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Granulomatosis in fish aquaculture: a mini review

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Abstract

Bacterial infections represent the greatest threat for fish culture, especially those causing granulomatous processes. Although there are experimental models used to elucidate the mechanisms of pathogenicity in granulomatous processes, most of these aimed to understand the infection in superior vertebrates like humans; therefore, there is still an urgent need to expand the number of infection models focused on aquaculture. Granulomatous infections are difficult to detect in early stages due to the lack of clinical signs and because these clinical signs have a great similarity to those generated by other pathogenic bacteria. Besides, there is no evidence or reports of granulomatous co-infections in aquaculture environments which is a plausible scenario either in culture systems or in the wild environment; co-infections could interfere with a correct diagnosis of any granulomatous disease, as reported for granulomatous infections in terrestrial species. This revision aims to highlight the state of the art in the knowledge of pathogenic bacteria associated with granulomatosis in fish aquaculture as well as analysing this information.

Key words: co-infections, fish, Francisella, granulomatous infections, Mycobacterium, Nocardia.

Introduction

Currently, about 597 aquatic animal species are belonging to different taxonomic groups that are cultivated around the world, most of which are secondary or tertiary consumers (~80%) (FAO 2016). The FAO reported total global production of 84.4 million tones of aquatic organisms in 2018 (FAO 2020), of which 45 million tones corresponded to fin-fish farming. Of the total of aquatic organisms produced worldwide by both aquaculture and fisheries, fish coming from the aquaculture industry represented up to 43%, and this has increased over the years.

Aquaculture has achieved a greater economic revolution and is considered as the fastest emerging activity worldwide in the food industry (Dadar *et al.* 2017). However, one of the most relevant threats in fish farming is the economic loss, due to outbreaks of infectious diseases causing high mortality rates. About 55% of fish infections are caused by bacteria (Dhar *et al.* 2014), because unlike the terrestrial environment, the aquatic environment favours the development of these microbes regardless of the host. Disease outbreaks in aquaculture are favoured by stocking intensification, which is a strategy to make the activity more profitable.

Diseases caused by bacteria in fish can be classified into two types, non-granulomatous and granulomatous (Pérez-Arellano & del Pozo 2013). These last ones pose a great threat to the aquaculture industry; herein, chronic and necrotizing granulomas can be caused in fish and shellfish by the genus *Francisella*, potentially leading to extensive chronic multifocal granulomatous response including multiple lesions throughout the affected organs which severely compromise the immune response of fish and ultimately its survival (Birkbeck *et al.* 2011).

Although mammals and fish show similar symptoms during these infections, the interactions between bacteria associated with granulomas formation and the underlying mechanisms of the host immune system remain unknown. The interactions are complex because different bacteria are associated with the formation of granulomatous lesions and sometimes clinical signs go unnoticed during early infection stages (Itano *et al.* 2006a; Gauthier & Rhodes 2009; Babalola 2015; Wang *et al.* 2017), making their detection a difficult task. Therefore, a variety of infection models are required not only to understand the infection mechanisms, but to develop prevention and control protocols (Wang *et al.* 2017).

The objective of this work is to carry out a review and discuss the current state of knowledge about granulomatous diseases in fish aquaculture and the most common bacteria associated with these outbreaks.

Granulomatous definition

A granuloma can be defined as a mass of granulation tissue that is regularly originated in response to microbial infections, inflammation or the presence of foreign material, and constitutes a strategy of the immune system to isolate microbes or material incapable to be eliminated. In this regard, granulomatous infections comprise a large family of pathologies exhibiting the histological denominator of granuloma formation. These can provoke acute or chronic clinical symptoms (James 2000; Alberts *et al.* 2010) caused by different factors, commonly of autoimmune or infectious origin. Granulomatous infections are characterized by a deficiency in the aetiological agent phagocytosis and are not easily controlled by other inflammatory mechanisms (Pérez-Arellano & del Pozo 2013).

Granulomas are constituted of agglomerations of heterogeneous, compact, well-organized and dynamic accumulation of immune cells, including infected and uninfected macrophages, epithelial cells and fibroblasts (Adams 1974). The phagocytosis of the foreign body is activated in the first instance, but if the process fails, the activation of T lymphocytes occurs, and these activate the macrophages or histiocytes that are the main components of the granuloma. In other words, when the cells of the immune system encounter the pathogen (including bacteria and fungi), a signalling cascade of anti- and pro-inflammatory nature is initiated. This triggers the recruitment and accumulation of macrophages and other leucocytes in tissue. Eventually, granulomas or granulomatous lesions are formed due to the complex interaction between the invading organism or prolonged antigenaemia, the activity of the macrophages, the response of Th1 cells, the hyperactivity of B cells and a wide range of biological mediators (James 2000; Myllymäki *et al.* 2018).

Granulomas can be detected as non-necrotic and necrotic granulomas. Commonly a non-necrotic granuloma is firstly formed, but it can progress to a necrotic phase. For example, Ruiz-Tagle *et al.* (2020) documented a non-necrotic granuloma consisting predominantly of lymphocytes, macrophages, giant cells and produced by mycobacteria. However, the granuloma progressed to a necrotic state registering changes in its microenvironment, macrophage apoptosis and the bacteria slowing their growth rate. The authors also reported that the lymphocyte cuff of the granuloma contained giant cells and isolated macrophages positive for the various mycobacterial genes. Necrotic granulomas usually have infectious causes but are usually not easily detected in fish, because most of the time their formation occurs internally.

Granulomatous infections in fish

The primary route of infection by pathogens in fish occurs via injured skin and gut (Austin 1999); however, eyes, gills and other vias have been discussed. Herein, the infection routes of agents associated with granulomatous lesions such as the bacterial genera *Mycobacterium*, *Nocardia*, *Francisella* and others are unknown, but it is hypothesized that this could occur through gills, muscle and other injured sites (Maekawa *et al.* 2018). This has been observed in the yellowtail (*Seriola quinqueradiata*) undergoing nocardiosis infections, causing injuries in gills, dermis and muscle (Itano *et al.* 2006). Noteworthy one of the factors influencing the mortality rates when these infections occur in fish is the infection route. For example, while intradermal infections tend to progress, the mortality and spread of the disease is greater in intraperitoneal or immersion infections (Itano *et al.* 2006a). In some cases, infections do not result in disease because some bacteria, such as *Mycobacterium marinum*, have developed evasion strategies to live in balance with the immune system, remaining dormant for long periods (Babalola 2015); however, a suppressed immune system accompanied by the appearance of lesions and favourable environmental conditions could trigger the proliferation of these bacteria, leading to granulomatous disease (Reavill & Schmidt 2012); this same pattern occurs in the transmission of granulomatous disease to humans, for instance Lewis *et al.* 2003 argued that disseminated infections of *M. marinum* in fish are rare in humans but almost always occur in immunosuppressed individuals.

Regarding disease development, there is consensus about the granuloma formation when the immune system cannot eliminate the pathogen (Evensen *et al.*, 2005; Swaim *et al.* 2006). In a latent infection, for example the pathogen is still not in high concentration, which facilitates the process to encapsulate the pathogenic cells by the action of infected and non-infected macrophages, and the granuloma begins to get defined (Diamant *et al.* 2000; Myllymäki *et al.* 2016; Figure 1). If the infection persists, the invasive bacteria are highly compacted within a defined core and surrounded by a thick fibrous capsule that maintains the pathogen in isolation; at this point, granulomas can be observed by the naked eye in different tissues. Finally, after a chronic infection, the reactivation and rapid replication of the encapsulated bacteria cause an overflow from the granuloma while bacteria disseminate, causing even more damage to the tissue and ultimately leading to death (Figure 2). For instance, this process has been observed in zebrafish infected with *Mycobacterium marinum*, which was used as a model to understand the tuberculosis disease in humans

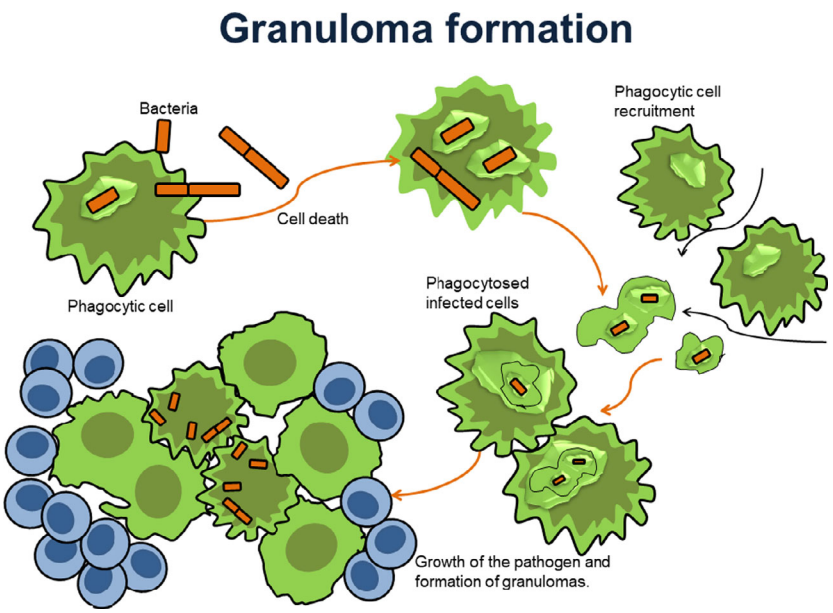

Figure 1 Basic granuloma formation. Bacteria such as *M. marinum* within phagocytic cells of zebrafish eventually trigger cell death. Infected cells recruit uninfected cells, and the latter engulf the infected cells. This provides a favourable niche or environment for the growth of the bacteria and allows the exit of infected cells to form new granulomas. Based on Davis and Ramakrishnan (2009).

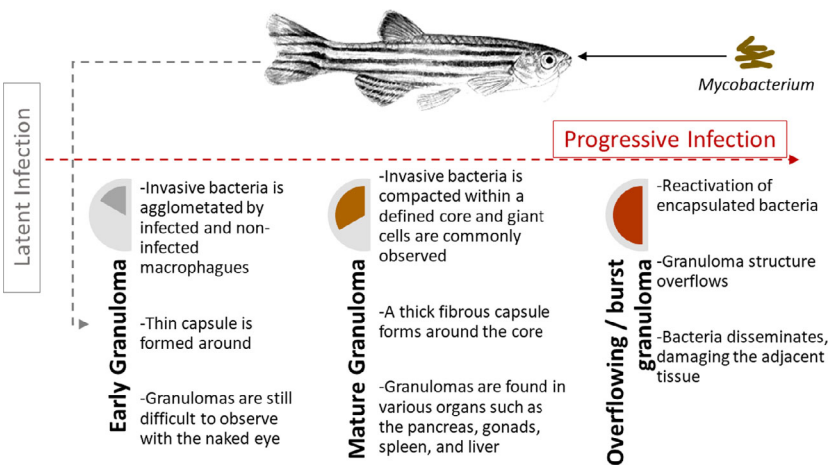

Figure 2 General scheme of granuloma formation in zebrafish infected with *Mycobacterium*.

because this species resembles the lesions caused by *Mycobacterium tuberculosis* (Myllymäki *et al.* 2016).

The most common bacteria associated with fish granulomatous infections belong to the genera *Mycobacteria* spp., *Nocardia* spp. and *Francisella* spp. (Pérez-Arellano & del Pozo 2013). These bacteria have been subject to further study, identified and used in experimental models to explain the underlying mechanisms in granulomatous processes; however, other bacteria apart of these can be also found in literature as etiological agents of granulomatuos diseases (Table 1); for example, *Lactococcus garvieae,* which is associated with fatal haemorrhagic septicaemia in farmed rainbow trout (Shahi *et al.* 2018), can also produce spleen granulomas while inducing antibody response by both IgM (+) and IgT (+) spleen B cells (Castro *et al.* 2019).

Finally, granulomatous infection in fish is not only relevant from the economic perspective or the ecological point of view in the case of open fish farms, but also the human health standpoint, because some of these pathogens can be transmitted from fish to humans (Kern *et al.* 1989; Hashish *et al.* 2018).

Mycobacterium spp.

Mycobacteria belong to the genus *Mycobacterium* (Order: *Actinomycetales*, suborder: *Corynebacterineae*, family: *Mycobacteriaceae*), and these are pleomorphic, non-spore forming, Gram-positive and aerobic bacteria, without motility in the form of bacilli, measuring 0.2–0.6 μm in diameter and 1–10 μm in length. These microbes have a unique cell wall that includes a long chain (60–90 carbons)

Table 1 Granulomatous infections caused by bacteria belonging to different taxonomic groups in diverse marine and freshwater fish species

Bacteria	Aquatic animal	Evaluated response	Reference
Edwarsiella tarda	West African lungfish (*Protopterus annectens*)	External ulcerative lesions and mortality after developing anorexia. Multifocal areas of necrosis and heterophilic and histiocytic inflammation in multiple tissues. Small numbers of intra- and extracellular monomorphic Gram-negative rod-shaped bacilli. Lung granuloma, kidney and testes showed heterophilic inflammation with phagocytosis of small monomorphic bacilli and some heterophils exhibiting cytoplasmic projections suggesting heterophil extracellular traps	Rousselet *et al*. (2018)
Francisella marina sp. nov.	Spotted Rose Snapper (*Lutjanus guttatus*)	Multifocal granulomatous lesions with small, pleomorphic coccobacilli predominantly in the peritoneum, spleen, kidneys, liver, pancreas, intestine and heart	Soto *et al*. (2018)
Francisella noatunensis subsp. *orientalis*	Nile tilapia (*Oreochromis niloticus* x *O. aureus*)	Numerous white granulomas in kidney, liver, heart and spleen. Head kidney and spleen were markedly swollen	Lin *et al*. (2016)
	Zebrafish (*Danio rerio*)	Granuloma-like structures containing small coccoid bacteria detected in the spleen. Some encapsulated granulomas were also observed even in previously immunized fish	Lagos *et al*. (2017)
	Tilapia (*Oreochromis* sp.)	Reduced appetite, lethargy, dark pigmentation and abnormal distension before death. Necropsy showed widespread of white multifocal nodules in the head kidney, spleen, liver and gill. Histopathology revealed granulomatous inflammation surrounded by numerous macrophages	Pulpipat *et al*. (2019)
Lactococcus garvieae	Rainbow trout (*Oncorhynchus mykiss*)	Haemorrhagic septicaemia was associated with this pathogen. Extensive degenerative and inflammatory changes in eye, kidney, gill and liver were observed.	Shahi *et al*. (2018)
Mycobacterium marinum	Zebrafish (*Danio rerio*)	This approach demonstrated that the zebrafish granuloma contains foam cells and the transdifferentiation of macrophages into foam cells is driven by the mycobacterial ESX1 pathogenicity locus	Johansen *et al*. (2018)
Mycobacterium leprae	Zebrafish (*Danio rerio*)	Rapid development of noncaseating granulomas, but infection was eventually eradicated. The rag1 mutant zebrafish lacking lymphocytes also formed noncaseating granulomas, but these controlled the infection more slowly. These results revealed the interplay between innate and adaptive immune determinants mediating leprosy granuloma formation and function	Madigan *et al*. (2017)
Mycobacterium gordonae	Ginbuna crucian carp (*Carassius auratus langsdorfii*)	Granulomatous responses consisted of central macrophage accumulation and surrounding lymphocytes. Ziehl-Neelsen-positive bacteria were detected in the trunk kidney of fish. The marginal lymphocytes were positive for CD4-1, and the IFNγ-producing cells surrounded the mycobacterial cell-laden phagocytes. CD4-1+ cells and IFNγ2 played important roles in the granulomatous inflammation	Kato *et al*. (2019)
Nocardia seriolae	Spotted butterfish (*Scatophagus argus*, Linn)	Enlargement of spleen, kidney and liver with white nodules varying in size. The pathogen caused systemic granulomas	Wang *et al*. (2014)
	Snubnose pompano (*Trachinotus blochii*)	Fish displayed paleness and lethargy, and exhibited skin haemorrhages and ulcers. Prominent white nodules varying in size were observed in the spleen, kidney and liver. Typical granulomatous lesions in these organs were observed	Vu-Khac *et al*. (2016)
	Tiger barb (*Puntius tetrazona*)	Green fluorescent protein-labelled *Nocardia seriolae* strain was injected in fish. Bacteria were phagocytized by leucocytes and proliferated within these cells, which in turn led to leucocyte aggregation, leucocyte death and granuloma formation. Bacteria could permanently colonize various tissues via leucocyte circulation, causing multi-organ infection	Wang *et al*. (2017)
	Japanese eel (*Anguilla japonica*)	Infected fish exhibited lethargy and skin ulcers. Pathiogen genoma was sequenced and assembled, revealing dozens of antibiotic resistance genes in the genome of *N. seriolae* strains; most of the antibiotics were involved in the inhibition of the biosynthesis of proteins or cell walls	Han et al. (2018)
Streptococcus iniae	Tilapia (*Oreochromis aureus*)	External signs included exophthalmia and cachexia, while internal signs were granulomatous septicaemia and interstitial nephritis, among others	Ortega *et al*. (2018)

of 3-hydroxy mycolic acid (Draper 1971; Gangadharam & Jenkins 1997). According to the Runyon classification scheme (1959), *Mycobacteria*, except for those species that cannot be cultivated *in vitro*, are functionally separated by growth time.

The genus *Mycobacterium* causes Mycobacteriosis (Gauthier & Rhodes 2009), which can affect humans, fish and other animals (Jacobs *et al*. 2009). It is a chronic disease that may not present symptoms and can be detected in cultured marine and freshwater fish (Novotny *et al*. 2010). The associated species with mycobacteriosis in either freshwater and marine fish are *M. marinum*, *M. salmoniphilum*, *M. fortuitum*, *M. shimoidei*, *M. chelonae*, *M. triplex*, *M. montefiorense* and *M. abscessus* (Gauthier & Rhodes 2009). Moreover, different non-tuberculous *Mycobacterium* species can cause co-infections in fish, even those associated with distant taxonomic groups; for example, *M. shimoidei*, *M. marinum*, *M. chelonae*, *M. septicum*, *M. peregrinum* and *M. porcinum* were isolated from the exotic goldfish Guppy in South Africa (Gcebe *et al*. 2018).

Mycobacterium affects all tissues including eyes, gills, visceral organs, muscles and scales. The external symptoms are not specific and may display loss of scales, skin ulcerations, changes in pigmentation, abnormal behaviour and spine malformations among other signs (Nigrelli & Vogel 1963; Ross 1970). The most severe signs include the enlargement of spleen, kidneys and liver, with the presence of white or grey nodules in internal organs.

Mycobacteriosis is also referred as piscine tuberculosis in both, human and fish; although not all *Mycobacterium* species are tuberculous and the disease is not associated with high mortality rates in fish caught by fisheries, it is associated with significant losses within aquaculture (Gauthier & Rhodes 2009) due to poor growth, lower survival and poor productive response in general. Mycobacteriosis seems to be a universal disease among the most different taxonomic classes of these vertebrates, with the aggravating circumstance that this genus easily generates resistance to antibiotics (Gauthier & Rhodes 2009).

Granulomatous infections in aquaculture could have severe impacts, because the cultured organisms are confined in high densities and are exposed to other acute and chronic stressors that could affect the immune system, making fish vulnerable to the disease.

The latency is one of the most important pathogenic aspects of the genus *Mycobacterium* and other bacteria that cause granulomatosis. *Mycobacterium marinum* has been reported as a pathogen not only able to remain dormant for years; however, when reactivated acts as a modulator of signalling pathways directed to cell death, promoting the dissemination and transmission of the bacteria (Myllymäki *et al*. 2016; Vemula *et al*. 2016). This bacterial capacity turns out to be relevant because it could be extrapolated to fish aquaculture.

The *Mycobacterium* genus has species that able to introduce, survive and even replicate in macrophages (Barker *et al*. 1997; Tobin & Ramakrishnan 2008), which is relevant to understand how this pathogen can adapt to evade the immune system. In this regard, evidence suggests that *Mycobacterium* can use the host´s macrophages and granulomas for its benefits, enhancing its expansion and dissemination (Volkman *et al*. 2010; Oksanen *et al*. 2013); as this process goes on, the pathogenic bacteria recruit new uninfected macrophages to the granuloma where these macrophages phagocytize infected dead cells and thus promoting the dissemination of the bacteria. Preventive strategies include DNA-based vaccines containing *Mycobacterium* antigens (Oksanen *et al*. 2013).

Nocardia spp.

Members of the *Nocardia* genus are Gram-positive, aerobic actinomycetes, motility-free, acid-alcohol-resistant bacteria in the form of pleomorphic bacilli (Luo *et al*. 2014). The genus contains more than 90 species recognized and widely distributed in aquatic and terrestrial habitats. Most of these species are known to cause nocardiosis in fish and a wide variety of animals including humans (Kandi 2015). *Nocardia* is considered a genus harbouring a group of pathogens with unique attributes and complicated properties (Mehta & Shamoo 2020). In this regard, *Nocardia* is clinically challenging to diagnose and treat. In humans, for example (also assumed in fish), *Nocardia* can survive as facultative intracellular parasites within macrophages and escape from being eliminated by neutrophils and monocytes. Infections can be asymptomatic and, combined with the slow growth rate of *Nocardia*, make them difficult to identify in clinical specimens. Four species of *Nocardia* have been commonly isolated from fish suffering from nocardiosis: *N. asteroides*, *N. seriolae*, *N. salmonicida* and *N. crassostreae* (Itano *et al*. 2006c).

Nocardiosis is considered a chronic, granulomatous and systemic disease where the lesions are localized in the skin and several internal organs including kidney, liver and spleen, with the typical nodular structure of granuloma formation. Observable symptoms usually do not manifest during the early stages of fish, but the invasion of tissue occurs gradually. This is the reason why nocardiosis is not rapidly detected in aquaculture until the mid-anaphase of breeding, resulting in economic losses for breeders (Wang *et al*. 2007; Elkesh *et al*. 2013).

Nocardia sp. infects a variety of fish around the world, including the Atlantic salmon (*Salmo salar*), the African catfish (*Clarias gariepinus*), the Snubnose pompano (*Trachinotus blochii*) (Bransden *et al*. 2000; Vu-Khac *et al*.

2016), the Transparent Tiger barb (*Puntius tetrazona*) (Wang *et al.* 2017), the Large yellow croaker (*Larimichthys crocea*) and the Largemouth Bass (*Micropterus salmoides*) (Chen *et al.* 2000; Wang *et al.* 2005), among several others. It is considered an opportunistic pathogenic bacterium infecting immunocompromised or injured fish (Wang *et al.* 2017).

With the increase in the intensification of culture methods in aquaculture, fish become more susceptible of being weakened by stress or physical injury. Although nocardiosis is increasing in cultured fish, pathogenesis and spread mechanisms are still poorly understood; however, these organisms can be latent in the environment and proliferating only when fish present accessible injuries (Miyoshi *et al.* 2019).

As described above, there are no methods for the early detection or control within infected populations. The genus is still an enigma in several ways; for this reason, *Nocardia* infections are difficult to identify and treat even in humans, in which most of the research has been focused (Mehta & Shamoo 2020). However, *Nocardia* sp. is phylogenetically related to *Mycobacterium* sp. (Shah *et al.* 2017) and the infectious lesions are also similar; therefore, these two genera may have similar infection mechanisms. In this regard, studies of genomic and transcriptomic sequence analysis (Imajoh *et al.* 2015; Xia *et al.* 2015a,b; Byadgi *et al.* 2016) as well as the development of vaccines and even experimental models (Itano *et al.* 2006b; Nayak & Nakanishi 2016) could provide relevant information to establish prevention and eradication measures to avoid *Nocardia* sp. infections while methods for early detection are developed (Maekawa *et al.* 2018).

Also, antigen markers of *Nocardia* species have not been studied as extensive; therefore, developing effective vaccines against fish nocardiosis is still a pending issue. In this regard, Chen *et al.* (2019) evaluated the antigenicity of whole-cell protein of three pathogenic *Nocardia* spp., and seven common immunogenic proteins were identified including molecular chaperone DnaK, 30 S ribosomal protein S1, molecular chaperone GroEL, FHA domain-containing protein, TerD family protein, 50 S ribosomal protein L7/L12 and PspA/IM30 family protein. From these, a DNA vaccine encoding the FHA gene against fish nocardiosis was developed showing promising results for the hybrid snakehead challenged with *N. seriolae*. Other DNA vaccines encoding ribosomal proteins (RplL and RpsA) have been developed and successfully tested against *Nocardia seriolae* infection in fish (Chen *et al.* 2020).

Francisella spp.

Francisella genus contains Gram-negative pleomorphic bacteria without motility (0.1–1.5 μm), and these are intracellular organisms some of which are strict aerobes while others may be facultative. However, this genus was first considered as a Rickettsia-like or Piscirickettsia-like organism (Chen *et al.* 1994; Colquhoun & Duodu 2011), but the pathogen was later confirmed as a γ-Proteobacteria in the family Francisellaceae, order Thiotrichales (Birkbeck *et al.* 2011). The genus also contains species that can cause disease to a wide range of hosts, including humans; the species that mostly affects fish include *Francisella asiatica* and *Francisella noatunensis* (Mikalsen *et al.* 2007), and two subspecies have been isolated from the last one, *F. noatunensis* subsp. *orientalis* which causes disease in fish of temperate climates including tilapia (*Oreochromis niloticus*), and *F. noatunensis* subsp. *noatunensis* which affects fish thriving in icy waters, such as the Atlantic salmon (*Salmo salar*) (Birkbeck *et al.* 2011).

Francisellosis is, therefore, a bacterial disease that mostly occurs in juvenile fish during winter months; according to Chong (2016), for tilapia, epizootics typically occur in cooler winter water temperatures with higher mortalities at 15°C than 30°C; while for the Atlantic cod, Francisellosis causes more mortalities as water temperatures increase towards 20°C. This disease provoked economic impacts in the culture of tilapia and Atlantic cod since registering the first cases three decades ago (Chong 2016); herein, the mortality rate of infected fish depends upon several factors; however, the Atlantic salmon undergoing francisellosis registered mortality of 20%, while mortality of 95% was reported for tilapia (Chern & Chao 1994; Ostland *et al.* 2006). Several *Francisella* species can be transmitted by direct contact between infected animals, through contaminated water or food, or by vectors (Colquhoun & Duodu 2011).

The main clinical signs observed in diseased tilapia are exophthalmia, erratic swimming, anorexia, ascites, skin ulcers at the base of the fins and pale gills. At the necropsy, multifocal granulomatous lesions are observed in kidney, spleen, and liver (Colquhoun & Duodu 2011; Soto *et al.* 2012; Leal *et al.* 2014; Assis *et al.* 2017). Extensive granulomatous inflammation with multiple granulomas (some liquid-filled) can be observed, and the granulomas are usually formed by hypertrophied foamy macrophages, fibroblasts and leucocytes, and in some cases with necrotic core (Colquhoun & Duodu 2011).

Several methods have been used for the diagnosis of *F. noatunensis* subsp. *orientalis* in diseased fish such as histopathology, PCR and bacteriological culture (Soto *et al.* 2010). The affected organs of fish present granulomas consisting of vacuolated macrophages with the *Francisella* cells, central necrosis and fibrous encapsulation (Camus *et al.* 2013).

Despite bacteriological culture is considered as the gold standard method for detection of *F. noatunensis* subsp.

orientalis (Colquhoun & Duodu 2011) studies are demonstrating that the use of real-time PCR (qPCR) in tilapia experimentally infected, has greater sensitivity and similar specificity (>99.2%) than bacteriological culture (Assis *et al*. 2017). However, there is scarce information about the detection of other species of the *Francisella* genus or the identification of these infections in other fish. Besides, there is no commercial vaccine for francisellosis, but several reports are documenting promising results for vaccines developed using outer membrane vesicles of the pathogen (Brudal *et al*. 2015) or inclusively mutants of *F. asiatica* as a live attenuated vaccine (Soto *et al*. 2011) that could serve as francisellosis regulator.

Co-infections

Co-infections in aquatic animals have received scarce attention despite these are more likely to occur either in aquaculture systems or in nature. Co-infections are caused by two or more genetically different pathogens, where each pathogen leads to different effects including harm to the host in syndemism with other pathogens (Cox 2001; Bakaletz 2004).

Most of the literature addresses single infections, classifying the other agent as opportunistic and mostly ignoring it (Kotob *et al*. 2017). However, once a host is infected and undergoing the disease, it is susceptible to the invasion of other pathogenic microbes aggravating its circumstance.

During co-infections, interactions occur between the infectious agents and the outcome will depend on the load of one or different pathogens, and even at this level, the competition among pathogens can occur, or not (Cox 2001). Natural environments in which animals thrive are diverse with a wide variety of heterogeneous micro-organisms including parasitic and non-parasitic species where co-infections are frequent. Currently, there is scarce information on granulomatous co-infections in aquatic environments; for example, a case report revealed that not all fish undergoing granulomatous disease present co-infection, and different co-infection (*Brevoortia tyrannus*) patterns may occur within the same population. Herein, a group of Atlantic menhaden undergoing granulomatosis registered not only three species of mycobacteria in spleen (*M. marinum*, *M. fortuitum* complex, and *M. gordonae*) (Stine *et al*. 2005), but also a 40% co-infection rate of *Mycobacterium* spp. and *Vibrio hollisae* was detected, while in other specimens also undergoing mycobacteriosis, *Photobacterium damselae* was isolated from the kidney, liver or brain; furthermore, another group of the same fish registered the three pathogens including *Mycobacterium* spp., *Vibrio hollisae* and *P. damsela*. It could be hypothesized that some granulomatous infections in aquaculture could be co-infections, considering the large percentage of non-culture bacteria in the aquatic environment that may go undetected or have not yet been studied (Martínez-Porchas & Vargas-Albores 2017). Such scenarios may cause simultaneous infections interfering with the correct diagnosis of any granulomatous disease as reported for bovine tuberculosis (Barry *et al*. 2011). Therefore, research about the interactions occurring between these species during mixed infections and the deleterious effects of multi-infections on fish disease pathogenesis, prognosis and treatment is required (Griffiths *et al*. 2011; Johnson & Hoverman 2012; Eswarappa *et al.,* 2012; Kotob *et al*. 2017).

Perspectives and conclusions

Currently, most of the existing experimental models only explain certain mechanisms of pathogenicity to extrapolate it to human diseases, such as tuberculosis and losing focus towards aquaculture. The few fish models are limited by poor susceptibility to certain pathogens or low reproducibility of the pathogenesis, and results display significant differences in their responses between models. Although some of them are already studying specific granulomatous diseases, there is an urgent need to expand the number of available and suitable models to explain not only specific immunological mechanisms of action but also to produce vaccines, develop prevention, and epidemiological control. New studies should focus on understanding how different species of bacteria induce granuloma lesions under aquaculture conditions (high-density growth, stress) in different experimental models and development of early detection methods especially if those models contain a great commercial interest for human consumption. Until now, the combined information from recent studies of vaccine development, molecular methods such as genomic and transcriptomic sequencing analysis could provide some protection against these diseases.

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