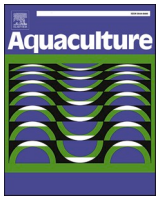
Aquaculture 580 (2024) 740363

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Review

Current knowledge of lactococcosis in rainbow trout: Pathogenesis, immune response and prevention tools

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ARTICLE INFO

Keywords:
Lactococcosis
Rainbow trout
Immune response
Infection
Vaccination

ABSTRACT

Lactococcosis caused by *Lactococcus garvieae* has emerged as one of the most devastating bacterial disease affecting rainbow trout (*Oncorhynchus mykiss*) worldwide. Understanding the immune response to this streptococcal pathogen is crucial for diagnosing and managing the associated disease and for developing vaccines and preventive measures, such as the selection of naturally resistant trout stocks. Over the past few decades, a significant number of studies have focused on several aspects of lactococcosis, including pathological features, immune responses and vaccination protocols. However, the disease's geographical distribution and its spread over time, the routes of entry and dissemination of the pathogen within the host's body, the progression of infection, and the subsequent inflammatory response of the host still need to be fully explored. This review describes the extant knowledge on pathogenesis, immune response and prevention tools for lactococcosis and identify areas that require further investigations and dedicated studies. Particular emphasis has been placed to describe the current understanding of how immune parameters and inflammatory mechanisms are affected in rainbow trout when they are exposed to natural or experimental infections or undergo vaccination treatments. Ultimately, a more comprehensive understanding of the immune interaction between *L. garvieae* and rainbow trout will contribute to developing effective disease prevention strategies in aquaculture settings.

1. Introduction

Among the freshwater fish species farmed in Europe, rainbow trout (*Oncorhynchus mykiss*) is the most widely cultured and has a high economic value (FEAP, 2022). Many countries around the world are involved in rainbow trout farming, but Iran and Turkey are the main producers, contributing with 22% and 13% of the world production respectively in 2019 (FAO, 2020). Concerning EU, France and Italy are the major contributors, providing 19% and 17% of the EU production in the year 2019 (FAO, 2020; FEAP, 2022). Rainbow trout's current intensive farming conditions increase its susceptibility to various pathogens, leading to significant losses (Janssen et al., 2017).

In the past few decades, *Lactococcus garvieae* has been identified as the cause of multiple outbreaks of lactococcosis. These episodes have resulted in significant mortality rates of up to 60–70% among rainbow trout populations (Meyburgh et al., 2017; Vendrell et al., 2006). So far, *L. garvieae* has been isolated from rainbow trout in several countries worldwide (Fig. 1) and its spread throughout Mediterranean Europe has been rapid. *L. garvieae* infections in trout were firstly recorded in Spain in 1991 (Doménech et al., 1993), and subsequently the pathogen was isolated in Italy (Ghittino and Prearo, 1992). Afterwards, infections in trout have also been registered or detected throughout the southern part of the European continent, including countries such as Turkey (Diler et al., 2002) and Portugal (Ravelo et al., 2003), as well as the Balkans (Eyngor et al., 2004). *L. garvieae* infections in trout have also involved Australia (Schmidtke and Carson, 1999), the Middle East (Eyngor et al., 2004) and more recently America (Nelson et al., 2016; Ortega et al., 2020). The information depicted in Fig. 1 is sourced from official literature. However, field data suggests that other countries such as Slovenia, Croatia, Bosnia and Herzegovina could also be affected (authors' observation). Recent genomic discoveries reassigned some past isolates of *L. garvieae* to the emerging and newly described species *L. petauri* (Goodman et al., 2017). Consequently, both bacterial species can be currently considered the etiological agents of lactococcosis in rainbow

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https://doi.org/10.1016/j.aquaculture.2023.740363
Received 27 August 2023; Received in revised form 27 October 2023; Accepted 13 November 2023
Available online 14 November 2023

trout (Altinok et al., 2022; Kotzamanidis et al., 2020).

Economic losses due to lactococcosis range from direct mortalities to reduced growth, increased labour costs and treatment expenditures (Shahin et al., 2022) and the economic impact in rainbow trout farming is considered very significant. The annual loss by infectious disease outbreaks in Iranian farmed trout is about USD 23 million (Halimi et al., 2018) and in other countries, such as Japan, the economic losses caused by infectious diseases including lactococcosis exceeded JPY 20 billion (USD 0.18 billion) prior to 1996 (Matsuura et al., 2019). When considering Italy, economic losses ascribable to lactococcosis typically account for 9 to 28% of the total turnover of an affected farm. Based on field data shared by Italian rainbow trout farmers, the estimated losses are distributed as follows: around one-third of the losses are attributed to the cost of medicated feed, approximately one-fourth of the losses are linked to mortality costs, which includes waste disposal, and the remainder is associated with compromised zootechnical performance, such as reduced feed conversion and growth. The severity of the losses hinges on the duration of disease impact throughout a typical annual cycle on the farm. Additionally, consequent sanitary costs may be ascribed to the occasional involvement of *L. garvieae* in human diseases (Chan et al., 2011). In recent years, there has been a rising incidence of *L. garvieae* infections in human, mainly where humans handle or consume raw fish. This trend has been identified as a significant risk factor in most clinical instances (Chan et al., 2011; Gibello et al., 2016), thus underscoring the emergence of *L. garvieae* as a significant zoonotic pathogen.

Review articles available have focused mainly on the pathogen and on the disease general features (Meyburgh et al., 2017; Vendrell et al., 2006), on the control of infection, based on the use of natural compounds or vaccination (Meyburgh et al., 2017; Soltani et al., 2021) and on the zoonotic potential of *L. garvieae* (Gibello et al., 2016). The available literature may not always offer complete and thorough information regarding the disease development and the particular relationship between *L. garvieae* and its vulnerable host, rainbow trout. In order to bridge this knowledge gap, we undertook a comprehensive review aimed at retrieving, updating, and delineating the current understanding of the selected topic. Our research is built upon the existing literature concerning immunological investigations related to *L. garvieae* infection

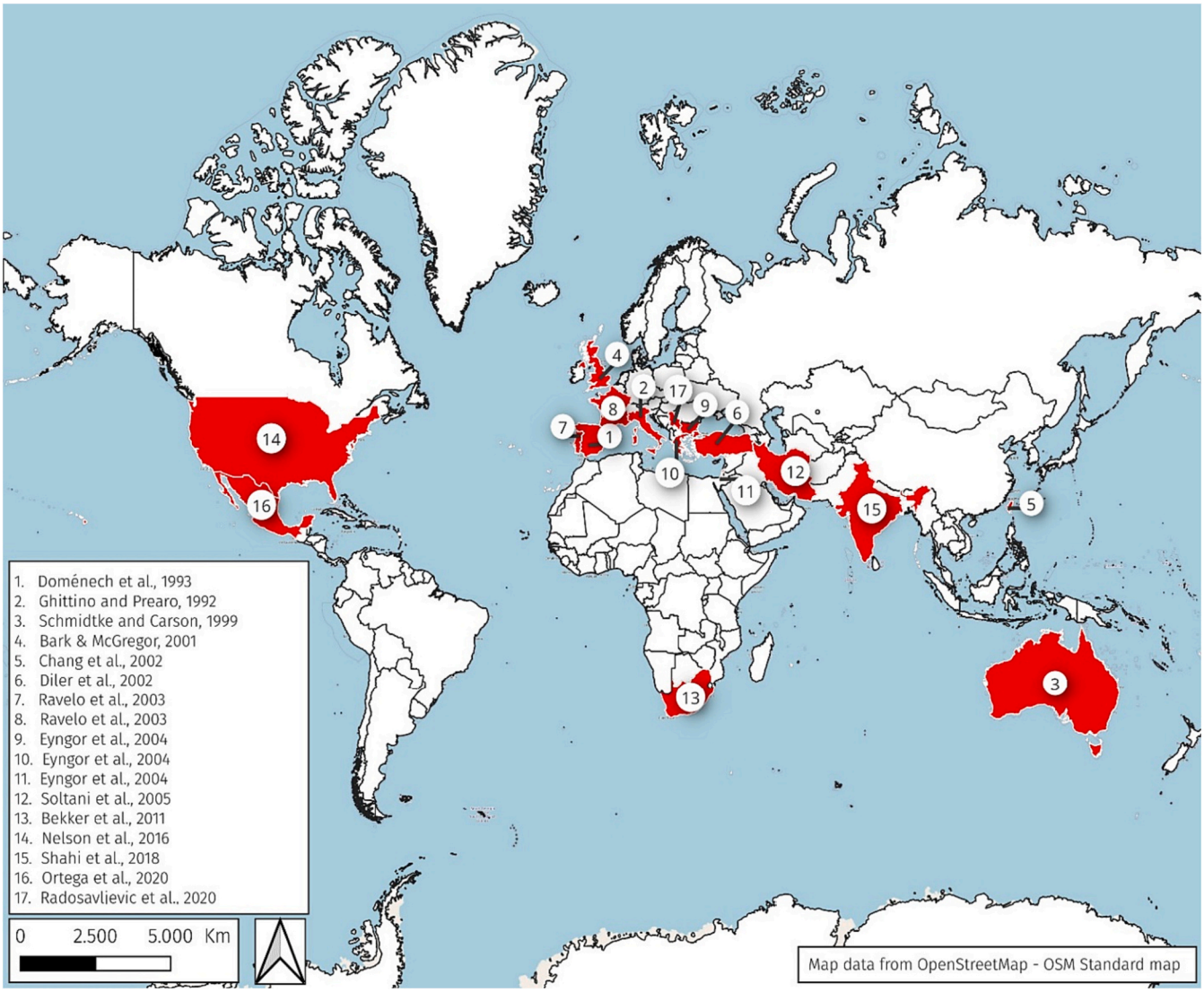

Fig. 1. Map showing the geographical distribution of lactococcosis due to L. *garvieae* in rainbow trout. In red colour, all the countries where outbreaks of the disease have been reported. Spain (Doménech et al., 1993), Italy (Ghittino and Prearo, 1992), Australia (Schmidtke and Carson, 1999), UK (Bark and McGregor, 2001), Taiwan (Chang et al., 2002), Turkiye (Diler et al., 2002), Portugal & France (Ravelo et al., 2003), Bulgaria, Greece and Israel (Eyngor et al., 2004), Iran (Soltani et al., 2005), South Africa (Bekker et al., 2011), USA (Nelson et al., 2016), India (Shahi et al., 2018), Mexico (Ortega et al., 2020) and Serbia (Radosavljević et al., 2020). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

or vaccination in rainbow trout. To accomplish our goal, we conducted a thorough analysis of published works related to the topic throughout the years. In our research, we concentrated solely on papers related to rainbow trout and *L. garvieae*. We identified these papers by conducting a preliminary assessment of titles and abstracts. Our primary objective was to investigate specific immunological and pathogenetic aspects of lactococcosis. To do this, we scanned abstracts for the keywords "immune" or "inflammatory response".

2. A brief focus on the host rainbow trout, on the pathogen *L. garvieae,* and on lactococcosis

Rainbow trout is a significant type of salmonid fish from an economic standpoint, and it is used as a model in biological studies. Researchers have dedicated numerous insights to study the morphology, ontogenesis, and function of its immune system (Bailey et al., 1996; Berthelot et al., 2014). Thorgaard et al. (2002) published a review paper specifically dedicated to genomic research on rainbow trout. Köllner et al. (2002) focused on the effect of environmental stressors on rainbow trout's immune functions. Salinas (2015) reviewed the mucosal-associated immunity, while Lulijwa et al. (2019) dealt with the purification of immune cells and their use for in vitro tests. Synthetically, what is currently stated on rainbow trout immunity is the presence of fully efficient primary and secondary lymphoid organs where the secondary lymphoid organs are not developed as in mammals (absence of lymph nodes, Peyer patches and germinal centers). The main sites where fish encounter pathogens or vaccines are the mucosal associated lymphoid tissues (MALT) such as nose, gill and skin (Salinas, 2015; Gomez et al., 2013). Moreover, we know the morphology and role of leukocyte populations (Perdiguero et al., 2021; Sunyer, 2012), the presence of diversified classes of Immunoglobulins (IgM: tetrameric molecules known as the most abundant class in serum, mainly synthesized by plasma cells located in the anterior kidney, and this synthesis is further triggered by infections or vaccination treatments (Magnadottir et al., 2005); IgT are mainly monomers and are detectable in mucus, so their action against pathogens takes place above all in the districts of mucosal immunity (Dong et al., 2020; Salinas, 2015); the IgD are still under investigation, and therefore their function is not fully defined (Ramirez-Gomez et al., 2012)), of pro- and anti-inflammatory cytokines (Valdés et al., 2022), of several immune receptors (Kutyrev et al., 2016; Secombes et al., 1998), as well as the sequences of immunologically relevant genes (IL1β, IL6, IL8, IL10, TCRβ, TNFα, IgM & IgT) (Ali et al., 2014; Phillips et al., 2013; Zuo et al., 2020). The activation of the immune response of trout against *L. garvieae* includes the modulation of several humoral and cellular mechanisms (i.e., lysozyme activity, bactericidal activity, antiprotease activity, phagocytosis, respiratory burst, natural and specific antibody synthesis, differential expression of immune-related genes) that show variability due to the lactococcal infection and to the vaccination with *L. garvieae* antigens (Bulfon et al., 2020; Castro et al., 2019; Halimi et al., 2020).

Lactococcus garvieae is a Gram-positive, lactic acid bacterium (LAB) which was first described as *Streptococcus garvieae* in 1983 (Collins et al., 1983) that infects a wide range of freshwater and marine animals (Gibello et al., 2016; Shiry et al., 2019; Vendrell et al., 2012). Since the first reports, *L. garvieae* has emerged as one of the most important streptococcal pathogens affecting trout and other cold, temperate, and warm water fishes across the globe (Gibello et al., 2016; Vendrell et al., 2012). The first isolation occurred from cases of bovine mastitis (Collins et al., 1983). However, it was later isolated in Spain during a lactococcosis outbreak in rainbow trout and classified as *L. garvieae* based on biochemical characteristics (Teixeira et al., 1996). Thanks to the advancement of molecular biology, the genome sequence was revealed and it confirmed the bacterium identification based on 16S ribosomal RNA (rRNA) sequencing (Bekker et al., 2011).

Trout Lactococcosis is a systemic hyperacute infection with widespread haemorrhaging (Austin and Austin, 2012). Outbreaks usually happen during the spring-summer season when the water temperature rises above 16–18 °C (Eldar and Ghittino, 1999). Based on field observations of natural outbreaks, it is clear that trout weighing between 150 and 600 g are highly susceptible to lactococcosis. Even juveniles between 10 and 80 g, when subjected to experimental infections, may develop symptoms and should be monitored closely (as shown in Table 1). The earliest symptoms of infection include anorexia, melanosis, lethargy, and erratic swimming. Typical external signs of infection include exophthalmia, haemorrhages in the periorbital/intraocular area, at the base of fins, in the perianal and buccal regions, swollen abdomen, and anal prolapse (Fig. 2) (Bekker et al., 2011; Eldar and Ghittino, 1999). Gross pathological findings may include exudative fluid within the coelomatic cavity, enlargement of the spleen, focal areas of necrosis in the liver and spleen, and yellowish exudate covering the brain surface (Eldar and Ghittino, 1999). Extensive haemorrhaging caused by vascular epithelium injury leading to haemorrhages and petechiae on internal and external surfaces can be observed (Eldar and Ghittino, 1999).

3. Pathogenesis of *L. garvieae* infection in rainbow trout

L. garvieae can be transmitted horizontally through the water and can infect healthy fish close to infected ones (Algöet et al., 2009; Soltani et al., 2021). Moreover, the pathogen can also be transmitted vertically, from infected broodstocks to their offspring and by contaminated equipment or personnel, and can survive in the environment for extended periods of time (Eyngor et al., 2004; Gibello et al., 2016). Several studies (as shown in Table 1) have contributed to our understanding of lactococcosis and its interaction with the host. These studies have focused on both natural outbreaks (Khalil et al., 2023a; Khalil et al., 2023b; Pastorino et al., 2019; Ortega et al., 2020) and experimental challenges. The latter provides more detailed and reliable information regarding the mode of infection (intraperitoneal or immersion), the dose of bacteria (CFU/ml), and the infection time-course (Avci et al., 2014; Bwalya et al., 2021; Shahi et al., 2018).

In early studies, virulence was thought to be essentially related to the bacterium ability to form a capsule (KG+ (non-capsulated and non-virulent) and KG- (capsulated and virulent) (Kawanishi et al., 2006), but the capsule gene cluster (CGC) is not the only virulent factor since even strains lacking the CGC are virulent for rainbow trout (Türe and Altinok, 2016). The virulence of *L. garvieae* may be rather regulated by a combination of genes (Shahi et al., 2018; Shahi and Mallik, 2020; Türe and Altinok, 2016). Hemolysin (hly1 and hly2), LPxTG-containing surface proteins, adhesins cluster, and several antimicrobial-resistant genes have also been implicated in the virulence of this bacterium (Shahi et al., 2018; Shahi and Mallik, 2020; Teker et al., 2018; Türe and Altinok, 2016). *L. garvieae* is an α-hemolytic bacterium causing the lysis of red and white blood cells by developing cellular membrane pores and damaging their cell membrane during infection (Eldar et al., 1995; Teker et al., 2018). Other genes, such as nadh oxidase, superoxide dismutase (sod), phosphoglucomutase (pgm), and enolase (eno) (Türe and Altinok, 2016), may also be implicated. Sod could contribute to the survival of the bacteria to respiratory burst and eno is a surface-expressed metalloenzyme that could contribute to the pathogenesis likewise stated for other bacteria such as *Aeromonas hydrophila* (Türe and Altinok, 2016). Furthermore, *L. garvieae*, similarly to other Gram-positive bacteria, may be able to bind host serum proteins, including the Fc component of immunoglobulins, what inhibits the activation of the complement by the classical pathway (Barnes et al., 2002). Finally, the bacterium may be capable of masking itself against specific antibodies and therefore avoid phagocytosis. All these factors can jointly contribute to bacterial adhesion to host tissues, tissue damage, systemic invasion, and host immunity evasion (Soltani et al., 2021).

L. garvieae primarily enters and spreads in rainbow trout through the gills and eyes as established through histological, immunohistochemical and biomolecular examination (bacterial DNA by PCR) in

Table 1
Post *L. garvieae* infection surveys carried out in rainbow trout (*O. mykiss*) as target host. The table includes examples of natural and experimental (challenge-based) infections, used to study the route of bacterial entry, the organs colonization timing (if available), as well as the techniques adopted to detect the bacteria or the bacterial DNA in the fish body.

Fish size (g)	Route of infection	Bacterial dose (CFU/ml)	Post-infection sampling time point (dpi)	Organs colonized	Techniques used for *L. garvieae* detection	Reference
Mode of infection: **Natural**						
500–600	n.d.	n.d.	n.d.	Blood	Stained blood smear	Khalil et al., 2023a
150–300	n.d.	n.d.	n.d.	Kidney and Spleen	PCR	Khalil et al., 2023b
200–500	n.d.	n.d.	n.d.	Eye, fins, skin, brain, liver, kidney and intestine	Bacteriology, histology and PCR	Ortega et al., 2020
250–300	n.d.	n.d.	n.d.	Kidney and brain	Bacteriology and PCR	Pastorino et al., 2019
500–600	n.d.	n.d.	n.d.	Kidney, spleen, liver, gonad and brain	PCR	Shahin et al., 2022
Mode of infection: **Experimental challenge**						
80 ± 10	IP IM	10^6 10^8	6 5&6	Gills, kidney, heart, liver Gills, kidney, heart, spleen	Histology & immunohistochemistry	Avci et al., 2014
80	IM	1.0×10^5	5	kidney, spleen	PCR	Bulfon et al., 2020
10	IP	100 µl of 2.65×10^3	5	Kidney & spleen	PCR	Castro et al., 2019
10 ± 0.6	IM	4.7×10^5	20	Kidney	PCR	Halimi et al., 2020
10 ± 0.6	IM	4.7×10^5	20	Kidney	PCR	Halimi et al., 2019
10 ± 0.6	IM	4.7×10^5	20	Kidney	PCR	Halimi et al., 2018
200–500	IP	10^2 and 10^4	4	Fins, brain, liver, kidney and spleen	Bacteriology, histology and PCR	Ortega et al., 2020
27 ± 3.7	IP, intramuscular & IM	100 µl of 2.6×10^5	15	Eye, kidney and liver	PCR	Shahi et al., 2018

dpi = days post infection; CFU = Colony forming unit; IP = Intraperitoneal injection; IM = Immersion infection; n.d. = not determined.

experimentally infected fish by intraperitoneal injection or immersion infection (Avci et al., 2014). Once the bacteria enter the fish's body, they can colonize and multiply in the gill tissue, leading to inflammation and damage. The spread of *L. garvieae* within the fish occurs through the bloodstream (Khalil et al., 2023a), allowing the bacteria to reach other organs such as the kidney, spleen and liver, leading to systemic infection. *L. garvieae* can also spread to the skin and fins, resulting in erosive lesions (Bwalya et al., 2021).

4. Infection with *L. garvieae*

4.1. Pathology of *L. garvieae* infection

Lactococcus garvieae can cause hyperacute and hemorrhagic septicemia characterized by high mortality and morbidity in both field and experimental cases. In rainbow trout (*Oncorhynchus mykiss)*, the typical clinical signs are similar to those described in other fish species, such as *Seriola quinqueradiata* (Kusuda et al., 1991), *Mugil cephalus* (Chen et al., 2002), *Salvelinus fontinalis* (Pastorino et al., 2019) and with a lower mortality rate in cyprinids (Algöet et al., 2009). Although the virulence factors of *L. garvieae* and the pathogenicity have been investigated at the genetic level, its underlying mechanisms are not fully understood (Austin and Austin, 2012; Shahi and Mallik, 2020; Vendrell et al., 2006).

Clinical manifestations and mortality associated with lactococcosis are heavily influenced by environmental factors, such as water temperature and quality (Algöet et al., 2009). In natural infections, the disease commonly appears with a sudden onset of anorexia, melanosis, lethargy, loss of orientation, and erratic swimming (Eldar and Ghittino, 1999; Vendrell et al., 2006), and mortality rates can range between 10 and 50% (Vendrell et al., 2006). Affected fish typically exhibit a range of external signs, including exophthalmia, corneal opacity, and haemorrhages in various regions, such as the periorbital, intraocular areas, fins, perianal area, opercula, and buccal areas (Shahin et al., 2022; Vendrell et al., 2006). Additionally, ascites and anal prolapse are common, and severe haemorrhaging in the skin and deep-muscle layers can occur in some cases (Ortega et al., 2020). *L. garvieae* has been found to cause damage to the vascular endothelium, leading to blood extravasation, which results in haemorrhages and petechiae on the surface of internal organs (Vendrell et al., 2006). Furthermore, kidney, spleen, eye fluid and brain wet mount preparations often reveal the presence of numerous pure cocci (Shahin et al., 2022). On the external surface, lactococcosis primarily affects the most vascularized tissues, such as the perianal or buccal area and fins. Interestingly, Eldar and Ghittino (1999) have described the macroscopic and microscopic tissue alterations that differentiate lactococcosis from *Streptococcus iniae* infection. Unlike lactococcosis, *S. iniae* infection does not typically exhibit external haemorrhages, pericardial lesions, or haemorrhage and congestion of internal organs and enteritis. In contrast, *L. garvieae*-infected fish often demonstrate serositis that extends to the myocardium and, less commonly, extensive superficial erosions with pseudomembrane-like formation in the intestinal tract and diffuse hepatic blood-filling spaces consistent with Peliosis Hepatis (Eldar and Ghittino, 1999). Reactive hyperplasia of the hematopoietic tissue is also evident in the kidneys of *L. garvieae*-infected fish (Eldar and Ghittino, 1999).

Numerous experimental studies have detailed the macroscopic and microscopic features of lactococcosis, induced through intra-peritoneal, intra-muscular, and immersion routes of infection (Avci et al., 2014; Chang et al., 2002; Eldar and Ghittino, 1999; Pereira et al., 2004; Shahi et al., 2018; Shahin et al., 2022). Mortality rates have been reported to be as high as 90–100% in these experimental settings. Avci et al. (2014) conducted a detailed study on the visible differences between the intraperitoneal and immersion groups. Intriguingly, intraperitoneal groups did not exhibit signs such as ascites, exophthalmia, or periorbital haemorrhage, but relatively more widespread and severe lesions affecting

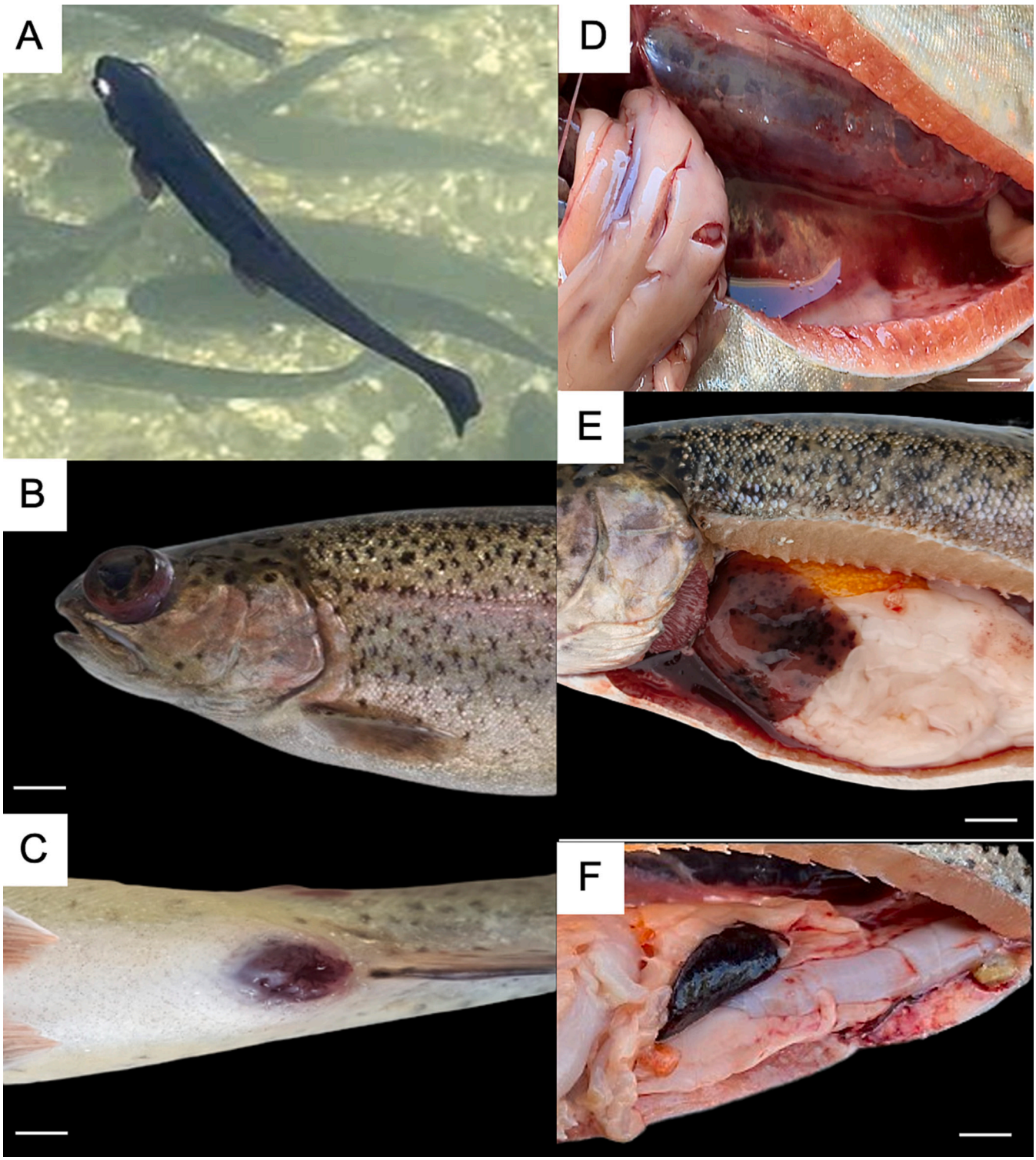

Fig. 2. Common gross pathological findings of lactococcosis in rainbow trout (*O. mykiss*) (A-F). Melanosis and bilateral exophthalmos (A), right exophthalmos with intraocular and periocular haemorrhage (B), anal prolapse and haemorrhage of anal and perianal tissues (C) can be frequent external signs. Necropsy findings can include serohaemorrhagic effusion within the coelomatic cavity and haemorrhages of the swim bladder (D), diffuse hepatic haemorrhages within the coelomatic cavity (E), splenomegaly and haemorrhage in the ceoelomatic cavity (F). Bar = 1 cm.

multiple organs, including the heart, gills, liver, swim bladder, peritoneum, and skin. In contrast, immersion groups showed lesions primarily in the eyes and gills and less commonly milder lesions in the heart, liver, swim bladder, and peritoneum. However, regardless of the route of infection, hyperemic and hemorrhagic pathologic changes are expected in several organs, such as the gills, pericardial cavity, swim bladder, peritoneum, and abdominal fat tissue. In addition, skin discolouration with petechial haemorrhages in the skin and skeletal muscles are present (Avci et al., 2014; Chang et al., 2002; Eldar and Ghittino, 1999; Shahin et al., 2022).

Moreover, vascular lesions around the anus are common and quite severe (Algöet et al., 2009; Shahin et al., 2022; Vendrell et al., 2006).

The spleen and kidney can be congested in both groups, while the intestine is less commonly affected (Avci et al., 2014; Chang et al., 2002). Hyperemia and haemorrhages, typically in the posterior intestinal segment, are observed predominantly in intra-coelomatic cavity experimental groups, with haemorrhages extending to the entire intestine, resulting in bloody content in the intestinal lumen (Avci et al., 2014; Shahin et al., 2022). Brain tissue was evaluated in other studies, and haemorrhages were found (Shahi and Mallik, 2020; Shahin et al., 2022).

Microscopically, histopathological features in experimental infection are reported for several organs and are usually compatible with generalized sepsis. Bacterial clusters are commonly seen with Gram stains and bacterial antigens, both in the free state and within macrophage cytoplasm, can be commonly seen with immuno-peroxidase and immunofluorescent staining methods (Avci et al., 2014). In experimental infections, branchitis, epicarditis, and peritonitis are predominantly seen (Avci et al., 2014), combined with gill vascular lesions and swelling in the secondary lamellar epithelium (Avci et al., 2014; Shahin et al., 2022) and the typical lesions in the ocular area. Haemorrhagic panophthalmitis, consisting of extensive fibroplasia with histiocytic inflammatory cell infiltration, is described (Chang et al., 2002; Shahin et al., 2022) and destruction of the anterior and posterior eye chambers, affection of the optic nerve papilla, and inflammation into retrobulbar fat and striated muscle are standard features (Avci et al., 2014; Chang et al., 2002; Eldar and Ghittino, 1999; Shahin et al., 2022).

Spleen and liver can be involved with fibrinous splenitis and splenic lymphoid necrosis, hepatitis with mononuclear cell infiltrations and hepatocellular necrosis. Liver lesions are similar in both immersion and intra-peritoneal groups of infection (Avci et al., 2014), and hepatocellular lipid depletion can be seen. The gastrointestinal system is generally less involved by microscopic features, including enteritis (Avci et al., 2014; Shahin et al., 2022), hyperaemia and haemorrhage in the intestines, and extensive superficial erosions with pseudomembrane-like formation. Degeneration and necrosis in the epithelium of the stomach glands can be observed (Avci et al., 2014). In the swim bladder, vessels can be hyperaemic, and serosa is quite oedematous with focal to multifocal haemorrhages (Avci et al., 2014). Tubular necrosis, renal hematopoietic necrosis, and interstitial nephritis are reported (Shahin et al., 2022) as renal melanomacrophage hyperplasia. Severe degeneration of the tubular epithelium, together with hyaline droplets and an increase in melanomacrophage centres, can be common findings (Avci et al., 2014; Shahin et al., 2022). Coelomitis with fat necrosis is reported (Shahin et al., 2022). Finally, meningitis is also commonly seen at the microscopic level (Chang et al., 2002; Eldar and Ghittino, 1999) in combination with intracranial oedema, cerebral and cerebellar meningitis, fibroplasia, and macrophage and lymphocyte infiltrations with colonies of Gram-positive cocci often widely distributed over the meningeal surface and within the Virchoff's spaces (Vendrell et al., 2006). These findings can explain neurological clinical signs of abnormal mentation and swimming pattern (Chang et al., 2002; Eldar and Ghittino, 1999). Generally, macrophages seem to play a crucial role in the immune response to this infection, as bacteria that undergo phagocytosis can spread to all tissues of the organism through histiocytes (Avci et al., 2014).

4.2. Immune responses of rainbow trout after infection by *L. garvieae*

Several studies have been performed targeting both natural outbreaks (Avci et al., 2010; Chang et al., 2002; Dıdınen et al., 2014; Khalil et al., 2023a; Khalil et al., 2023b; Shahi et al., 2018) and experimental challenges (Avci et al., 2014; Bilen et al., 2019; Castro et al., 2019; Eldar and Ghittino, 1999; Khosravi et al., 2018; Korun et al., 2017; Mohammadian et al., 2019; Pérez-Sánchez et al., 2011; Shahin et al., 2022), but only 6 of them (reported in Table 2) specifically focused on understanding the immune and inflammatory responses of rainbow trout in the course of *L. garvieae* infection. Based on current knowledge, during natural exposure the bacterium can enter the trout body throughout the main mucosal sites (gills, eye, gut) (Pastorino et al., 2019). In contrast, the entry route for challenged fish has been either intracoelomatic cavity (IP) or mucosal (IM and cohabitation). In all cases, the bacteria can subsequently spread to several organs using the bloodstream (Avci et al., 2014; Bulfon et al., 2020; Ortega et al., 2020). Among these, immune organs such as the head kidney and spleen are colonized. Based on the available evidence, the immune system can be activated in different ways, such as mucosal or systemic, specific or nonspecific, and humoral or cellular. There are several methods to study its response to the infection, with the most common ones being the evaluation of immune genes and blood/mucus parameters (refer to Table 2). Fish's resident mucosal immune cells play an important role in regulating local

Table 2
Post *L. garvieae* infection (natural and experimental challenges) surveys carried out in rainbow trout (*O. mykiss*) as target host, in order to study the inflammatory/immune response parameters modulation. Variations of single parameters are indicated by arrows, in terms of comparison between infected and healthy fish.

Type of infection	Fish Size (g)	Route of infection	Dose (CFU/ml)	Time of Sampling	Immune/inflammatory responses		References
					Immunological parameters (referred to blood)	Gene expression (referred to HK, spleen, intestine)	
Natural	500–600	n.d.	n.d.	n.d.	APA↑, POA↑, TP↑, TIgM↑; SIgM↓; LA↔, BA↔; TC↑, LC↓, NG↔, MC↔	–	Khalil et al., 2023a
	150–300	n.d.	n.d.	n.d.	–	IL-1β↑, IL-8↑, IL-10↑, TCR-β↑, IgT↑; MHC-II↓; IL-6↔, TNF-α↔, TLR5↔, MHC-I↔, IgM↔	Khalil et al., 2023b
Experimental	80	IM	10^5	5 dpi	LA↑, RBA↑	IL-8↑, MHC-II↑, IgM↑	Bulfon et al., 2020
	10	IP	100 μl of 2.65×10^3	3 dpi	–	IgM↑, IgT↑, MHC-I&II ↑; TLR5↓, TLR13↓, TLR22↓, IFN1↓	Castro et al., 2019
	20 ± 5	IP	3×10^8	1, 3, 14 & 21dpi	CRP↑, BA↑, LA↑, POA↑, SIgM↑, CA↑, WBC↑; HC↓, HG↓	–	Khosravi et al., 2018
			3×10^7		CRP↑, BA↑, LA↑, SIgM↑, WBC↑; HC↓, HG↓		
	26	cohabitation	n.d.	2 wks post infection	–	IL-1β↑, TNF- α↑, TLR5↑, IgT↑	Pérez-Sánchez et al., 2011

↑ = up-regulation; ↓ = down-regulation; ↔ = no expression; IP = Intraperitoneal injection; IM = Immersion infection; CFU = Colony Forming Unit, HK = head kidney; dpi = days post infection; LA = lysozyme activity; RBA = Respiratory Burst Activity; APA = Antiprotease activity; POA = Peroxidase activity; CA = Complement activity; TP = Total protein; TIgM = Total Immunoglobulin, BA = Bactericidal activity; SIgM = Specific anti-*L. garvieae* IgM; CRP = C-reactive protein; TC = Thrombocyte; LC = Lymphocyte; NG = Neutrophil granulocyte; MC = Monocyte; WBC = White blood cell; HC = Hematocrit; HG = Hemoglobin; n.d. = not determined.

immunity by releasing various immune-regulating substances and cytokines. Additionally, the intestinal and gill epithelia may play a crucial role in initiating and regulating mucosal immunity to bacteria, as per the studies conducted by Gomez et al. (2013) and Salinas (2015). Moreover, when considering the studies specifically dedicated to the reactivity of the rainbow trout abdominal adipose tissue (Korytář et al., 2013; Veenstra et al., 2019), there is evidence of immune cells activation to bacteria or bacterial antigens by determining the dynamics of the recruitment of myeloid cell population, T and B lymphocytes when they are administered intra-peritoneally.

Studies performed in the zebrafish model have demonstrated that *L. garvieae* can be uptaken by phagocytes and non-phagocytic cells (hepatocytes and enterocytes) during the early post-infection phase (Aguado-Urda et al., 2014). Macrophages are the cells responsible for bacterial killing, although they alternatively allow intra-cellular bacterial survival promoting subsequent systemic spreading (Avci et al., 2014). Barnes et al. (2002) have also investigated (in vitro) the role of rainbow trout antibodies as opsonins in promoting the effectiveness of phagocytosis of *L. garvieae*. As regards the profile of humoral factors in trout affected by lactococcosis, a significant positive correlation between the C-reactive protein level in blood and some haematological (WBC, hematocrit and hemoglobin) and immunological indices (lysozyme, peroxidase, complement and specific IgM) has been observed in *O. mykiss* intraperitoneally infected (Khosravi et al., 2018). Increased serum lysozyme activity in rainbow trout exposed to *L. garvieae* may be reliably associated with macrophage activation after exposure to bacterial antigens (Magnadottir, 2010). Further non-specific humoral factors such as complement, opsonins, antiproteases, acute phase proteins (APPs), and antibacterial peptides have been associated with serum bacterial killing ability in rainbow trout (Fevolden et al., 1992; Hollebecq et al., 1995).

Antibody synthesis is an integral part of bony fishes' humoral immune response, which prevents bacterial pathogens' growth and colonization by neutralization, complement activation and opsonization to enhance phagocytosis. So far, the most frequent immunoglobulin in teleost serum is IgM, which is responsible for systemic immunity (Semple and Dixon, 2020). Differences in total and specific antibodies (IgM) have been detected in rainbow trout during infection with *L. garvieae* (Khalil et al., 2023a; Khosravi et al., 2018). Symptomatic fish showed low levels of specific IgM compared to asymptomatic fish which is reasonable as symptomatic fish had compromised immune system (Khalil et al., 2023a).

Rainbow trout immune genes related to inflammation and immune response have been evaluated in several studies after exposure to *L. garvieae (*Bulfon et al., 2020; Castro et al., 2019; Khalil et al., 2023b*;* Pérez-Sánchez et al., 2011; Shahi et al., 2018). The main target organs submitted to evaluation were the spleen and head kidney (HK) (Bulfon et al., 2020; Castro et al., 2019; Khalil et al., 2023b), but the intestine was also evaluated by Pérez-Sánchez et al. (2011). On the contrary, no authors dedicated attention to gene expressions at the level of gills/skin/adipose tissue (Table 2). A significant increase of IL-1β, IL-8, IL-10, MHC-II and IgT transcripts was observed in spleen and HK of infected rainbow trout (Bulfon et al., 2020; Castro et al., 2019; Khalil et al., 2023b; Pérez-Sánchez et al., 2011). However, interestingly, the exposure to *L. garvieae* does not seem to have influenced the expression of genes encoding for IL-6, TNF-α, TLR-5, TCR-β, MHC-I and IgM in head kidney (Khalil et al., 2023b). IL-1β promotes the recruitment of defensive cells during the early stages of disease and IL-10 modulates the regression of the inflammatory process after pathogen elimination (Secombes et al., 1998; Zou and Secombes, 2016), whereas TNF- α (pro-inflammatory cytokine) plays an important role in inflammation and immune homeostasis maintaining (Valdés et al., 2022). On the other hand, IL-8 is a chemokine which plays a key role in the recruitment of monocytes, neutrophils, and lymphocytes into fish tissues and in the activation of phagocytosis (Zou and Secombes, 2016), found to be over expressed in the study of Khalil et al., 2023b. Major histocompatibility complex (MHC I & II) are glycoproteins expressed on the cell membrane, they are able to present antigenic peptides to T lymphocytes, therefore initiating immune response towards invading pathogens (Yamaguchi and Dijkstra, 2019). Toll-like receptors are known to be over-expressed immediately after cell interaction with PAMPs, and TLR-5 is usually up-regulated following a flagellin-based stimulation (Palti, 2011). The TLR-5 did not show expression as reported by Castro et al. (2019) and Khalil et al. (2023b), most probably due to the fact that *L. garvieae* is not a flagellated bacterium. Among the Ig isotypes identified in fish, IgM is more prevalent at the systemic level, whereas IgT (also called teleost-specific Ig) is more prominent in the mucosal compartment (Salinas et al., 2021; Yu et al., 2020). Based on our findings and other authors observations mainly IgT gene resulted upregulated in response to lactococcosis rather than IgM gene (Castro et al., 2019; Khalil et al., 2023b; Pérez-Sánchez et al., 2011). Normally the IgT gene is considered as parameter of mucosal specific response but the detection of its increased expression in HK suggests that IgT might be functional for both mucosal and systemic immune responses.

4.3. The immune response of other susceptible aquatic species to *L. garvieae*

Apart from rainbow trout, *L. garvieae* can infect a wide range of species in warm-water, cold-water, freshwater and marine aquaculture, including Japanese eel (*Anguilla japonica*) (Kusuda et al., 1991), freshwater prawn (*Macrobrachium rosenbergii*) (Chen et al., 2001), grey mullet (*Mugil cephalus*) (Chen et al., 2002), red sea wrasse (*Coris aygula*) (Colorni et al., 2003), catfish (*Silurus glanis*) (Ravelo et al., 2003), olive flounder (*Paralichthys olivaceous*), amberjack (*Seriola dumerili*), yellowtail (*Seriola quinqueradiata*) (Kawanishi et al., 2006), Nile tilapia (*Oreochromis niloticus*), Pintado (*Pseudoplathystoma corruscans*) (Evans et al., 2009), Turbot (*Psetta maxima*), black sea trout (*Salmo trutta labrax*) and sea bass (*Dicentrarchus labrax*) (Türe et al., 2014), bottlenose dolphin (*Tursiops truncates*) (Evans et al., 2006), and common octopus (*Octopus vulgaris*) (Fichi et al., 2015) worldwide. Being this revision specifically dedicated to rainbow trout, we focused our dissertation to its specific response to the pathogen, but relevant information can be comparatively gained also exploring the wide literature dedicated to the above mentioned susceptible species. Among those listed, mullet (*M. cephalus*), yellowtail (*S. quinqueradiata*) and tilapia (*O. niloticus*) have been specifically targeted by refined studies on *L. garvieae*- induced immune response (Byadgi et al., 2016; Ooyama et al., 1999; Ooyama et al., 2002; Su et al., 2022). In mullet, the host response to *L. garvieae* has been studied after intraperitoneal, biofilm and planktonic based experimental infections, by the evaluation of DIGs (differentially expressed genes) in the head kidney/spleen (Byadgi et al., 2016; Su et al., 2022). In the yellowtail, Ooyama et al. (1999); Ooyama et al. (2002) studied the response after an immunization with *L. garvieae* bacterins obtained from capsulated versus uncapsulated strains. The researchers investigated the host response in terms of specific/agglutinating antibodies synthesis, as well as in terms of phagocytosis activity. Moreover, the antigenicity of selected bacterial surface molecules was studied. Recently, in Nile tilapia, Bwalya et al. (2020) studied the specific antibody response and the post-challenge bacterial clearance in the fish organs, after an intraperitoneal vaccination with a *L. garvieae* vaccine.

5. Vaccination against *L. garvieae*

5.1. Immune responses of rainbow trout after vaccination

In the light of bibliographic evidences, the vaccination against lactococcosis should be typically carried out when the trout reach around 50 g in weight and the water temperature falls within the range of 12 to 14 °C (Vendrell et al., 2006). However, Khalil et al., 2023a, thanks to an infield survey, observed that farmers are administering vaccines even to

larger trout weighing up to 500–600 g in Italian farming settings. Vaccination is classically performed by intra-celomatic injection of inactivated bacteria (either autovaccines or commercial formulations). The vaccines tested up to date are mostly composed of formalin killed whole bacteria so apparently they do not contain bacterial fractions or extracellular products (Table 3). There are no examples of formulations composed of bacterial selected antigens, purified or produced by recombinant technologies. A significant limit to the efficacy of lactococcosis vaccines lies in the relatively short duration of immunity/protection they confer, lasting approximately 3–4 months. This limited span poses a primary hurdle for the success of these vaccines, as it fails to cover the entirety of the warm season when water temperatures exceed 15 °C and most lactococcosis outbreaks tend to occur (Meyburgh et al., 2017; Ravelo et al., 2006). Some studies reported the efficacy against rainbow trout lactococcosis of a combined strategy consisting in a primary immunization with an aqueous bacterin followed by a booster with the same vaccine (de Ruyter et al., 2023; Romalde et al., 2004). Another approach includes adjuvants in the vaccine formulation enabling a better duration of protection (approximately 4–8 months) (Anderson, 1997; Meyburgh et al., 2017), without need of revaccination. Concerning oral or bath vaccination in the field practice (not experimental), we didn't find literature dedicated. There are papers available on oral

Table 3
Post *L. garvieae* vaccination surveys carried out in rainbow trout (*O. mykiss*), aiming at the study the immune response parameters modulation. Variations of single parameters are indicated by arrows, in terms of comparison between vaccinated and control fish.

Type of vaccine	Fish weight (g) & water temperature (°C)	Route of vaccination	Vaccine formulation	Dose	Time of sampling (dpv or wpv or mpv)	Immune/inflammatory responses		References
						Immunological parameters	Gene modulation	
Experimental (lab formulation)	W: 12 ± 2.5 WT: 14 ± 1°	IP	BIVALENT FK *L. garvieae* + FK *A. hydrophila*	0.1 ml of 1 × 10^8 CFU/fish	15 & 30 dpv 90 dpv	SIgM ↑ SIgM ↔	– –	Bastardo et al., 2012
	W: 10 ± 0.6 WT: n.m	Oral pellet coated with bacterin + EUDRAGIT, 14 days adm.	Bivalent FK *L. garvieae* + FK *S. iniae*	1 × 10^{10} CFU/ml used for coating	20 dpv 40 dpv 60 dpv	BA ↑; LA↔, CA↔, SIgM↔, RBA↔, TIgM↔, TP↔ LA↑, SIgM ↑; BA↓; RBA↔, LA↔, CA↔, TIgM↔, TP↔ LA↑, SIgM ↑; BA↓; RBA↔, CA↔, TIgM↔, TP↔	IL-6, IgM ↑	Halimi et al., 2018
	W: 10 ± 0.6 WT: n.m	Oral pellet coated with bacterin + Chitosan-alginate, 14 days adm.	Bivalent FK *L. garvieae* + FK *S. iniae*	2.4 × 10^9 cells/ml used for coating	20 dpv 40 dpv 60 dpv	LA ↑; BA↓; CA↔, TIgM↔, TP↔ LA↑, SIgM↑; BA↓; CA↔, TIgM↔, TP↔ LA↑, SIgM↑; BA↓; CA↔, TIgM↔, TP↔	IL-6, IgM ↑	Halimi et al., 2019
	W: 10 ± 0.6 WT: n.m	Oral pellet coated with bacterin + Chitosan-alginate, 14 days adm.	Bivalent FK *L. garvieae* + FK *S. iniae*	2.4 × 10^9 cells/ml used for coating	20 dpv 40 dpv 60 dpv	BA↑, CA↑; RBA↔, TIgM↔, TP↔, LA↔ SIgM ↑; BA↓, CA↓; RBA↔, TIgM↔, TP↔, LA↔ SIgM ↑; BA↓, CA↓; RBA↔, TIgM↔, TP↔, LA↔	IL-6, IgM ↑	Halimi et al., 2020
Commercial (available in the market)	W: 20 WT: 12°	IP	FK *L. garvieae* with or without adjuvant	0.1 ml of 1 × 10^{10} CFU/fish	30 dpv 75 & 90 dpv	SIgM↑ SIgM↓	–	Kubilay et al., 2008
	W: 72 ± 3 WT: 14 ± 1°	IP	FK *L. garvieae*	0.2 ml of 2 × 10^8 CFU/fish	2, 4, 6 & 8 wpv	LA↑, CA↑, SIgM↑	–	Zaheri-Abdevand et al., 2021
	W: 10 WT: 14°	IP	Lacto-Fish Vax (Fatro, Italy)	0.1 ml/fish	4 & 8 wpv	SIgM↑	–	Bulfon et al., 2019
	W: 150–200 WT: n.m.	IP	Inactivated *L. garvieae* with adjuvant	0.1 ml/fish	3 wpv 3 mpv	SIgM↑ SIgM↔	–	Bulfon et al., 2016
	W: 50 ± 5 WT: 15 ± 1°	IP IM 90 s	Polyvalent FK *L. garvieae* + *S. iniae* + *Y. ruckeri* (ACECR, Iran)	0.1 ml of 4 × 10^8 & 1 × 10^9 CFU/ml	20, 40 & 60 dpv	LC↑, LA↑, CA↑, SIgM ↑	–	Erfanmanesh et al., 2023
	W: 30 ± 1.7 WT: n.m.	IP + IM booster after 30 days	Bivalent FK *L. garvieae* + FK *S. iniae*	0.1 ml/fish	14, 30, 45 & 60 dpv	SIgM↑	–	Karami et al., 2019
	W: 500–600 WT: 20–22°	IP	Icthiovac® LG Hipra	0.1 ml/fish	10 wpv	APA↑, POA↑, TP↑, TIgM↑, SIgM ↑, LA↔, BA ↔	–	Khalil et al., 2023a
	W: 120 ± 6.7 WT: 17.09 ± 1.5°	IP	Bivalent FK *L. garvieae* + *S. iniae* (ACECR, Iran)	0.1 ml of 1.7 × 10^7 CFU/fish	2, 4 & 6 wpv	SIgM ↑	–	Raissy et al., 2018

W = Weight; WT = Water temperature; ↑ = up-regulation; ↓ = down-regulation; ↔ = no expression; IP = Intraperitoneal injection; IC = Intracoelomic injection; IM = Immersion infection; CFU = Colony Forming Unit, LA = lysozyme activity; RBA = Respiratory Burst Activity; APA = Antiprotease activity; POA = Peroxidase activity; CA = Complement activity; TP = Total protein; TIgM = Total Immunoglobulin, BA = Bactericidal activity; LC = Leukocyte; SIgM = Specific anti-*L. garvieae* IgM; n.m. = not mentioned; dpv = days post vaccination; wpv = weeks post vaccination; mpv = months post vaccination; FK = formalin killed; adm = administration.

vaccination only describing experimental trials (Altun et al., 2010; Halimi et al., 2018, 2019, 2020). Another evidence from the bibliographic research summarised in the Table 3 is that some experiments have been performed by administering bivalent or event trivalent formulations, in which *L. garvieae* has been alternatively combined with *S. iniae*, *A. hydrophila*, and *Y. ruckeri*.

Vaccination leads to the activation of specific and non-specific immune responses, as well as to the reduction of specific mortality (de Ruyter et al., 2023; Erfanmanesh et al., 2023; Halimi et al., 2020) (Table 3). One of the main immunological pieces of evidence of effective vaccination against lactococcosis is the synthesis of specific antibodies (IgM) that are able to recognize and neutralize *L. garvieae* (Kubilay et al., 2008; Zaheri-Abdevand et al., 2021). The presence of these antibodies (IgM) in serum and mucus can be detected using techniques such as ELISA, agglutination and western blot (Karami et al., 2019; Zaheri-Abdevand et al., 2021). Concerning specific antibody titer against the antigens in the vaccinated groups, a significant increase compared to the control group was reported in several studies (Bastardo et al., 2012; Bulfon et al., 2019; Erfanmanesh et al., 2023; Karami et al., 2019; Kubilay et al., 2008; Raissy et al., 2018; Zaheri-Abdevand et al., 2021) and this may foster the protection of fish. Regarding the non-specific immune parameters potentially triggered by vaccination Khalil et al. (2023a) identified an elevation in antiprotease activity, peroxidase activity, total protein, and total immunoglobulin levels in farmed trout vaccinated intraperitoneally using the Icthiovac® LG Hipra commercial vaccine. Halimi et al. (2020) noted the activation of humoral factors within the innate immune system. In their study, bactericidal and complement activity in serum demonstrated a noteworthy rise at 20 days' post oral vaccination. In trout vaccinated orally against *L. garvieae* demonstrated that bactericidal activity and specific antibody titer found to be a noteworthy incremented on day 20, 40 and 60 of investigation in fish immunized by an oral vaccine covered pellets with alginate/chitosan compared to control (Halimi et al., 2019). In both studies, vaccination was bivalent (formalin killed-*L. garvieae* and *S. iniae*) and the vaccine-charged feed was coated with Chitosan-alginate and feed them orally.

Vaccination leads to the activation and recruitment of immune cells such as macrophages, neutrophils and lymphocytes that play a crucial role in the host's defence against bacterial infection (Erfanmanesh et al., 2023). Erfanmanesh et al. (2023) considered the increase in the number of circulating leukocytes at 20, 40 and 60 days' post vaccination as a reason for improving the non-specific and specific immunity of the fish immunized by the formalin killed-*L. garvieae* vaccine (both IP and IM). A significant upregulation of the lysozyme activity in vaccinated rainbow trout has been reported in several studies even if the protocols of administration and formulation of vaccines were different (Erfanmanesh et al., 2023; Halimi et al., 2019, 2020; Khalil et al., 2023a). Considering the antibacterial role of lysozyme and the significant increase of serum lysozyme activity in the vaccinated group it can be assumed that vaccination can be effective also by the promotion of aspecific antibacterial molecules (Erfanmanesh et al., 2023).

Vaccination also leads to the synthesis/expression of cytokines and immune receptors, which are able to modulate the immune response by the recruitment, activation, and differentiation of immune cells. Concerning cytokines, only IL-6 has been evaluated and found significantly upregulated in trout vaccinated by an oral bivalent vaccine (Halimi et al., 2018, 2019, 2020). As regards to immunoglobulin expression, only IgM has been evaluated and found to be upregulated significantly in vaccinated fish in the same trials (Halimi et al., 2018, 2019, 2020).

Efficient delivery of vaccines is crucial in transferring immunogenic constituents to the recognition and effector components of the fish immune system, as highlighted in studies by Altun et al. (2010) and Palm Jr. et al. (1998). A comparative analysis of immune response and survival rate by Erfanmanesh et al. (2023) recommended the injection method as more effective and suitable than immersion. To achieve long-term protection, a booster dose is recommended by de Ruyter et al. (2023) and Romalde et al. (2004). Adding adjuvants to vaccine formulation provides longer protection (4–8 months) without revaccination (Anderson, 1997; Meyburgh et al., 2017). The size of the fish (typically 50 g) and water temperature (12–15 °C) during vaccination are also important factors to consider when aiming for targeted protection against lactococcosis, as highlighted by Meyburgh et al. (2017) and Vendrell et al. (2006).

5.2. Side-effects of the vaccination against lactococcosis in rainbow trout

The current prophylactic strategies against bacterial diseases affecting rainbow trout are primarily based on intraperitoneal injection of bacterins (commercial or stabulogenic) formulated with mineral oil adjuvants. This strategy promotes a significant antibody response and a tangible protection against pathogens, but determines a variety of injection-site lesions, such as adhesions, granulomas and melanisation of the coelomic viscera and fat (Midtlyng, 1997; Veenstra et al., 2017; Villumsen et al., 2015; Villumsen et al., 2017). These phenomena affect trout welfare but also the quality of its products. Edible parts of the fish and the fillet must be discarded due to inappreciable alterations consequent to superficial and deep lesions.

The literature lacks studies specifically addressing the potential side effects of vaccinations against *L. garvieae* in rainbow trout. Nevertheless, occasional field observations have revealed gross and microscopic pathological findings following intraperitoneal injection of adjuvant-containing vaccines. Among these findings, we observed a severe and locally diffused necro-haemorrhagic dermatitis and myositis, extending towards serosal layer of the coelomatic cavity, primarily localized on the ventral area of the fish (Fig. 3, A-B). Microscopically, a significant monocytic inflammatory infiltrate is evident, diffusely affecting the hypodermis and muscle layers. Inflammatory cells also infiltrate the dermis compactum and spongiosum (Fig. 3, C-D-E). Within these tissue sections, vaccine oil droplets appear as empty vacuoles with well-defined borders. Surrounding these vacuoles, lympho-histiocytic infiltrate, along with haemorrhage and necrosis, can be observed (Fig. 3, C—D). Additionally, similar occurrences of these oil droplets can occasionally be found in other organs, such as the spleen (Fig. 3, E).

Rainbow trouts in Italian farms are injected either by hand, using "injection guns" operated by trained professionals, or by automated delivery machines. The recommended injection site is at the abdominal midline, slightly posterior to the pelvic fins. If operators work too quickly or imprecisely, or if machines are not adjusted to the size of the fish, the recommended injection site may be missed. This can result in internal organ injuries, occasionally leading to bleeding or potential bacterial entry into the tissues. When implementing this preventive measure, it is important to strike a balance between producing sufficient protection and minimizing side-effects.

6. Conclusions and future perspectives

Lactococcosis outbreaks are a major concern in the global aquaculture industry, particularly in rainbow trout farming. However, the exact economic impact of these outbreaks has not been adequately quantified yet. A comprehensive analysis that takes into account direct factors such as reduced fish production, as well as indirect consequences such as expenses related to disease prevention, treatment protocols, and potential zoonotic implications, can help in developing effective policies and strategies to mitigate the multifaceted impacts of this infection. In this regard, the conservation of fish stocks is of paramount importance.

Based on an extensive analysis of scientific literature related to lactococcosis in rainbow trout over the past four decades, we have created a geospatial representation that highlights the disease's significant prevalence. This is particularly noticeable in temperate regions with a high concentration of rainbow trout aquaculture, where the water temperature in summers is often above the critical level of 18–20 °C. We are concerned that global climate change could cause the spread of diseases

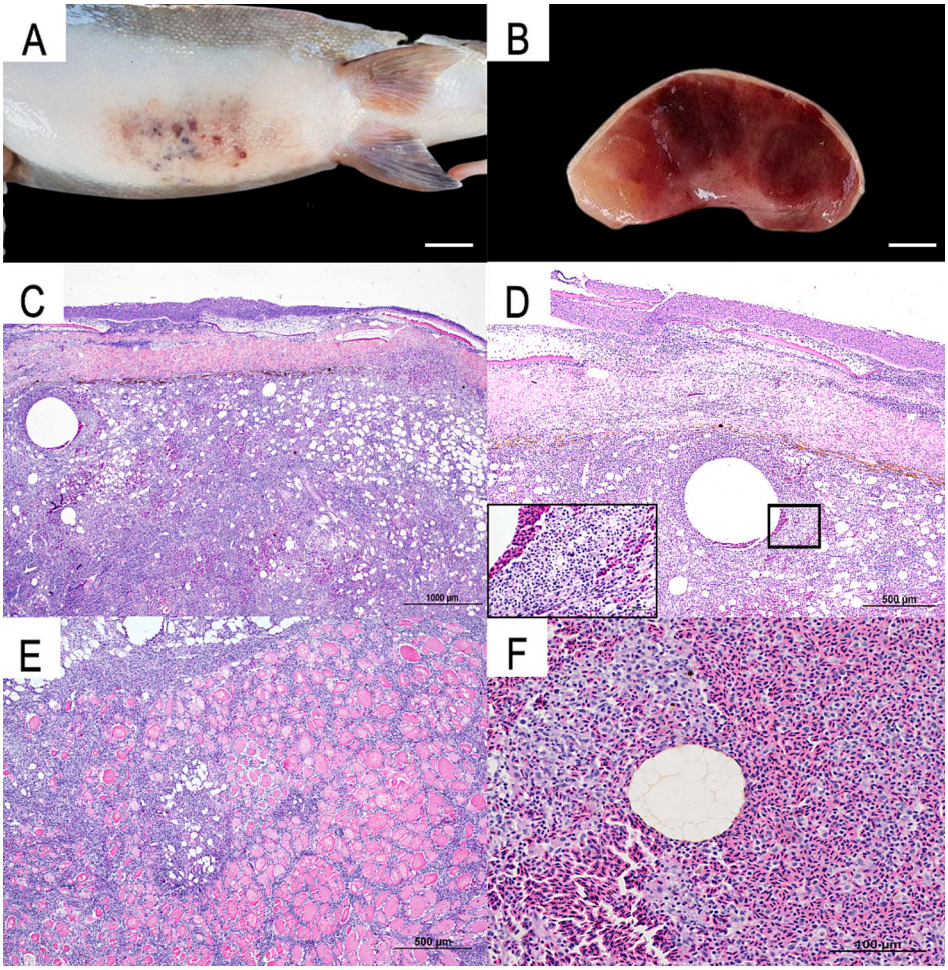

Fig. 3. Gross and microscopic pathological findings occasionally observed after intraperitoneal injection of adjuvant-containing vaccines against *L. garvieae* in farmed rainbow trout. Severe and locally diffused necro-haemorrhagic dermatitis and myositis localized on the ventral area (A). Cut section (B) of the same area is the serosal layer of the coelomatic cavity. Microscopically, the severe monocytic inflammatory infiltrate is predominantly and diffusely involving the hypodermis and muscle layers (C-E). Nevertheless, the infiltration of inflammatory cells involves the dermis compactum and spongiosum (C, D). Vaccine oil droplets are visible as empty vacuoles with even margins (C, D), surrounded by lympho-hystiocitic infiltrate, haemorrhage, and necrosis (D and inset). These can be occasionally found in other organs, such as the spleen (F). H & E sections. Bar = 1 cm.

like lactococcosis to regions that have not previously been exposed to them. Rising temperatures could exacerbate certain fish illnesses and increase mortality rates, which could lead to the introduction of new pathogens into ecosystems or hosts that have not been affected before. This is especially worrisome in areas located at lower latitudes.

The studies reviewed in this article aimed to explore the impact of *L. garvieae* infection and vaccination on rainbow trout. These investigations have not only identified the physical and microscopic lesions caused by the pathogen, but they have also highlighted the effects on innate and adaptive immune parameters. However, there are still gaps in our understanding, particularly with regards to the pathogen's entry routes and the temporal dynamics of bacterial dissemination, which are affected by physiological conditions, variable fish sizes, and elevated water temperatures. Additionally, there is limited research on the characterization of inflammatory cells recruited post-infection or post-vaccination, and a lack of in vitro studies to eliminate animal testing. Using rainbow trout cell lines or primary cell cultures from immune organs could be a useful tool to study immunological mechanisms such as phagocytosis, killing, antigen presentation, and proliferation, which could lead to the identification of bacterial proteins that are potentially immunogenic for the development of vaccines against lactococcosis.

Moving forward, it is crucial to address these gaps in our knowledge to gain a better understanding of the dynamics between *L. garvieae* and rainbow trout. Future research should focus on exploring the impact of temperature on these dynamics, and not just the immune system's response to infection or vaccination.

Author statement

All authors have confirmed to submit the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Sarker Mohammed Ibrahim Khalil: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing – review & editing. **Massimo Orioles:** Formal analysis, Writing - original draft, Writing – review & editing. **Paolo Tomé:** Formal analysis, Writing – review & editing. **Marco Galeotti:** Supervision, Validation of the article. **Donatella Volpatti:** Conceptualization, Formal analysis, Writing - original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors wish to acknowledge the field vets providing some valuable information/images useful to shape this manuscript. They also wish to mention the reviewed papers authors', allowing the collection of relevant information on the selected topic.

References

Aguado-Urda, M., Rodríguez-Bertos, A., de las Heras, A.I., Blanco, M.M., Acosta, F., Cid, R., Fernández-Garayzábal, J.F., Gibello, A., 2014. Experimental *Lactococcus garvieae* infection in zebrafish and first evidence of its ability to invade non-phagocytic cells. Vet. Microbiol. 171 (1–2), 248–254. https://doi.org/10.1016/j.vetmic.2014.03.029.

Algöet, M., Bayley, A.E., Roberts, E.G., Feist, S.W., Wheeler, R.W., Verner-Jeffreys, D.W., 2009. Susceptibility of selected freshwater fish species to a UK *Lactococcus garvieae* isolate. J. Fish Dis. 32 (10), 825–834. https://doi.org/10.1111/j.1365-2761.2009.01058.x.

Ali, A., Rexroad, C.E., Thorgaard, G.H., Yao, J., Salem, M., 2014. Characterization of the rainbow trout spleen transcriptome and identification of immune-related genes. Front. Genet. 5, 348. https://doi.org/10.3389/fgene.2014.00348.

Altinok, I., Ozturk, R.C., Ture, M., 2022. NGS analysis revealed that *Lactococcus garvieae* Lg-per was *Lactococcus petauri* in Türkiye. J. Fish Dis. 45 (12), 1839–1843. https://doi.org/10.1111/jfd.13708.

Altun, S., Kubilay, A., Ekici, S., Didinen, B.I., Diler, Ö., 2010. Oral vaccination against Lactococcosis in rainbow trout (*Oncorhynchus mykiss*) using sodium alginate and poly (lactide-co-glycolide) carrier. J. Facul. Vet. Med. 16, 211–217. University of Kafkas, Kars (Turkey).

Anderson, D.P., 1997. Adjuvants and immunostimulants for enhancing vaccine potency in fish. Dev. Biol. Stand. 90, 257–265.

Austin, B., Austin, A.D., 2012. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish, 5th ed. Springer/Praxis Publishing.

Avci, H., Aydogan, A., Tanrikul, T.T., Birincioglu, S., 2010. Pathological and Microbiological ınvestigations in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) naturally infected with *Lactococcus garvieae*. J. Facul. Vet. Med. Kafkas University 16, S313–S318.

Avci, H., Birincioglu, S.S., Tanrikul, T.T., Epikmen, E.T., Metin, N., Avsever, M.L., 2014. Experimental *Lactococcus garvieae* infection in rainbow trout, *Oncorhynchus mykiss*, Walbaum 1792: a comparative histopathological and immunohistochemical study. J. Fish Dis. 37 (5), 481–495. https://doi.org/10.1111/jfd.12132.

Bailey, G.S., Williams, D.E., Hendricks, J.D., 1996. Fish models for environmental carcinogenesis: the rainbow trout. Environ. Health Perspect. 104 (Suppl. 1), 5–21. https://doi.org/10.1289/ehp.96104s15.

Bark, S., McGregor, D., 2001. The first occurrence of lactococcosis in farmed trout in England. Trout News 31, 9–11.

Barnes, A.C., Guyot, C., Hansen, B.G., Horn, M.T., Ellis, A.E., 2002. Antibody increases phagocytosis and killing of *Lactococcus garvieae* by rainbow trout (*Oncorhynchus mykiss*, L.) macrophages. Fish Shellfish Immunol. 12 (2), 181–186. https://doi.org/10.1006/fsim.2001.0372.

Bastardo, A., Ravelo, C., Castro, N., Calheiros, J., Romalde, J.L., 2012. Effectiveness of bivalent vaccines against *Aeromonas hydrophila* and *Lactococcus garvieae* infections in rainbow trout *Oncorhynchus mykiss* (Walbaum). Fish Shellfish Immunol. 32 (5), 756–761. https://doi.org/10.1016/j.fsi.2012.01.028.

Bekker, A., Hugo, C., Albertyn, J., Boucher, C.E., Bragg, R.R., 2011. Pathogenic gram-positive cocci in south African rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 34 (6), 483–487. https://doi.org/10.1111/j.1365-2761.2011.01259.x.

Berthelot, C., Brunet, F., Chalopin, D., Juanchich, A., Bernard, M., Noël, B., Bento, P., Da Silva, C., Labadie, K., Alberti, A., Aury, J.M., Louis, A., Dehais, P., Bardou, P., Montfort, J., Klopp, C., Cabau, C., Gaspin, C., Thorgaard, G.H., Boussaha, M., Quillet, E., Guyomard, R., Galiana, D., Bobe, J., Volff, J.N., Genêt, C., Wincker, P., Jaillon, O., Roest, Crollius H., Guiguen, Y., 2014. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nat. Commun. 22 (5), 3657. https://doi.org/10.1038/ncomms4657.

Bilen, S., Sirtiyah, A.M.A., Terzi, E., 2019. Therapeutic effects of beard lichen, *Usnea barbata* extract against *Lactococcus garvieae* infection in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 87, 401–409. https://doi.org/10.1016/j.fsi.2019.01.046.

Bulfon, C., Poli, M., Sarti, M., Volpatti, D., 2016. Specific antibody response of rainbow trout (*Oncorhynchus mykiss*) after a vaccination treatment against lactococcosis performed during the autumn. Ittiopatologia 13, 75–88.

Bulfon, C., Pacorig, V., Sarti, M., Luzzana, U., Galeotti, M., Volpatti, D., 2019. Protec™ improves innate immune response and specific antibody response against *Lactococcus garvieae* in rainbow trout (*Oncorhynchus mykiss*). Vet. Immunol. Immunopathol. 213, 109885 https://doi.org/10.1016/j.vetimm.2019.109885.

Bulfon, C., Prearo, M., Volpatti, D., Byadgi, O., Righetti, M., Maniaci, M.G., Campia, V., Pastorino, P., Pascoli, F., Toffan, A., Biolatti, C., Acutis, P.L., Colussi, S., 2020. Resistant and susceptible rainbow trout (*Oncorhynchus mykiss*) lines show distinctive immune response to *Lactococcus garvieae*. Fish Shellfish Immunol. 105, 457–468. https://doi.org/10.1016/j.fsi.2020.06.040.

Bwalya, P., Hang'ombe, B.M., Gamil, A.A., Munang'andu, H.M., Evensen, Ø., Mutoloki, S., 2020. A whole-cell *Lactococcus garvieae* autovaccine protects Nile tilapia against infection. PLoS One 15 (3), e0230739. https://doi.org/10.1371/journal.pone.0230739.

Bwalya, P., Hang'ombe, B.M., Evensen, Ø., Mutoloki, S., 2021. *Lactococcus garvieae* isolated from Lake Kariba (Zambia) has low invasive potential in Nile tilapia (*Oreochromis niloticus*). J. Fish Dis. 44 (6), 721–727. https://doi.org/10.1111/jfd.13339.

Byadgi, O., Chen, Y.C., Barnes, A.C., Tsai, M.A., Wang, P.C., Chen, S.C., 2016. Transcriptome analysis of grey mullet (*Mugil cephalus*) after challenge with *Lactococcus garvieae*. Fish Shellfish Immunol. 58, 593–603. https://doi.org/10.1016/j.fsi.2016.10.006.

Castro, R., Coll, J., Blanco, M.D.M., Rodriguez-Bertos, A., Jouneau, L., Fernández-Garayzábal, J.F., Gibello, A., 2019. Spleen and head kidney differential gene expression patterns in trout infected with *Lactococcus garvieae* correlate with spleen granulomas. Vet. Res. 50 (1), 32. https://doi.org/10.1186/s13567-019-0649-8.

Chan, J.F., Woo, P.C., Teng, J.L., Lau, S.K., Leung, S.S., Tam, F.C., Yuen, K.Y., 2011. Primary infective spondylodiscitis caused by *Lactococcus garvieae* and a review of human *L. garvieae* infections. Infection. 39 (3), 259–264. https://doi.org/10.1007/s15010-011-0094-8.

Chang, P.H., Lin, C.W., Lee, Y.C., 2002. *Lactococcus garvieae* infection of cultured rainbow trout, *Oncorhynchus mykiss*, in Taiwan and associated biophysical characteristics and histopathology. Bull. Eur. Assoc. Fish Pathol. 22, 319–327.

Chen, S.C., Lin, Y.D., Liaw, L.L., Wang, P.C., 2001. *Lactococcus garvieae* infection in the giant freshwater prawn *Macrobranchium rosenbergii* confirmed by polymerase chain reaction and 16S rDNA sequencing. Dis. Aquat. Org. 45 (1), 45–52. https://doi.org/10.3354/dao045045.

Chen, S.C., Liaw, L.L., Su, H.Y., Ko, S.C., Wu, C.Y., Chaung, H.C., Tsai, Y.H., Yang, K.L., Chen, Y.C., Chen, T.H., Lin, G.R., Cheng, S.Y., Lin, Y.D., Lee, J.L., Lai, C.C., Weng, Y. J., Chu, S.Y., 2002. *Lactococcus garvieae*, a cause of disease in grey mullet, *Mugil cephalus* L., in Taiwan. J. Fish Dis. 25, 727–732.

Collins, M.D., Farrow, J.A., Phillips, B.A., Kandler, O., 1983. *Streptococcus garvieae* sp. nov. and *streptococcus plantarum* sp. nov. J. Gen. Microbiol. 129, 3427–3431.

Colorni, A., Ravelo, C., Romalde, J.L., Toranzo, A.E., Diamant, A., 2003. *Lactococcus garvieae* in wild Red Sea wrasse *Coris aygula* (Labridae). Dis. Aquat. Org. 56 (3), 275–278. https://doi.org/10.3354/dao056275.

de Ruyter, T., Littman, E., Yazdi, Z., Adkison, M., Camus, A., Yun, S., Welch, T.J., Keleher, W.R., Soto, E., 2023. Comparative evaluation of booster vaccine efficacy by Intracoelomic injection and immersion with a whole-cell killed vaccine against *Lactococcus petauri* infection in rainbow trout (*Oncorhynchus mykiss*). Pathogens 12, 632. https://doi.org/10.3390/pathogens12050632.

Dıdınen, B.I., Yardımcı, B., Onuk, E.E., Metın, S., Yıldırım, P., 2014. Naturally *Lactococcosis garvieae* infection in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792): new histopathological observations, phenotypic and molecular identification. Rev. Med. Vet. 165, 12–19.

Diler, Ö., Altun, S., Adiloglu, A.K., Kubilay, A., Isıklı, B., 2002. First occurrence of Streptococcosis affecting farmed rainbow trout (*Oncorhynchus mykiss*) in Turkey. Bull. Eur. Assoc. Fish Pathol. 22 (1), 21–26.

Doménech, A., Prieta, J., Fernández-Garayzábal, J.F., Collins, M.D., Jones, D., Domínguez, L., 1993. Phenotypic and phylogenetic evidence for a close relationship between *Lactococcus garvieae* and *enterococcus seriolicida*. Microbiologia. 9 (1), 63–68.

Dong, F., Yin, G.M., Meng, K.F., Xu, H.Y., Liu, X., Wang, Q.C., Xu, Z., 2020. IgT plays a predominant role in the antibacterial immunity of rainbow trout olfactory organs. Front. Immunol. 11, 583740 https://doi.org/10.3389/fimmu.2020.583740.

Eldar, A., Ghittino, C., 1999. *Lactococcus garvieae* and *streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: similar, but different diseases. Dis. Aquat. Org. 36 (3), 227–231. https://doi.org/10.3354/dao036227.

Eldar, A., Bejerano, Y., Livoff, A., Horovitcz, A., Bercovier, H., 1995. Experimental streptococcal meningo-encephalitis in cultured fish. Vet. Microbiol. 43 (1), 33–40. https://doi.org/10.1016/0378-1135(94)00052-x.

Erfanmanesh, A., Beikzadeh, B., Khanzadeh, M., 2023. Efficacy of polyvalent vaccine on immune response and disease resistance against streptococcosis/lactococcosis and yersiniosis in rainbow trout (*Oncorhynchus mykiss*). Vet. Res. Commun. https://doi.org/10.1007/s11259-023-10081-6.

Evans, J.J., Pasnik, D.J., Klesius, P.H., Al-Ablani, S., 2006. First report of *Streptococcus agalactiae* and *Lactococcus garvieae* from a wild bottlenose dolphin (*Tursiops truncatus*). J. Wildl. Dis. 42 (3), 561–569. https://doi.org/10.7589/0090-3558-42.3.561.

Evans, J.J., Klesius, P.H., Shoemaker, C.A., 2009. First isolation and characterization of *Lactococcus garvieae* from Brazilian Nile tilapia, *Oreochromis niloticus* (L.), and pintado, *Pseudoplathystoma corruscans* (Spix & Agassiz). J. Fish Dis. 32 (11), 943–951. https://doi.org/10.1111/j.1365-2761.2009.01075.x.

Eyngor, M., Zlotkin, A., Ghittino, C., Prearo, M., Douet, D.G., Chilmonczyk, S., Eldar, A., 2004. Clonality and diversity of the fish pathogen *Lactococcus garvieae* in Mediterranean countries. Appl. Environ. Microbiol. 70 (9), 5132–5137. https://doi.org/10.1128/AEM.70.9.5132-5137.2004.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action, Rome. https://doi.org/10.4060/ca9229en.
FEAP, 2022. European Aquaculture Production Report 2015–2021. https://feap.info/wp-content/uploads/2023/04/2023-04-05-production-report-2023.pdf.
Fevolden, S.E., Refstie, T., Røed, K.H., 1992. Disease resistance in rainbow trout (*Oncorhynchus mykiss*) selected for stress response. Aquaculture 104 (1–2), 19–29.
Fichi, G., Cardeti, G., Perrucci, S., Vanni, A., Cersini, A., Lenzi, C., De Wolf, T., Fronte, B., Guarducci, M., Susini, F., 2015. Skin lesion-associated pathogens from *Octopus vulgaris*: first detection of *Photobacterium swingsii*, *Lactococcus garvieae* and betanodavirus. Dis. Aquat. Org. 115 (2), 147–156. https://doi.org/10.3354/dao02877.
Ghittino, P., Prearo, M., 1992. Report of Streptococcosis in rainbow trout (*Oncorhynchus mykiss*) in Italy: preliminary note. Boll. Soc. Ital. Pathol. Ittica. 8, 4–9.
Gibello, A., Galán-Sánchez, F., Blanco, M.M., Rodríguez-Iglesias, M., Domínguez, L., Fernández-Garayzábal, J.F., 2016. The zoonotic potential of *Lactococcus garvieae*: an overview on microbiology, epidemiology, virulence factors and relationship with its presence in foods. Res. Vet. Sci. 109, 59–70. https://doi.org/10.1016/j.rvsc.2016.09.010.
Gomez, D., Sunyer, J.O., Salinas, I., 2013. The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. 35 (6), 1729–1739. https://doi.org/10.1016/j.fsi.2013.09.032.
Goodman, L.B., Lawton, M.R., Franklin-Guild, R.J., Anderson, R.R., Schaan, L., Thachil, A.J., Wiedmann, M., Miller, C.B., Alcaine, S.D., Kovac, J., 2017. *Lactococcus petauri* sp. nov., isolated from an abscess of a sugar glider. Int. J. Syst. Evol. Microbiol. 67 (11), 4397–4404. https://doi.org/10.1099/ijsem.0.002303.
Halimi, M., Alishahi, M., Abbaspour, M.R., Ghorbanpoor, M., Tabandeh, M.R., 2018. Efficacy of a Eudragit L30D-55 encapsulated oral vaccine containing inactivated bacteria (*Lactococcus garvieae/streptococcus iniae*) in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 81, 430–437. https://doi.org/10.1016/j.fsi.2018.07.048.
Halimi, M., Alishahi, M., Abbaspour, M.R., Ghorbanpoor, M., Tabandeh, M.R., 2019. Valuable method for production of oral vaccine by using alginate and chitosan against *Lactococcus garvieae/streptococcus iniae* in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 90, 431–439. https://doi.org/10.1016/j.fsi.2019.05.020.
Halimi, M., Alishahi, M., Abbaspour, M.R., Ghorbanpoor, M., Tabandeh, M.R., 2020. High efficacy and economical procedure of oral vaccination against *Lactococcus garvieae/streptococcus iniae* in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 99, 505–513. https://doi.org/10.1016/j.fsi.2020.02.033.
Hollebecq, M.G., Faivre, B., Bourmaud, C., Michel, C., 1995. Spontaneous bactericidal and complement activities in serum of rainbow trout (*Oncorhynchus mykiss*) genetically selected for resistance or susceptibility to furunculosis. Fish Shellfish Immunol. 5 (6), 407–426.
Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2017. Impact of selective breeding on European aquaculture. Aquaculture 472 (1), 8–16. https://doi.org/10.1016/j.aquaculture.2016.03.012.
Karami, E., Alishahi, M., Ghorbanpour, M., Tabandeh, M., Mohammadian, T., 2019. Serum antibody response of rainbow trout, *Oncorhynchus mykiss* to two species of pathogenic bacteria; *streptococcus iniae* and *Lactococcus garviae*. Iranian Scientific Fisheries Journal 28 (1), 1–8. https://doi.org/10.22092/isfj.2019.118605.
Kawanishi, M., Yoshida, T., Yagashiro, S., Kijima, M., Yagyu, K., Nakai, T., Murakami, M., Morita, H., Suzuki, S., 2006. Differences between *Lactococcus garvieae* isolated from the genus *Seriola* in Japan and those isolated from other animals (trout, terrestrial animals from Europe) with regard to pathogenicity, phage susceptibility and genetic characterization. J. Appl. Microbiol. 101 (2), 496–504. https://doi.org/10.1111/j.1365-2672.2006.02951.x.
Khalil, S.M.I., Bulfon, C., Galeotti, M., Acutis, P.L., Altinok, I., Kotzamanidis, C., Vela, A.I., Fariano, L., Prearo, M., Colussi, S., Volpatti, D., 2023a. Immune profiling of rainbow trout (*Oncorhynchus mykiss*) exposed to *Lactococcus garvieae*: evidence in asymptomatic versus symptomatic or vaccinated fish. J. Fish Dis. 46 (7), 731–741. https://doi.org/10.1111/jfd.13782.
Khalil, S.M.I., Sacca, E., Galeotti, M., Sciuto, S., Stoppani, N., Acutis, P.L., Öztürk, R.C., Kotzamanidis, C., Blanco, M.M., Fariano, L., Prearo, M., Colussi, S., Volpatti, D., 2023b. In field study on immune-genes expression during a lactococcosis outbreak in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 574, 1–9. https://doi.org/10.1016/j.aquaculture.2023.739633.
Khosravi, M., Mohammadian, T., Tahmasebifard, M., Boroujeni, M.P., 2018. Correlation between C-reactive protein level, immunology, and hematology of a *Oncorhynchus mykiss* infected with *Lactococcus garvieae*. Aquac. Int. 26, 1415–1425.
Köllner, B., Wasserrab, B., Kotterba, G., Fischer, U., 2002. Evaluation of immune functions of rainbow trout (*Oncorhynchus mykiss*)-how can environmental influences be detected? Toxicol. Lett. 131 (1–2), 83–95. https://doi.org/10.1016/s0378-4274(02)00044-9.
Korun, J., Timur, G., Yardimci, E., Balci, B., 2017. Histopathological changes of rainbow trout after experimental infection with *Lactococcus garvieae*. J. Adv. VetBio Sci. Tech. 2 (3), 12–20. https://doi.org/10.31797/vetbio.336097.
Korytář, T., Jaros, J., Verleih, M., Rebl, A., Kotterba, G., Kühn, C., Goldammer, T., Köllner, B., 2013. Novel insights into the peritoneal inflammation of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 35 (4), 1192–1199. https://doi.org/10.1016/j.fsi.2013.07.032.
Kotzamanidis, C., Malousi, A., Bitchava, K., Vafeas, G., Chatzidimitriou, D., Skoura, L., Papadimitriou, E., Chatzopoulou, F., Zdragas, A., 2020. First report of isolation and genome sequence of *L. petauri* strain from a rainbow trout Lactococcosis outbreak. Curr. Microbiol. 77 (6), 1089–1096. https://doi.org/10.1007/s00284-020-01905-8.
Kubilay, A., Altun, S., Ulukoy, S., Ekici, S., Diler, O., 2008. Immunization of rainbow trout (*Oncorhynchus mykiss*) against *Lactococcus garvieae* using vaccine mixtures. Israeli J. Aquacul. - Bamidgeh 60 (4), 268–273.
Kusuda, R., Kawai, K., Salati, F., Banner, C.R., Fryer, J.L., 1991. *Enterococcus seriolicida* sp. nov., a fish pathogen. Int. J. Syst. Evol. Bacteriol. 41, 406–409.
Kutyrev, I., Cleveland, B., Leeds, T., Wiens, G.D., 2016. Proinflammatory cytokine and cytokine receptor gene expression kinetics following challenge with *Flavobacterium psychrophilum* in resistant and susceptible lines of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 58, 542–553. https://doi.org/10.1016/j.fsi.2016.09.053.
Lulijwa, R., Alfaro, A.C., Merien, F., Meyer, J., Young, T., 2019. Advances in salmonid fish immunology: A review of methods and techniques for lymphoid tissue and peripheral blood leucocyte isolation and application. Fish Shellfish Immunol. 95, 44–80. https://doi.org/10.1016/j.fsi.2019.10.006.
Magnadottir, B., 2010. Immunological control of fish diseases. Mar. Biotechnol. (N.Y.) 12 (4), 361–379. https://doi.org/10.1007/s10126-010-9279-x.
Magnadottir, B., Lange, S., Gudmundsdottir, S., Bøgwald, J., Dalmo, R.A., 2005. Ontogeny of humoral immune parameters in fish. Fish Shellfish Immunol. 19 (5), 429–439. https://doi.org/10.1016/j.fsi.2005.03.010.
Matsuura, Y., Terashima, S., Takano, T., Matsuyama, T., 2019. Current status of fish vaccines in Japan. Fish Shellfish Immunol. 95, 236–247. https://doi.org/10.1016/j.fsi.2019.09.031.
Meyburgh, C.M., Bragg, R.R., Boucher, C.E., 2017. *Lactococcus garvieae*: an emerging bacterial pathogen of fish. Dis. Aquat. Org. 123 (1), 67–79. https://doi.org/10.3354/dao03083.
Midtlyng, P.J., 1997. Vaccinated fish welfare: protection versus side-effects. Dev. Biol. Stand. 90, 371–379.
Mohammadian, T., Nasirpour, M., Tabandeh, M.R., Heidary, A.A., Ghanei-Motlagh, R., Hosseini, S.S., 2019. Administrations of autochthonous probiotics altered juvenile rainbow trout *Oncorhynchus mykiss* health status, growth performance and resistance to *Lactococcus garvieae*, an experimental infection. Fish Shellfish Immunol. 86, 269–279. https://doi.org/10.1016/j.fsi.2018.11.052.
Nelson, M.C., Varney, J.S., Welch, T.J., Graf, J., 2016. Draft genome sequence of *Lactococcus garvieae* strain PAQ102015-99, an outbreak strain isolated from a commercial trout farm in the northwestern United States. Genome Announc. 4, e00781 https://doi.org/10.1128/genomeA.00781-16.
Ooyama, T., Kera, A., Okada, T., Inglis, V., Yoshida, T., 1999. The protective immune response of yellowtail *Seriola quinqueradiata* to the bacterial fish pathogen *Lactococcus garvieae*. Dis. Aquat. Org. 37 (2), 121–126. https://doi.org/10.3354/dao037121.
Ooyama, T., Hirokawa, Y., Minami, T., Yasuda, H., Nakai, T., Endo, M., Ruangpan, L., Yoshida, T., 2002. Cell-surface properties of *Lactococcus garvieae* strains and their immunogenicity in the yellowtail *Seriola quinqueradiata*. Dis. Aquat. Org. 51 (3), 169–177. https://doi.org/10.3354/dao051169.
Ortega, C., Irgang, R., Valladares-Carranza, B., Collarte, C., Avendaño-Herrera, R., 2020. First identification and characterization of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) cultured in mexico. Animals (Basel) 10, 1609. https://doi.org/10.3390/ani10091609.
Palm Jr., R.C., Landolt, M.L., Busch, R.A., 1998. Route of vaccine administration: effects on the specific humoral response in rainbow trout *Oncorhynchus mykiss*. Dis. Aquat. Org. 33 (3), 157–166. https://doi.org/10.3354/dao033157.
Palti, Y., 2011. Toll-like receptors in bony fish: from genomics to function. Dev. Comp. Immunol. 35 (12), 1263–1272. https://doi.org/10.1016/j.dci.2011.03.006.
Pastorino, P., Vela Alonso, A.I., Colussi, S., Cavazza, G., Menconi, V., Mugetti, D., Righetti, M., Barbero, R., Zuccaro, G., Fernández-Garayzábal, J.F., Dondo, A., Acutis, P.L., Prearo, M., 2019. A summer mortality outbreak of lactococcosis by *Lactococcus garvieae* in a raceway system affecting farmed rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Animals (Basel) 9 (12), 1043. https://doi.org/10.3390/ani9121043.
Perdiguero, P., Morel, E., Tafalla, C., 2021. Diversity of rainbow trout blood B cells revealed by single cell RNA sequencing. Biology (Basel) 10 (6), 511. https://doi.org/10.3390/biology10060511.
Pereira, F., Ravelo, C., Toranzo, A., Romalde, J., 2004. *Lactococcus garvieae*, an emerging pathogen for the Portuguese trout culture. Bull. Eur. Assoc. Fish Pathol. 24, 274–279.
Pérez-Sánchez, T., Balcázar, J.L., Merrifield, D.L., Carnevali, O., Gioacchini, G., de Blas, I., Ruiz-Zarzuela, I., 2011. Expression of immune-related genes in rainbow trout (*Oncorhynchus mykiss*) induced by probiotic bacteria during *Lactococcus garvieae* infection. Fish Shellfish Immunol. 31 (2), 196–201. https://doi.org/10.1016/j.fsi.2011.05.005.
Phillips, R.B., Ventura, A.B., Dekoning, J.J., Nichols, K.M., 2013. Mapping rainbow trout immune genes involved in inflammation reveals conserved blocks of immune genes in teleosts. Anim. Genet. 44 (1), 107–113. https://doi.org/10.1111/j.1365-2052.2011.02314.x.
Radosavljević, V., Radanović, O., Zdravković, N., Savić, B., Stanković, M., Maksimović Zorić, J., Veljović, L., Nešić, K., 2020. The first outbreak of lactococcosis caused by *Lactococcus garvieae* in Serbia. Arch. Vet. Med. 13 (1), 53–68. https://doi.org/10.46784/e-avm.v13i1.78.
Raissy, M., Hashemi, S., Roushan, M., Jafarian, M., Momtaz, H., Soltani, M., Pirali Kheirabad, E., 2018. Effects of essential oils of *Satureja bachtiarica* and *Nigella sativa* on the efficacy of lactococcosis vaccine in rainbow trout (*Oncorhynchus mykiss*). Iran. J. Fish. Sci. 17 (1), 95–106.
Ravelo, C., Magarinos, S.B., Lopez-Romalde, S., Toranzo, A.E., Romalde, J.L., 2003. Molecular finger printing of fish-pathogenic *Lactococcus garvieae* strains by random amplified polymorphic DNA analysis. J. Clin. Microbiol. 41, 751–756.
Ramirez-Gomez, F., Greene, W., Rego, K., Hansen, J.D., Costa, G., Kataria, P., Bromage, E.S., 2012. Discovery and characterization of secretory IgD in rainbow

trout: secretory IgD is produced through a novel splicing mechanism. J. Immunol. 188 (3), 1341–1349. https://doi.org/10.4049/jimmunol.1101938.
Ravelo, C., Magariños, B., Herrero, M.C., Costa, L., Toranzo, A.E., Romalde, J.L., 2006. Use of adjuvanted vaccines to lengthen the protection against lactococcosis in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 251 (2–4), 153–158.
Romalde, J.L., Luzardo-Alvárez, A., Ravelo, C., Toranzo, A.E., Blanco-Méndez, J., 2004. Oral immunization using alginate microparticles as a useful strategy for booster vaccination against fish lactoccocosis. Aquaculture 236 (1–4), 119–129.
Salinas, I., 2015. The mucosal immune system of teleost fish. Biology (Basel) 4 (3), 525–539. https://doi.org/10.3390/biology4030525.
Salinas, I., Fernández-Montero, Á., Ding, Y., Sunyer, J.O., 2021. Mucosal immunoglobulins of teleost fish: A decade of advances. Dev. Comp. Immunol. 121, 104079 https://doi.org/10.1016/j.dci.2021.104079.
Schmidtke, L.M., Carson, J., 1999. Induction, characterisation and pathogenicity in rainbow trout *Oncorhynchus mykiss* (Walbaum) of *Lactococcus garvieae* L-forms. Vet. Microbiol. 69 (4), 287–300. https://doi.org/10.1016/s0378-1135(99)00120-0.
Secombes, C., Zou, J., Daniels, G., Cunningham, C., Koussounadis, A., Kemp, G., 1998. Rainbow trout cytokine and cytokine receptor genes. Immunol. Rev. 166, 333–340. https://doi.org/10.1111/j.1600-065x.1998.tb01273.x.
Semple, S.L., Dixon, B., 2020. Salmonid antibacterial immunity: an aquaculture perspective. Biology 9 (10), 331. https://doi.org/10.3390/biolo gy910 0331.
Shahi, N., Mallik, S.K., 2020. Emerging bacterial fish pathogen *Lactococcus garvieae* RTCLI04, isolated from rainbow trout (*Oncorhynchus mykiss*): genomic features and comparative genomics. Microb. Pathog. 147, 104368 https://doi.org/10.1016/j.micpath.2020.104368.
Shahi, N., Mallik, S.K., Sahoo, M., Chandra, S., Singh, A.K., 2018. First report on characterization and pathogenicity study of emerging *Lactococcus garvieae* infection in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), from India. Transbound. Emerg. Dis. 65 (4), 1039–1048. https://doi.org/10.1111/tbed.12843.
Shahin, K., Veek, T., Heckman, T.I., Littman, E., Mukkatira, K., Adkison, M., Welch, T.J., Imai, D.M., Pastenkos, G., Camus, A., Soto, E., 2022. Isolation and characterization of *Lactococcus garvieae* from rainbow trout, *Onchorhyncus mykiss*, from California, USA. Transbound. Emerg. Dis. 69 (4), 2326–2343. https://doi.org/10.1111/tbed.14250.
Shiry, N., Shomali, T., Soltanian, S., Akhlaghi, M., 2019. Comparative single-dose pharmacokinetics of orally administered florfenicol in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) at health and experimental infection with *streptococcus iniae* or *Lactococcus garvieae*. J. Vet. Pharmacol. Ther. 42 (2), 214–221. https://doi.org/10.1111/jvp.12736.
Soltani, M., Sh, J., Sharifpour, I., 2005. Streptococcosis caused by *streptococcus iniae* in farmed rainbow trout (*Oncorhynchus mykiss*) in Iran: biophysical characteristics and pathogensis. Bull. Eur. Assoc. Fish Pathol. 25, 95–107.
Soltani, M., Baldisserotto, B., Hosseini Shekarabi, S.P., Shafiei, S., Bashiri, M., 2021. Lactococcosis a re-emerging disease in aquaculture: disease significant and phytotherapy. Vet. Sci. 8 (9), 181. https://doi.org/10.3390/vetsci8090181.
Su, F.J., Periyasamy, T., Chen, M.M., 2022. Comparative transcriptomic immune responses of mullet (*Mugil cephalus*) infected by planktonic and biofilm *Lactococcus Garvieae*. Front. Cell. Infect. Microbiol. 12, 887921 https://doi.org/10.3389/fcimb.2022.887921.
Sunyer, J.O., 2012. Evolutionary and functional relationships of B cells from fish and mammals: insights into their novel roles in phagocytosis and presentation of particulate antigen. Infect. Disord. Drug Targets 12 (3), 200–212. https://doi.org/10.2174/187152612800564419.
Teixeira, L.M., Merquior, V.L., Vianni, M.C., Carvalho, M.G., Fracalanzza, S.E., Steigerwalt, A.G., Brenner, D.J., Facklam, R.R., 1996. Phenotypic and genotypic characterization of atypical *Lactococcus garvieae* strains isolated from water buffalos with subclinical mastitis and confirmation of *L. garvieae* as a senior subjective synonym of *enterococcus seriolicida*. Int. J. Syst. Bacteriol. 46 (3), 664–668. https://doi.org/10.1099/00207713-46-3-664.
Teker, T., Albayrak, G., Akayli, T., Urku, C., 2018. Detection of Haemolysin genes as genetic determinants of virulence in *Lactococcus garvieae*. Turk. J. Fish. Aquat. Sci. 19 (7), 625–634. https://doi.org/10.4194/1303-2712-v19_7_09.
Thorgaard, G.H., Bailey, G.S., Williams, D., Buhler, D.R., Kaattari, S.L., Ristow, S.S., Hansen, J.D., Winton, J.R., Bartholomew, J.L., Nagler, J.J., Walsh, P.J., Vijayan, M. M., Devlin, R.H., Hardy, R.W., Overturf, K.E., Young, W.P., Robison, B.D., Rexroad, C., Palti, Y., 2002. Status and opportunities for genomics research with rainbow trout. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 133 (4), 609–646. https://doi.org/10.1016/s1096-4959(02)00167-7.
Türe, M., Altinok, I., 2016. Detection of putative virulence genes of *Lactococcus garvieae*. Dis. Aquat. Org. 119 (1), 59–66. https://doi.org/10.3354/dao02981.
Türe, M., Haliloglu, H.I., Altuntas, C., Boran, H., Kutlu, I., 2014. Comparison of experimental susceptibility of rainbow trout (*Oncorhynchus mykiss*), turbot (*Psetta maxima*), black sea trout (*Salmo trutta labrax*) and sea bass (*Dicentrarchus labrax*) to *Lactococcus garvieae*. Turk. J. Fish. Aquat. Sci. 14, 507–513.
Veenstra, K.A., Wang, T., Alnabulsi, A., Douglas, A., Russell, K.S., Tubbs, L., Arous, J.B., Secombes, C.J., 2017. Analysis of adipose tissue immune gene expression after vaccination of rainbow trout with adjuvanted bacterins reveals an association with side effects. Mol. Immunol. 88, 89–98. https://doi.org/10.1016/j.molimm.2017.05.026.
Valdés, N., Cortés, M., Barraza, F., Reyes-López, F.E., Imarai, M., 2022. CXCL9-11 chemokines and CXCR3 receptor in teleost fish species. Fish Shellfish Immunol. Rep. 3, 100068 https://doi.org/10.1016/j.fsirep.2022.100068.
Veenstra, K.A., Alnabulsi, A., Tubbs, L., Ben Arous, J., Secombes, C.J., 2019. Immunohistochemical examination of immune cells in adipose tissue of rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal vaccination. Fish Shellfish Immunol. 87, 559–564. https://doi.org/10.1016/j.fsi.2019.02.001.
Vendrell, D., Balcázar, J.L., Ruiz-Zarzuela, I., de Blas, I., Gironés, O., Múzquiz, J.L., 2006. *Lactococcus garvieae* in fish: a review. Comp. Immunol. Microbiol. Infect. Dis. 29 (4), 177–198. https://doi.org/10.1016/j.cimid.2006.06.003.
Vendrell, D., Serarols, L., Balcázar, J.L., de Blas, I., Gironés, O., Múzquiz, J.L., Ruiz-Zarzuela, I., 2012. Accumulation and depletion kinetics of erythromycin in rainbow trout (*Oncorhynchus mykiss*). Prev. Vet. Med. 105 (1–2), 160–163. https://doi.org/10.1016/j.prevetmed.2012.02.017.
Villumsen, K.R., Koppang, E.O., Raida, M.K., 2015. Adverse and long-term protective effects following oil-adjuvanted vaccination against *Aeromonas salmonicida* in rainbow trout. Fish Shellfish Immunol. 42 (1), 193–203. https://doi.org/10.1016/j.fsi.2014.09.024.
Villumsen, K.R., Koppang, E.O., Christensen, D., Bojesen, A.M., 2017. Alternatives to mineral oil adjuvants in vaccines against *Aeromonas salmonicida* subsp. salmonicida in rainbow trout offer reductions in adverse effects. Sci. Rep. 7 (1), 5930. https://doi.org/10.1038/s41598-017-06324-7.
Yamaguchi, T., Dijkstra, J.M., 2019. Major histocompatibility complex (MHC) genes and disease resistance in fish. Cells. 8 (4), 378. https://doi.org/10.3390/cells8040378.
Yu, Y., Wang, Q., Huang, Z., Ding, L., Xu, Z., 2020. Immunoglobulins, mucosal immunity and vaccination in teleost fish. Front. Immunol. 11, 567941 https://doi.org/10.3389/fimmu.2020.567941.
Zaheri-Abdevand, L., Soltani, M., Shafiei, S., 2021. Adjuvant effect of licorice (*Glycyrrhiza glabra*) extract on the efficacy of lactococcosis vaccine in rainbow trout (*Oncorhynchus mykiss*). Iran. J. Fish. Sci. 20 (3), 646–662.
Zou, J., Secombes, C.J., 2016. The function of fish cytokines. Biology (Basel) 5 (2), 23. https://doi.org/10.3390/biology5020023.
Zuo, S., Karami, A.M., Ødegård, J., Mathiessen, H., Marana, M.H., Jaafar, R.M., von Gersdorff Jørgensen, L., Abdu, M., Kania, P.W., Dalsgaard, I., Nielsen, T., Buchmann, K., 2020. Immune gene expression and genome-wide association analysis in rainbow trout with different resistance to *Yersinia ruckeri* infection. Fish Shellfish Immunol. 106, 441–450. https://doi.org/10.1016/j.fsi.2020.07.023.