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Placemat and rotational culturing: A novel method to control *Cryptocaryon irritans* infection by removing tomonts

B. Jiang [a,b], Y.W. Li [c], A.Y. Abdullahi [a], X.M. Dan [c], H. Gong [d], Q.Z. Ke [e], A.X. Li [b,*], G.Q. Li [a,*]

[a] College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, PR China
[b] State Key Laboratory of Biocontrol, Key Laboratory for Aquatic Products Safety of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, PR China
[c] College of Animal Science, South China Agricultural University, Guangzhou 510642, PR China
[d] Institute of Biotechnology, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian Province 350003, PR China
[e] Ningde Fufa Fisheries Co., Ltd., Ningde 352002, PR China

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ABSTRACT

Cryptocaryoniasis is a severe parasitic disease caused by ciliate *Cryptocaryon irritans* in farmed marine fish. To control this disease, we established a method to interrupt the life cycle of *C. irritans* via the removal of its tomonts. Marine fish *Plectorhinchus cinctus* were divided randomly into four groups, including placemat removal group (Group I), rotational culturing group (Group II), infection control group (IF group) and blank control group (Control); the former three groups were infected with *C. irritans* at low dose. A daily food consumption (DFC), relative infection intensity (RII) and survival rate of each group were observed. At 4-week post infection, some fish were challenged with *C. irritans* theronts at a lethal dose. Results showed that the fish' DFC in IF Group, Group I and II decreased significantly on 2nd day post infection, and gradually restored food intake when the trophonts exited the fish on the 5th day. However, their DFC in IF Group decreased significantly on 7th day, and eventually stopped feeding. Trophonts on fish' body of the three groups began to reduce from the 4th day and those of Group I and II almost disappeared on the 7th day, conversely the trophonts of IF group increased ten times. At 2-week post infection, the survival rates of Group I, II, IF and Control were 97%, 98%, 0%, and 100%, respectively. The survival rates of Group I, II and Control were 88.3%, 93.3%, 66.7%, respectively after challenge. Results demonstrate that placemat and rotational culturing method to remove tomonts can effectively control *C. irritans* infection.

Statement of relevance

Cryptocaryoniasis is a severe parasitic disease caused by ciliate *Cryptocaryon irritans* in farmed marine fish. To control this disease, we established a method to interrupt the life cycle of *C. irritans* via the removal of its tomonts. Marine fish *P. cinctus* were divided randomly into four groups, including placemat removal group (Group I), rotational culturing group (Group II), infection control group (IF group) and blank control group (Control); the former three groups were infected with *C. irritans* at low dose. A daily food consumption (DFC), relative infection intensity (RII) and survival rate of each group were observed. At 4-week post infection, some fish were challenged with *C. irritans* theronts at a lethal dose. Results showed that the fish' DFC in IF Group, Group I and II decreased significantly on 2nd day post infection, and gradually restored food intake when the trophonts exited the fish on the 5th day. However, their DFC in IF Group decreased significantly on 7th day, and eventually stopped feeding. Trophonts on fish' body of the three groups began to reduce from the 4th day and those of Group I and II almost disappeared on the 7th day, conversely the trophonts of IF group increased ten times. At 2-week post infection, the survival rates of Group I, II, IF and Control were 97%, 98%, 0%, and 100%, respectively. The survival rates of Group I, II and Control were 88.3%, 93.3%, 66.7%, respectively after challenge. Results demonstrate that placemat and rotational culturing method to remove tomonts can effectively control *C. irritans* infection. This method is an mechanical preventive one, not only provides an ideal means for controlling *C. irritans* in small scale culture systems, but also solves food safety problems.

1. Introduction

Cryptocaryon irritans parasitizes tropical and subtropical marine teleost fish (Colorni and Burgess, 1997). This ectoparasitic protozoan rapidly proliferates and severely impairs the physiological functions of

* Corresponding authors.
E-mail addresses: lianxing@mail.sysu.edu.cn (A.X. Li), gqli@scau.edu.cn (G.Q. Li).

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its skin, eyes and gills, causing a lethal "white spot disease" (Nigrelli and Ruggieri, 1966). *C. irritans* has a quadriphasic life cycle including four developmental stages: theront, trophont, protomont, and tomont (Colorni, 1985). The trophont is the parasitic stage, which can get its nutrition by feeding on tissue debris and body fluids. It becomes free-swimming protomont when it exits the host, which moves along the substrate and rock for 2–8 h (Burgess and Matthews, 1994a). Protomont adheres to the hard surface, sheds its cilia and encysts forming the reproductive stage tomont, which divides over 3–28 days (Colorni, 1985). After several divisions, the tomont ruptures, releasing about 300 free-swimming theronts. The latter must find a suitable host within 48 h to enter the next life cycle, or they will die (Burgess and Matthews, 1994b; Colorni, 1985).

The technological advances in aquaculture are continuously improving water quality and feeds. In intensive aquaculture systems with high fish density *C. irritans* can cause severe infection with high mortality and significant economic losses, but it lacks effective control (Chen et al., 2011). The ultimate goal of a prevention or control program is to disrupt the life cycle of the parasite and stop future infections. The infected fish can acquire immunity against *C. irritans* and the vaccination can effectively prevent *C. irritans* parasitism. However, it is difficult to obtain a large amount of antigen because *C. irritans* cannot multiply as bacteria in vitro (Dan et al., 2013; Luo et al., 2007). Hirazawa et al. (2001) found that feeding medium-chain fatty acids (octanoic acid) can significantly reduce the amount of trophonts on red sea bream at seawater temperature 17 °C. Recently, Goto et al. (2015) suggested that the water-soluble extract from *Sophora flavescens* could effectively control *C. irritans* infections. However, it is not suitable for large-scale farming production because of high production cost. Other methods such as heat treatment, freshwater immersion, drying treatment, ozone treatment and ultraviolet (UV) sterilization to kill theronts have proven to be effective, but most of them have some shortcomings. For example, theronts that are not exposed to UV radiation and remain in the tank or holding areas will be unaffected. Likewise, encysted tomonts within the tank or holding area would not be affected. Ozonation is a highly effective method for disinfection of water, but is more complicated and may affect water quality, especially with regard to reaction products in salt water (Colorni, 1987; Colorni and Burgess, 1997; Gratzek et al., 1983; Hirazawa et al., 2003; Wilkie and Gordin, 1969; Yanong, 2009). Currently, various chemicals such as copper, hyposalinity, chloroquine, and to less extent formalin (Yanong, 2009) have been used to prevent and control outbreaks with *C. irritans*. Nevertheless, this approach is usually not suitable for commercial farming due to environmental pollution, toxicity to fishes and chemical residues in their flesh (Hirazawa et al., 2003; Pironet and Jones, 2000).

The chemotherapy of captive reared stocks and their benefits are part of an integrated system of pest management. Many literatures have been documented on the control and management of other pathogenic ciliates of cultured species. In particular, there is a significant body of work on *Ichthyophthirius multifiliis* (Matthews, 1994, 2005) which has looked at chemical treatments (Picón-Camacho et al., 2012) and non-chemical control methods including the manipulation of water speeds (Bodensteiner et al., 2000), mechanical devices (e.g. UV, electric grids, filtration, vacuum systems and mechanical wipers) (Gratzek et al., 1983; Aihua and Buchmann, 2001; Buchmann et al., 1999; Shinn et al., 2009; McRobbie and Shinn, 2011) and biological control using cleaner fish (Picón-Camacho et al., 2012). Some of these studies are important as they also address the removal of cysts and tomonts from culture systems to prevent proliferation of the parasite.

A system of culture container rotation could control *C. irritans* propagation process. However, few investigations have been reported on interrupting the route of transmission to control outbreaks with *C. irritans*. In this study, a new method (placemat and rotational culturing) was designed to remove *C. irritans* tomonts or to interrupt its life cycle. Marine fish *P. cinctus* were infected with *C. irritans* theronts at low dose firstly and challenged at lethal dose later. We assessed their control effects after different treatments by observing the fish's daily food consumption (DFC), the relative infection intensity (RII) and survival rate.

2. Materials and methods

2.1. Fish

Marine fish *Plectorhinchus cinctus* (weight: 27.79 ± 3.07 g) were purchased from Ningde Marineland, Fujian, China. Firstly, ten fish were randomly checked to ensure that there was no *C. irritans* on the gill and skin. Then, every 30 healthy *P. cinctus* were reared in a 1000-L round plastic tanks to acclimatize for two weeks. They were fed twice daily with commercial pellet feed and their feces were siphoned before feeding. The amount of commercial feed fed per day was equal to 3.5% of the fish' body weight in total and residual feed was recorded 2 h after feeding. During the trial, sand-filtered seawater was used at salinity of 29–31‰, 28 ± 1 °C water temperature, 5.0–7.0 mg/L dissolved oxygen, <0.2 mg/L inorganic nitrogen and 100 L/h water flow rate.

2.2. Parasite propagation

The *C. irritans* GD1 strain was used in the experiments, which was established by the State Key Laboratory of Biocontrol at Sun Yat-sen University. Parasites were propagated by infecting the animal model large yellow croaker (50 ± 8.37 g) according to Dan et al. (2006).

2.3. Placemat selection and adhesion test

The twelve artificial substrate liners (composite polyethylene colorband cloth, PVC waterproof cloth, silicone cloth, PE waterproof cloth, Oxford cloth, cotton canvas, linen canvas, polypropylene spunbond nonwovens, polyester rain tarpaulin, PVC waterproof soft glass transparent tablecloth, soft gauze and bolting silk) were purchased from Guangzhou International Textile City, Guangdong, China. Placemat was selected based on their adhesion to tomont and cohesion resistance. To detect tomont's adhesion rate of selected artificial substrate liners under different flow rates, the artificial substrate liner (5 cm × 5 cm) was put at the bottom of fish tank on third day post infection and was removed on the next day, and placed them parallel and perpendicular to the water flow for 3 min respectively, then recorded the number of tomonts before and after impacted by water flow. Flow velocity was set into 5 different levels of 0.33 m/s, 0.47 m/s, 0.92 m/s, 1.24 m/s, 2.53 m/s, repeated triplicate at every level.

$$\text{Adhesion rate} = \text{the number of tomonts after impact} / \text{the number of tomonts before impact} \times 100\%$$

2.4. Tomont inactivation

After using the placemat, tomonts were removed and dried at room temperature for different time intervals: 0 min, 10 min, 30 min, 60 min, 120 min, and 240 min. Afterwards they were incubated in the seawater at 27 °C, which were changed with the sterilized seawater everyday. The survival rate was represented as hatching rate, and was recorded by observing number of empty tomonts within 144 h with aid of stereoscope.

$$\text{Survival rate} = \text{total number of empty tomonts within 144 h} / \text{total number of tomonts} \times 100\%$$

2.5. Control test

Fish were divided randomly into four groups: placemat removal group (Group I), rotational culturing group (Group II), infection control group (IF group) and blank control group (Control). Each group contained