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Occurrence and Distribution of *Aeromonas* Spp. In Goldfish (*Carassius auratus*) Farms in Guilan Province, Iran

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Abstract: The bacteriological study on goldfishes (*Carassius auratus*) that were obtained from commercial goldfish farms of the Guilan Province in Iran, both in healthy fishes and fishes with cutaneous ulcerative signs, was conducted in all seasons of the year 2009. Fishes were examined for existence of bacteria especially *Aeromonas* spp. Fishes from all the examined farms were infested by gram negative and gram positive bacteria. One of the bacteria separated from fish kidney in all seasons was an atypical, often late-pigmenting strain of *Aeromonas salmonicida.* This organism was isolated from 8 (6%) of the total 118 cultured samples. The second isolated bacteria was *Aeromonas hydrophila,* which was separated from fishes in four seasons from 12 (10%) of the total cultured samples. Also *Aeromonas* spp. in 20 (16%) of the total cultures were detected. From this study it was concluded that the highest prevalence of bacteria was in seasons which goldfishes were under the most stress. These opportunistic bacteria can cause ulcerative signs and mortality in commercial farms under the unsuitable conditions.

Key words: Goldfish · Guilan · Opportunistic bacteria · Aeromonad · Disease

INTRODUCTION

Every year, close to the New Year ceremony in Iran, goldfish selling and trade which is an Iranian tradition for nowrouz celebration, is started. Because of the high mortality of these fishes in this special period, we decided to evaluate the prevalence of *Aeromonas* spp. which is an opportunistic bacteria that can cause sickness under unsuitable maintenance conditions in goldfish farms. Because many bacteria isolated from fish with hemorrhagic septicemias in fish were often misidentified, it is now recognized that certain isolations of bacteria ascribed to the genera *Pseudomonas, Proteus, Bacillus, Aerobacter* and *Achromobacter* actually belonged to the genus Aeromonas. Consequently, Aeromonads are often referred to as a complex of disease organisms that are associated with bacterial hemorrhagic septicemias and other ulcerative conditions in fishes. So far 15 species of Aeromonas are described in fishes [1].

Aeromonas salmonicida is a significant pathogen of many freshwater and marine species of fishes and has been reported to occur in most countries around the world. A typical form of *Aerornonas salmonicida* is the cause of goldfish ulcer disease (GUD), an ulcerative dermatitis prevalent aquarium fishes [2]. *Aeromonas hydrophila* and other motile aeromonads are ubiquitous gram-negative, rod-shaped and among the most common bacteria in freshwater habitats throughout the world, these bacteria are also normal inhabitants of the gastrointestinal tract of fish and frequently cause disease among cultured and feral fishes [3, 4].

Fishes infected with Aeromonas may have many different clinical signs. These include from sudden death in apparently healthy fishes to inappetence, swimming abnormalities, pale gills, bloat and skin ulcerations. The skin ulcers may occur at any site on the fish body and often they are surrounded by a bright red rim of tissue. Because of the variability of these clinical signs, the diagnosis of this disease based only upon the clinical presentations of the fishes is highly unreliable and eventually can be economically disastrous to the fish producers [5].

MATERIALS AND METHODS

The majority of the fishes used in this study were obtained from commercial goldfish farms in the Guilan Province in north of Iran and both healthy fishes and

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fishes with a cutaneous ulcerative signs, were studied. Fishes from all sources were examined immediately after arrival to the laboratory for bacteria.

As one of the best methods of bacteriologic studies in fish is by bacteriologic culture of affected organs, so cultures were made from lesions or kidneys or both from 20 goldfish in spring, 33 goldfish in summer, 33 goldfish in autumn and 32 goldfish in winter.

Skin lesions were cultured by inserting a sterile bacteriologic loop beneath the scales in affected areas. Kidney cultures were done after disinfecting the surface of the fish with 70% ethanol and opening the body cavity with sterile scissors. Culture media used included nutrient agar (NA) with 5% defibrinated sheep blood for the isolation of fastidious organisms and trypticase soy agar (TSA) as general isolation media.

All cultures were incubated at 18-22°C for at least 7 days. Growth characteristics and gram stain characteristics were recorded for the predominant colony types on each plate. Organisms which were among the predominant observed types were sub-cultured for 48 to 72 h on the same medium and characterized by the form and size of colonies and biochemical reactions (gram stain, oxidase, vibriostatic reagent 0/129, motility, catalase, ureas, OF, indole, nitrate, gelatin and fermentation of carbohydrates such as arabinose, glucose, lactose, salicin, cellobiose, sucrose, maltose, mannitol,…) for bacterial identification. Oxidation-fermentation test (OF) with basal medium supplemented with 1% glucose, as a critical reaction that differentiates the motile aeromonads from species of *Pseudomonas* [6, 7], also was done.

Eventually Polymerase chain reaction (PCR) also was used for 3 samples to confirm the data available from phenotypic observations and biochemical reactions.

RESULTS

Cultures on the Mediums Showed:

- Discrete rounded cream colonies which, on prolonged incubation, produced a brownish coloration and their size 2-3 mm. The organisms were small rods (which showed some pleomorphism), occurred singly or in pairs and some had rounded ends, gram-negative, oxidase positive, resistant to vibriostatic reagent 0/129. For these findings the organism was identified as *Aeromonas* [1]. Motility was difference between *Aeromonas salmonicida* and motile aeromonads. Differentiation of common motile aeromonads was done by Buller [1].
- Indiscrete small grey colonies with undulating edges produced a blue-black coloration signifying oxidase production, with the addition of phenylenediamine to the surface of the Trypticase soy agar. The organisms were gram negative, motile rods, which occurred separately and long. These bacilli fermented glucose without any gas production. On these colonies the organism was identified as a member of the *Pseudomonas* spp.

Results of oxidation fermentation were interpreted as follows: yellow coloration in both tubes (one tube with a plug of sterile petrolatum and one tube without petrolatum) indicated acidic fermentation of glucose typical of *Aeromonas,* whereas yellow coloration only in the tube without petrolatum indicated oxidation of glucose that is a characteristic of *Pseudomonas.* Results of this study are shown in Table 1.

Bacteria were isolated from 72% (86 of 118) of the fish kidneys. Among identified bacteria, *Aeromonas salmonicida* in 8 (6%), *Aeromonas hydrophila* in 12 (10%) and *Aeromonas* spp. in 20 (16%) of the total cultures were detected. Other organisms commonly isolated were bacteria presumptively identified as *Pseudomonas* spp. This bacteria was isolated from three seasons and 5 (4%) of the cultures. Miscellaneous organisms during the course of this study were lumped under the classification mixed gram-negative rods and gram-positive bacteria. These bacteria were isolated with more frequency (16% in gram-negative rods and 18% in gram-positive bacteria) from cultures. The highest prevalence of bacteria was in summer and then in the early spring, that goldfishes were under the most stresses. The least frequency of bacteria was in winter. Results of this study were

Table 1: Isolated bacteria from goldfishes in the present study

	Number of infected fish with cultured bacteria (% positive)			
Bacterial types most frequently isolated from each fish	Spring	Summer	Autumn	Winter
Aeromonas salmonicida	2/20 (10%)	2/33 (6%)	1/33 (3%)	3/32 (9%)
Aeromonas hydrophila	3/20 (15%)	5/33 (15%)	3/33 (9%)	1/32 (3%)
Aeromonas spp.	5/20 (25%)	7/33 (21%)	5/33 (15%)	3/32 (9%)
Pseudomonas spp.	0/20 (0%)	1/33 (3%)	3/33 (9%)	1/32 (3%)
Unidentified gram negative rods	7/20 (35%)	7/33 (21%)	3/33 (9%)	2/32 (6%)
Unidentified gram positive bacteria	2/20 (10%)	10/33 (30%)	5/33 (15%)	5/32 (15%)

confirmed by C.V.L (central veterinary laboratory) belongs to I.V.O (Iran veterinary organization). Also polymerase chain reaction on 3 samples identified as *Aeromonas salmonicida*, confirmed the identification of this organism.

DISCUSSION

Goldfish (*Carassius auratus*), Cyprinidae family was introduced from China to Iran several years ago. Every year millions of goldfishes are used in nowrouz traditional ceremony in Iran. Although this fish is very resistant to bad conditions, but every year lots of them die and this makes a great economic losses.

Lack of enough space that decreases water oxygen, direct sunlight, unusual noises, overcrowd and pollution can cause stress to fishes and this can weaken fish immune system and activate water opportunistic microorganisms like bacteria. In these conditions, bacteria like *Aeromonas* spp. are findable in internal organs.

The intestinal tract or epidermal abrasions are the possible portals of bacterial entry. Internally, the liver and kidneys are target organs of an acute septicemia. The liver may become pale or have a greenish coloration. These organs are apparently attached by bacterial toxins [8].

Aeromonads are ubiquitous and occur in most fresh water environments. They are adapted to environments that have a wide range of conductivity, turbidity, pH, salinity and temperature [9]. These bacteria cause diverse pathologic conditions that include acute, chronic and covert infections. Severity of disease is influenced by a number of interrelated factors, including bacterial virulence, the kind and degree of stress exerted on a population of fish, the physiologic conditions of the host and the degree of genetic resistance inherent within specific populations of fishes [10].

Pathologic conditions attributed to members of the aeromonads complex may include dermal ulceration, tail or fin rot, ocular ulcerations, erythrodermatitis, hemorrhagic septicemia, red sore disease, red rot disease and scale protrusion disease. In the acute form of disease, a fatal septicemia may occur so rapidly that fish die before they have time to develop anything but a few gross signs of disease. When clinical signs of infection are present, affected fish may show exophthalmia, reddening of the skin and an accumulation of fluid in the scale pockets [9].

Although aeromonads appropriately receive much notoriety as pathogens of fish, it is important to note that these bacteria also compose part of the normal intestinal microflora of healthy fishes [11]. Therefore, the presence of these bacteria, by itself, is not indicative of disease and consequently, stress is often considered to be a contributing factor in outbreaks of disease caused by these bacteria. Such stressors are most commonly associated with environmental and physiological parameters that adversely fish under intensive culture.

In the present study, we used kidney tissue as an internal source of bacteria for isolating *Aeromonas* spp. Most of fishes were clinically normal and many of them had ulcer signs. Eventually in both group of fishes, *A. salmonicida* in 8 (6%), *A. hydrophila* in 12 (10%) and *Aeromonas* spp. in 20 (16%) of the total cultures were detected.

As in the goldfish cases, mortality and severe infections were not seen, so isolated *A. salmonicida* are thought to belong to the subspecies nova. *Aeromonas* spp. has been reported to occur in most countries around the world. It was shown that aeromonad isolates along the River Porma, Leon Province (Spain) can be grouped within three species, *A. hydrophila, A. sobria* and *A. caviae* [12]. Isolated *Aeromonas salmonicida* from skin ulcerations of goldfish has been subsequently characterized as a typical biotype [13]. However atypical *A. salmonicida* has been cultured from skin ulcers of other cyprinid species [14]. The occurrence of *A. salmonicida* in Australia was formally reported in another study [11]. Events associated with the establishment of atypical *A. salmonicida* in goldfishes (*Carassius auratus*) in Australia have been documented [15]. Following introduction in 1974 with broodstock goldfish (*Carassius auratus*) imported from Japan, *A. salmonicida* became enzootic in the commercial goldfish farms [16]. In 1992, atypical *A. salmonicida* was isolated from cutaneous ulcers on silver perch (*Bidyanus bidyanus*) with ulcerative dermatitis [16]. Severe eye pathology and heavy mortality have been reported among yearling and older rainbow trout accompanying a severe outbreak of motile aeromonad septicemia. This study showed that the gills may hemorrhage and ulcers may develop on the dermis [17].

Knowledge of the true affinities of bacterial isolates from different sources enables meaningful assessment of the likely means of disease transmission and may assist in the formulation of disease control strategies. Under conditions of stress, it is even likely that some strains of motile aeromonads that are ordinarily part of the normal gut flora become pathogenic.

Results of our study in four seasons of the year indicated that, in spring and summer which fishes were under most stress, highest prevalence of opportunistic bacteria is seen.

It was described that infection occurs in winter, when fish are relatively inactive and that the disease breaks out in spring, but aquarium fishes, which are usually maintained at constant water temperature, can develop this disease at any time [18]. Recent studies have shown that *A. hydrophila* can infect internal organs through the digestive tract or through uninjured skin under conditions of crowding (13.1 g of fish/L) and unsuitable temperature in catfish. Such infections did not occur when catfishes were held at a lower density (5.2 g of fish/L) and optimal temperature (18°C) [19]. Also another study has indicated that increasing water temperatures increases metabolism, decreases overall condition and make stress for the fishes. Production of corticosteroids is increased in fishes under stress, which in turn increases their susceptibility to infection [20]. Diseases associated with motile aeromonads are most severe among fishes that are propagated under conditions of intensive culture; these bacteria may also affect feral fishes and are common in the intestinal flora of apparently healthy fishes [11].

Aeromonas species is transmissible from animal sources such as fish to human, especially children. In one study on children, *Aeromonas* species has been seprated from 53 children with diarrhea and it was shown that these bacteria can cause acute onset of watery diarrhea with fever and vomiting. *Aeromonas* was also isolated from 2 (0.5%) of 380 asymptomatic children [21]. Also occurrence of *Aeromonas* spp. in feces of children with diarrhea has been reported in another study [22].

As the prevalence of opportunistic bacteria is more in bad conditions of culture, so effective management is the best approach to avoid infections and subsequent epizootics caused by these bacteria. Motile aeromonad septicemias are generally mediated by stress. For example elevated water temperature, decrease in dissolved oxygen concentration or increases in ammonia and carbon dioxide concentrations have been shown to promote stress in fish and trigger motile aeromonad infections [9]. So the monitoring of environmental variables can therefore enable one to forecast stressful situations and possibly avoid problems before they arise.

With intensive fish farming systems, whether these systems are outdoor ponds or indoor aquaria and tanks, predisposing factors are primarily responsible for the precipitation of some diseases. Stress is the most important predisposing factor associated with diseases [23]. Stress can be caused due to poor management and/or poor water quality. Management factors include: nutrition, handling, transportation and over-crowding of fish. Controlling water quality is an excellent way to prevent this disease. This means that the dissolved oxygen (DO), pH, temperature and alkalinity of the water must be satisfactory and that the ammonia, nitrite and CO levels must be kept to a minimum level.

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