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Research Article

Edwardsiella ictaluri Infection in Rainbow Trout (*Oncorhynchus mykiss*)

Oktay KESKİN
Department of Microbiology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa - TURKEY
Selçuk SEÇER
Department of Fisheries and Aquaculture, Faculty of Agriculture, Ankara University, Ankara - TURKEY
Müjgan İZGÜR
Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY
Süheyla TÜRKYILMAZ
Department of Microbiology, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın - TURKEY
Rajhab Sawasawa MKAKOSYA
Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY

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Abstract: The aim of this study was to isolate *Edwardsiella ictaluri* from diseased rainbow trout obtained from a commercial farm in the Ankara region, Turkey. Growth of the microorganism after 36 h of incubation was higher and purer at 28 ºC than at 37 ºC on all the organs (liver, spleen, kidney) of the 10 fish examined. Three groups, each consisting of 3 fish were used for experimental infection. All the fish in Group I, which received a pure culture suspension, died after treatment. There were no clinical symptoms of the disease or deaths in Group II treated with 1/50 concentration of the pure culture or in Group III (control group) treated with 0.85% saline. The isolation of *E. ictaluri* from non-ictalurid fish and the fact that similar bacteria have been isolated from non-ictalurid fish in different countries indicate that the organism is a widespread pathogen. The results from this study show that rainbow trout are susceptible to *E. ictaluri* and there is a possibility of natural infection.

Key Words: *Edwardsiella ictaluri*, *Oncorhynchus mykiss*, natural infection

Gökkuşağı Alabalığında (*Oncorhynchus mykiss*) *Edwardsiella ictaluri* İnfeksiyonu

Özet: Bu çalışmada, Türkiye'de Ankara yöresindeki özel bir balık üretim işletmesinden getirilen hasta gökkuşağı alabalıklarından etken izolasyonu amaçlandı. İncelenen 10 adet balığa ait organlardan (karaciğer, dalak, böbrek) yapılan ekimler sonucunda, 36 saatte 28 ºC'lik inkubasyonda 37 ºC'lik inkubasyondan daha fazla ve saf olarak *Edwardsiella ictaluri* izole edildi. Deneysel infeksiyon oluşturmak amacıyla herbir grupta 3'er balık olacak şekilde 3 grup kullanıldı. Saf kültür verilen Grup I'deki balıkların tamamı uygulama sonrası öldüler. 1/50 yoğunlukta saf kültür uygulanan Grup II ve % 0,85'lik fizyolojik tuzlu su uygulanan Grup III (Kontrol Grubu)'te herhangi bir klinik semptom veya ölüm görülmedi. Yayın balıkları ailesinden olmayan balık türlerinde de *E. ictaluri* infeksiyonlarının görülmesi ve etkene benzer bakterilerin bu balık türlerinden değişik ülkelerde izole edilmiş olması, bakterinin tüm dünyada yaygın bir patojen olabileceğini düşündürmektedir. Bu çalışmada elde edilen sonuçlar, Gökkuşağı alabalıklarının da *E. ictaluri*'ye duyarlı olduklarını ve doğal infeksiyonların oluşabileceğini göstermiştir.

Anahtar Sözcükler: *Edwardsiella ictaluri*, *Oncorhynchus mykiss*, doğal infeksiyon

Introduction

Edwardsiella ictaluri is the causative agent of enteric septicemia in channel catfish (1,2). The organism was first isolated in Georgia and Alabama in the United States in 1976 and has subsequently been frequently isolated from diseased fish (1).

Organisms in the genus Edwardsiella exhibit typical characteristics of the Enterobacteriaceae. They are Gram negative, coccoid, measuring 1 x 2-3 µm, non-spore forming and motile by peritrichous flagella. *E. ictaluri* shows weak motility when grown at 25-30 °C, and becomes completely immotile when grown at higher temperatures. Microorganisms in the genus are facultatively anaerobic, catalase positive, oxidase negative and they ferment glucose and many other carbohydrates thereby producing acid and gas. They are frequently

isolated from spring waters and cold-blooded animals. They are also found in warm-blooded animals and may cause various disorders in man as opportunistic pathogens (3). *E. tarda* and *E. ictaluri* cause serious infections in fish. *E. tarda* causes septicemia in warmwater fish (catfish and eels) and the infected fish tend to cause gastroenteritis and rarely other disorders in man. *E. ictaluri* is the causative agent of enteric septicemia with 10-50% mortality in catfish. The microorganism has 1-3 plasmids whose role has not been elucidated. The microorganisms produce small punctiform colonies on solid media in 36-48 h. The organism grows well at 28-30 °C, but weak growth is exhibited when grown at 37 °C (2).

Enteric septicemia appears in acute as well as chronic forms. In the acute form, death is seen in 4-12 days. Generalized septicemia elicits necrosis and granulomatous inflammation on the skin, small intestinal mucosa, liver, kidneys and nostril mucosa of the fish. In the chronic form, the symptoms of the disease (granulomatous meningoencephalitis and skin lesions that lead to abscess or ulcer) manifest after several weeks or months following infection. The disease is also known as "holed head" because of an open lesion in the head (1). However, such lesions are caused by many other pathogens.

Catfish are a naturally susceptible host to the infection. So far, natural infections have been reported in channel catfish (*Ictalurus punctatus*), brown bullhead (*I. nebulosus*), blue catfish (*I. furcatus*), danio (*Danio devario*), green knifefish (*Eigemannia virens*), walking catfish (*Clarias batrachus*) and white catfish (*I. catus*) (4-8). Experimental studies have been carried out to correlate *E. ictaluri* and non-ictalurid fish species infection (9,10). Following an experimental work Baxa et al. (8) reported that *E. ictaluri* is a potential pathogen for salmonid fishes.

The aim of this study were to isolate *E. ictaluri* from diseased rainbow trout obtained from a commercial farm and to determine its pathogenicity.

Materials and Methods

Fish

Ten 1-year-old, moribund rainbow trout (*Oncorhynchus mykiss*) weighing between 95 and 110 g were brought to the Department of Microbiology of the Veterinary Faculty at Ankara University from a commercial farm rearing fish in net cages at Kesikköprü Dam Lake. Externally petechial hemorrhage was observed in the skin and organs (liver, spleen, kidney), which were aseptically collected for bacterial isolation following the necropsy. The case occurred in the early summer and water temperature was from 17 to 25 °C.

Media

Seven percent sheep blood agar, MacConkey agar (Oxoid), EMB agar (Oxoid), Simon's Citrate agar (Oxoid), Edwards agar (Oxoid) and Brain Heart Infusion Broth (Oxoid) were used for isolation and identification purposes.

Experimental animals

Nine 18 month-old rainbow trout supplied by the Department of Fisheries And Aquaculture, Faculty of Agriculture, University of Ankara, weighing 115 and 125 g, were used to determine the pathogenicity of the isolated organisms.

Isolation process

Organs (liver, spleen, kidney) collected after the postmortem examination were streaked on 2 7% sheep blood agar plates and 2 MacConkey agar plates followed by incubation at 25 °C and 37 °C for 48 h.

Identification process

Colonies forming after 36 h of incubation were identified using the conventional cultural, morphological, biochemical and enzymatic tests and API 20 E test.

For cultural and morfological examinations, ability to grow at 25 °C and 37 °C, oxidation/fermentation (O/F), indole and H_2S production and motility characteristics were investigated. The biochemical characteristics investigated were carbohydrate fermentation (adonitol, arabinose, dextrose, fructose, galactose, glucose, inositol, xylose, lactose, maltose, mannitol, mannose, mellibiose, raffinose, rhamnose, salicine, sorbitol and sucrose), nitrate reduction, Methyl Red/Voges-Proskauer (MR/VP), use of citrate and malonate and hydrolysis of esculine and gelatine. For the enzymatic characterization of the isolates, the presence of oxidase, catalase, tryptophane deaminase (TDA), lysine decarboxylase (LDC), arginine dihydrolase (ADH), ornithine decarboxylase (ODC), β-Galactosidase (ONPG) and urease were investigated. Additionally, sensitivities of the isolates to polymyxin B were determined (11-14).

With the API 20 E system, the organisms' abilities on ONPG, ADH, LDC, ODC, citrate, H_2S, urease, TDA, indole, VP, gelatine and carbohydrate fermentation (glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, mellibiose, amigdalin and arabinose) were evaluated (15,16).

Pathogenicity test

Three groups, each consisting of 3 fish were used for experimental infection. Each fish in Group I was injected with 0.5 ml of a pure culture suspension (approximately 10^6 cell/ml according to the McFarland No: 1 standard) through the muscles surrounding the dorsal fin. The fish in Group II were given a 1/50 concentration of the pure culture. The control group (Group III) was treated with 0.5 ml 0.85% physiological saline water (PSW). The trial was conducted at water temperature (25 °C).

Results

Isolation and Identification results

Growth of the microorganism after 36 h of incubation was higher and purer at 28 °C than at 37 °C on all the organs of the 10 fish examined. The organisms stained Gram negative appeared rod shaped. The microorganisms were identified as *E. ictaluri* using classic biochemical tests and the API 20E tests (Table).

Pathogenicity test results

Externally, petechial hemorrhage was observed in the skin in Group I. One of the fish in the same group died 3 days after the inoculation and the other 2 died on the fourth day. *E. ictaluri* was re-isolated and identified from the organs (liver, spleen, kidney) of the dead fish. The fish in Groups II and III did not develop any clinical signs of the disease and remained alive.

Table . Results obtained from the classic biochemical and the API 20E tests.

Test	Classic method	API 20E	Textbook characteristics**	Test	Classic method	API 20E	Textbook characteristics**
Growth at 37 ºC	+	*		Sensitivity to polymyxine	+	*	
Growth at 25 ºC	+	*		Acid production from			
Motility at 37 ºC	-	*	-	Adonitol	-	*	-
Pigmentation	-	*	-	Amygdalin	*	-	
Oxidase	-	-	-	Arabinose	-	-	-
Catalase	+	*	-	Dextrose	+	*	
O/F (H&L)	+/+	*	+/+	Dulcitol	+	*	-
Citrate	-	-	-	Fructose	+	*	
Nitrate reduction	+	*	+	Galactose	+	*	
Gelatine hydrolysis	-	-	-	Glucose	+	+	+
Malonate	-	*	-	Inositol	-	-	-
Esculin hydrolysis	-	*	-	Lactose	-	*	-
Urease	-	-	-	Maltose	+	*	+
H_2S production	-	-	-	Mannitol	+	+	-
Indol	-	-	-	Mannose	+	*	+
ONPG	-	-	-	Mellibiose	+	+	-
LDC	+	+	+	Raffinose	-	*	-
ADH	-	-	-	Rhamnose	-	-	-
ODC	+	+	+	Salicin	+	*	-
TDA	-	-		Sorbitol	-	-	-
MR	+	*	-	Sucrose	-	-	-
VP	-	-	-	Xylose	-	*	-

* These tests were not used in this system.
**Textbook characteristics of *E. ictaluri* (Bergey's Manual of Determinative Bacteriology).

Discussion

E. ictaluri is the causative agent of enteric septicemia in channel catfish and is known as a specific pathogen of ictalurid fishes. The agent causes serious diseases in fish and was found to be responsible for 50% of the deaths that occurred in Mississippi in 1985-1986 and the epizootic infections that emerged in California in the summer of 1987 (8). Although the agent is said to be specific for ictalurid fishes it has been isolated from nonictalurid fish species such as green knifefish (*Eigemannia virens),* walking catfish (*Clarias batrachus)* and danio (*Danio devario)* during natural epidemic outbreaks (4-7). Following experimental infection with an intraperitoneal injection, although tilapia were found to be susceptible, golden shiners, bighead carp and largemouth bass were resistant to the agent (9).

Although susceptible hosts of *E. ictaluri* are not well defined, the host range of the agent may be wider than is thought (2). So far, natural infections with the agent have been reported in channel catfish, brown bullhead, blue catfish, danio, green knifefish, walking catfish and white catfish (*I. catus*) (2). Although Plumb and Sanchez (9) reported that only catfish are susceptible to the agent, they did not use trout in their study. Baxa et al. (8) studied the susceptibility of channel catfish and 3 other non-ictalurid fish species and found that the agent is a potential pathogen for salmonids. While studying an experimental infection with *E. ictaluri* in chinook salmon (*Oncorhynchus tshawytscha*), white sturgeon (*Acipenser transmontanus*) and striped bass (*Morone saxatilis*), the researchers also investigated the LD_{50} value of the agent in chinook salmon and rainbow trout. They found this value to be 3.4×10^7 cfu/ml for chinook salmon but could not provide a value for rainbow trout because death occurred in the fish inoculated with 7.9×10^8, otherwise no death was noticed with dilutions containing 7.9×10^7 and 7.9×10^6 microorganisms. In the current study, *E. ictaluri* was isolated and identified from naturally infected trout and the results seem to be compatible with the results reported in an experimental study by Baxa et al (8). In this study 300×10^6 and 0.6×10^6 concentrated suspensions were used and deaths were seen in the fish injected with 300×10^6. This concentration falls between the fatal and non-fatal concentrations reported by Baxa et al. (8).

E. ictaluri and similar bacteria from non-ictalurid fish have been isolated in different countries. For that reason, these organisms are a potential pathogen world-wide.

The results of this study show that rainbow trout are susceptible to natural infection with *E. ictaluri*. It is concluded that further studies must be carried out on *E. ictaluri* in other regions of Turkey where farming of rainbow trout has an important role in human nutrition.

References

1. Newton, J.C., Bird, C., Blevins, W.T., Wilt, G.R., Wolfe, L.G.: Isolation, characterization, and molecular cloning of cryptic plasmids isolated from *Edwardsiella ictaluri.* Am. J. Vet. Res. 1988; 49: 1856-1860.
2. Roberts, R.J., Bromage, N.R.: Enterobactericeae Part 2, Chapter 4 In: Bacterial Diseases of Fish. Ed. Inglis V., The University Press, Cambridge, UK, 1994.
3. Holt, J.G., Noel, R.K.: Bergey's Manual of Determinative Bacteriology. 9th Edition, Williams and Wilkins, Baltimore, Maryland, USA, 1994.
4. Blazer, V.S., Shotts, E.B., Waltman, W.D.: Pathology associated with *Edwardsiella ictaluri* in catfish *(Ictalurus punctatus)* and danio *(Danio devario).* J. Fish Biol., 1985; 27: 167-176.
5. Kasornchandra, J., Rogers, W.A., Plumb, J.A.: *Edwardsiella ictaluri* from walking catfish, *Clarias batrachus* L., in Thailand. J. Fish Dis., 1987; 10: 137-138.
6. Kent, M.L., Lyons, J.M.: *Edwardsiella ictlauri* in the green knife fish, *Eigemannia virescens.* Fish Hlth. News 1982; 2: 2.
7. Waltman, W.D., Shotts, E.B., Blazer, V.S.: Recovery of *Edwardsiella ictaluri* from danio *(Danio devario).* Aquaculture, 1985; 46: 63-66.
8. Baxa, D.V., Groff, J.M., Wishkovsky, A., Hedrick, R.P.: Susceptibility of nonictalurid fishes to experimental infection with *Edwardsiella ictaluri.* Dis. Aquat. Organ., 1990; 8: 113-117.
9. Plumb, J.A., Sanchez, D.J.: Susceptibilitiy of five species of fish to *Edwardsiella ictaluri.* J. Fish Dis., 1983; 6: 261-266.
10. Baxa, D.V., Hedrick, R.P.: Two more species are susceptible to experimental infections with *Edwardsiella ictaluri.* Fish Health Section/*American Fisheries Society Newsletter,* 1989; 17: 4.
11. Waltman, W.D., Shotts, E.B., Hsu, C.: Biochemical characteristics of *Edwardsiella ictaluri.* Appl. Environ. Microbiol., 1986; 51: 101-104.
12. Plumb, J.A., Vinitnantharat, S.: Biochemical, biophysical, and serological homogenicity of *Edwardsiella ictaluri.* J. Aquat. Anim. Health, 1989; 1: 51-56.

13. Bisping, W., Amtsberg, G.: Colour Atlas for the Diagnosis of Bacterial Pathogens in Animals. Paul Parey Scientific Publishers, Berlin, 1988.

14. Quinn, P.J., Carter, M.I., Markey, B.K., Carter, G.R.: Clinical Veterinary Microbiology. Wolf Publishing, Spain, 1994.

15. Taylor, P.W., Crawford, J.E., Shotts, E.B. Jr.: Comparison of two biochemical test systems with conventional methods for the identification of bacteria pathogenic to warmwater fish. J. Aquat. Anim. Health, 1995; 7: 312-317.

16. API: API 20E Instruction Manual Version D, Analytical Profile Index. API Computer Service, İstanbul, 1994.