

## Enteric redmouth disease: Past, present and future: A review

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### Abstract

Enteric red mouth disease (also known as Yersiniosis) is one of the most significant bacterial infections in coldwater fish farms that cause significant mortalities and economical losses in the salmonids fish farms, especially in rainbow trout (*Oncorhynchus mykiss*). ERM is caused by the gram negative pathogen bacteria *Yersinia ruckeri* that has five O-serotypes (O1, O2, O5, O6 and O7), five outer membrane protein types (OMP types 1–5) and two biotypes 1 and 2. The disease has a wide geographical distribution in various fresh or sea water fish. More than twenty species mainly of Salmonide origin have been affected and this number is likely to rise in the future following the introduction of new species and the increase of aquaculture trade. The disease can affect fish of all age classes but is most acute in small fish up to fingerling size. Affected fish may reveal different clinical symptoms depending on species, age and temperature. The most characteristic and common clinical signs of the disease include lethargic behavior and inactivity, swimming near the surface, anorexia, and darkening of the skin. The reddening of the throat and mouth, caused by subcutaneous haemorrhaging and exophthalmos are commonly present. Different diagnostic methods have been used for *Y. ruckeri*, including culturing, serological, biochemical tests, histopathological studies and molecular techniques. This review summarizes the past, present and future state of yersiniosis with emphasis on status of this disease in Iran. Also, some criteria in diagnosis, control and prevention of ERM were discussed.

**Keywords:** Enteric redmouth disease, *Yersinia ruckeri*, Diagnosis, Control and prevention

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## Introduction

Enteric redmouth disease (ERM) or Yersiniosis is a systemic bacterial infection and one of the most significant diseases in farmed fish, especially salmonids (Gregory *et al.*, 2010). Yersiniosis is caused by Gram-negative bacteria that belonged to a member of the family Enterobacteriaceae with the name of *Yersinia ruckeri* (Argenton *et al.*, 1996; Soltani *et al.*, 2014). The pathogen was first isolated from rainbow trout *Oncorhynchus mykiss*, in the Hagerman Valley of Idaho, USA, in the 1950s (Rucker, 1966). At present, Yersiniosis has been isolated from different continent including the USA (Arias *et al.*, 2007; Bastardo *et al.*, 2011; Haig *et al.*, 2011), Europe (Austin *et al.*, 2003; Fouz *et al.*, 2006; Tobback *et al.*, 2007; Wheeler *et al.*, 2009; Welch *et al.*, 2011; Chettri *et al.*, 2013), Australia (Carson and Wilson, 2002; Keeling *et al.*, 2012), Africa (Hussein *et al.*, 1997; Eissa *et al.*, 2008) and Asia (Zorriehzahra *et al.*, 2002; Akhlaghi and Sharifi, 2008; Adel *et al.*, 2013; Soltani *et al.*, 2014; Fadaeifard *et al.*, 2014). Yersiniosis is the second bacterial disease in coldwater fish farms in Iran and 15 epidemic outbreaks of this bacterial disease were reported in the country's provinces especially from Mazandaran, Tehran, Lorestan and Chaharmahal Bakhtiari provinces during 2012-2013 (Zorriehzahra *et al.*, 2012; Soltani *et al.*, 2014). The bacterium responsible for economic losses is numerous in salmonid and non-

salmonid fish species, reared in both fresh and marine waters, especially rainbow trout (Austin and Austin, 2003; Fadaeifard *et al.*, 2011).

## Aetiology

### *Morphological and genomic characteristics*

*Y. ruckeri* is a gram-negative rod with rounded ends of  $0.5-0.8 \times 1.0-3.0 \mu\text{m}$  in size (Gregory *et al.*, 2010). This bacterium does not form endospores, a capsule is not present, but often has flagella and *Y. ruckeri* strains show variable motility (Bastardo *et al.*, 2011). It was demonstrated that *Y. ruckeri* isolates had a G + C ratio of 47.5-48.5% (Ewing *et al.*, 1978).

Several studies showed that *Y. ruckeri* is a highly clonal and biochemically homogeneous species (Wheeler *et al.*, 2009). At present, five O-serotypes (O1, O2, O5, O6 and O7), five outer membrane protein types (OMP types 1–5) and two biotypes of *Y. ruckeri* are recognized. Romalde *et al.* (1993) proposed that serovar O1 can be subdivided into two subgroups O1a (previously serovar I) and O1b (serovar III) and serovar O2 (serovar II) into three subgroups O2a, O2b and O2c. Biotype 1 strains are positive for motility and lipase activity, whereas biotype 2 strains are negative for both tests (Strom-Bestor *et al.*, 2010; Kumar *et al.*, 2015).

Results showed that, most epizootics and mortality of Yersiniosis are caused by biotype 1 strains (Garcia *et al.*, 1998; Arias *et al.*, 2007). In addition, *Y.*

*ruckeri* strains can be grouped into clonal types on the basis of biotype, serotype and outer membrane protein (OMP) type into 6 major clonal groups. Clonal group 5 isolates (include the Hagerman type strain) are the most prevalent cause of Yersiniosis in mainland Europe and the US, while clonal group 2 isolates are responsible for ERM in the UK (Davies, 1991; Fouz *et al.*, 2006).

It was confirmed that, clonal groups 2 and 5 are predominantly associated with rainbow trout. Strains of serotypes O1a (classic serovar I) and O2b (classic serovar II) cause most epizootic outbreaks, and serotype O1a (heat-stable O1 antigen) is predominant in cultured salmonids (Arias *et al.*, 2007). Annotated whole genome sequences of two strains of *Y. ruckeri* are currently available: the motile CSF007-82 strain, isolated from diseased rainbow trout and the motile O1b 37551 strain, isolated from vaccinated Atlantic salmon (*Salmo salar*) (Navas *et al.*, 2014; Nelson *et al.*, 2015). In previous studies, several virulence mechanisms of *Y. ruckeri* including: extra-cellular products (such as the iron-regulated Serratia-like haemolysin YhlA, azocasein protease, 47 kDa metalloprotease Yrp1), yrpA and yrpB (Secades and Guijarro, 1999) and a heat sensitive factor (HSF) have been identified (Navais *et al.*, 2014).

#### *Taxonomic characterization and phylogenetic analysis*

The taxonomic position of *Y. ruckeri* has been subject to debate since its first classification in the genus *Yersinia* by Ewing *et al.* (1978). This study demonstrated that *Y. ruckeri* isolates had a G+C ratio of 47.5 - 48.5%, and on this basis it would be included in the genus *Yersinia*. Other researchers found that the biochemical characteristics of *Y. ruckeri* have more similarities with *Serratia* and *Salmonella* (Llewellyn, 1980). This bacterium with 47.5–48% G + C (Fouz *et al.*, 2006) differs clearly from *Serratia* species (52–60% G+C) and is closer to other *Yersinia*e (46–50% G+C).

Kotetishvili *et al.* (2005) used multilocus sequence typing (MLST), and considered that *Y. ruckeri* was the most genetically distant species within the genus *Yersinia*, and that the taxonomic status possibly needs to be reassessed (Wheeler *et al.*, 2009). From the results of many studies and the similarities with many other members of the Enterobacteriaceae, *Y. ruckeri* could possibly belong to its own separate genus. DNA studies based on guanine plus cytosine percentage content of total DNA initially resulted in classification of this organism as a new species of *Yersinia* (Kotetishvili *et al.*, 2005). Several studies demonstrating heterogeneity in biochemical (Daly *et al.*, 1986) and antigenic characteristics resulted in *Y. ruckeri* isolates being classified (Davies

and Frerichs, 1989) into five serovars (Wheeler *et al.*, 2009).

Molecular analysis based on nucleic acid hybridization and sequence analysis of 16S rDNA of the *Y. ruckeri* genome has been conducted to determine the phylogeny of this organism (Ibrahim *et al.*, 1993). Based on DNA hybridization and G 1+C ratios, *Y. ruckeri* was found to be only 30% related to other *Yersiniae* and *Serratia*, however, 16S rDNA similarities between *Y. ruckeri* and other *Yersiniae* was as high as 98.3% (Ibrahim *et al.*, 1993). Dot-blot DNA hybridization assays also demonstrated a high level of genetic relatedness among *Y. ruckeri* serovars (Kotetishvili *et al.*, 2005).

#### *Hosts and geographical distribution*

Yersiniosis is one of the most common diseases in salmonids, especially in juvenile rainbow trout, but it was also reported from seawater in several other fish species, and in both farmed and wild fish. Some of the host species of *Yersinia ruckeri* are shown in Table 1.

*Y. ruckeri* is highly contagious and has been reported in several fish species in North and South America, Australia, New Zealand, Africa, Europe and Asia especially in Iran and Turkey (Tobback *et al.*, 2007; Keeling *et al.*, 2012; Soltani *et al.*, 2014).

#### *Clinical signs*

Redmouth disease takes place as acute to chronic forms (Woo and Bruno, 2011; Kumar *et al.*, 2015). Yersiniosis

is most acute in small fish up to fingerling size (Tobback, 2009; Şeker *et al.*, 2011). Pre-acute to acute infection usually occurs in the spring and in the beginning of summer, during periods of rising water temperatures (Eissa *et al.*, 2008; Şeker *et al.*, 2011). Acute to sub-acute infections usually appear in yearling fish in the fall and early winter with declining water temperatures (Eissa *et al.*, 2008). The acute epizootics can result in severe mortalities (30-70%) depending on the size of fish, stress condition, water temperature and individual susceptibility and are also associated with strain virulence (Furones *et al.*, 1993; Noga, 2010).

Like other bacterial septicemia there are no specific early signs of disease for ERM except in small fry, where mortality may be seen without clinical signs (Altinok *et al.*, 2001). Observable signs (Fig. 1) of the disease include lethargic behavior and inactivity (Tobback *et al.*, 2007; Austin and Austin, 2012), swimming near the surface (Keeling *et al.*, 2012), anorexia (Carson and Wilson, 2002), darkening of the skin (Woo and Bruno, 2011). Prolapse and hemorrhages in the anus has been observed in many cases and could be counted as a specific sign for ERM (Şeker *et al.*, 2011). Fish infected with *Y. ruckeri* are often jaded and found in areas of low flow.

**Table 1: Some of the host species of *Yersinia ruckeri*.**

Common name	Scientific name	Reference
Rainbow trout	<i>Oncorhynchus mykiss</i>	(Chettri <i>et al.</i> , 2013; Fadaeifard <i>et al.</i> , 2014; Soltani <i>et al.</i> , 2014, etc.)
Arctic charr	<i>Salvelinus alpinus</i>	(Tinsley, 2010)
Atlantic salmon	<i>Salmo salar</i>	(Rintamäki <i>et al.</i> , 1986; Valtonen <i>et al.</i> , 1992; Bastardo <i>et al.</i> , 2011)
Cod	<i>Gadus morhua</i>	(Tinsley, 2010)
Coalfish	<i>Pollachius virens</i>	(Michel <i>et al.</i> , 1986)
Sturgeon	<i>Acipenser sturio</i>	Vuillaume <i>et al.</i> , 1987
Amur Sturgeon	<i>Acipenser schrencki</i>	(Shaowu <i>et al.</i> , 2013)
Sole	<i>Soleidae sp.</i>	(Michel <i>et al.</i> , 1986)
Nile tilapia	<i>Oreochromis niloticus</i>	(Eissa <i>et al.</i> , 2008)
Brown trout	<i>Salmo trutta</i>	Mc Daniel, 1971)
Gudgeon	<i>Gobio gobio</i>	(McDaniel, 1971)
Turbot	<i>Scophthalmus maximus</i>	(Michel <i>et al.</i> , 1986)
Goldfish	<i>Carassius auratus</i>	(McArdle and Dooley-Martyn, 1985)
Common carp	<i>Cyprinus carpio</i>	(Fuhrmann <i>et al.</i> , 1983; Berc <i>et al.</i> , 1999)
Indian major carp	<i>Cirrhinus mrigala</i>	(Manna <i>et al.</i> , 2003)
Eel	<i>Anguilla Anguilla</i>	(Fuhrmann <i>et al.</i> , 1983)
Pike	<i>Esox lucius</i>	(McDaniel, 1971)
African catfish	<i>Clarias gariepinus</i>	(Abd-El Latief, 2001)
Minnnows	<i>Pimephales promelas</i>	(Michel <i>et al.</i> , 1986)
Channel catfish	<i>Ictalurus punctatus</i>	(Danley <i>et al.</i> , 1999)
Sea bass	<i>Dicentrarchus labrax</i>	(Savas and Ture, 2007)
Char	<i>Salvelinus leucomaenis</i>	(Sakai <i>et al.</i> , 2012)
Whitespotted char	<i>Salvelinus leucomaenis</i>	(Sakai <i>et al.</i> , 2012)



**Figure 1: Hemorrhage and red spots in mouth cavity (A), darkening and exophthalmia (B), hemorrhage and abdominal distention (C, d) of naturally *Yersinia ruckeri* infected rainbow trout (Zorriehzahra *et al.*, 2002; Adel *et al.*, 2015).**

Also, fish with chronic Yersiniosis may display dark pigmentation (Fig. 1) or depigmentation in the skin (Tobback *et al.*, 2007).

The reddening of the throat and mouth cavity resulted from subcutaneous haemorrhaging and from which it has led to its present name (Carson and Wilson, 2002; Woo and Bruno, 2011). Haemorrhages are found on the external surface, at the base of the fins, at the gill tips, and around the lateral line (Bastardo *et al.*, 2011; Huang *et al.*, 2013). The involved fins in the haemorrhagic congestion comprise the pectoral and pelvic, anal caudal fins (Carson and Wilson, 2002; Seker *et al.*, 2011). Shaowu *et al.* (2013) observed that the disease in Amur sturgeon (*Acipenser schrencki*) fish showed obvious hemorrhages around the mouth, at the lower jaw, the base of the pectoral fins, the abdomen, and the urogenital pore as the main external signs.

In atypical infections of hemorrhages are observed on the mouth and gill operculum (Bullock and Cipriano, 1990; Woo and Bruno, 2011); fish sometimes show abnormal behavior and may become dark and swim near the surface (Bullock and Cipriano, 1990) thus lack of classic 'redmouth' does not deny infection with *Y. ruckeri* (Tobback *et al.*, 2007). There are frank patches of haemorrhagic congestion on the iris of the eye, is a feature for the epithet salmonid blood spot disease (Carson and Wilson, 2002; Keeling *et al.*, 2012).

#### *Tissue tropism*

Similar to other septicemic bacterial diseases, *Y. ruckeri* causes generalised haemorrhaging over the external and internal organs. Tissues tropism of yersiniosis has seen in hematopoietic organs including anterior and posterior kidney, liver and spleen. But in the chronic or acute forms of Yersiniosis, histopathological changes were observed in other organs such as stomach, intestine, pyloric caecae, pancreas, swim bladder, body muscles and etc. (Zorriehzahra *et al.*, 2009; Fadaeifard *et al.*, 2014).

#### *Transmission routes*

Transmission is primarily horizontal from fish to fish by direct contact with infected fish or carriers. The role of carrier fish is important during *Y. ruckeri* infections. Different studies showed that wild animals, such as birds, wild fish, invertebrates and even humans could act as vectors for *Y. ruckeri* (Tinsley, 2010). Hunter *et al.* (1980) discussed the importance of stress induced transmission of *Y. ruckeri* from asymptomatic carriers to healthy fish. It was found that the organism was shed in the faeces over 36-40 day cycles. The organism may remain for up to 2 months in pond mud (Tinsley, 2010). *Y. ruckeri* has been recovered from the aquatic environment, including water, faeces and sewage sludge and from human bile (Farmer *et al.*, 1985; Coquet *et al.*, 2002). Results showed while asymptomatic fish can remain carriers

for an indefinite period of time, major stressors, such as an increase in temperature, can trigger an increase of pathogen shedding and increase the probability of horizontal transmission to healthy fish (Hunter *et al.*, 1980).

Vertical transmission has not been demonstrated (Tinsley, 2010), and probably does not occur. Although, *Y. ruckeri* from chinook salmon (*Oncorhynchus tshawytscha*) eggs was reported, highlighting that vertical transmission could be a possibility; further evidence of this issue has not yet been provided (Tinsley, 2010). Although, the presence of *Y. ruckeri* DNA in ovarian fluids and unfertilized eggs of Chinook salmon (*Oncorhynchus tshawytscha*) indicates that vertical transmission of the *Y. ruckeri* from females to their progeny is possible (Richard *et al.*, 2014).

#### *Diagnostic methods*

Different diagnostic methods have been used for *Y. ruckeri*, including culturing, serological, biochemical tests, histopathology studies and molecular techniques (Altinok *et al.*, 2001; Kubilay and Timur, 2001; Bastardo *et al.*, 2012; Kumar *et al.*, 2015).

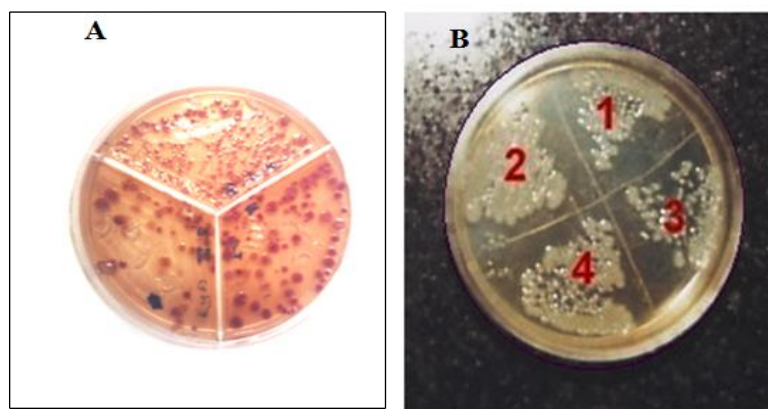
#### *Media culture isolation*

*Y. ruckeri* can be grown on simple culture media such as blood agar and Tryptone Soya Agar (TSA) that is are commonly media for identification of bacteria (Fig. 2). After incubation for 48 h at 25°C, off-white, opaque colonies of approximately 2-3 mm in

diameter were observed. Also, *Y. ruckeri* grows readily on MacConkey agar and Xylose Lysine Desoxycholate (XLD) agar. In addition, this bacterium can tolerate bile salts, the selective component in a number of media used for the isolation of enteropathogenic *Enterobacteriaceae*.

The Shotts-Waltman medium is a semi-selective indicator medium for *Y. ruckeri*, with inhibitory properties equivalent to MacConkey agar (Shotts, 1991). The indicator is based on the ability of *Y. ruckeri* to hydrolyse Tween 80, and inability to produce acid from sucrose (Shotts, 1991). In secondary infection with other bacteria, samples should also be cultured on plates of XLD agar (Oxoid CM469) or preferably Ribose Ornithine Desoxycholate agar (ROD), a moderately selective indicator medium for *Y. ruckeri* (Tinsley, 2010). Also, ROD can be useful for the detection of *Y. ruckeri* in faeces of carrier fish and can detect both biotypes 1 and 2 (Tinsley, 2010). The density of *Y. ruckeri* in faecal samples tends to be low in carriers and, typically, few colonies of *Y. ruckeri* will be evident on culture plates.

Yersinia Selective Agar is a selective and differential medium supporting good growth of *Y. ruckeri* and some *Yersinia* spp such as *Y. enterocolitica* (Fig. 2).



**Figure 2:** Pure culture of *Yersinia ruckeri* on Yersinia selective agar (CIN Agar Base/Schiemann Medium Base) (A) and Tryptic Soy Agar (B) after incubation at 25°C for 48 hours (Zorriehzahra *et al.*, 2013).

Yersinia Selective Agar was first described by Schiemann (1979) as an alternative to MacConkey Agar and other commonly used media for isolation of *Y. ruckeri*, a causative agent of Yersiniosis.

#### *Histopathology assay*

The histopathology assay could be a supportive diagnosis method regarding the lack of pathognomonic lesions in Yersiniosis. According to obtained findings in recent years, the most important histopathological changes in varied tissues could be distinguished as follows:

#### *Skin:*

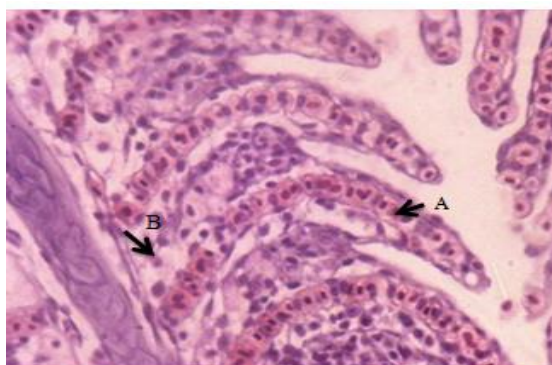
Skin of infected rainbow trout shows an increase in mucus cells that has a relation with marked epidermal hyperplasia. Also, thinning of the stratum spongiosum and red muscle degradation was observed (Tobback *et al.*, 2009).

#### *Gills:*

In gill tissue often hypotrophy and hyperplasia of the epithelial cells on the filaments and lamellae were observed along with lamellae destruction that leads to fusion and clubbing (Fig. 3). Furthermore, capillary dilatation of the lamellae and diffused haemorrhages in both the lamellae and filaments could be observed (Adel *et al.*, 2014).

#### *Kidney:*

Obvious changes could be found in the posterior kidney. Pathological sections were revealing large amounts of congestion with blood suggesting loss of haemopoietic tissue. Noticeable changes in kidney tubule structure in sample later into the infection are due to slight interstitial haemopoietic hyperplasia. So, glomerular nephritis, melanin deposits and destruction of the renal tubules could occur. Also, severe necrosis may be seen in the haemopoietic tissues of anterior and posterior parts of the kidney (Tobback *et al.*, 2009; Woo and Bruno, 2011).



**Figure 3: Hyperplasia (A) necrosis and lifting of the pillar cell (B) in gill tissue of rainbow trout infected by yersiniosis (Adel *et al.*, 2014, H&E. X40).**

Furthermore, pyknotic cells in the lumen and glomerulus are observed, suggesting that the cells are going through apoptosis. Basophilic immature kidney tubules are often visible, highlighting signs of regeneration. Melano-macrophages cells in the kidney often increase throughout the course of the infection (Mahjoor and Akhlaghi, 2012).

#### *Liver:*

Liver sections often displayed marked areas of necrosis with diffused areas lipidosis of hepatocytes. So, hyperplasia of necrotic foci and blood congestion should be anticipated (Tinsley, 2010).

#### *Heart:*

Pathological observations in rainbow trout heart originated from infection procedure. So, hyperplasia of the epicardium and myocardial necrosis are observed in acute infectious cases (Avci *et al.*, 2005).

#### *Enzyme-Linked Immunosorbent assays (ELISA)*

Different diagnostic methods have been developed for *Y. ruckeri*, including

culturing, serological tests and molecular biological techniques (Toback, 2009). Some methods may be used in detecting *Y. ruckeri* such as enzyme-linked immunosorbent assay (ELISA), agglutination test and immunofluorescence antibody technique (IFAT), based on serological characteristics (Toback, 2009; Woo and Bruno, 2011).

Since leukocyte phagocytosis, antibody production and lymphocyte proliferation are activated in fish infected by *Y. ruckeri* (Toback *et al.*, 2007), ELISA can be important to detect and determine amount of specific antibodies in fish. Antigen detection has been utilised either in studies or commercially in plate using specific antibodies in ELISA formats (Romalde *et al.*, 1995).

Enzyme linked immunosorbent assays (ELISA) have been improved to detect antibodies against *Y. ruckeri* in rainbow trout. The tests have proved to be more sensitive than the agglutination test (Olesen, 1991; Furones *et al.*, 1993; Toback *et al.*, 2007).

In a study, the quick diagnosis of clinical cases of ERM using a monoclonal antibody-based ELISA indicated value of a novel approach using a dipstick-ELISA which was a method easily adaptable for field use. However, it was investigated the technique was used for the confirmation of clinical cases of ERM and didn't have usage for finding sub-clinically infected or carrier fish. Probably because of this lack of sensitivity, the ELISA method has not been greatly used in the diagnostic field (Furones *et al.*, 1993).

#### *Immunofluorescence antibody test (IFAT)*

Immunofluorescence antibody test is an important method for screening of *Y. ruckeri* (Kubilay and Timur, 2001). In this method by preparing histopathological sections from the kidney or other tissues such as spleen, liver, and staining with specialized Immunofluorescence technique indicates the localized bacteria in kidney and haematopoietic tissues (Fig. 4). The binding of antibodies to target tissues, cells or organisms can be visualized if those antibodies are directly coupled to a fluorochrome or indirectly bound by a fluorescent reagent. Fluorochromes emit visible light (of an 'emission' wavelength) when exposed to light of a different wavelength, usually in the ultraviolet range (Kubilay and Timur, 2001).

#### *Molecular assays*

Nowadays, Polymerase Chain Reaction (PCR) has been able to represent a widely-used alternative to traditional identification methods (Eissa *et al.*, 2008). Many of them are based on the amplification of 16S ribosomal RNA (16S rRNA) genes (Fig. 5), to find out various bacterial fish pathogens (Bastardo *et al.*, 2012; Fadaeifard *et al.*, 2014). This PCR assay is able to demonstrate low amounts of *Y. ruckeri* and provides the possibility to find asymptomatic carrier fish, this matter is highly important in order to inhibit the transmission and release of ERM into the environment (Tobback *et al.*, 2007).

It has been recommended that isolation in pure culture, afterwards identification, by amplification and sequencing of the 16S rRNA gene using the universal bacterial primers 27F and 1492R as the gold standard provide a very strong diagnostic method (Woo and Bruno, 2011). Also 16S rDNA PCR assay has been employed to identify or confirm the presence of *Y. ruckeri* in several studies (Akhlaghi and Sharifi, 2008; Bastardo *et al.*, 2012).

Altinok *et al.* (2001) evaluated a PCR method for detecting *Y. ruckeri*, in the blood of rainbow trout. In this study, the authors demonstrated that a non-lethal blood sample can be used with PCR to find *Y. ruckeri*. Şeker *et al.* (2011) extracted DNA from blood samples and carried out PCR amplification based upon a pair of *Y. ruckeri* specific primers, as well.

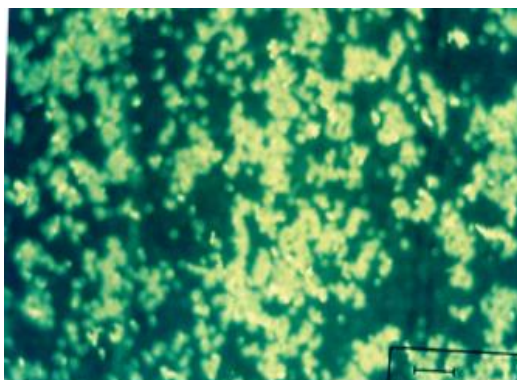


Figure 4: IFAT assay to diagnosis of *Yersinia ruckeri* isolated from rainbow trout (x 40, Zorriehzahra *et al.*, 2013).

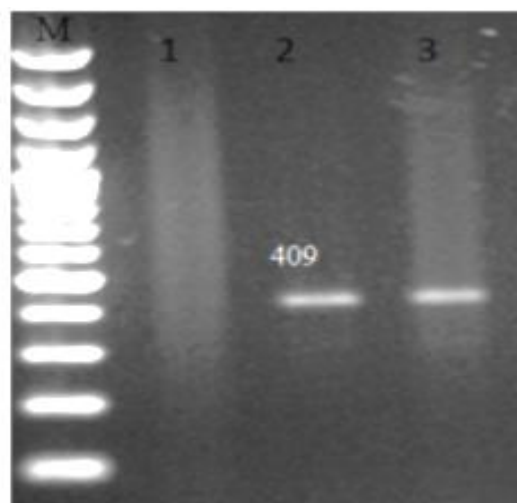


Figure 5: Electrophoretic analysis (2% agarose gel) of DNA amplified fragments from 8 isolates in this experiment. M: marker (100 bp), Lane 1: negative control, Lane 2: the isolated bacteria (409 bp), Lane 3: positive control, (Adel *et al.*, 2013).

However, the quantitative PCR (qPCR) method is speedily replacing other molecular techniques for detecting the nucleic acids of pathogens (Purcell *et al.*, 2011). This method is able to detect and quantify DNA targets by scanning PCR product accumulation in the beginning of exponential phase of amplification, shown by increased fluorescence (Bastardo *et al.*, 2012). QPCR assays can be used to detect the pathogen within fish tissues, which can be useful to investigate a continuous carrier state or the beginning of an epizootic by comparison with threshold

levels of pathogen load prior to significant losses (Taylor *et al.*, 2009). Thus, real-time qPCR methods would be used for the detection of several fish pathogens such as *Y. ruckeri* (Glenn *et al.*, 2011; Bastardo *et al.*, 2012). Several researchers have developed, a real-time primer/probe set for detecting and quantifying the amount of *Y. ruckeri* DNA found in various samples of fish exposed to *Y. ruckeri* (Glenn *et al.*, 2011; Zorriehzahra *et al.*, 2013).

Intraspecific genetic diversity among Chilean *Y. ruckeri* strains can be indicated using genotyping analyses by

enterobacterial repetitive intergenic consensus (ERIC-) and repetitive extragenic palindromic (REP-) PCR methods (Bastardo *et al.*, 2011). Huang *et al.* (2013) in another study, characterized *Y. ruckeri* originated from rainbow trout by biochemical profiling, 16S rDNA sequencing, repetitive sequence-based PCRs, including (GTG)<sub>5</sub>-PCR, BOX-PCR, ERIC-PCR and REP-PCR.

#### *Treatment, control and prevention methods*

##### *Antimicrobial compounds*

The use of antimicrobial compounds has been implemented in the treatment of *Y. ruckeri* infections in fish since several years ago (Gudding and Van Muiswinkel, 2013). Rucker (1966) described treatment with sulphamethazine for 5 days, followed by three days of chloramphenicol or oxytetracycline administration. Indeed, sulphonamide therapy has been of great value in the treatment of both experimental and natural cases (Bullock *et al.*, 1983). Furthermore, Rodgers and Austin (1983) used oxolinic acid for prophylaxis and treatment of Yersiniosis in rainbow trout. Although *Y. ruckeri* is susceptible to various antibiotics, there is acquired resistance of *Y. ruckeri* strains to many antimicrobial compounds. Post (1987) reported the absolute resistance of some isolates of *Y. ruckeri* in the USA to therapeutic levels of both sulphamerazine and oxytetracycline. By exposing bacteria to inadequate or

variable dosage, failure to complete the recommended course or by repeated short term treatment, will probably lead to selection for drug resistance (Rodgers, 2001).

##### *Vaccination*

Vaccines against ERM are one of the success scenarios of disease control in aquaculture, with the first commercial product released in 1976 and are composed of formalin-killed whole bacterial cells. Soltani *et al.* (2013) expressed to establish a new native vaccine against *Y. ruckeri* that could protect sensitive rainbow trout in Iran. The level of mortality in immunized groups was in the range of 10-20% within 10 weeks after vaccination, in contrast to those of control group was in range 56.7-73.3% ( $p < 0.05$ ).

Tinsley *et al.* (2011) and Deshmukh *et al.* (2012) developed a bivalent vaccine using formalin inactivated biotypes 1 and biotype 2 *Y. ruckeri* strains that provides good protection against the biotype 2 strains in rainbow trout. New vaccines have been developed that are based on the *Y. ruckeri* Yrp1 protease, *aroA* gene, extracellular product and lipopolysaccharide and these provide good protection against *Y. ruckeri* biotype 1 strains which are administered by several routes such as immersion, injection and oral (Deshmukh *et al.*, 2014; Ispir and Dorucu, 2014). Nowadays, some non-motile strains seem to be unaffected by commercial vaccines (Kim and Austin,

2006; Fouz *et al.*, 2006). Thus, novel ways based on subunits or DNA vaccines might be an additional approach to eliminate or minimize this disease. Recently, attention to the use of live attenuated vaccines against bacterial pathogens in fish has increased, because they can provide effective protection. Totally, they reduce a greater cell-mediated response than bacterins, although the higher immunity caused by attenuated bacteria in comparison with that provided by killed organisms is likely due to the induced expression of stress proteins (Temprano, 2005).

#### *Immunostimulants*

Recently, interest in the use of natural immunostimulants in the protection of fish against bacterial fish pathogens has increased. In addition, resistance to bacterial pathogens can be improved by administration of immunostimulants to increase the effectiveness of vaccines.  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) is a breakdown product of the amino acid leucine which is an essential building unit of proteins in all tissues and provided a positive immunostimulatory effect after *in vivo* immunization of rainbow trout with anti-*Y. ruckeri* vaccine (Raida, 2003). Immunostimulating effects against *Y. ruckeri* were also observed in rainbow trout using levamisole bathing (Ispir and Yonar, 2007). Nowadays, herbal plants as immunostimulant agents are used in many countries such as Iran, India, China, etc. against fish

pathogens. The findings of Adel (2014) demonstrated that the use of *M. piperita* extract, particularly at the rate of 3% can increase the survival rate of rainbow trout when exposed to *Y. ruckeri*. Some researchers have examined antibacterial activity of *Zataria multiflora*, *Nigella sativa*, *Scutellaria multicaulis*, *Punica granatum*, *Olea europaea*, *Echinacea purpurea* (Alishahi, 2012) *Lavandula officinalis*, *Melissa officinalis*, *Ocimum basilicum*, *Origanum vulgare*, *Rosmarinus officinalis* and *Salvia officinalis* on *Y. ruckeri* in *in vitro* condition (Bulfon, 2014).

Application of certain probiotics to reared trout enhanced their survival when exposed to *Y. ruckeri*. Administration of feeds supplemented with *Bacillus subtilis* and *B. licheniformis* ( $4 \times 10^4$  spores  $g^{-1}$  feed) raised resistance in rainbow trout against withering caused by *Y. ruckeri* (Raida, 2003). Similarly, feed supplemented with *Carnobacterium maltaromaticum* or *C. divergens* dosed at  $>10^7$  cells  $g^{-1}$  feed also induced protection against challenge with *Y. ruckeri* because these cultures improved cellular and humoral immune defences (Kim and Austin, 2006). When rainbow trout fed with  $10^8$  cells *Enterobacter cloacae* and *Bacillus mojavensis*  $g^{-1}$  feed for 60 days, it led to a significant enhanced survival of the fish after bath challenge with *Y. ruckeri* (Capkin and Altinok, 2009). Moreover, Abbas (2010) showed that biotype 2 infections of *Y. ruckeri* could be inhibited by

cellular components of certain probiotic bacteria.

#### *Future prospects*

Despite passing almost 50 years after the first report of the ERM disease (Rucker, 1966), some important issues still remain unresolved or not fully understood, particularly those concerned with the mechanisms of transmission, disease spreading and the role of asymptomatic carrier fish. Furthermore, in some cases of *Y. ruckeri* occurrence, the possibility of vertical transmission has been suggested; nevertheless it has not yet been definitively demonstrated whether it is a real vertical transmission or, rather, a phenomenon of egg shell contamination which is more probably what will happen. If so, it would be just a matter of finding an effective disinfection procedure, which can inhibit the transmission of the disease from affected broodfish to their offspring and produced eggs (green eggs and eyed eggs).

Otherwise, in the presence of a true vertical transmission, the only choice to prevent infection in the larval and juvenile stages will be based exclusively on the identification and elimination of subclinical infected broodfish, as well as the need for disinfection of water coming into the hatchery and cultured fish farms. Generally, good health management strategy could play the most important role in control and prevention. Furthermore, good farm management

consists of appropriate water quality, high quality feeding, quarantine requirements, reduction of stress and fish density, use of effective vaccine, probiotics, prebiotics and immunostimulants that should be considered as practical important parameters in the control and prevention of Yersiniosis in the fish hatchery and rearing farms.

More attention should also be addressed in the future to interactions and exchanges of pathogens, between farmed and wild populations, to assess the risk of transmission of infection from one environment to another.

Despite all attempts that can be performed and the more restrictive measures taken, it is estimated that the optimal solution can be acquired only when an effective and commercial vaccine will be available. So, more studies are needed on the molecular epidemiological sights to produce effective protection against ERM. Moreover, using suitable health management strategy, besides development of educational programs should be considered to share information and awareness of fish farmers to new developmental equipment and achievements.

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