, also known as enteric septicemia of catfish (ESC) or emphysematous putrefactive disease of catfish (EPDC) [10]. Aquaculture occurs an infection most frequently involves through gills and occasionally the skin [11]. In this phenomenon, here we applied an *in vivo* imaging of Rhodamine B (RhoB) labeled *E. tarda* by immersed catfish. An addition, we planned to develop an *in vivo* imaging of pathogen distribution in the shrimp model.

Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

* Corresponding author at: Translational Research Platform for Veterinary Biological (TRPVB), 2nd floor, Central University Laboratory Building, TANUVAS, Madhavaram Milk Colony, Chennai 600051, Tamil Nadu, India.
E-mail addresses: ramvel82@gmail.com, dhinakarrajg@tanuvas.ac.in (G. Dhinakar Raj).

https://doi.org/10.1016/j.ijvsm.2017.09.003
2314-4599/

Moreover, the several epidemiological agents are responsible for vibriosis in shrimp, a disease characterized by slow growth, loss of appetite, and high shrimp mortality [12]. In order to understand complex biological phenomena, to injection and topically acquired bacterial infections caused by *V. alginolyticus* and *V. harveyi*, from aquaculture species, and how to noninvasively track them. Substantial evidence supports the importance of infection in the physiopathology of these diseases; however, its exact organ specificity of *in vivo* imaging is still poorly understood. So, the preliminary information was claimed that aquaculture production and the consumption rate of aquaculture product's increase; the possibility of contracting zoonotic infections from either handling or ingesting these products also increased day by day [13]. Outbreaks are often related to management factors, such as the quality and quantity of nutrients to the water and high stocking density, which can increase bacterial loads on the external surface of the aquatic species [14]. In diseased aquatic species are more likely to transmit an infection to humans. While many clinical applications of optical imaging have been explored, basic research can also benefit tremendously from imaging techniques that provide information about living small animals [15].

Preclinical development *in vivo* imaging approaches for applying three-dimensional fluorescence images of the internal structures, especially small animals, are procured [16]. *In vivo* imaging system made a sensitive camera to detect fluorescence emission from different fluorophores in whole-body live small animals [17]. Recent developments *in vivo* live imaging make it a promising tool for non-invasive imaging of the various internal organs. Therefore, this experiment was planned to describe new imaging methodology, present several ubiquitous and pathogens with specific fluorescent dyes labeling, which we have termed an aquatic species.

2. Materials and methods

2.1. Bacterial culture

The bacterial strains used in this study were *E. coli* (B2), *E. tarda*, *V. alginolyticus* and *V. harveyi*. These strains were cultivated in different media, including Luria Bertani (LB, used for inoculate *E. coli*), Tryptic Soy Broth (TSB, for *E. tarda*), and Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS, for both *vibrio strains*). LB medium supplemented with 2% NaCl was used for Vibrio pure culture separation. Among the four strains, *E. coli* stock was utilized from our laboratory Translational Research Platform for Veterinary Biologicals (TRPVB, Chennai, India). *E. tarda* was obtained from the Tamil Nadu Fisheries University (Chennai, India), and both *V. alginolyticus* and *V. harveyi* were obtained from Central Institute of Brackishwater Aquaculture (CIBA; Chennai, Tamil Nadu, India). Cryopreserved bacterial strains from 10% glycerol stocks were aseptically inoculated in appropriate broth kept at overnight incubation; then the bacterial strains were plated and incubated for 24 h. The single colonies were subsequently transferred and grown in agar plate by overnight incubation (37 °C). The pure bacterial colonies were then transferred to sterile saline and centrifuged at 5000 rpm for 15 min. The pellets were washed twice and finally resuspended in sterile saline. The bacterial densities were determined spectrophotometrically at an optical density (OD) of 600 nm assuming that an optical density value 1.0 corresponds to 10^9 CFU/mL. As a control, saline was used.

2.2. Catfish and shrimp aquaculture

Healthy white and Albino catfish (length: 12–15 cm and weight: 25–50 g) were obtained from Tamil Nadu Fisheries University in Chennai, India. The fish were kept in quarantine plastic tanks with a follow-through water volume of 500 L (dissolved oxygen 6.0 ± 0.5 mg/L; temperature 28 ± 1 °C) with additions of the aerator and fed with commercial diet twice daily. Adult white shrimp *Litopenaeus vannamei* (weight 18–20 g and length: 10–12 cm) were randomly obtained from a farm at Central Institute of Brackishwater Aquaculture (CIBA; Chennai, Tamil Nadu, India). The temperature, dissolved oxygen, pH and salinity of the sampling sites were 28 °C, 6.0 mg/L, 8.20 and 20–25 ppt, respectively. All fish and shrimp were acclimatized for one week prior to the experiment.

2.3. Method of bacterial labeling

An amount of 1×10^9 CFU/mL of *E. coli, E. trada, V. alginolyticus* and *V. harveyi* was added 10 mL of PBS, pH 7.4 in separate test tubes. After mixing, the bacteria were centrifuged at 5000 rpm for 10 min. FITC (Ex:495; Em:519 nm), Rhodamine B (Ex:571; Em:591 nm), Quantum dots (Ex:425; Em:605 nm) and AUNCs (Ex:560; Em:710 nm) were used 50 μg/mL concentration into each strain of the bacterial pellets and incubated at 4°C for 2 h with continuous shaking. The bacteria were washed thrice with PBS, to confirm the binding stability of the fluorescent probes with these bacterial strains. The OD and fluorescence intensity were measured at 600 nm and its excitation, emission nanometers, respectively. A number of labeled cells should be approximately 10^9 CFU/mL, which measures around 1 OD.

2.4. Vaccination of fish with E. Tarda

Intraperitoneal (i.p.) injection of 0.2 mL of PBS (control group) and 0.2 mL of inactive *E. tarda* lysate (vaccination group) was carried out in the selected catfish. Secondary vaccinations were performed to obtain an optimal immune response with the same method and dosage after two-weeks of the first vaccination. After 2-weeks of the secondary vaccination (s.v.), the fishes were used in the experiment by immersion (10^9 and 10^5 CFU) of *E. tarda*-RhoB labeled bacteria.

2.5. In vivo imaging of catfish and shrimps

The IVIS Lumina LT series III (Caliper, MA) was used to determine the fluorescence intensities during the imaging an appropriate excitation and emission filters. Two-dimensional images had the background signal subtracted, and image scaling was normalized by converting total radiance efficiency. Fluorescence intensity was represented by a multicolor scale ranging from blue (least intense) to red (most intense). Signal intensity images were superimposed over gray scale reference photographs for anatomical representations. Scales were manually set to the same values for comparable images to normalize the intensity of the fluorescence across time points. Fluorescence intensity within specific regions of individual animals was quantified using the region of the interest (ROI) tools in the Live Image 4.5 software (PerkinElmer).

For instance, *in vivo* imaging, two different routes such as immersion, and intraperitoneal injections were used in catfish and shrimp. The immersion route, 10^9 CFU bacteria were suspended in 1L of brackish water and others two (intramuscular and intraperitoneal) used an injection of 100 μL containing 10^9 CFU fluorescent probes-labeled bacteria. The both fish and shrimp were exposed for different time points and anesthetized by immersion of Eugenol (50 mg/L; Himedia, India). After imaging, the fishes were sacrificed; the organs were harvested and performed the distribution of fluorescent intensity in fluorescent-probes labeled bacteria. The *in vivo* transmission of fluorescent intensity was analyzed by using live imaging software (PerkinElmer).