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A Review on Rainbow Trout Fry Syndrome (RTFS)

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Abstract: The present knowledge about rainbow trout fry syndrome (RTFS), a serious bacterial disease affecting cultivated rainbow trout (*Oncorhynchus mykiss*) fry and fingerling in Europe is reviewed. The aetiologic agent of this disease is *Flavobacterium psychrophilum*, a Gram negative, filamentous, chromogenic rod shaped bacterium. The disease usually occurs in the spring when water temperatures are below 10°C. Gross pathological signs of the disease include lethargy, darkening of the skin, bilateral exophthalmia, abdominal distension and periocular hemorrhaging. Internally ascites, swollen spleen, pallor of the liver and anorexia can also be observed. Histologically the most consistent and prominent changes occur in the spleen characterized by peripheral layering due to fibrous infiltration, loss of border definition and generalized intracellular oedema. The diagnosis of RTFS is generally confirmed by isolation of the bacterium from internal organs of fish showing the characteristics signs of RTFS. No effective control methods for RTFS are currently available to fish farmers. The control of RTFS largely depends on the use of antibiotics, such as oxytetracycline and amoxycillin.

Key words: Rainbow trout fry syndrome, RTFS, *Falvobacterium psychrophilum*

Introduction

Rainbow trout fry syndrome or RTFS is a systemic bacterial disease affecting hatchery reared rainbow trout (*Oncorhynchus mykiss*) fry and fingerlings in many parts of Europe during the last few decades (Bernardet *et al.*, 1988; Lorenzen *et al.*, 1991, Santos *et at.*, 1992; Toranzo and Barja, 1993; Wiklund *et al.*, 1994). Rainbow trout are considered as one of the important freshwater food fish species produced in Europe. The disease RTFS is now recognized as a one of the most important factors preventing a thriving rainbow culture industry in Europe. It has also been reported from Australia (Schmidtke and Carson, 1995) and Chile (Bustos *et al.*, 1994). The financial impact of RTFS on world aquaculture is difficult to quantify. The recent estimates of actual losses of rainbow trout fry and fingerlings due to RTFS in the UK alone are 10 million fry per annum. These fish have a potential value of nearly £500,000 and represent up to 22% of all fry produced (Rangdale, 1997). The syndrome appears to affect rainbow trout in the weight range of 0.2 to 10.0 g at water temperatures below 10°C (Rangdale, 1995). Fry losses are often between 10 to 30 % within a single batch of fry (Scott, 1989), but can rise to 70% as the disease rapidly spreads amongst stock (Chua, 1991; Santos *et al.*, 1992).

The disease was first recognized in 1984, when a novel systemic disease of unknown aetiology was seen in rainbow trout hatcheries across the UK (Chua, 1991; Casey, 1993; Rangdale, 1995). The condition was characterized by certain behavioural traits including lethargy, cessation of feeding, swimming close to water inlets/outlets and at the side of the tanks. External gross clinical signs comprised exophthalmia, swollen abdomen, darkening of skin, reddening of the vent and occasional raised epidermal lesions. Internal clinical signs included enlarged friable spleen, hemorrhage of the liver and the posterior and anterior kidney, severe anaemia and ascites. Due to the gill pallor of affected fish, and the inability to detect any known viral, bacterial, fungal or parasitic causal agent with any consistency, the condition was known as rainbow trout anaemia syndrome (Rangdale, 1995). In the present review a detailed account of RTFS, its aetiology, pathology, diagnosis and control measures are discussed in relation to published data.

Synonyms: There has been considerable confusion surrounding the different names applied to the condition. In Denmark, where it is known as fry mortality syndrome, the disease has been reported to cause up to 60% moralities (Lorenzen *et al.*, 1991). In France, it is known as a visceral form of bacterial cold-water disease (BCWD) since the gross pathological changes were typically found internally, in contrast to BCWD (Baudin-Laurencin *et al.*, 1989). The condition has also been diagnozed in Italy where it became visceral myxobacteriosis (Sarti *et al.*, 1992), while in the UK it is known as rainbow trout fry anaemia or RTFS (Santos *et al.*, 1992).

Aetiology: The aetiologic agent of RTFS is *Flavobacterium psychrophilum* (formerly known as *Flexibacrter psychrophilus* and also *Cytophaga psychrophila*). This bacterium was recently transferred into the genus *Flavobacterium* (Bernardet *et al.*, 1996), which includes the causal agent of columnaris disease (*F. columnare*) and *F. branchiophilum*, implicated in bacterial gill disease (BGD). *F. psychrophilum* was originally isolated from Coho salmon (*O. kisutch*) in USA in 1948 (Borg, 1960). This bacterium has also been associated with a systemic bactereamia in salmonid fishes in USA and Canada known as bacterial cold water disease (BCWD).

Cells of *F. psychrophilum* are weakly refractile, Gram negative, strictly aerobic, slender and flexible rods. Reports on the size of the bacterium vary in the literature. Cells from young broth culture (12-48 h) have been reported to be approximately 0.75 µm (Pacha, 1968), 0.3-0.5 µm (Bernardet and Grimont, 1989) and 0.3-0.75 µm (Holt *et al.*, 1993) in diameter. In length, cells have been estimated to be between 1.5 and 7.5 µm (Pacha, 1968), 1.5 –5.0 µm (Bernardet and Grimont, 1989) and 2.0-7.0 µm (Holt *et al.*, 1993).

The bacteria display gliding motility (active movement over surfaces without the aid of flagella). The nature of the mechanism responsible for gliding is unknown. Although there is some strain variation in this ability, it is generally considered that the gliding movement of *F. psychrophilum* is slow and weak, and is only noticed after prolonged observation (Bernardet and Grimont, 1989). Dalsgaard (1993) reported that the production of copious amount of extracellular polysaccharides or slime, facilitates this gliding motion. Poor gliding ability is a notable characteristic of *F. psychrophilum* when compared with other fish pathogens of the order Cytophagales, such as *F. columnare* (Bernardet, 1989) and *F. maritimus* (Wakabayashi *et al.*, 1986).

A low nutrient medium, devised by Anacker and Ordal (1959) is the most commonly used medium for the isolation and cultivation

of *F. psychrophilum*. This medium is also frequently referred to as *Cytophaga* medium (Holt, 1987). Isolates of *F. psychrophilum* incubated for 48-96 h at 15-20°C on this medium produce bright yellow, convex, smooth and glossy colonies with regular margins of 1 to 5 mm in diameter (Bernardet and Kerouault, 1989). On *Cytophaga* medium, the bacterium usually exhibits slow and fastidious growth. Therefore, several authors have reported improved growth performance by changing the medium formulation e.g. increasing the amount of tryptone (Bernardet and Grimont, 1989), adding 10% foetal calf serum (FCS) or 5% FCS and/or 0.5% tryptone (Lorenzen and Karas, 1992). In a recent study, Daskalov *et al.* (1999) reported an improved growth medium for *F. psychrophilum* by supplementing *Cytophaga* medium with 0.5 gl^{-1} each of D (+) galactose, D (+) glucose, L-ramnose and skimmed milk. Generally, growth is limited on tryptone soya agar (TSA). Faruk (2000) reported that auto-agglutination in broth was a common feature amongst the *F. psychrophilum* isolates obtained from outbreaks of RTFS.

Growth of *F. psychrophilum* generally occurs between 4 and 23°C, but not at 30 C° (Holt, 1987). The optimum growth temperature of the bacterium quoted by different authors appears to vary. Pacha (1968) found optimum growth at 20°C. Holt *et al.* (1993) reported optimum growth at 15°C with a generation time of 2 h, while in a recent study by Uddin and Wakabayashi (1997) optimum growth of the bacterium was reported to be at 19.6 ± 0.5° C.

The fastidious and thermo-sensitive nature of *F. psychrochilum* limits its sub-cultivation even when an improved medium is used. Faruk (2000) found that temperature was the most likely cause for the problems related to the sub-culturing of *F. psychrophilum*. Michel *et al.* (1999) mentioned that the cells of *F. psychrophilum* are highly susceptible to osmotic conditions. Moreover, Michel *et al.* (1999) believed that both improvement in the medium formulation and careful handling of the bacteria whilst in isotonic solutions should improve the overall viability and growth of the bacterium.

Isolates of *F. psychrophilum* are unable to utilize either simple or complex carbohydrates, but are actively proteolytic with the capacity to degrade gelatin, casein, and tyrosine (Dalsgaard, 1993; Holt *et al.*, 1993). Using API ZYM galleries (BioMerieux), Bernardet and Kerouault (1989) reported that the bacteria lacked the enzymes necessary for carbohydrate metabolism. Holt *et al.* (1993) reported that *F. psychrophilum* was devoid of cytochrome oxidase activity. Other studies have found varying degrees of activity from weakly positive to readily detectable levels (Bustos *et al.*, 1994; Schmidtke and Carson, 1995). The colonies of the bacterium do not absorb Congo red (Bernardet and Grimont, 1989).

Electrophoretic analysis of the whole cell preparation of *F. psychrophilum* showed that protein and carbohydrate banding patterns of different isolates of *F. psychrophilum* were similar (Faruk, 2000). Moreover, Faruk (2000) observed that the bacterium possess a substantial amount of carbohydrates and glycoprotein in its cellular and extracellular products.

Genetic diversity between *F. psychrophilum* isolates has recently been reported (Cipriano *et al.*, 1996; Lorenzen *et al.*, 1991; Chakroun *et al.*, 1998). Cipriano *et al.* (1996) found that the isolates of *F. psychrophilum* they examined were phenotypically and serologically homogenous, but varied in their ribosomal RNA gene restriction pattern (ribotypes). Chakroun *et al.* (1998) also reported different ribotypes and plasmid profiles between different *F. psychrophilum* isolates, and found that some ribotypes were associated with a particular fish species from which they were isolated.

Pathogenicity: Little information exists in the published literature describing virulence mechanisms associated with the pathogenicity of *F. psychrophilum*. It is speculated that components present in the extracellular products (ECPs), and in the cell wall or on the surface of the bacterium, such as outer membrane proteins (OMPs) lipopolysaccharides (LPS), proteases and iron acquisition system are involved with the infection and virulence. It has been suggested that the proteolytic activity of *F. psychrophilum* may play a role in the pathogenicity of the bacterium (Dalsgaard, 1993). Some potential virulence factors have already been identified for the bacterium, for example extracellular protease activity (Bertolini *et al.*, 1994), although their role and significance in the disease process is not fully understood. Several authors have examined the proteolytic activity of different isolates of the bacterium and different proteolytic activities have been reported for the bacterium (Madsen and Dalsgaard, 1998; Faruk, 2000).

Typical gross pathology: Gross pathological signs of the disease from other European countries appeared similar to those observed in UK including lethargy, swimming close to water surface, darkening of the skin, bilateral exophthalmia, abdominal distension and periocular hemorrhaging (Toranzo and Barja, 1993; Rangdale, 1995). Mortality patterns in fry are typical of an acute systemic bacterial infection. Fingerlings (≈5.0 g) usually show a broader range of signs. The most pronounced signs are blindness, moderate anaemia, visceral petecheae, skin lesions in the region of dorsal fin or on the flanks and abnormal swimming behaviour (Bruno, 1992; Santos *et al.*, 1992). Internally ascites, swollen spleen, pallor of the liver and anorexia can also be observed (Rangdale, 1996). Larger and older fish are infected with a more chronic form of the disease. Typical gross signs are skin ulcers, in particular behind the dorsal fin and on the peduncle. Ulceration on the peduncle can lead to total erosion and loss of the caudal fin (Bruno and Poppe, 1996). Larger trout and particularly brood fish can be asymptomatic carriers of *F. psychrophilum* and act as a reservoir of infection for younger or previously uninfected fish (Rangdale, 1995).

Typical histopathological changes: There are few studies of RTFS describing the histopathological changes, which occur during natural or experimental infection. Chua (1991) reported that the most consistent and prominent changes in naturally affected fish occur in the spleen. The splenic pathology is characterized by peripheral layering due to fibrous infiltration, loss of border definition, generalized intracellular oedema and the presence of swarms of weakly stained long slender Gram negative bacteria, interspersed throughout the organ. Severe congestion of the splenic stroma, depletion of the haemopoetic tissue components, and pyknosis and karyorrhexis in the sinusoids has also been reported (Chua, 1991; Rangdale, 1995).

Bruno (1992) observed weakly stained, Gram negative, filamentous rods within the spleen, liver, kidney and trabecular layer of the heart. He also reported that lateral skin lesions showed necrosis, collapse, pyknosis and lymphocytic infiltration of the dermis and underlying muscle block. Chua (1991), however, noted intracellular oedema of malpighian cells in the entire thickness of the dermis, but reported that deeper layers of the skin were unaffected in RTFS. Rangdale (1995) observed some degree of pericarditis in both naturally and experimentally infected fry, but the changes were not severe and were limited to localized areas of cellular infiltration and vacuolation of the heart muscle. Evensen and Lorenzen (1996) reported that *F. psychrophilum* can

be detected by immunohistochemistry, and showed localization of bacteria in the monocyte-macrophage system, in skin lesions, in the retina and the choroid gland of the eye.

Route of transmission: The route of transmission of *F. psychrophilum* has not been fully established. Nothing is known about the portal of entry of *F. psychrophilum* in fish during infection, the way it adheres to and penetrates the animal, its spread through the animal to target tissues, and its survival within the animal. It has been suggested that *F. psychrophilum* is transmitted both horizontally and vertically (Brown *et al.*, 1997). Dalsgaard (1993) suggested that *F. psychrophilum* probably enters the fish via the gills or through the skin, but they also enter by oral or gastrointestinal routes (Faruk, 2000). The bacterium has been isolated from both brood fish and sexual products from several species of salmonids (Brown *et al.*, 1997; Ekman *et al.*, 1999) indicating transmission of the bacterium from brood fish to their offspring. The bacterium has also been isolated from the inside of fertilized eggs (Brown *et al.*, 1997).

Diagnosis: The diagnosis of RTFS is generally confirmed by isolation of the bacterium from internal organs, particularly the spleen of fry and fingerling showing the characteristic signs of RTFS. Isolation and identification on culture are both time consuming and may be confused with other *Flavobacterium* species. Even serodiagonistic methods using antibody probes (both polyclonal and monoclonal antibodies) are not always useful for diagnosis because of the presence of several serotypes of the bacterium (Faruk, 2000). Recently, molecular techniques have been developed for the diagnosis of *F. psychrophilum*. Polymerase chain reaction (PCR) has been developed to amplify *F. psychrophilum* DNA and has proven useful in diagnostic application (Toyama *et al.*, 1994). PCR-based method to identify and detect *F. psychrophilum* in infected tissue. Ribotyping and plasmid profiling of *F. psychrophilum* showed that these methods, used alone or in combination with other typing techniques, are powerful tools in epizootiological studies of *F. psychrophilum* (Chakroun *et al.*, 1998). These techniques have limited application, as they do not establish if the bacterium is viable and furthermore the presence of the bacterium does not assure a diseased state in the animal.

Prevention and control measures: The bacterium and the response it induces in fish are poorly understood, and as a result no effective control methods for RTFS are currently available to fish farmers. The control of RTFS largely depends on the use of antibiotics, such as oxytetracycline and amoxycillin, either for treating diseased fish or as a preventive measure. There is a risk of drug resistance developing after prolonged use, however. The use of antibiotics is not ideal for the treatment of RTFS, not only because of the development of antibiotic resistant strains, but also because it does not eliminate the source of infection, which is likely to be infected brood stock fish (Rangdale, 1997). There has been a decrease in bacterial susceptibility to these drugs over recent years, and the numbers of resistant *F. psychrophilum* isolates are increasing (Lorenzen, 1994; Rangdale, 1996). There are very few alternatives to the drugs currently used, since very few antibiotics have been registered for use in aquaculture in the European Community. A long-term aid for the control of RTFS and one that would reduce the increasing use of antibiotics, is the development of an effective vaccine.

Vaccination is theoretically an ideal way for controlling many fish diseases. However, no commercial vaccine is currently available to protect against *F. psychrophilum* infection. The development of vaccines against RTFS is complicated by the fact that mortality due to *F. psychrophilum* generally occurs in juvenile fish ranging from 0.5 to 5.0 g in weight. Firstly, it has not yet been fully established exactly when the immune system of rainbow trout fry is sufficiently mature for vaccination to be successful and it may not be possible to vaccinate small fry against the disease (Rangdale, 1999). Secondly, a reproducible and standardized model of experimental infection is currently difficult to achieve and this is necessary to investigate the potency of vaccine preparations. Thirdly, there are technical problems associated with the production of large amounts of this fastidious bacterium under laboratory condition (Bernardet, 1997). Finally, it has been found that serotype differences exist between the different isolates of *F. psychrophilum*, although the exact number have not yet been fully established.

Disinfection of eggs with iodophors to prevent vertical transmission has been attempted, but was reported to be unsuccessful in preventing RTFS (Lorenzen, 1994). Rangdale (1997) reported some success in reducing fry mortality attributable to RTFS after treating eggs with either hydrogen peroxide or glutaraldehyde at concentrations of between 100 and 400 ppm for 10 min. Careful hygiene and stock monitoring within a fish farm is vital in preventing cross infection between different holding tanks.

Future methods for the control of RTFS are likely to include a combination of several methods, including improving the general hygiene of the farms, careful management, disinfection of eggs, vaccination and chemotherapy using antibiotics and the use of immunostimulants.

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