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# Dynamics of co-infection in fish: A review of pathogen-host interaction and clinical outcome

Ekemini Moses Okon [a,*], Reuben Chukwuka Okocha [b,c], Adesina Babatunde Taiwo [d], Falana Babatunde Michael [b,d], Adeniran Moji Bolanle [b]

[a] Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[b] Department of Animal Science, College of Agricultural Sciences, Landmark University, P.M.B. 1001 Omu-Aran, Kwara State, Nigeria
[c] Climate Action Research Group, Landmark University SDG 13, Nigeria
[d] Life Below Water Research Group, Landmark University SDG 14, Nigeria

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ABSTRACT

Co-infections can affect the transmission of a pathogen within a population and the pathogen's virulence, ultimately affecting the disease's dynamics. In addition, co-infections can potentially affect the host's immunological responses, clinical outcomes, survival, and disease control efficacy. Co-infections significantly impact fish production and can change several fish diseases' progression and severity. However, the effect of co-infection has only recently garnered limited attention in aquatic animals such as fish, and there is currently a dearth of studies on this topic. This study, therefore, presents an in-depth summary of the dynamics of co-infection in fish. This study reviewed the co-infection of fish pathogens, the interaction of pathogens and fish, clinical outcomes and impacts on fish immune responses, and fish survival. Most studies described the prevalence of co-infections in fish, with various parameters influencing their outcomes. Bacterial co-infection increased fish mortality, ulcerative dermatitis, and intestinal haemorrhage. Viral co-infection resulted in osmoregulatory effects, increased mortality and cytopathic effect (CPE). More severe histological alterations and clinical symptoms were related to the co-infection of fish than in single-infected fish. In parasitic co-infection, there was increased mortality, high kidney swelling index, and severe necrotic alterations in the kidney, liver, and spleen. In other cases, there were more severe kidney lesions, cartilage destruction and displacement. There was a dearth of information on mitigating co-infections in fish. Therefore, further studies on the mitigation strategies of co-infections in fish will provide valuable insights into this research area. Also, more research on the immunology of co-infection specific to each fish pathogen class (bacteria, viruses, fungi, and parasites) is imperative. The findings from such studies would provide valuable information on the relationship between fish immune systems and targeted responses.

## 1. Introduction

Aquaculture plays a vital role globally, particularly in satisfying the growing need for high-quality animal-source protein. The sector accounts for about 44% of global fish production and has continued to expand in recent years [1]. However, most fish grown in aquaculture are intended for human consumption [2,3] and are sometimes associated with diverse challenges. As a result, these challenges need to be addressed for the industry to fulfil the market's requirements. These challenges include environmental impacts, inadequate water quality, and diverse diseases [4–6].

The current approach to increasing aquaculture output focuses on intensifying the production of aquaculture goods and increasing their commercialization [7–9]. Nevertheless, pursuing quick intensification in the sector may have unintended consequences, such as spreading disease [10], a significant barrier to expansion [11,12]. Furthermore, disease outbreaks often associate with economic repercussions, as many aquatic species, such as fish, suffer considerable losses [7]. Consequently, the production output, market supply, and availability of quality and nutritious fish may be affected.

Fish infections frequently occur due to one or more pathogens: parasites, fungi, viruses, bacteria, or a combination of two or more. Among these pathogens, bacterial fish infections are the aquaculture enterprise's most significant challenge [13]. Therefore, managing fish health

* Corresponding author.
*E-mail address:* Ekemini.Okon@UGent.be (E.M. Okon).

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is integral to disease prevention and sustainable production. Furthermore, effective health management strategies are beneficial in minimizing the majority of losses caused by infections [13,14].

Fish diseases pose significant challenges to sustainable production as they undermine regular fish supply worldwide [15,16]. In addition, diseases and other environmental conditions are often responsible for mass mortalities in farmed and wild fishes [16]. However, despite the prevalence of such infections in the natural world, the topic of multiple pathogens simultaneously infecting fish species has garnered little attention [17].

Co-infections are infections of a host by two or more genetically distinct pathogens. Each pathogen is responsible for its pathogenic effects and contributes to the overall damage to the host when combined with other pathogens [18,19]. When two or more pathogens infect the same host, they can compete for resources or target areas within that host. Alternately, one pathogen can sometimes change the host's immune response against subsequent infections by other pathogens by either priming the immune system or suppressing it, although it rarely occurs [20,21]. This alteration can affect the host's susceptibility level to future infections. It can also impact the dynamics between the pathogen and the host, the physiological condition, the degree of the infection, the infection period, and the host's pathology [21,22].

Consequently, the interactions between coexisting pathogens can be antagonistic or synergistic [18,23]. For example, the host's first pathogen that promotes immunosuppression and impairs the immune response to future infections can lead to synergistic effects [21,23]. Synergistic effects can increase disease severity and their associated mortality rates. However, antagonistic effects can arise from direct pathogenic competition for resources and locations. These effects can influence pathogens' populations and sometimes modify the infection site [24]. In some instances, the antagonistic effects are brought about by the first pathogen triggering and modulating the host's immune response, making it more difficult for the second pathogen [25].

Co-infections significantly impact fish health and can potentially change several fish diseases' progression and severity [26]. Despite these challenges, there is a dearth of evidence on this topic. This review article, therefore, presents an in-depth summary of the co-infection of fish pathogens, the interaction of pathogens and fish, clinical outcomes, impacts on fish immune responses, fish survival, and mitigation strategies.

## 2. Methodology

In this review, the Web of Science, Scopus, and Google Scholar databases were used to extract data records on the co-infection of fish pathogens, the interaction of fish pathogens and host, its impacts on fish immune responses, clinical outcomes, survival, and mitigation strategies. The keywords used were 'fish', 'co-infection', 'co-infection', 'mixed infection', 'simultaneous infection', 'multiple infection', 'concurrent infection', 'concomitant infection', 'polymicrobial infection', 'multiple-parasitisms', 'poly-parasitism'. The search period was set at "All years" to capture all possible articles with no language restriction. An initial search of the databases yielded 1131 articles after 194 duplicate reference items were removed. A manual screening was conducted by reading the abstracts to determine their suitability for this study. To ensure that important articles are included, further screening was conducted by evaluating their impact and relevance to the topic of this article. Some of the most important articles were selected, and relevant information related to fish co-infection was extracted to complete this review.

This review answered the following questions: (1) What are the concepts of co-infection dynamics and fish pathogens? (2) How do fish pathogens interact with fish? (3) What are the available incidences of co-infections and clinical outcomes in fish? (4) What are the interactions between co-infection and immunology in fish? (5) What are the future research directions on co-infections dynamics in fish?

## 3. The concept of co-infection dynamics and fish pathogens

Co-infection is equivalent to multiple-parasitisms, poly-parasitism, concurrent, concomitant, multiple, mixed, and simultaneous infections of an individual host [27–30]. In co-infection, infectious pathogens from different taxonomic levels and genetic variants of the same contagious agents are simultaneously present [31]. Compared to mono-infections, the likelihood of a co-infection negatively influencing the host's health is much higher (Fig. 1A). In addition, a high pathogen abundance and the interaction of several pathogens could substantially impact infection dynamics by altering host vulnerability and enhancing co-infection chances [32].

During co-infection, the host may suffer tissue damage either indirectly induced by an inflammatory response not effectively addressed or directly triggered by the toxicity of the pathogen. As a result, a tolerance mechanism is used as a defensive approach to reduce the detrimental effect of various kinds of stress, hence decreasing the corresponding impact. A tolerance mechanism employs a method known as "tolerance building." If this tolerance is not established, there is a risk of a significant shift in the clinical outcome of secondary infections, and this shift is independent of the pathogen load [33]. One example is the gill tissue damage in Nile tilapia (*Oreochromis niloticus*) due to *Branchiomycosis* co-infection with *Ichthyophonus* sp. and *Saprolegnia* spp pathogens [34].

Co-infections affect the transmission of a pathogen within a population and the pathogen's virulence, ultimately affecting the disease's dynamics [38]. Recent research has shown that a single host is frequently infected by several pathogens [39,40], with diverse effects on the host's immunological responses, clinical outcomes, host survival, and disease control efficacy [41–44]. For instance, a secondary pathogen could lower the resistance to a primary infection, of which such resistance may be inherited [45]. These highlight the importance of empirical investigations of the effects of co-infection on disease dynamics, virulence, and their significance for many diseases [40].

## 4. Interactions between co-infecting fish pathogens, the host, and environmental influences

Co-infecting pathogens could interact between themselves, the host, and the environment. These complex interactions can produce positive (synergistic) or adverse (antagonistic) effects. Synergistic interactions, which are beneficial, occur when one pathogen's existence amplifies the development or virulence of another pathogen. For example, a viral infection may compromise the fish's immune system, rendering it more vulnerable to a bacterial infection that is ordinarily within its capacity to resist. Alternatively, the co-infection of the two pathogens may lead to synergistic pathogenicity, resulting in more severe tissue damage in the fish compared to a single infection. In rainbow trout (*Oncorhynchus mykiss*), co-infection with the bacterium *Yersinia ruckeri* and *Myxobolus cerebralis* can lead to higher mortality rates than infection with either pathogen alone [46].

Antagonistic interactions involve inhibiting the growth or virulence of one pathogen in the presence of another. Certain bacterial strains can synthesize substances that exhibit bacteriostatic or bactericidal properties, thereby impeding the proliferation of other bacterial species. This phenomenon is a protective mechanism against the onset of a secondary bacterial infection in fish already infected by a primary bacterial infection. Alternatively, the immune response triggered by one pathogen could also help to eliminate a second pathogen from the host system. For example, the co-infection of fish with the bacterium *Aeromonas hydrophila* and ISKNV decreased the disease severity in Chinese perch (*Siniperca chuatsi*) [47].

Co-infecting pathogens may also come up against three different kinds of competition: seeming competition, interference competition, or resource rivalry [48]. First, competition for those resources might ensue when coinfecting conspecific strains have overlapping resource needs. Pathogenic strains that can utilize nutrients more effectively in a limited

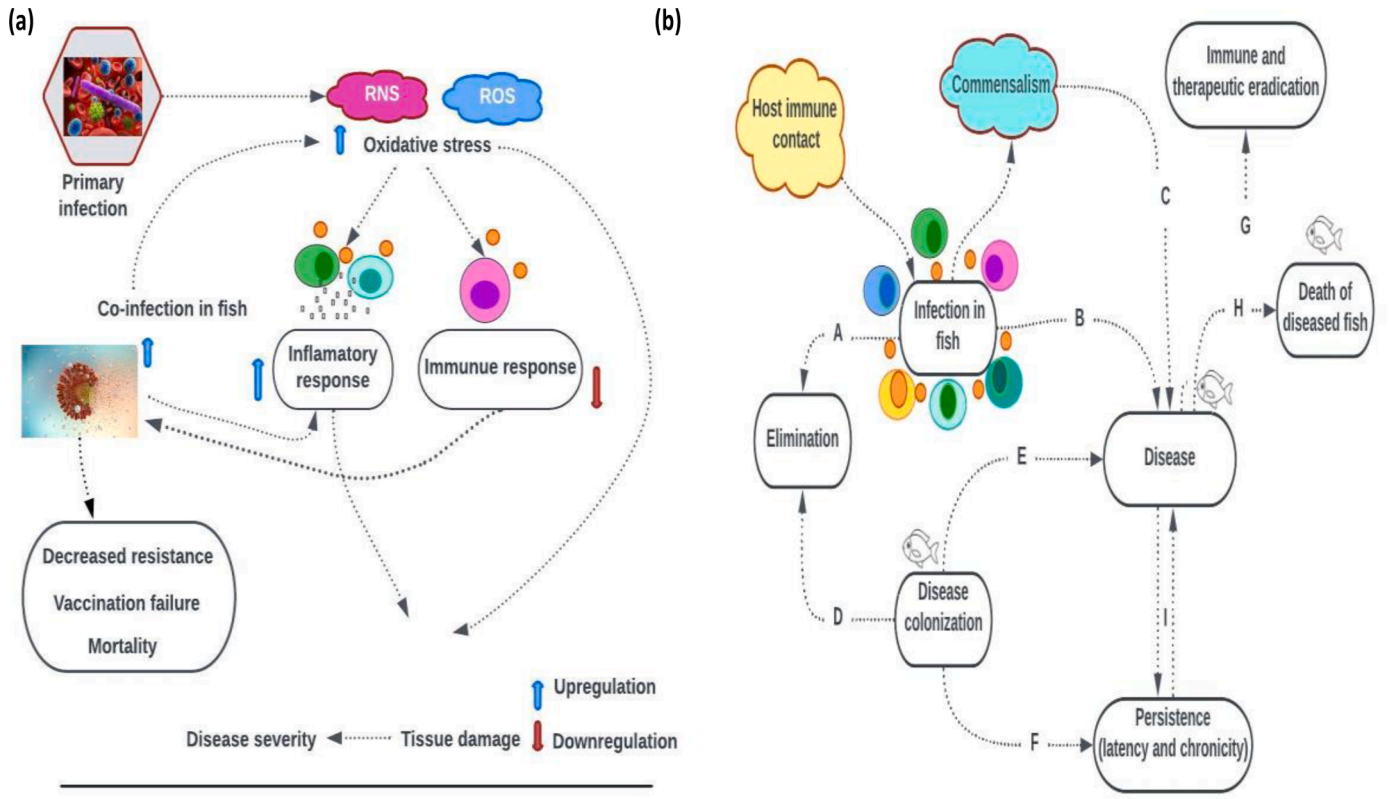


**Fig. 1.** **(A)** The conceptual framework of co-infection in fish. First, the primary infection enhances oxidative stress, further heightened by co-infection. Eventually, the immune response is dysregulated, resulting in tissue damage and disease severity (after Devi et al., [35]). RNS - Reactive Nitrogen Species, ROS - Reactive Oxygen Species. **(B)** The conceptual framework of the interactions between fish pathogens and hosts (based on the damaged framework [36,37]). A – Physical defences or immune mechanisms of fish; B – Disease occurrence and damage; C – Alteration of fish host microbial flora or disturbance of commensalism due to immune impairment; D – Immune response in fish; E – Colonization of pathogens due to damage; F – Inability of immune response to eradicate the disease despite consistent damage; G- Eradication of infection from therapy or immune response (earlier damage may be irreversible); H – High damage resulting to fish death; I – Overt disease due to reactivation of persistent infections.

supply of nutrients will likely overrun the rival strains [49]. Second, coinfecting strains engage in interference competition, in which one strain secretes harmful chemicals to the other competing strains, such as bacteriocins [50]. The limited activity range of bacteriocins results in interference and competition between conspecific strains, which seem more common than between distantly related bacteria [51]. Third, apparent competition results when the host immune response indirectly excludes the co-infecting strains. The indirect exclusion is stimulated by the growth of a strain acting on both competing strains [48,52].

The interactions of fish pathogens and host can be influenced by the multiplication rate, capability to harm tissue, presence of animal reservoir, ease of spread to new hosts, and medication therapy. These factors may alter due to the presence of another fish pathogen [53]. During co-infections in fish, two or more pathogens can coexist within the same host and compete for resources or target areas [17,45]. These intricate interactions between fish pathogens and hosts can be beneficial or harmful, depending on the disease duration, disease severity, infection biology, fish-pathogen dynamics, fish susceptibility to infection, and level of fish pathology [17].

The manifestation of these interactions in a co-infected fish depends on the relationship between the host and co-infecting strains (Fig. 1B). Also, the genetic relatedness of the strains determines whether their interactions would be competitive, neutral, or cooperative [54], including the interaction type [55]. Thus, fish pathogens closely related to one another are more likely to work together and economically exploit their hosts to maximize their transmission. In contrast, fish pathogens that are distantly related to one another are more likely to compete, resulting in increased virulence and mortality of the host [55, 56].

Environmental factors can also impact the incidence and severity of co-infections in fish. These include water quality, temperature, precipitation patterns, pH, salinity, and dissolved oxygen levels in the water. Changes in these factors can produce more or less favourable conditions for the growth and survival of various pathogens, affecting the likelihood and severity of co-infections. Poor water quality, for example, can physiologically stress fish and increase their susceptibility to infection. Low dissolved oxygen levels can also impair fish's immune function, making them more susceptible to disease [57].

These factors can impact the life cycles of diverse pathogens, leading to modifications in their prevalence and interactions with other pathogens [58]. With climate change, temperature and precipitation patterns can affect the distribution and abundance of pathogens and their hosts, potentially increasing or decreasing co-infection risk. The fish's immune response may alter at warmer temperatures and increased humidity, increasing their risk of co-infections [59].

The presence of non-native species, which can introduce new pathogens into the ecosystem [60], is another crucial factor influencing co-infections in fish. Specifically, poor aquaculture management and the unintentional release of exotic species can contribute to spreading of pathogens and co-infections. In addition, aquatic pollutants, including heavy metals, pesticides, and industrial chemicals, can impact fish's immune systems and increase their vulnerability to diseases [57,59]. Consequently, understanding these is critical for fish disease management and prevention.

## 5. Incidence of co-infections and clinical outcome in fish

### 5.1. Incidence of bacterial co-infections and clinical outcome in fish

There is a dearth of information regarding bacterial co-infections compared to single bacterial infections. Bacterial co-infections induce severe effects, including increased severity of other diseases, increased mortality rates, a shift in host vulnerability, and an increased infection period [17]. Unfortunately, farmers typically fail to report bacterial co-infections, which results in a lack of data relating to outbreaks [61]. This lack of data includes diagnostic information, the host's immunological response, and clinical indicators [17]. In addition, there is limited knowledge regarding which pathogen could be specifically attributable to a particular infection symptom. Thus, it can be challenging to differentiate between the clinical signs that result from co-infections [61].

Consequently, most research focuses on primary pathogen infections rather than secondary infections or opportunistic diseases caused by other infectious agents that accompany primary pathogens [61]. In addition, co-infections alter the sensitivity of fish to many diseases, leading to outbreaks that result in high mortality rates [44]. Hence, pathogen interactions can cause variability in bacterial load. In such cases, the bacterial loads can increase, suppress, or potentially suppress while the other increases [61].

Various studies have described the incidence of bacterial co-infections in fish. In Nile tilapia (*Oreochromis niloticus*), *Francisella noatunensis orientalis* (Fno), and *Streptococcus agalactiae* co-infection showed that exposure to chronic hypoxia predisposed the fish to co-infection [62]. In Barramundi (*Lates calcarifer*), co-infection of *Streptococcus iniae* with *Shewanella algae* (an opportunistic pathogen) resulted in cutaneous ulcers and systemic disease in the fish. The most common clinical signs in the visceral organs were hyperaemia and haemorrhage, as systemic streptococcosis appears to be a risk factor for *Shewanella* skin penetration in the fish, resulting in ulcer development [63]. In zebrafish (*Danio rerio*), co-infection with *Aeromonas hydrophila* and *Aeromonas veronii*, resulted in tubular cell necrosis, kidney shrinkage of tubules, skin lesions and increased fish mortality (Figs. 2 and 3). However, single infections resulted in lower mortality than co-infections [64]. It, therefore, suggests that co-infections of *Aeromonas veronii* and *Aeromonas hydrophila* are more harmful than single infections.

Bacterial vibrio isolates are potentially dangerous to fish and are responsible for the recent disease epidemic. Co-infection with *Vibrio alginolyticus* and *Vibrio harveyi* resulted in a more catastrophic effect on cultured fish. The histological abnormalities and clinical symptoms observed in an artificially infected Asian seabass (*Lates calcarifer*) were comparable to those observed in the naturally infected hybrid groupers. In contrast, fish infected with both pathogens at the same time displayed more severe histological alterations and clinical symptoms than single-infected fish [65]. Furthermore, a co-infective pathogen challenged with new *Flavobacteriaceae* isolates revealed more significant mortality (up to 92%) in Rainbow trout (*Oncorhynchus mykiss*) than treatments with a single isolate [66]. In Cobia (*Rachycentron canadum*), a co-infective challenge with *Photobacterium damselae* and *Vibrio harveyi* increased fish mortality (up to 100%) to single infection [67]. Another co-infection in Rainbow trout (*Oncorhynchus mykiss*) with *Pseudomonas fluorescens* and *Y. ruckeri* increased mortality (up to 40%) [68]. In Koi carps (*Cyprinus carpio* var. *koi*), co-infection with *Vibrio cholerae* and *Aeromonas veronii* presented clinical symptoms, including liver, spleen, and intestine lesions intestinal haemorrhage (Fig. 4) in the fish [69].

The highlighted studies have provided a good understanding of bacterial co-infections in fish. The studies reveal that bacterial co-infection could increase mortality due to increased virulence and synergistic effects with diverse clinical signs. Thus, it can be built upon to improve fish health within production and health management. In addition, it may be possible to tailor treatment protocols better to achieve more clinical success. Treatment protocols could rely on understanding the underlying infections, virulence processes, and the significant pathogens observed in diagnostic casework.

### 5.2. Incidence of viral co-infections and clinical outcome in fish

The most typical result of co-infection is viral interference, which occurs when one virus reduces the reproduction of the other co-infecting viruses through competitive inhibition. Viral co-infections may also stimulate an increase in viral reproduction. In a few additional scenarios, co-infections do not influence virus reproduction. Hence, most coinfecting viruses can coexist (accommodation) [70]. They may modify the virulence of the virus and the death of cells, consequently affecting the severity of the disease and its epidemiology (Fig. 5). Besides the highlighted outcome of viral co-infection (Fig. 5), different factors could influence the outcome of co-infections in fish. These include the fish's age, the infection route, cell types, virus dose and the time lag between co-infecting viruses, rate of viral replication and cytopathic effect.

Different studies have reported cases of viral co-infections in fish. For instance, in Atlantic salmon (*Salmo salar*), ISAV could be avirulent in a co-infection of a togavirus-like virus and infectious salmon anaemia (ISA) [71]. The finding suggests that the togavirus could significantly affect disease propagation [71]. On the other hand, co-infection with snakehead retrovirus (SnRV) increased both the cytopathic effects (CPE) and the infection titre of Grouper Nerve Necrosis Virus (GNNV) [72]. In Sockeye salmon *Oncorhynchus nerka* smolts, co-infection with the infectious hematopoietic necrosis virus (IHNV) and salmon louse *Lepeophtheirus salmonis* (V/SL+) increased fish mortality attributed to the osmoregulatory effects of the sea lice infections, and exacerbated by the

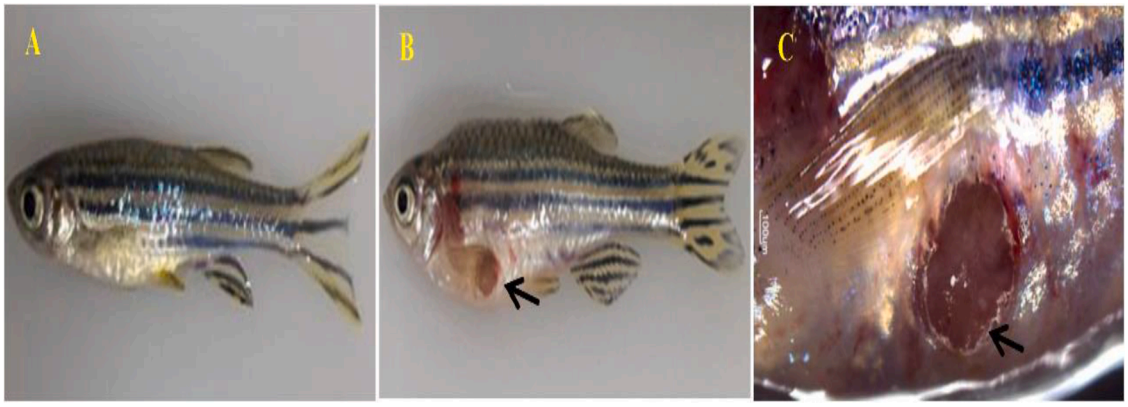


**Fig. 2.** Gross clinical signs of *A. hydrophila* and *A. veronii* co-infected zebrafish. **A**) Healthy fish; **B**) infected fish with deep skin lesion (arrow); **C**) enlarged skin lesion area of infected fish-white ragged margin (arrow). Figure and caption from [64] with permission.

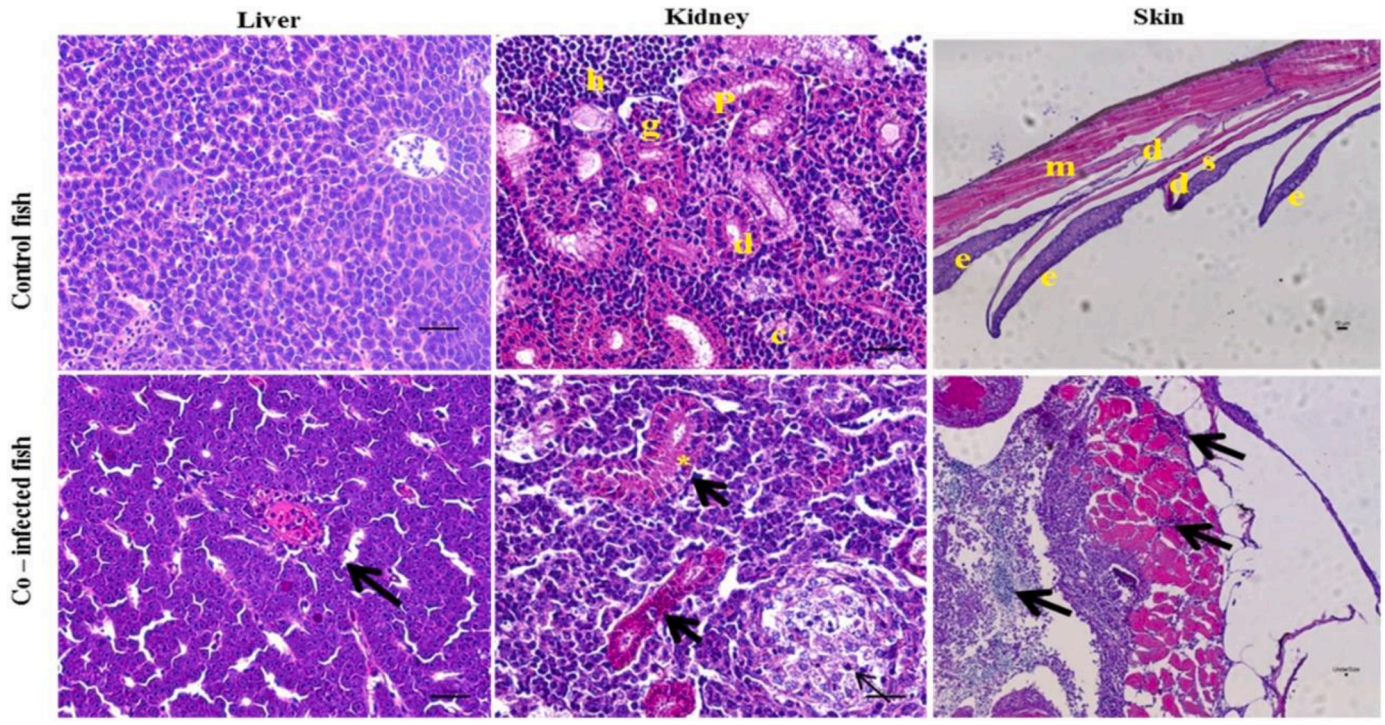


**Fig. 3.** Histological comparison of *A. hydrophila* and *A. veronii* co-infected zebrafish with healthy fish. The healthy liver architecture is shown as prominent hepatocytes radiating from the central vein and marked sinusoidal dilation (arrow) in infected fish liver. Healthy zebrafish kidney is shown with normal glomerulus (**g**), proximal tubule (**p**), distal tubule (**d**), collecting tubules (**c**) and hematopoietic tissue (**h**). In co-infected kidney shrinkage of tubules (thick arrows), tubular cell necrosis (star) and enlarged glomerulus (thin arrows) are prominent. Healthy skin is shown with epidermis (**e**), dermis (**d**), scales (**s**), and muscle layer (**m**). In co-infected zebrafish lymphocyte infiltration within epidermis, dermis and deep muscle layers (thick arrows) are shown. Figure and caption from [64] with permission.

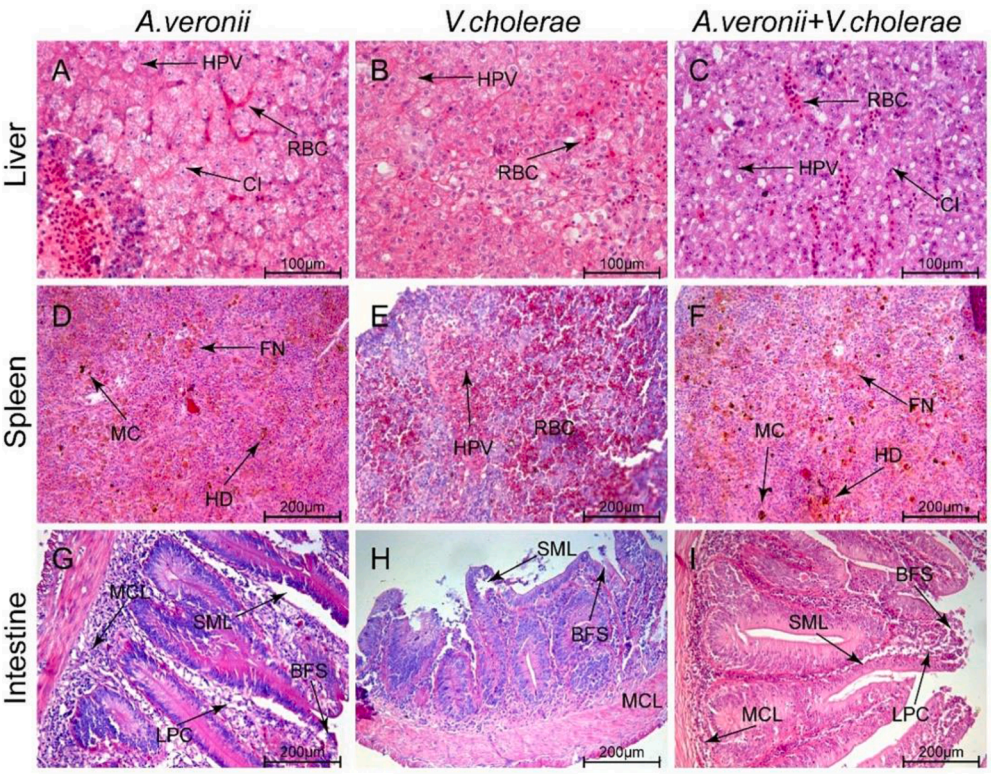


**Fig. 4.** Histopathological changes of the liver, spleen and intestine from the infected koi carp. A-C: histological lesion of liver (scale bar = 100 μm); D-F: histological lesion of spleen (scale bar = 200 μm); G-I: histological lesion of intestine (scale bar = 200 μm). A, D, and G, infected with *Aeromonas veronii*; B, E, and H, infected with *Vibrio cholerae*; C, F, and I, infected with *Aeromonas veronii* and *Vibrio cholerae*. HPV, cellular vacuolation; CI, indistinguishable cellular outline; RBC, red blood cell; MC, melano-macrophage centre; HD, hemosiderin deposition; FN, focal necrosis; SML, separation between mucosa and lamina propria; BFS, blunted, fused, or shed intestinal villi; LPC, collapsed lamina propria; MCL, loosened mucous layer and connective tissues. Figure and caption from [69] with permission.

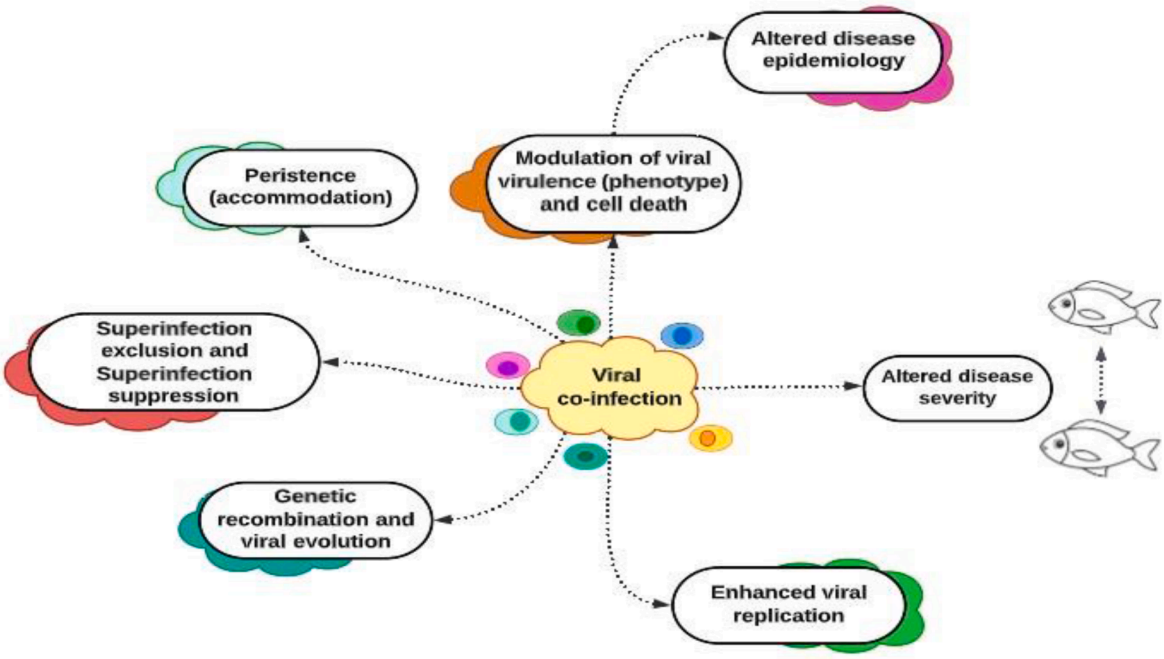


**Fig. 5.** Viral co-infection with different virological consequences based on [70].

disease with IHNV [73].

### 5.3. Incidence of parasitic co-infections and clinical outcome in fish

Parasites and their hosts frequently coexist in a state of dynamic equilibrium. However, shifts in environmental conditions can disrupt this balance, leading to increased disease transmission [17]. Co-infections caused by more than one parasite significantly impact the ecology of the host-parasite relationship [74]. Also, there is a significant negative correlation between *Tetracapsuloides bryosalmonae* and *Chloromyxum schurovi*. This relationship is especially noticeable in the kidney, as both parasites use the organ as a target site. Therefore, an infection caused by one parasite may reduce the likelihood of disease caused by the other parasite due to competition for the same target organ [75].

Studies on parasitic co-infections in fish indicate diverse clinical signs and impacts. For example, in Atlantic salmon, *Lepeophtheirus salmonis* (a vector for transmitting *Neoparamoeba perurans*) influenced the epizootiology of the disease and increased fish mortalities [76]. Co-infection involving the myxozoan species (*Myxobolus spp*., *Chloromyxum truttae, Chloromyxum schurovi, Sphaerospora truttae*, and *Tetracapsuloides bryosalmonae*) affected Brown trout kidneys, particularly *Tetracapsula bryosalmonae, Salmo truttae*, and *Chloromyxum Schurovi* [77]. Co-infection by *Nucleospora cyclopteri* and *Kudoa islandica* resulted in 65% mortality in cultured lumpfish. In addition, severe necrotic alterations occurred in the kidney, spleen, and liver, along with intracellular *Nucleospora cyclopteri* in the damaged tissues [78].

Rainbow trout (*Oncorhynchus mykiss*) initially infected with *Myxobolus cerebralis* and then co-infected with *Tetracapsuloides bryosalmonae* revealed aggravated clinical alterations by both parasites with a greater mortality rate than uninfected fish [17]. Compared to the pathological changes in fish following single infections with *Tetracapsuloides bryosalmonae* or *Myxobolus cerebralis*, fish with multiple infections with either pathogen had more severe cartilage displacement, cartilage damage, and a higher (grade 4) kidney swelling index [17]. On the other hand, fish initially infected with *Tetracapsuloides bryosalmonae* and then co-infected with *Myxobolus cerebralis* showed pathology-related changes related to both parasites. However, co-infection had a lower mortality rate than a single infection with either *Tetracapsuloides bryosalmonae* or *Myxobolus cerebralis*. In concurrent myxozoan infections, the co-infection outcome was primarily determined by the underlying pathogen infecting the host, which can change the secondary infection results. The initial condition with *Myxobolus cerebralis*, followed by infection with *Tetracapsuloides bryosalmonae*, had a significantly more severe effect and prompted a synergistic interaction [17].

Also, in Atlantic salmon, a high level of *Moritella viscosa* co-infection caused a high mortality rate and more severe skin lesions. Positive results for *Moritella viscosa* growth were associated with the skin lesions observed. However, these lesions could infrequently be found in environments associated with lice. This shows that a single infection with *Moritella viscosa* can cause skin lesions in salmon. However, co-infection with a high number of lice can amplify this effect and significantly reduce the ability of these lesions to heal, leading to an increased risk of mortality [79].

### 5.4. Incidence of parasitic and bacterial co-infections and clinical outcome in fish

In aquaculture, co-infection with bacteria and parasites is a common occurrence which can lead to reduced growth or even mortality in fish, depending on the primary pathogen load (parasite or bacteria). Infections with parasites not only increase the risk of secondary bacterial diseases but also have the potential to function as a vehicle for the transmission of disease-causing bacteria [77]. Findings from [77] indicated the presence of myxozoan species in the blood, intestinal tissues, and kidney in farmed *Salmo trutta* (Fig. 6), suggesting different entry routes for these species.

Experimental studies have proven bacteria and parasites co-infection and indicated higher mortality rates in fish co-infected with [80,81]. On the one hand, the synergistic effect is explained by the stress caused by parasites decreasing fish's resistance to other secondary bacterial infections [82]. On the other hand, the damaging effects caused by parasites provide invading bacteria with an entry route influencing this phenomenon. Although the parasites sometimes carry the bacteria, they invariably transmit it to their host while feeding on their host [82].

*Dactylogyrus intermedius* has been reported to cause high mortality and increase bacterial loads in fish tissues than in un-parasitized fish

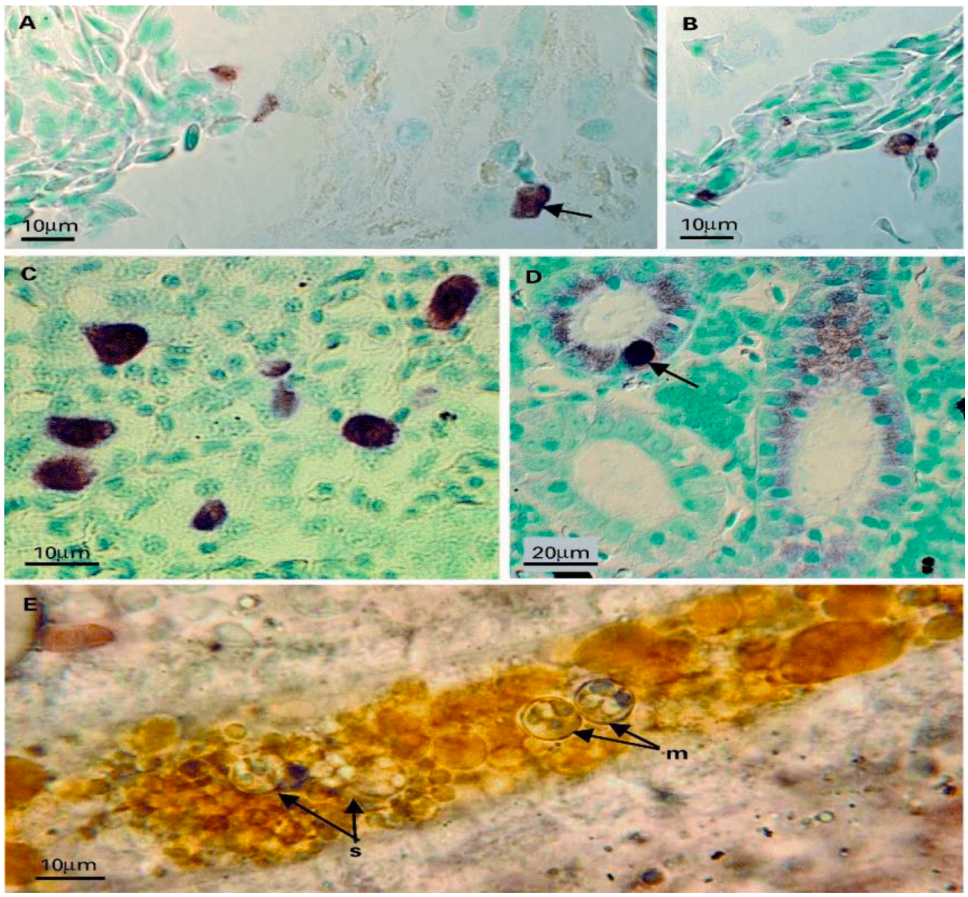


**Fig. 6.** (A–D) *Tetracapsuloides bryosalmonae*, ISH with parasite RNA/DNA signalling in purple. (**A, B**) Intravascular developmental stages in the heart; arrow indicates a stage which is attached to the endothelium. (**C**) Clinical PKD in the kidney of 0+ brown trout with numerous parasites in the interstitial tissue. (**D**) Kidney of a 1+ brown trout showing a *T. bryosalmonae* stage within the tubular epithelium (arrow) and DNA/RNA remains in the cytoplasm of previously infected epithelial cells. (**E**) *Chloromyxum schurovi*, fresh kidney smear showing a renal tubule filled with sporogonic stages (s), 2 mature spores (m) and extracellular material containing yellow pigment. Figure and caption from [77] with permission.

[83]. In goldfish (*Carassius auratus*), *Dactylogyrus intermedia* increased bacterial invasion following the development of host immune suppression and the downregulation of immune genes such as transforming growth factor (TGF)- and complement 3 in kidneys and gills; thus, allowing it to control the response of the host immune system [83]. This synergy could be explained by the intensity of infestation, indicating that the downregulation of immune genes may benefit parasites,

In Rainbow trout, the mortality rates in the co-infected group with *Y. ruckeri* and *Myxobolus cerebralis* were more significant than in the group not infected with *Myxobolus cerebralis* [46]. The mortality rates were connected to the reduction of proliferative lymphocyte responses and the immunomodulatory effects that *Myxobolus cerebralis* exerts through inhibiting lymphocyte blastogenesis. Consequently, a more robust bactericidal activity potentially influenced secondary infections caused by the *Y. ruckeri* bacteria [46]. In channel catfish (*Ictalurus punctatus*), the fish's vulnerability to *Streptococcus agalactae* or *Streptococcus iniae* may considerably enhance co-infection with *Trichodina* sp. This could be explained by the high fish mortalities (100%) due to the synergistic co-infection effects between the bacteria and parasites. Hence, the invasion of *Streptococcus agalactae or Streptococcus iniae* enhanced disease severity after fish exposure [84]. It has been demonstrated that co-infections can occur in intensive farming of Nile tilapia (*Oreochromis niloticus*), and is often associated with mortality. A concurrent experimental infection model with *Gyrodactylus niloticus* and *Streptococcus iniae* using Nile tilapia indicated 42.2% mortality within the first two weeks following exposure. Compared to the group infected only by *Streptococcus iniae* (6.7% mortality rate), no mortalities were observed in the *Gyrodactylus niloticus* besides the infected fish group. This shows that *Gyrodactylus niloticus* is a gateway for pathogenic bacteria to invade fish populations by causing mechanical damage to the epithelial tissue of the fish [85].

Furthermore, a co-infection model with *Streptococcus iniae* and *Ichthyophthirius multifiliis* in Nile tilapia (*Oreochromis niloticus*) observed a strong relationship between the parasite load, its developmental size, and fish mortality. In addition, an extended period between exposures to both pathogens during the co-infection enhanced *Ichthyophthirius multifiliis* to produce large, fully developed trophonts [86]. It, therefore, suggests that more significant damage could occur to the fish epithelial tissue with an increased bacterial load. Moreover, fish mortality could be higher than when the fish are presented with immature, tiny trophonts.

Co-infection with *Piscirickettsia salmonis* and the sea lice *Caligus rogercresseyi* in Atlantic salmon significantly decreased resistance to *Piscirickettsia salmonis* in non-vaccinated fish co-infected with sea lice *Caligus rogercresseyi*. After 53 days, the fish mortality rate from a single infection with *Piscirickettsia salmonis* increased to 50% compared to co-infection with a medium or high load of *Caligus rogercresseyi* with 100% mortality [45]. This symbiotic relationship might be explained by the sea louse's ability to lower the resistance of Atlantic salmon to *Piscirickettsia salmonis*. Furthermore, *Caligus rogercresseyi* has been hypothesized to affect the skin, making it easier for germs to infiltrate and ultimately leading to more mortalities [87]. As observed in Rainbow trout, fish louse, *Argulus coregoni*, dramatically enhanced the susceptibility of fish to *Flavobacterium columnare*. Thus, the cumulative mortality was much greater in the co-infected group than in the single-infected group [80].

In freshwater fish populations, a ciliated ectoparasitic fish protozoan (*Ichthyophthirius multifiliis*) is a major factor that significantly impacts fish health worldwide. Damage to the epithelium of the gills and skin can lead to an increased risk of bacterial invasion and increased mortality in fish [88]. Besides mortality, alteration of parameters has been linked to *Ichthyophthirius multifiliis* loads. Channel catfish exposed one

day earlier to *Edwardsiella ictaluri* co-infection had a much more significant bacterial burden in various organs and a significantly higher mortality rate than the single-infected group [89]. Another co-infection experiment involving *Ichthyophthirius multifiliis* with *Aeromonas hydrophila* in Channel catfish increased the fish mortality (80%), with a higher load of *Aeromonas hydrophila* in their internal organs [90]. This is similar to what has been reported in Rainbow trout *Oncorhynchus mykiss*, which in addition to increased mortality, had much higher cortisol levels, which inhibits the fish's immune system and synergistic impact [91,92].

Co-infection of Channel catfish with fluorescent *Edwardsiella ictaluri* and *Ichthyophthirius multifiliis* indicated that 100% of the tomonts tested carried the luminous bacterium [97]. This suggests that the *Edwardsiella ictaluri* could live and proliferate inside the tomonts, increasing the cumulative mortality rate amongst infected fish [93]. *Ichthyophthirius multifiliis* theronts possesses carbohydrates on its surfaces, such as N-acetylgalactosamine, D-glucose, D-mannose, and D-galactose molecules, which *Edwardsiella ictaluri* can bind and attach itself to [94,95]. In Atlantic salmon, co-infection with *Piscirickettsia salmonis* and *Caligus rogercresseyi* in saltwater conditions significantly increased blood parameters such as the $pCO_2$ levels, plasma glucose, and haematocrit. This suggests that a very modest parasite load, such as 4–11 parasites per fish, might be sufficient to affect fish physiology significantly [96].

Different physiological effects have been linked to parasitic and bacterial co-infection of fish tissues. For instance, there is evidence of adverse effects of co-infection on fish tissues vaccinated against *Piscirickettsia salmonis* in Atlantic salmon [97]. Single infection of *Piscirickettsia salmonis* resulted in an accumulated survival of 42.7% and a specific growth rate of 0.21%. However, co-infection resulted in a lower specific growth rate (0.05%) and accumulated survival (5.2%) of vaccinated fish. This indicates the possibility of reducing the efficiency of vaccinations in co-infection, and more research is needed to understand the relationship better.

In goldfish (*Carassius auratus*), co-infection of *Argulus* spp. with *Aeromonas hydrophila* resulted in excessive mucus discharge, detached scales, and severe bleeding at the operculum and the fins. In addition, congestion and haemorrhages were observed in the internal organs. The result suggests a correlation between the degree of parasite infestation and a downward trend in haemoglobin, pack cell volume, and red blood cell levels without co-infection. In addition, lower neutrophils, monocytes, and white blood cells were observed in the higher parasite group co-infected with a sub-lethal bacteria dose compared to other co-infected groups [98]. In this case, an increased dosage of parasitic infection could increase bacterial colonization in fish, inhibiting the innate immune system and increasing mortality.

### 5.5. Incidence of parasitic and viral co-infections and clinical outcome in fish

There has been quite some research on parasitic and viral co-infection in fish, with ongoing efforts to understand the mechanisms and consequences of these interactions. One study showed that Atlantic salmon with wounds from *Lepeophtheirus salmonis* could become more vulnerable to secondary infections with *Aeromonas salmonicida* and infectious salmon anaemia (ISA agent). It further suggests that ISAV epidemic could result from the co-infection of fish with sea lice and ISAV [99].

In whiting (*Merlangius merlangus euxinus*), the relationship between viral haemorrhagic septicaemia virus (VHSV) and *Trichodina* ectoparasite has been documented [100]. The affected fish exhibited *Trichodina* spp. in VHSV infection than uninfected VHSV fish. This suggests that ectoparasite loads considerably influence the incidence of VHSV in fish, potentially in combination with other variables such as spawning or water temperatures [100]. Generally, only limited research has been conducted, and there is a significant need for further studies.

### 5.6. Incidence of bacterial and viral co-infections and clinical outcome in fish

The incidence of bacterial and viral co-infections is not uncommon. Infected fish may face several complications due to concurrent bacterial and viral infections. These pathogens can potentially exacerbate clinical symptoms, heighten mortality risk, and complicate the diagnosis and treatment protocol [101]. In many cases, viruses are thought to cause subsequent bacterial co-infections (e.g., by *Staphylococcus* sp.), which can later develop into potentially fatal fish diseases [102]. It, therefore, requires effective disease management to curb the underlying disease-causing conditions. Though fish are adapted to survive in environments rich in bacteria, such as water, it becomes challenging to identify interactions between viral and bacterial pathogens, especially during viral infection [101]. For instance, several outbreaks of the rainbow trout fry syndrome (RTFS), caused by the Gram-negative bacteria *Flavobacterium psychrophilum,* have been observed, leading to a significant increase in the mortality of rainbow trout fry [103–105]. However, it has been difficult to acknowledge this synergistic interaction between those two infections and to distinguish whether the virus is the principal cause of mortality outbreaks [103].

Co-infection with infectious pancreatic necrosis virus (IPNV) and *Vibrio carchariae* in grouper (*Epinephelus sp*.) indicated mortalities induced by subsequent exposure to *Vibrio carchariae* [106]. In Japanese flounder, there are interactions between aquabirnavirus (ABV) and other diseases, including viral haemorrhagic septicaemia virus (VHSV), *Edwardsiella tarda,* and *Streptococcus iniae*. The synergistic interaction between ABV and *Edwardsiella tarda* or *Streptococcus iniae* amplified the secondary bacterial infection, leading to more significant mortalities (84%). On the other hand, the interaction between viral haemorrhagic septicaemia (VHS) and ABV can be hostile. In such cases, fewer mortalities may be observed in fish than those infected with only VHSV [107].

Based on existing scientific literature, there is evidence of flavobacteria and carp oedema virus (CEV) co-infections in fish, resulting in the increased effect of Koi sleepy disease (KSD). KSD is becoming increasingly important in common carp aquaculture globally. Although CEV is most likely the primary cause of KSD, the disease frequently manifests as a multifactorial condition with other bacteria and parasites on the gills, skin, or internal organs [108]. In other cases, initial infection of Atlantic salmon (*Salmo salar*) with infectious pancreatic necrosis virus (IPNV) and challenged with either infectious salmon anaemia virus (ISAV) or *Vibrio salmonicida* led to mortalities. The collective mortality was higher in the IPNV–*Vibrio salmonicida* co-infected group than in the group of IPNV-free fish challenged with *Vibrio salmonicida* only [109]. The study confirmed the synergistic interaction between both viruses as mortalities started sooner in the co-infected group (3–4 days), contrasting with fish infected with *Vibrio salmonicida* (8 days). On the other hand, secondary exposure of acutely infected Atlantic salmon (*Salmo salar*) with IPNV and ISAV lowered the mortality rate more than only ISAV-infected fish. This finding indicates IPNV's antagonistic action against ISAV, which offered some immunity against ISAV development by producing interferon (IFN) or IFN-like agents in reaction to acute IPNV infection [109].

In Tilapia, it has been demonstrated that the co-infection of Tilapia Lake Virus (TiLV) and bacteria (*Streptococcus, Aeromonas*, and *Flavobacterium*) increased fish mortality rates by up to 90% [110]. In addition, other *Aeromonas* genus species, including *Aeromonas Ichthiosmia, Aeromonas veronii, Aeromonas hydrophila*, and *Aeromonas enteropelogenes*, were found in the TiLV- infected Tilapia as they are not immune to the disease [111,112]. The pathogenesis and immunology of TiLV co-infection in fish are not well understood. Therefore, there is a need to explore this area of study further.

Furthermore, post-coinfection with *Aeromonas hydrophila* and TiLV increased the severity of the infection in Tilapia, underscoring the requirement for developing techniques to reduce the risk of co-infection.

Moreover, fish subjected to both challenges showed severe histopathological changes, including severe loss of hepatic sinusoid, red blood cell depletion, glycogen storage, vacuolation of lymphocytes in the spleen, and syncytial hepatitis (Figs. 7 and 8). Comparing bacteria levels in fish co-challenged with those only exposed to *Aeromonas hydrophila* indicated that the fish co-challenged had a much higher bacteria. Thus, infectious agents, such as *Aeromonas spp*., appear to synergize the severity of TiLV in Tilapia [113].

In Rainbow trout, the number of infectious hematopoietic necrosis virus (IHNV) plaque-forming units and *Flavobacterium psychrophilum* colony-forming units in tissues increased due to co-infection [114]. This further proves that both infections are retrieved from tissues in co-infected fish more effectively when both are present simultaneously. In addition, elevated systemic pathogen load was paralleled by an increase in the number of pathogen genes significantly enhanced in co-infected groups. The haematopoietic tissue showed significant tissue necrosis, with many intracellular and extracellular pathogens. The pathogens and necrosis were more noticeable in co-infected fish than in a single infection, which most certainly contributed to the worsened clinical symptoms and higher mortality rate.

In Chinese perch (*Siniperca chuatsi*), there is a somewhat complicated relationship between *Aeromonas hydrophila* and infectious spleen and kidney necrosis virus (ISKNV) for various infection patterns (Fig. 9). Specifically, *Aeromonas hydrophila* and ISKNV were reported to work complementarily or competitively. Thus, the co-infected group indicated a higher mortality rate than the single-infected groups or with secondary infections [47]. This indicates that co-infection with ISKNV and *Aeromonas hydrophila* has a synergistic and potentially lethal effect.

The highlighted studies have described various bacterial and viral co-infections and their clinical outcomes in fish. There are possibilities to build upon this research to understand their dynamics better. However, future perspectives could be directed to explore their pathogenesis, immunology, interaction mechanisms, diagnosis and sensitivities.

### 5.7. Incidence of fungal and bacterial co-infections and clinical outcome in fish

Fungal co-infections can affect farmed freshwater and marine fish species, including the wild. One of the most common consequences of fungal and bacterial co-infection in fish is the development of skin and fin rot. Bacteria can damage the fish's skin and fins, making them more susceptible to fungal infections. Fungi, in turn, can further damage the already weakened tissue, making it easier for bacteria to proliferate. In Discus fish (*Symphysodon*), fungal and bacterial co-infection showed sudden onset of mortalities with extreme body mucus, ascites, eye cloudiness and tail rot, which harbour various fungi such as *Fusarium moniliform, Fusarium oxysporum* and *Fusarium solani*. In addition, the bacterium *Aeromonas hydrophila* was re-isolated from 60%, and the dinoflagellate fish parasite *Spironucleus* spp. was re-isolated from 80% of the studied cases infected [115]. This shows that the organisms responsible for Discus fish mortalities are an accumulation of numerous pathogens, such as parasites, fungi, and bacteria.

In Nile tilapia (*Oreochromis niloticus*), histological examination revealed severely congested hepatopancreas and necrotic foci in the hepatic tissue due to *Fusarium oxysporum* and *Aeromonas hydrophila* co-infection. Clinical signs showed skin granuloma, head lesions, and skin lesions in the affected fish (Fig. 10). Specifically, *Fusarium oxysporum* caused tissue degradation, making it easier for *Aeromonas hydrophila* to invade the fish, increasing the mortality rate [116].

The highlighted studies suggest that fungal and bacterial co-infections can lead to more severe disease than either infection alone, resulting in significant economic losses in fish production. A summary of co-infection incidences in fish is presented in Table 1.

Fish pathogens can affect immunity in various ways, including susceptibility, the capacity to evade or harm immune systems, and whether or not vaccination is appropriate [117]. For instance, *Aeromonas hydrophila*-infected tilapia showed a considerably increased white blood cell count compared to the healthy group [118]. This indicates that pathogens can compromise the immune system of fish. This effect depends on the manner of co-infection, the sequence in which infection occurs, and the time of disease [47]. Even while a bacterial infection always follows a viral infection, it may affect the anti-bacterial immune response, making the host more vulnerable to infections caused by bacteria [47].

### 6.1. Factors affecting immune response to co-infection in fish

A variety of factors have the potential to affect fish infection and immunity. For example, the invulnerability of most warm-water fish to the virus may be due to a lack of viral receptors or other mechanisms

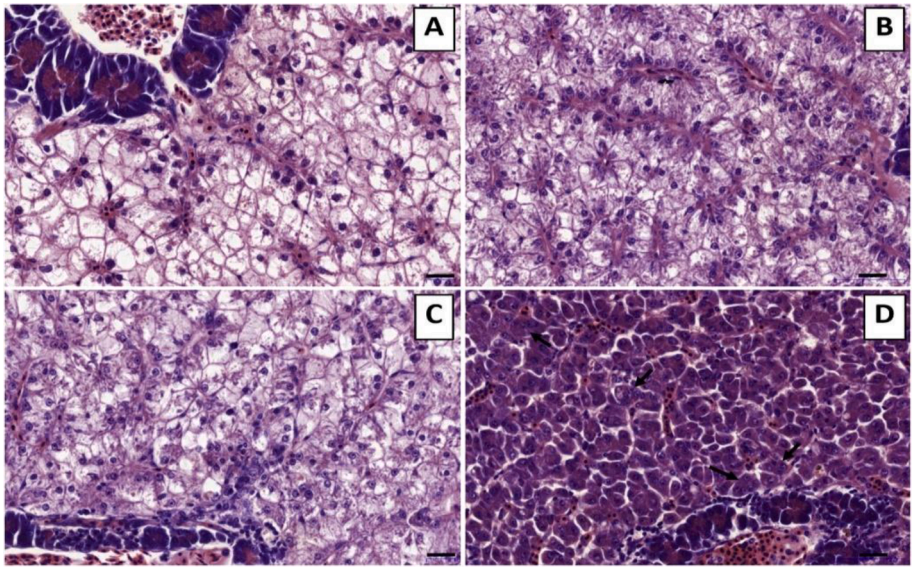


**Fig. 7.** Histopathological appearance of liver tissue from representative fish from the stipulated challenge groups (H&E staining). (**A**) Control (**B**) *A. hydrophila* single infection at 107 CFU/fish (**C**) TiLV single infection (**D**) Co-infection of TiLV-*A hydrophila* at 107 CFU/fish. The arrows indicate areas of syncytial cell formation in co-infected fish. Figure and caption from [113] with permission.

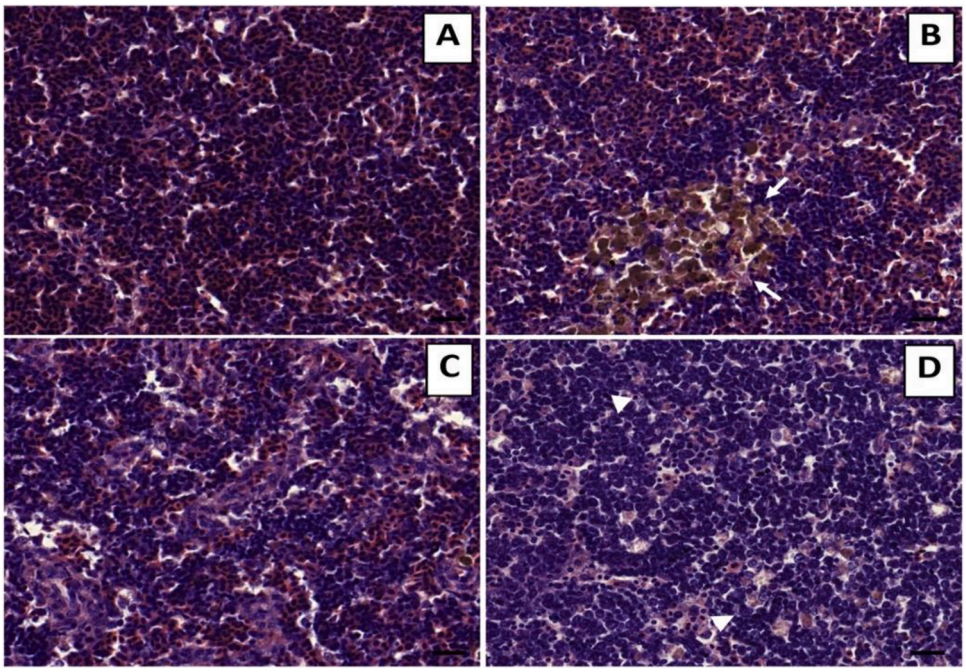


**Fig. 8.** Histopathological appearance of spleen tissue from representative fish from the specified challenge groups (H&E staining). (**A**) Control (**B**) A. hydrophila at 107 CFU/fish, accumulation of melanomacrophage centre (arrows) (**C**) TiLV single infection (**D**) Co-infection of TiLV-*A. hydrophila* at 107 CFU/fish, lymphocytes showing large and vacuolated nucleus (arrow heads). Figure and caption from [113] with permission.

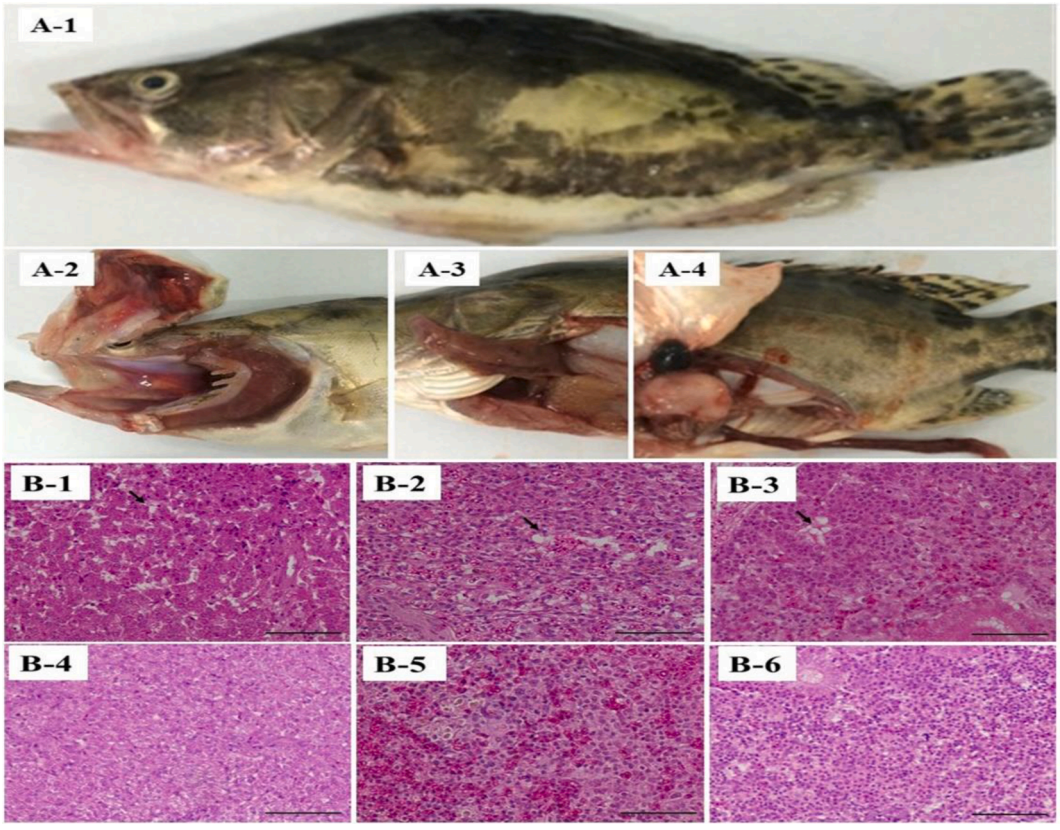


**Fig. 9.** Clinical symptoms and histopathological analysis of *Siniperca chuatsi* co-infected with *A. hydrophila* and ISKNV. (**A-1**) Diseased fish after co-infection, (**A-2**) gill haemorrhage, (**A-3**) liver haemorrhage, **(A-4)** splenomegaly and intestinal inflammation. (**B-1**) diseased liver, (**B-2**) diseased spleen, (**B-3**) diseased kidney, (**B-4**) non-infected liver, (**B-5**) non-infected spleen, (**B-6**) non-infected kidney. Scale bar = 50 μm. Figure and caption from [47] with permission.

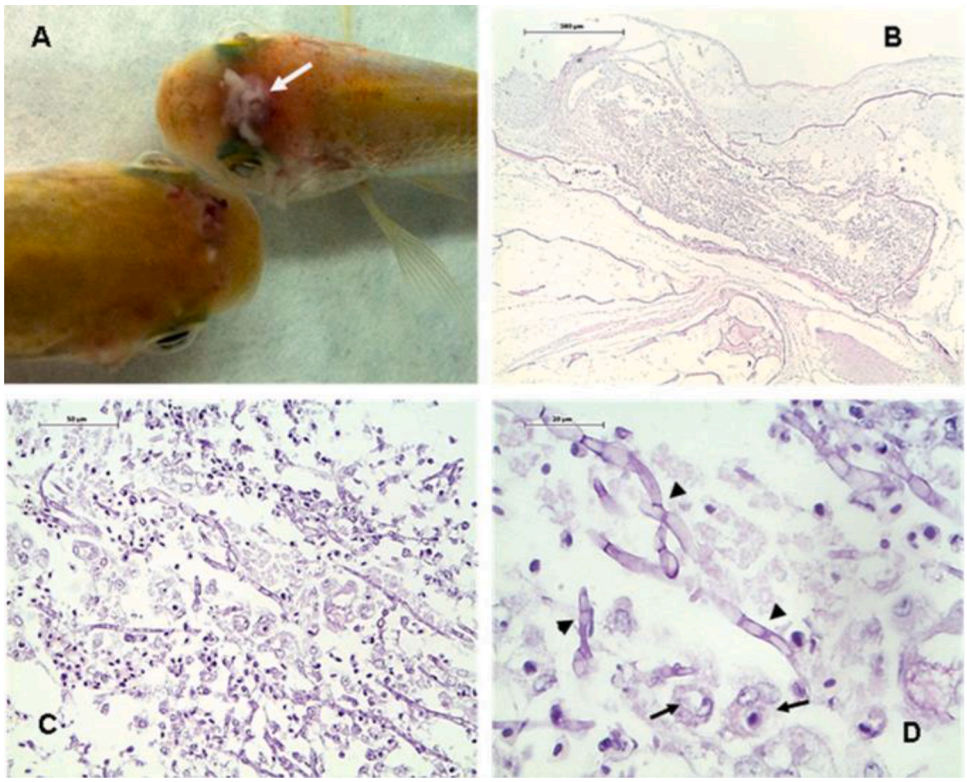


**Fig. 10.** (**A**) Gross appearance of the head and skin lesions of fish: soft creamy and yellowish nodules with hyphae and haemorrhagic subcutaneous spot; (**B**) histological appearance of nodules with low magnification. H&E. 2,5 × –Bar – 500 μm; (**C**) skin granuloma formation composed of numerous foamy macrophages, numerous neutrophils and fungal formations compatible with septate hyphae and conidia. PAS. 10 × – Bar – 50 μm. (**D**) Dermal fungal structures with high magnification: septate hyphae (head arrows) and intracytoplasmatic conidia (arrows) into the macrophages. PAS. 40 × – Bar – 20 μm. Figure and caption from [116] with permission.

that enable virus replication [53]. In bacteria, *Aeromonas hydrophila*, for instance, is an opportunistic pathogen that can act as a secondary invader in fish already infected by other diseases [119]. Specific virulence factors allow *Aeromonas hydrophila* to cling to, penetrate, and kill host cells, evading the host's immunological response [120].

The innate immune system of fish is a vital defence mechanism against various pathogens. This system can be affected by various factors, including the environment. Temperature is a significant factor that can influence the performance of this innate immune system during co-infection. The modulation of various innate immune system components in fish, such as cytokines, lysozyme, and the complement system, can be influenced by alterations in temperature. For instance, the complement system exhibits more significant activity in certain fish species at higher temperatures than in lower ones [121]. Consequently, the expression of cytokines during co-infection in fish can be either upregulated or downregulated, depending on the temperature.

Temperature can also influence the severity of co-infection in fish by affecting the activity of the innate immune system. An excessively high or low temperature may weaken the natural immune system, leaving the fish more vulnerable to infection [122]. Hence, temperature changes can alter the virulence of pathogens, which can also affect co-infection severity. Temperature is significant in modulating the innate immune system and infection pattern and severity of co-infection with *Argulus* and *Aeromonas hydrophila* in goldfish. An increase in temperature accelerates co-infection intensity and adversely affects immunological and physiological parameters [122]. These effects highlight the importance of the concurrent occurrence of temperature and co-infection in the immune responses of fish.

### 6.2. Gene regulation of immune response to co-infection in fish

Immune response to co-infection in fish involves the upregulation of genes associated with innate immunity. Innate immunity is the first line of defence against pathogens, characterized by a rapid and non-specific response. Various cell types, such as macrophages, neutrophils, and natural killer cells, mediates this response, which recognizes and eliminates pathogens by expressing various genes. In some cases, this interaction can result in the downregulation of genes associated with adaptive immunity [123]. The downregulation of genes associated with adaptive immunity can occur for several reasons. One possible explanation is that co-infected pathogens may interfere with the host's immune response, reducing the expression of genes associated with adaptive immunity. Alternatively, co-infected pathogens may overcome the host's immune response, weakening the adaptive immune response [124]. In Barramundi (*Lates calcarifer*), co-infection of Lates calcarifer Herpes virus (LCHV) and Scale Drop Disease Virus (SDDV) resulted in the downregulation of genes associated with adaptive immunity and upregulation of genes associated with innate immunity. Consequently, a severe inflammatory response in the fish affected the spleen, followed by the kidney [123].

Co-infections in fish can elicit various alterations in the expression of immune genes in fish, which may include the upregulation of specific genes. In Chinese perch (*Siniperca chuatsi*), co-infection *with Aeromonas hydrophila* and ISKNV showed higher gene expression [47]. In rainbow trout, co-infection *Flavobacterium psychrophilum* and *Aeromonas salmonicida* led to increased expression of immune-related genes such as interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) compared to single infections [125].

### 6.3. Mechanism of immune response to co-infection in fish

Interferons (IFNs) (class II helical cytokines) are the defining characteristic of vertebrate immune function to viruses [126–129]. IFNs are of three families (type I, II, and III) based on their receptors and functional and structural properties [126,129]. Based on the pathogen class and the tissue milieu, activated immune cells can polarise toward functionally and phenotypically distinct cell populations. The polarization could produce several types of immune responses, the most prevalent being type I and type II [70,130]. An inflammatory cytotoxic response to combat cancer cells and intracellular infections

**Table 1**
An overview of incidences of co-infection in fish and the pathogens, mortality rate, parts affected and immune responses.

| Host species | Pathogens | | Mortality (%) | Parts affected | Immune impact | References |
|---|---|---|---|---|---|---|
| | Bacteria | Bacteria | | | | |
| Nile tilapia | *Francisella noatunensis orientalis* (Fno) | *Streptococcus agalactiae* | 100% | Kidney, brain, spleen, and liver | Not indicated | [62] |
| Barramundi | *Streptococcus iniae* | *Shewanella algae* | Not known for this study | Skin, muscle and visceral organs | Not indicated | [63] |
| Zebrafish | *Aeromonas hydrophila* | *Aeromonas veronii* | Up to 87% | Kidney, muscle, and liver | Induced immune response | [64] |
| Hybrid groupers | *V. alginolyticus* | *Vibrio harveyi* | Up to 100% | Kidney, brain, spleen, and liver | Not indicated | [65] |
| Rainbow trout | *Flavobacteriaceae* isolates | | Up to 92% | Kidney, brain, gills, skin, spleen, and liver | Not indicated | [66] |
| Cobia | *Photobacterium damselae* | *Vibrio harveyi* | Up to 100% | Liver, spleen, kidney, gills, and stomach | Not indicated | [67] |
| Rainbow trout | *Y. ruckeri* | *Pseudomonas fluorescens* | Up to 40% | Liver, spleen, kidney, skin, and intestines | Not indicated | [68] |
| Koi carp | *V. cholerae* | *Aeromonas veronii* | Up to 100% | Spleen, liver, and intestines | Not indicated | [69] |
| | Virus | Virus | | | | |
| Atlantic salmon | ISAV | togavirus-like virus | Up to 100% | Not indicated | The fish response did not provide complete protection against infection | [71] |
| Sockeye salmon | IHNV | *Lepeophtheirus salmonis* | Up to 10% | Kidney, liver, spleen, and skin | Induced immune response | [73] |
| | Parasites | Parasites | | | | |
| Brown trout | *Tetracapsuloides bryosalmonae* | *Chloromyxum schurovi* | Not indicated | Kidney | Not indicated | [75] |
| Atlantic salmon | *Lepeophtheirus salmonis* | *Neoparamoeba perurans* | Not indicated | Not indicated | Not indicated | [76] |
| Brown trout | ** | | Not indicated | Kidney | Not indicated | [77] |
| Lumpfish | *Nucleospora cyclopterid* | *Kudoa islandica* | 65% | Kidney, spleen, liver, heart, and gill | Not indicated | [78] |
| Rainbow trout | *Myxobolus cerebralis* | *Tetracapsuloides bryosalmonae* | Up to 40% | Kidney and spleen | Not indicated | [26] |
| Atlantic salmon | *Lepeophtheirus salmonis* | *Moritella viscosa* | 33.3% | Skin and kidney | Not indicated | [79] |
| | Parasites | Bacteria | | | | |
| Rainbow trout | *Myxobolus cerebralis* | *Yersinia ruckeri* | Up to 33% | Kidney | Reduced proliferative lymphocyte responses and inhibited lymphocyte blastogenesis | [51] |
| Nile tilapia | *Gyrodactylus niloticus* | *Streptococcus iniae* | 42.2% | Skin | Not indicated | [85] |
| Nile tilapia | *Ichthyophthirius multifiliis* | *Streptococcus iniae* | 88% | Skin | Not indicated | [86] |
| Atlantic salmon | *Caligus rogercresseyi* | *Piscirickettsia salmonis* | Up to 100% | Skin | Not indicated | [45] |
| Rainbow trout | *Argulus coregoni* | *Flavobacterium columnare* | Up to 46% | Skin | Not indicated | [82] |
| Channel catfish | *Ichthyophthirius multifiliis* | *Edwardsiella ictalurid* | 71.1% | Brain, gill, kidney and liver | Induced immune response | [89] |
| Channel catfish | *Ichthyophthirius multifiliis* | *Aeromonas hydrophila* | 80% | Spleen, skin, kidney, liver and gill | Not indicated | [92] |
| Channel catfish | *Ichthyophthirius multifiliis* | *Edwardsiella ictalurid* | 91.7% | Brain, gill, kidney and liver | Not indicated | [93] |
| Atlantic salmon | *Caligus rogercresseyi* | *Piscirickettsia salmonis* | Not indicated | Blood parameters | Not indicated | [96] |
| Atlantic salmon | *Caligus rogercresseyi* | *Piscirickettsia salmonis* | 94.6% | Kidney, liver, and gills | Not indicated | [97] |
| Goldfish | *Argulus* spp. | *Aeromonas hydrophila* | 84.2% | Skin and haematological parameters | Lowered white blood cells and inhibited the innate immune system | [98] |
| | Parasite | Virus | | | | |
| Whiting | *Trichodina* ectoparasite | VHSV | Not indicated | Kidney, spleen and liver | Not indicated | [100] |
| | Bacteria | Virus | | | | |
| Grouper fish | IPNV | *Vibrio carchariae* | | | | [106] |
| Japanese flounder | ABV | *Edwardsiella tarda,* and *Streptococcus iniae* | 84% | Heart, brain, kidney, gill, and spleen | Not indicated | [107] |
| Common carp | CEV | Flavobacteria | Up to 100% | Not indicated | Not indicated | [108] |
| Tilapia | TiLV | *Aeromonas Ichthiosmia, Aeromonas veronii, Aeromonas hydrophila,* and *Aeromonas enteropelogenes* | 20 - 90% | Not indicated | Not indicated | [110] |
| Rainbow trout | IHNV | *Flavobacterium psychrophilum* | 76.2 – 100% | Liver, spleen, kidney, muscle, and skin | Not indicated | [114] |
| Chinese perch | ISKNV | *Aeromonas hydrophila* | Up to 72.4% | Gill, liver, spleen, intestine, kidney, and fin base | Activated the host immune system resulting in host inflammation | [47] |
| | Fungi | Bacteria | | | | |
| Discus fish | *Fusarium moniliform, Fusarium oxysporum* and *Fusarium solani* | *Aeromonas hydrophila* | Up to 100% | Gall bladder, kidney, spleen, and liver | Not indicated | [115] |
| Nile tilapia | *Fusarium oxysporum (fun)* | *Aeromonas hydrophila* | Not indicated | Skin | Not indicated | [116] |

** Myxobolus spp., Chloromyxum truttae, Chloromyxum schurovi, Sphaerospora truttae, and Tetracapsuloides bryosalmonae

*Legend:* ISAV - Infectious Salmon Anaemia Virus; IHNV - Infectious Hematopoietic Necrosis Virus; VHSV - Viral haemorrhagic septicaemia Virus; IPNV - Infectious Pancreatic Necrosis Virus; ABV - Aquabirnavirus; CEV - Carp Oedema Virus; TiLV - Tilapia Lake Virus; ISKNV - Infectious Spleen and Kidney Necrosis Virus.6. Co-infection and immune response in fish.

characterizes type 1 immunity. Type II immunity refers to host defence responses against helminthic infections, including tissue repair and immune suppression activities. Switching between type I and II immunity can substantially alter how the immune system responds to a specific clinical situation and, as a result, the course of the disease [123,131].

The ability to create interferons (IFN-I) and express their receptor is present in virtually all cells, including fish [132,133]. Type I Interferons (IFN-I) generated during co-infection can potentially affect several immunological activities, either negatively or favourably, leading to either protective or harmful phenotypes. The IFN-I are vital in antiviral defence; nevertheless, since these cytokines have various effects on a broad range of immune cells, they can influence many other diseases in many ways. Specifically, fish type I IFN has impressive diversity, most likely representing an adaptation to diverse viral methods to avoid the host's innate immunity [70,127]. Type II IFNs (produced by T helper-1 cells and activated natural killers) play essential roles in adaptive and innate immunity, particularly against intracellular bacteria. Viral infections significantly activate types I and III interferons and are essential in the early innate response to viruses [70,132].

During co-infection, the host may suffer tissue damage that is either directly caused by the toxicity of the pathogen or indirectly induced by an inflammatory response that has not been effectively addressed [70]. As a result, a tolerance mechanism is used as a defensive strategy to minimize the detrimental effect of various kinds of stress, hence minimizing tissue damage [134]. This activity is accomplished through the utilization of an adaptive response. If this tolerance is not established, there is a potential risk of a significant shift in the clinical outcome of secondary infections. Moreover, this shift is independent of the pathogen load. Several lines of evidence show that exposure to infectious agents, such as viruses, parasites, or bacteria, can either favourably or adversely influence the clinical outcome of fish co-infections [70].

Fish co-infection is associated with immunological consequences. For instance, the stage at which a future infection is encountered in viral infections is critical in determining the immunological result. First, Antigen-presenting cells (APCs) become active when exposed to a primary virus. The subsequent infection after APC maturation results in effective antigen presentation. This infection may lead to immunopathology or a protective immune response, which depends on the immune response. Immune responses to fish infections that have previously been encountered can alter immune reactions to different pathogens [44,135]. This condition is known as heterologous immunity, and it may happen between viruses that are related or unrelated, as well as between viruses and other diseases [135,136]. Depending on various circumstances, the heterologous immune response may either result in immunopathology or protective immunity of the fish. After that, when activated APCs come into contact with antigen, the cells release cytokines, eventually affecting T-cell differentiation. These polarised T helper cells mediate bystander protection if a newly arriving fish pathogen comes into contact with existing polarised T helper cells [70].

On the other hand, coming into contact with a regulatory T cell that is polarised can inhibit immune responses against a new pathogen. Furthermore, when subsequent heterologous infection occurs during an active effector $CD8^+$ T-cell response, bystander protection against IFN-generation may also be mediated. In fish, $CD8^+$ T cells are responsible for antigen-specific cell-mediated cytotoxicity. Finally, the consequence of cross-reactivity (protective or pathogenic), a remodelled T-cell receptor repertoire, or a changed immunodominance hierarchy can occur when a new virus infects a host with an established memory $CD8^+$ T-cell pool as a result of a past viral infection [137,138].

In fish, activated immune cells can polarise toward phenotypically and functionally divergent cell populations depending on the kind of pathogen and the tissue condition [33,139]. The activated immune cell creates many types of immunological responses, with types 1 and 2 being the most prevalent. An inflammatory and cytotoxic response is what is meant by type 1 immunity. This reaction is built to combat intracellular infections [140]. The functions of immunosuppression and tissue healing are a part of type 2 immunity, which refers to reactions essential for the host's resistance to helminthic infections. Changing from type 1 to type 2 immunity can significantly impact how a fish's immune system responds to a particular clinical environment and, as a result, how the infection develops [33,141].

## 7. Conclusions and future directions

The transmission of a pathogen within a population and the pathogen's virulence can be affected by co-infections, which will ultimately influence the dynamics of the disease. In addition, the immune responses of the host, the clinical outcome, the host's chances of survival, and the effectiveness of disease control can all be impacted by co-infection.

The disease manifests in co-infected fish due to the complicated interactions between the host and coinfecting fish pathogens. The environment is also a significant part of this interaction, determining disease occurrence. Fish pathogens can substantially influence the severity and spread of disease. Fish pathogens can also affect the multiplication rate, the potential to cause tissue damage, the presence of an animal reservoir, the ease of disease spread, and drug therapy. The presence of another fish disease may also influence these parameters. The genetic similarity of the strains influences whether their interactions are competitive, neutral, or cooperative, as well as the type of interaction. Closely related fish pathogens are more likely to collaborate, exploit the hosts, and maximize transmission. Indistinctly related fish infections, conversely, are more likely to compete with one another, resulting in higher virulence and reduced spread due to host mortality.

Most studies reported co-infection prevalence in fish, with various parameters influencing their outcomes. These include the fish's age, the infection channel, cell types, virus dose and time lag between co-infecting viruses, viral replication rate, and cytopathic effect. Although not within the scope of this review, environmental factors such as climatic factors (e,g., ambient temperature) and management (e.g., feeding) are also significant. In addition, fish co-infection has been linked to immunological effects. Activated immune cells can polarise functionally and phenotypically different cell populations, resulting in numerous types of immunological responses, the most common of which are type I and type II.

In this review, the dynamics of co-infection in fish were highlighted. However, there was a dearth of information on mitigating co-infections in fish. Therefore, further studies on the mitigation strategies of co-infections in fish will provide valuable insights into the subject. Furthermore, there is a dearth of information on co-infection in tropical fish species. Consequently, future investigations in this area would be imperative. Also, more investigations on the immunology of co-infection specific to each fish pathogen class (bacteria, viruses, fungi, and parasites) would be helpful. The findings from such studies would provide valuable information on the relationship between fish immune systems and targeted responses.

## Data availability statement

The article content includes the original contributions summarised in this review. Any further questions should be forwarded to the

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## CRediT authorship contribution statement

**Ekemini Moses Okon:** Conceptualization, Methodology, Writing – original draft. **Reuben Chukwuka Okocha:** Writing – review & editing. **Adesina Babatunde Taiwo:** Writing – review & editing. **Falana Babatunde Michael:** Writing – original draft. **Adeniran Moji Bolanle:** Writing – original draft.

## 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.

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