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Case Report

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Isolation and characterization of *Vibrio (Listonella) anguillarum* from catfish

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Abstract: *Vibrio anguillarum* was isolated from several organs of some aquarium catfish in a fish population with high mortality. Macroscopic examination of the affected catfish revealed ascites in the abdomen, site-petechiae, and dorsal erection. Although there are some variations in phenotypic characterization among different isolates of *Vibrio anguillarum*, the majority show a common biochemical profile with distinct microscopic appearance that can provide presumptive identification of *Vibrio anguillarum*. Biochemical tests were used for identification of *Vibrio anguillarum* in aquarium catfish in this report.

Key words: *Vibrio anguillarum*, catfish, bacteriology, isolation

Introduction

Vibriosis is a major disease occurring in marine culture and characterized by haemorrhagic septicemia. Within the genus, *Vibrio (Listonella) anguillarum* possesses a wide distribution throughout the world causing a typical haemorrhagic septicaemia in a great variety of fish species (1,2). Although up to a total of 23 O serotypes are known to occur among *V. anguillarum* isolates, only serotype O1, O2, and to a less extent, serotype O3 have been associated with mortalities in farmed and feral fish throughout the world. The remaining serotypes are considered to be environmental strains and only on rare occasions they are isolated as the cause of vibriosis in fish. The bacterium is gram-negative, short rod-shaped and motile; it can grow in water with concentrations of NaCl in the range of 0.5%-7%. The optimum concentration is about 1%. When the pathogen exposed to sterilized aged lake water, it lost its culturability without losing respiratory activity (3). In the study conducted by Schiewe et al. (4), it was revealed that the 2 biogroups of pathogenic vibrios are readily distinguishable by as many as 17 routine biochemical tests. A medium for differentiation of *Vibrio anguillarum* was described by Alsina et al. (5). The presence of bile salts and the high pH and NaCl concentration select mainly for vibrio species, and sorbitol fermentation differentiates among those vibrios still able to grow (5). In the study of Demircan and Candan (2), PCR method was used to detect the *rpoN* gene, which is specific for the diagnosis of *V. anguillarum* strains. To the best of our knowledge, the

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case described here is the first report of *V. anguillarum* causing death in aquarium catfish.

Case history

In December 2007, several aquarium catfish from a fish population brought to the Microbiology Laboratory, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad. Macroscopic examination of these specimens revealed ascites in the abdomen, site-petechiae, and dorsal erection.

Blood agar plates (7% defibrinated bovine blood) and McConkey agar plates were used for primary isolation of the bacterium. It was recovered from different organs, including liver, spleen, and kidney, after incubation at room temperature (approximately 25 °C) for 48 h. Standard bacteriological methods were used for the following tests: oxidation-fermentation, motility, indole, urease, citrate, nitrate, Voges-Proskauer, and carbohydrates utilization. The requirement for salt was determined using nutrient medium contained 7% NaCl. Sensitivity to antibiotics was determined with different antibiotic disks using disk diffusion agar. The antibiotic disks used were chloramphenicol, novobiocin, oxytetracycline, enrofloxacin, penicillin, and trimetoprim-sulfamethoxazol.

Table 1. Biochemical characteristics of the isolate.

Tests	Results
Catalase	+
Oxidase	+
O/F	Fermentative
Indole	-
Urease	-
Nitrate reduction	-
Voges-Proskauer	+
Simmons citrate	-
Growth on nutrient medium contained 7% NaCl	+
Growth at 37 °C	+
Growth on MacConkey agar	+
Glucose	+
Sucrose	+
Manitol	+
Sorbitol	+
Fructose	+
Maltose	+

Table 2. Antibiotic sensitivity test of the isolate.

Discs of antibiotic	Results
Chloramphenicol	Sensitive
Novobiocin	Sensitive
Oxytetracycline	Sensitive
Enrofloxacin	Sensitive
Penicillin	Resistant
Trimetoprim-sulfamethoxazol	Sensitive

Results and discussion

The results showed pure cultures of a small gram-negative curved rod that were isolated from all organs of the fish. On the blood agar there were circular, raised, yellow-brown opaque colonies of 3 to 5 mm. These became green in heavy growth after 72 h. Colonies were revealed complete hemolysis after 24 h. On MacConkey agar colonies of lactose negative appeared.

Tables 1 and 2 show the results of biochemical and antibiotic sensitivity results of the isolate, respectively. On the basis of the obtained results, the isolated bacterium was identified as *Vibrio anguillarum*.

Vibriosis caused by *Vibio anguillarum* has been reported in various kinds of fish and is known as one of the most serious diseases in salmonid cultures (3). The bacterium is gram-negative, short curved rod shaped, and motile. It can grow in water with concentrations of NaCl in the range of 0.5%-7% (3). In this study this bacterium was isolated from catfish in a fish population with high mortality. Identification of this isolate was achieved by a variety of biochemical tests. Many methods have been applied by some researchers around the world. Because of high mortality of affected catfish, diagnosis is extremely important in reducing the mortality. Some

These criteria were used to place the isolate in the genus Vibrio and the species anguillarum.

biochemical properties that were defined for this genus of bacteria are cytochrome oxidase positive, fermentation of glucose with production of acid but no gas, and positive motility. Our isolate dose not ferment lactose, hence lactose negative colonies appeared on MacConkey agar. Strains fermenting lactose were also described (6). Our findings on biochemical characteristics are similar to the findings described in the literature (1,2,4,7,8). Some biochemical variations among *V. anguillarum* strains were noted by Schiewe et al. (4). They showed that *V. anguillarum* and *V. ordalii* (*V. anguillarum* biotype 2) differ in cultural and biochemical characteristics (4). In the study of Powell and Loutit (1), it was shown that from the original 37 isolates, 25 were New Zealand strains of *V. anguillarum*. These strains were closely phenotypically similar to the salmon pathogenic strain 775, but not to strain 2028, which is pathogenic for cod (1). Difficulty in identification of isolates because of their biochemical heterogeneity required a wide range of tests to ensure correct identification.

A medium for differentiation of *V. anguillarum* is described by Alsina et al. (5), which contains bile salts and high Nacl and pH concentration. This medium selects mainly vibrio species, and sorbitol fermentation differentiates among those vibrios still able to grow (5). In the study of Cisar and Fryer (7) various biochemical tests were used for identification of isolates isolated from Chinook salmon. They reported all of these isolates as *V. anguillarum* type A, according to Nybelin classification, which described 2 types of this bacterium (7). In the study of DiSalvo et al. (9), it was shown that *V. anguillarum* was isolated as a pathogen in the commercial culture of oyster spat. An exotoxin extracted from cultures of the isolates inhibited larval swimming and contributed to larval mortality. Production of toxins by the bacteria that inhibit larval swimming may be the cause of spotting phenomena seen in the hatchery, where a large number of larvae aggregate on the bottoms of cultures tanks, so larval feces and sedimented feed algae stimulate bacterial growth and promote larval infection (9). In the study of Demircan and Candan (2), PCR method was used to detect the *rpoN* gene, which is specific for diagnosis of *V. anguillarum* strains. It seems that PCR can be used as a confirmatory test, especially if some isolates show very different biochemical characteristics, other than those that are common for the majority of isolates.

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