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The prevalent bacterial fish diseases in fish hatcheries of Latvia

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Abstract

To evaluate the bacterial background, pathological examination of fish was performed in seven state fish hatcheries and three private farms from 1997 to 2009. A total of 3334 individual fish were examined. Bacteriological material from ulcers of the surface, gills, heart, liver, kidney, spleen and muscles was collected from individuals with visible clinical signs and from clinically healthy fish. Bacteriological samples were inoculated on plates with specific medium. Representative colonies were cultivated for further biochemical characterization and identification. Strains were identified by using commercial bacterial identification test strips. The study showed that the prevalent isolated pathogenic bacteria were *Flexibacter* spp., *Aeromonas salmonicida*, and *Aeromonas hydrophila*. Bacteriological examination revealed that 15.3% of tests were positive for aeromonosis,16.5% tests for myxobacteriosis and 68.2% tests demonstrated negative results. Greater attention should be directed to fish bacterial diseases, as they have a significant impact on cultured and occasionally wild populations.

Key words: Aeromonosis, Baltic salmon, carp, hatchery, myxobacteriosis, rainbow trout.

Introduction

Bacteria are important pathogens for both cultivated and wild fish, and are responsible for serious economic losses. Some bacteria cause only surface diseases as skin or gill infections, especially flexibacteria, but some inflict systemic disease (Inglis et al. 2001). The prevalent fish diseases in fish farms are usually initiated by bacteria.

There are basically two types of bacteria producing disease – obligate pathogens and facultative pathogens. Facultative pathogens can survive indefinitely in water and, when environmental conditions are conductive, infectious fish diseases may spread. Many potentially pathogenic bacteria of fish normally exist in a commensal association with the host or live free in the environment. Both these types of bacteria become pathogenic when the fish is immuno-compromised by some form of stressor (Kirjusina et al. 2007).

Fish bacterial infections can occur as a bacteremia, which implies the presence of bacterial organisms in the bloodstream without clinical signs. Others occur as a septicemia, which indicates that bacteria and toxins are actually present in the circulatory system and usually precipitate disease and clinical signs. Inflammation, hemorrhage and necrosis are clinical signs associated with septicemia. Gram-negative bacteria can produce either exotoxins or endotoxins, which consist of proteolytic enzymes that kill host cells and cause necrosis or can make blood vessels more porous and cause hemorrhage (Kirjusina et al. 2007).

Fish in husbandry are exposed to a multitude of stressors. Among the important stress-inducing factors are those with strong psychological components that cause fright, excitement and discomfort. Stress can be induced by such activities as handling, transport and weighing. Moreover, crowding at high densities also produces a variety of stimuli that cause stress (Peters et al. 1998). Outbreaks of diseases are associated with depressed oxygen levels. Predisposing risk factors include also overcrowding, organic pollution and hypoxia (Kirjusina et al. 2007). In fish farms, mainly salmon farms, outbreaks are typically associated with stress, especially, high temperatures.

The dominant fish bacterial diseases are furunculosis and myxobacteriosis of salmonids and aeromonosis of carps. Myxobacteriosis is a common bacterial disease that affects the skin or gills of freshwater fish. *Flexibacter columnaris* is the most prevalent member of this group, which has a worldwide distribution and can probably infect most freshwater fish. It is an important fish pathogen and can rapidly infect a population and cause large mortalities (Kirjusina et al. 2007).

Aeromonas spp. are universally distributed and can be isolated from a wide range of environmental and water samples. The most abundant pathogenic bacteria isolated from the environment is *Aeromonas hydrophila* (Briede, Medne 2004). However, furunculosis caused by *Aeromonas salmonicida* can produce massive fish mortality and is becoming a serious problem in fishery as fish of all ages are susceptible. The disease symptoms vary according to

the stage of infection. In peracute infections, the disease is characterized by a sudden death without any premonitory clinical signs. In its acute form, the disease is characterized by septicemia with external and internal hemorrhagic lesions; the chronic form of the disease is characterized by formation of external furuncle-like lesions and congestion of visceral organs (Cipriano, Bullock 2001; Kirjusina et. al. 2007).

As aeromonosis has a significant impact on cultivated fish and, occasionally, on wild fish populations, more attention needs to be directed on studies of various aspects of the disease. Future investigations will include wild fish population examination, both for aeromonosis and furunculosis.

The aim of this study was to investigate the bacteriological background of fish diseases in farms.

Materials and methods

Baltic salmon and trout from larvae (0+) to smolt stage were collected from fish hatcheries, where they were reared in 800 litre tanks and supplied with aerated fresh water from a river basin. Routine pathological examinations of fish were carried out from 1997 to 2009. In the period from 1997 to 2000, bacteriological investigations were performed for myxobacteriosis of salmonids and since 2001, the bacteriological tests included also aeromonosis of salmonids and cyprinids fish. Sampling was conducted in seven state fish hatcheries ("Brasla", "Dole", "Karli", "Kegums", "Pelchi", "Salaca" and "Tome") and three private farms (two lakes in Latgale district and "Sillakas"). A total of 3334 individuals of salmon and trout from larvae (0+) to smolt stage, 10 individuals of rainbow trout (2+), two individuals of carps (*Cyprinus carpio*), and four individuals of pike perch breeders (*Stizostedion lucioperca*) were investigated. Bacteriological material from ulcers of the surface, gills, heart, liver, kidney, spleen and muscles were collected both from individuals with visible clinical signs as well as from clinically healthy fish. Bacteriological samples were inoculated on plates with specific medium. Strains of *Aeromonas* spp. were plated on nutrient agar plates; strains of *Flavobacterium* spp. were plated on plates with Anackel-Ordal medium and incubated for four to five days at 18 to 25 °C (Balcázar et.al. 2007). Representative colonies were cultivated for further biochemical characterization and identification. Strains were identified using commercial bacterial identification test strips API-20E (Bio-Mèrieux, France) (Janda et al. 1996; Wiklund et al. 1999).

Fish mortality and water temperature were estimated daily in each fish hatchery.

Results

The investigations from 1997 to 2009 demonstrated that the prevalent fish bacterial diseases were myxobacteriosis and furunculosis of salmonids and individual cases of aeromonosis of carps and pike-perch. In summer, water temperature varied between 18 and 26 °C, with the highest peak in July, when the majority of disease outbreaks occurred. During this period, several outbreaks with massive fish mortality were registered.

Since 2004, investigations were focused also on aeromonosis because these bacteria *Aeromonas* spp. were isolated from clinically health fish and positive results were not always related with disease outbreaks. In winter *Aeromonas* spp. and *Flexibacter* spp. were isolated only

Table 1. Positive results for aeromonosis and myxobacteriosis from river basins

Year	Lakes in Latgale	River basin								Total number of analyses
		Daugava		Gauja		Venta		Salaca		
	Aero-monosis	Aero-monosis	Myxo-bacteriosis	Aero-monosis	Myxo-bacteriosis	Aero-monosis	Myxo-bacteriosis	Aero-monosis	Myxo-bacteriosis	
1997			9		1			1	1	29
1998			5						2	20
1999		2	1	2	1				1	28
2000		2	3							32
2001		5	2	4	2					24
2002		2	5	1		1	1			24
2003	1	2	4	1	4					21
2004		4	1	1						26
2005	1	2		2	3					30
2006		1	1	2	2					29
2007		2	2	4	2					33
2008		1		4		1	1			30
2009			1	2	1	1	1			19
Total	2	23	34	23	16	3	3	1	4	345

Table 2. Positive results of aeromonosis and myxobacteriosis for investigated fish species

Year	Lakes in Latgale	River basin							
		Daugava		Gauja		Venta		Salaca	
	Aero-monosis	Aero-monosis	Myxo-bacteriosis	Aero-monosis	Myxo-bacteriosis	Aero-monosis	Myxo-bacteriosis	Aero-monosis	Myxo-bacteriosis
Salmon		17	32	18	12	3	2	1	4
Trout		4	2	5	4	0	1	0	0
Rainbow trout		1							
Carps	2								
Pike perch		1							
Total	2	23	34	23	16	3	3	1	4

from fish with disease signs, but in summer also from clinically healthy fish. Better growth for both bacteria occurs at higher water temperature, with optimal water temperature at 20 to 24 °C. The majority of positive results on bacteriological tests for *Aeromonas* spp. were closely associated with disease outbreaks.

More often, these bacteria were isolated from fish with clinical signs of disease. The prevalent isolated pathogenic bacteria were *Flexibacter* spp., *Aeromonas salmonicida*, and *Aeromonas hydrophila.* There were three cases of other facultative pathogens – *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Samples with *Pseudomonas* spp. also contained *Aeromonas* spp.

Fish collected from hatcheries located on the Daugava and Gauja river basins produced more positive results for both aeromonosis and myxobacteriosis than samples from other river basins (Table 1). Based on aeromonosis and myxobacteriosis cases, Baltic salmon was more infected than the other fish species (Table 2). Of all tests, 15.3% were positive for aeromonosis, 16.5% tests for myxobacteriosis and 68.2% were negative (Table 3).

Several acute disease outbreaks were registered, in 1997, 2001, and 2003, when massive fish mortality was observed (Fig. 1). The disease agent was isolated both from fish and water. In other years, only individual cases of disease were observed without massive mortality.

Discussion

In fish farming, mortality due to infectious diseases has been considered to be a significant factor contributing to reduced production and profits. Furunculosis was more frequent during the summer and early autumn. The disease incidence varied with water temperature, being highest in August and lowest in February (Lillehaug et al. 2003). In Latvia, the disease outbreaks were commonly in spring – summer, when water temperature increased to above 20 °C.

In Norway four most important bacterial infections in salmonids are furunculosis, cold-water vibriosis, vibriosis and winter ulcer disease (Lillehaug et al. 2003). The most significant fish diseases in Latvia fish hatcheries are myxobacteriosis of salmonids, furunculosis of salmonids and aeromonosis of carp. During the period from 1997 till 2009 the most common isolated bacteria were *Flexibacter* spp., *Aeromonas salmonicida, Aeromonas hydrophila* and and there were three cases of other facultative pathogens – *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. The fact that *Pseudomonas* spp. occurred in the same samples as *Aeromonas* spp., suggests that these bacteria normally exist in a commensal association with the fish or sometimes free in the environment.

The motile aeromonads are widespread in natural environment – fresh water, brackish water and sea environments. Aeromonosis and myxobacteriosis are called "dirty water illness" because they appear in various unfavourable conditions (Bychkova, Yukhimenko 2007). Bacteriological findings demonstrated that fish collected from the Daugava and Gauja river basins produced more positive results for both aeromonosis and myxobacteriosis than samples from other river basins. Perhaps, the Daugava and Gauja river basins were more contaminated with bacteria, which could raise disease risk. In the Daugava river

Table 3. Positive results of aeromonosis and myxobacteriosis in different fish species)

Site	Fish age	Aeromonosis (%)	Myxobacteriosis (%)
Baltic salmon	0+	2.60	7.23
Baltic salmon	1+	8.67	7.23
Trout	0+	1.16	1.16
Trout	1+	1.73	0.87
Rainbow trout	2+	0.29	-
Carp	n/d	0.58	-

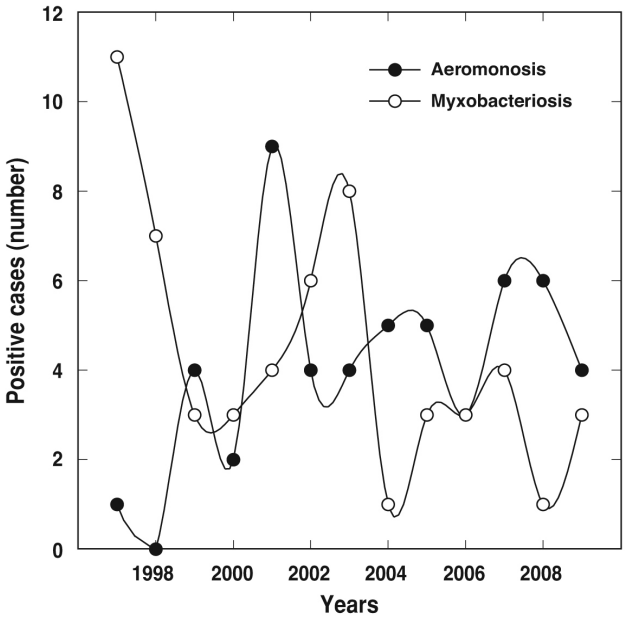

Fig. 1. Positive bacteriological results for aeromonosis and myxobacteriosis during 1997 – 2009

there are three salmonid fish hatcheries downstream one after another, amd thus a disease agent can be transfered from one hatchery to another, which might be the reason of disease outbreaks in distal hatcheries. On the Gauja river basin there are two hatcheries, but on different tributaries which suggests that infection can not be transmitted.

A. salmonicida has been isolated from a wide range of cultivated and wild fish species, non-salmonids as well as salmonids, inhabiting fresh water, brackish water and marine environments (Wiklund, Dalsgaard 1998). Since 2004, the investigations were focused also to aeromonosis because *Aeromonas* spp. was isolated from clinically healthy fish and from the water supply. The obtained results showed that positive results for this bacteria were not always related with disease outbreaks.

The disease problems are more conspicuous in farmed fish than in wild fish. Some of the *A. salmonicida* strains affect salmonids and therefore may represent a significant risk to farmed fish, both salmonids and non-salmonids (Wiklund, Dalsgaard, 1998). In the present study, salmon produced more significant positive results than other examined fish species.

Studies on the role of the environment as a reservoir of pathogenic bacteria are of particular importance. Therefore greater attention will be directed to fish diseases, as they have a significant impact on cultured and occasionally wild populations. Future investigations will examine wild fish populations for aeromonosis and myxobacteriosis.

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