ABSTRACT

Aquaculture play central role in socio-economic development of rural and coastal countries. Human interfere displace aquatic animals from their natural ecosystems to artificial stressed environment which often results in emergence & spread of pathogens and new diseases. Many severe pathogens have been emerged in aquaculture important species; especially the viral ones. Some economically important species are Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss*), Sea bream (*Pagrus major*), Yellow tail (*Seriola quinquergiata*), Gilthead sea bream (*Sparus aurata*), Ayu (*Plecoglossus altivelis*), Sea bass (*Dicentrarchus labrax*), Turbot (*Scophthalmus maximus*), Flounder (*Paralichthys olivaceus*) and shrimps (*Penaeus monodon* & *P. vannamei*). Permanent eradication of these pathogens from major aquaculture producing countries is very necessary; for which the causative etiological agent must be known. So main aim of this article is to describe these pathogens and developed diseases. Permanent vaccines against all pathogens are not developed yet as they change their pathogenicity via enhanced virulence. Additionally, available vaccines are unable to raise strong and long term immune response. Amongst these pathogens, viral ones are discussed in detail as they are most contagious and can wipe out whole farms.

**Key words:** Aquaculture, pathogens, diseases, vaccines, viral pathogens.
INTRODUCTION

Fish farming is an ancient practice known to human civilization [1]. From 20th century, husbandries were started to establish for salmon, trout and shrimp species, and thereafter aquaculture became a major animal food producing sector [2]. Aquaculture species are farmed in fresh, brackish and marine water environments in which they are reared for optimal production [3]. These species are diverse in geographically mean, viz. from tropical to arctic & subarctic, from lakes & rivers to estuaries. Principal farming species are salmon, trout, carp, oyster and shrimps. Countries like UK, Canada, Norway, Chile and USA are dominant producers of salmon and trouts whereas Asian countries are involved for carp, oyster and shrimps [4]. In previous decades many serious diseases have been emerged in aquatic animals due to anthropogenic global movement of animals & their products. These diseases are listed as ‘notifiable’ by world organization of animal health. Among the fish pathogens, diseases caused by viruses are of great concern as they wipe out whole aquaculture farms. Brood stocks from wild fisheries are significant healthy carrier of viral pathogens [5] and attempts are in progress to confront these carriers using RNA interference approach [6]. Fish cell lines are suitable subjects for modern fish pathology especially for virology to isolate the viral agent in vitro [7]. Bacteria also cause severe diseases in fish and economic losses in aquaculture (Table 1). Some of the bacterial pathogens among these are known to infect (via food or contact) humans also [8]. Death outbreaks associated with usually occur due to consumption of raw or improperly processed infected fish. Many bacterial pathogens have been reported for histamine poisoning which occur by decarboxylation of histidine [9]. These histamine formers grow optimally at 25 °C. Symptoms of histamine poisoning are diarrhea, dyspnoea, pruritus, hypertension and metallic or peppery taste in mouth [9]. Like fishes, shrimps also cover a major part of aquaculture industry and as demanding sea food. Shrimps comprise 15-20% of total fishery products worldwide [10]. Out of various species, two principal species namely Black tiger shrimp (Penaeus monodon) and White pacific shrimp (Penaeus vannamei) dominate the shrimp production as animal food. Pathogens emerge in shrimps also, especially viruses (Table 2); producing mass mortality in the farm [11]. Some immunity components have been described in shrimps like toll like receptors [12], RNAi [13] and vaccination attempts are also in progress [14]. So in this review we have summarized the diseases in the light of expanding geographical host range, virulence diversity, clinical signs and vaccination schemes and emphasized on the viral pathogens. Complete diagnosis of these pathogens can’t be fully described as they require histopathological, immunological & molecular diagnostics, in situ hybridization and cell culture diagnosis [15].

Bacterial diseases in fish

Diseases due to Aeromonads

Aeromonads are the most common bacterial pathogens in fishes throughout the world. Species included in this group are Aeromonas hydrophila, A. veronii and A. salmonicida which cause serious diseases in aquatic animals and in humans too [16]. Among these A. hydrophila causes motile aeromonas septicemia (MAS) or red sore disease [17] and A. salmonicida causes furunculosis [18]. The most common clinical signs are hemorrhages in skin, fins, oral cavity and muscles with superficial ulceration of epidermis [19]. Exophthalmia, ascites, swollen kidneys, splenomegaly, necrosis in spleen and kidneys are also observed. A. salmonicida is antigenically homogenous and can be diagnosed by immunodiagnostics and PCR [20]. Expression profiling and virulence factors have been described in A. salmonicida species [21,22]. Bacteriovorax sp. has recently been identified as biocontrol against A. veronii [23,24].
Bacterial kidney disease (BKD)

Aetiological agent for BKD or Overt disease is *Renibacterium salmoninarum* which mainly affect salmonids [25]. Infection symptoms are exophthalmia, petechial hemorrhages, enlargement & necrosis in kidneys with granulomatous lesions [26]. Biochemically and antigenically *R. salmoninarum* is homogenous [27] and PCR, IFAT and ELISA diagnostic methods have been developed against the pathogen [28,29]. This pathogen spread horizontally or vertically [30,31] and transmission dynamics & phylogenetic history have been recently demonstrated with single nucleotide polymorphism (SNP) analysis [25].

Mycobacteriosis (Fish Tuberculosis)

Fish are convincing host of mycobacteriosis [32]. Many mycobacterium species (*M. marinum, M. fortuitum, M. chelonae, M. avium, M. gordonae, M. abscessus, M. aurum, M. parafortuitum, M. poriferae* and *M. triplex*) have been recognized as causative agents for this chronic disease [33] but *M. marinum* is notable amongst these. Its host range is salmons, turbot, sea bass, striped bass, sod and halibut [34]. Disease symptoms include exophthalmoses, keratitis, change in pigmentation, skin ulcerations, granulomas in liver, spleen & kidney and hemorrhagic lesions in muscles [35]. Histopathology and PCR detection procedures have been developed to detect the pathogen [36].

Vibriosis

Pathogenic species in this group are *Vibrio anguillarum, V. ordalii, V. vulnificus* Biotype 2, *V. harveyii* and *Alivibrio salmonicida* within the family Vibrionaceae [37]. *V. anguillarum* is antigenically heterogeneous and approximate 20-25 ‘O’ serotypes has been identified in contrast to other *Vibrio* species [38]. Among these only O1, O2 and O3 serotypes are implicated in mortality cases. Complete genome of *V. anguillarum* has been sequenced recently [39]. Clinical signs observed with vibriosis are hemorrhagic septicemia on the base of fins, edema tous lesions, corneal opacity and exophthalmia [40]. *V. salmonicida* causes Hitra disease in salmonids and cods and contain marker protein VS-P1 on surface [41]. Many species have been reported to affect humans also. *V. alginolyticus* reported for otitis media [42]; *V. vulnificus* for chronic hepatic diseases, immunodeficiency symptoms, septicemia [43], purpuras, necrotizing dermo-hypodermatitis and fasciitis [44] in patients; *V. parahaemolyticus* causes diarrhea, gastroenteritis and septicemia [45,46]; and *V. cholerae* reported for cholera [47,48]. For diagnosis and identification many molecular techniques (e.g. 16S rRNA, FISH, AFLP and RAPD) have been developed for these species [49,50].

Flexibacteriosis

It is also named as Eroded mouth syndrome and Black patch necrosis and caused by *Tenacibaculum maritimum* which has a wide host range, viz. turbot, gilthead sea bream, sea bass, red sea bream, black sea bream sole, and salmonids [51]. Clinical symptoms are eroded and hemorrhagic mouth, ulcerative skin lesions, frayed fins and tail rot [51]. *T. maritimum* is antigenically and biochemically homogenous but two serotypes has been identified. Serological identification, PCR based detection methods [52] and vaccination attempt have been made but an effective vaccine is still awaited [53].

Pseudomonadiasis

*Pseudomonas anguilliseptica* caused mass mortality in eels of Japan, Taiwan, Scotland and Denmark [54]. Its host range includes eels, black sea bream, ayu, wild herrings, gilthead sea bream
and turbot. This pathogen causes red spot disease with clinical symptoms of abdominal distention and hemorrhagic petechia in skin & lesions [55]. *P. anguilliseptica* has two ‘O’ serotypes, one of eel and another of gilthead sea bream, turbot [55]. Clinical diagnostics and detection of the pathogen by immunohistochemical and PCR methods is available [56,57]. Oil-adjuvant bacterins were developed but any permanent inactivation is still awaited.

**Streptococcosis**

Cocci like *Lactococcus garvieae*, *L. piscium*, *Streptococcus iniae*, *S. agalactiae*, *S. parauberis* and *Vagococcus salmoninarum* infect fishes throughout the world [58]. They infect species in Japan (yellowtail, flounder); Spain, France, Italy, Israel (rainbow trout); USA (tilapia) and Australia (barramundi). These cocci damage central nervous system with symptoms of exophthalmia and meningo-encephalitis [59]. In humans *Streptococcus iniae* has been identified for cellulitis, endocarditis, meningitis and septic arthritis [60]. Serotype studies revealed two serogroups (KG and KG+) in *L. garvieae* and two serotypes (I and II) in *S. iniae* [61]. *S. agalactiae* was reported for illness and deaths in doctor fish (*Garra rufa*) [62]. Immunodiagnostic procedures are available for identification of these cocci [63,64].

**Diseases due to Flavobacteria**

Flavobacteria are important aetiological pathogens represent by *Flavobacterium psychrophilum*, *F. branchiophilum* and *F. columnare*, and cause bacterial coldwater disease (BCWD), bacterial gill disease and columnaris disease respectively [65]. These species has wide host and geographic ranges. CWD produce serious mortalities with clinical signs as increased mucus secretion, epidermal hyperemia, splenomegaly and hepatomegaly [66,67]. Bacterial gill disease primarily occurs in juvenile salmonids in spring season. Affected fish display lethargicity, high in water column and gasping for air at surface. In columnaris disease pathological signs are erotic lesions or deep ulcers on skin and fins. Proteome analysis of *F. columnare* has been performed that exhibited virulence diversity in the species [68,69].

**Edwardsielllosis**

Two pathogenic species are in this group; *Edwardsiella tarda* and *E. ictaluri*. Among these *E. tarda* has wide host range and reported to infect humans also [70] whereas *E. ictaluri* limited to fishes only. These species cause systemic hemorrhagic septicemia and enteric septicemia in catfishes [71]. A characteristic symptom observed is the presence of a raised or open ulcer on the head. *E. tarda* causes loss of pigmentation, swelling on abdominal surface and petechial hemorrhages in fins and skin [72,73]. Clinical diagnostic kits (*i.e.* ELISA, PCR and LAMP) are available commercially to identify these species [74,75].

**Enteric red mouth (ERM)**

*Yersinia ruckeri* causes ERM which is responsible for critical economic losses. This bacterium belongs to Enterobacteriaceae family and produce yersiniosis or ERM disease in fishes. Its common host is *Salmo gairdneri* [76]. Clinical manifestations of this disease are exophthalmia, ascites and hemorrhage & ulceration in jaw, palate, gills and operculum. Biochemically this bacterium is very diverse and has two biotypes (1 & 2) grouped in many ‘O’ serogroups [77]. The disease spread via direct contact with infected fish or by carriers. Progression of the pathogen in infected rainbow trout has been demonstrated through bioluminescence imaging [78]. Recently genetic component for holomycin resistance has been identified [79] and some probiotics and
bacterins have also been developed for prevention of yersiniosis but permanent eradication still is the matter of research [80,81].

**Photobacteriosis**

Also known as pasteurellosis, photobacteriosis is characterized by white nodules in spleen and kidneys [82]. *Photobacterium damselae* subsp. *piscicida* is the causal agent. Mortality incidences due to the photobacterium reported from Chesapeake Bay (white perch and striped bass), Japan (yellowtail), Mediterranean countries of Europe (gilthead sea bream, sea bass and sole) and USA [hybrid striped bass; 83]. Clinical diagnostics and laboratory identification (*i.e.* ELISA, PCR) are available for photobacterium for disease control in farms [84].

**Piscirickettsiosis**

*Piscirickettsia salmonis* is responsible for piscirickettsiosis in rainbow trouts and salmons principally in Coho salmon [85]. Observed clinical signs of this septicemic condition are nodules in liver, darkening of the skin, lethargy, anorexia and respiratory distress in affected fish [86]. Commercial ELISA kits, PCR based protocols for diagnoses are available in the market [87].

**Winter ulcer**

It’s a winter season problem characterized by skin ulcers at scale covered surface; mainly occur in Atlantic salmons with few incidences reported in plaice and rainbow trouts also [88]. Causal pathogen for winter ulcer is *Moretella viscosa* which is represented by two groups: first one is Norway group and second one is Iceland group [89,90].

**Table 1: Bacterial pathogens in fishes.**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Genome size (Mb)</th>
<th>Disease</th>
<th>Main host(s)</th>
<th>Vaccine(s) if any</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio anguillarum</em> [91]</td>
<td>4.12</td>
<td>Vibriosis</td>
<td>Salmonids, cods, red sea bream</td>
<td><em>Vibrio anguillarum-ordalii- Yersinia rackeri</em> Bacterin</td>
</tr>
<tr>
<td><em>V. ordalii</em> [92]</td>
<td>3.41</td>
<td>Vibriosis</td>
<td>Salmonids</td>
<td><em>Vibrio anguillarum-ordalii-Yersinia rackeri</em> Bacterin</td>
</tr>
<tr>
<td><em>V. vulniificus</em> [93]</td>
<td>5.26</td>
<td>Vibriosis</td>
<td>Eel, tilapia</td>
<td>-----</td>
</tr>
<tr>
<td><em>Aliivibrio salmonicida</em> [41]</td>
<td>4.65</td>
<td>Vibriosis</td>
<td>Atlantic salmon</td>
<td><em>Aliivibrio salmonicida</em> Bacterin</td>
</tr>
<tr>
<td><em>Flavobacterium psychrophilum</em> [67]</td>
<td>2.86</td>
<td>Coldwater disease</td>
<td>Salmonids, carp</td>
<td>-----</td>
</tr>
<tr>
<td><em>F. branchiophilum</em> [94]</td>
<td>3.56</td>
<td>Bacterial gill disease</td>
<td>Reared fishes</td>
<td>-----</td>
</tr>
<tr>
<td><em>F. columnare</em> [95]</td>
<td>3.2</td>
<td>Columnaris disease</td>
<td>Cyprinids, salmonids</td>
<td>-----</td>
</tr>
<tr>
<td><em>Edwardsiella tarda</em> [96]</td>
<td>3.76</td>
<td>Edwardsiellosis</td>
<td>Salmons, catfish, carp</td>
<td>-----</td>
</tr>
<tr>
<td><em>Edwardsiella ictalauri</em> [97]</td>
<td>3.81</td>
<td>Enteric septicemia</td>
<td>Catfish</td>
<td><em>Edwardsiella ictalauri</em> Bacterin</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em> [98]</td>
<td>4.74</td>
<td>Motile aeromonas septicemia</td>
<td>Salmonids, non salmonids</td>
<td><em>Aeromonas hydrophila</em> Bacterin</td>
</tr>
<tr>
<td><em>A. salmonicida</em> [18]</td>
<td>4.70</td>
<td>Furunculosis</td>
<td>Salmons</td>
<td><em>A. salmonicida</em> Bacterin</td>
</tr>
<tr>
<td><em>A. veronii</em> [99]</td>
<td>4.55</td>
<td>Epizootic ulcerative syndrome</td>
<td>Salmonids, non salmonids</td>
<td>-----</td>
</tr>
<tr>
<td><em>Renibacterium salmoninarum</em> [25]</td>
<td>3.15</td>
<td>Bacterial kidney disease</td>
<td>Salmonids</td>
<td>-----</td>
</tr>
</tbody>
</table>
Fungal diseases in fish

Fungal diseases are quite rare than bacterial and viral ones in mass mortality means. Fungi can infect fishes if they are given environmental stress conditions like poor environmental conditions (water with low circulation, low dissolved oxygen or high ammonia or high organic loads), poor nutrition or injury. In most cases fungal infection occurs as secondary infection in association with bacterial or viral. Common fungal infections are due to water molds (Saprolegnia). This cotton wool fungus belongs to Saprolegniaceae family and causes saprolegniasis in fishes and fish eggs [105]. Saprolegnia grow optimum at temperature ranges 59° to 86° F [106]. In most cases saprolegnia attacks on existed injured tissue and spread to healthy tissues [107]. These saprophytes transmit via motile zoospores and detritus of bottom. Saprolegniasis symptoms are superficial and observed as fluffy tufts of cottony growth on the skin and gills in affected fish [108].

Another common fungal disease in fishes is gill rot (Branchiomycosis) that occurs mainly in carp and eels. Its causative agents are Branchiomycyes sanguinis and B. demigrans. These species invades fish in water of low pH, low dissolved oxygen and high algal bloom. Infected fish manifest respiratory distress, thrombosis in blood vessels of gills and necrosis in gills [109]. Another fungal problem in fish is Icthyophonus disease caused by Icthyophonus hoferi [110]. Icthyophonus manifest itself internally and primarily attack liver and kidney [111]. Disease is transmitted via fungal cysts released by faeces and via cannibalism of infected fish [112]. A fish fungus is Exophiala [113] which is represented by two important pathogenic species namely E. salmonis and E. psychrophila [114]. These fungi produce round yellow to white granulomas in liver, kidneys and spleen with enlargement of posterior kidney [115]. To manage fungal diseases sanitation of aquatic system, avoidance of overcrowding to minimize injury, use of anti-fungal agents and good nutrition are things to keep in mind.

Viral diseases in fish

Infectious hematopoietic necrosis (IHN)

Aetiological agent of IHN is infectious hematopoietic necrosis virus (IHNV). Its principle host is rainbow trout (Oncorhynchus mykiss) and this virus causes IHN in trouts and salmonids. IHNV, endemic in salmons of North America, emerged as pathogen in farmed rainbow trouts in USA in 1970 [116]. Thereafter, it expanded its geographical and host range by increasing virulence and pathogenicity [117] and spread worldwide via contaminated eggs. It is an enveloped bullet shaped virus that belongs to genus Novirhabdovirus with in Rhabdoviridae family [118] and contain –ve sense ssRNA of approximate 11000 nucleotide length [119]. Its genome contain six genes (N-, P-
, M-, G-, NV- and L-genes) located from 3’ to 5’ [120]. IHNV infects many economically important species of aquaculture; principally of genus *Oncorhynchus*, several salmonids and non-salmonid species [121]. This virus proliferates in vascular endothelial tissues, nephron cells and hematopoietic tissues which results in viraemia, impairment of osmotic balance, edema and hemorrhages. IHNV produced severe aquaculture losses in USA, Canada, Japan, Russia, Austria, Czech Republic, France, Germany, Italy, Netherlands, Poland and Spain through infected eggs and/or fish [122]. Phylogenetic studies identified 5 genogroups (U, M, L, E and J) and multiple subtypes exist in each genogroup that produce diversity in virulence and pathogenicity of virus [123]. This virulence diversity has been demonstrated in rainbow trout [117]. In North America U, M and L genogroups occurs [124]. Molecular epidemiology and evolution of IHNV has been assessed using Bayesian analyses of N, G and NV genes [125]. Diagnostic and identification methods have been developed and validated using reverse transcriptase PCR of nucleocapsid gene [126]. Transmission of virus takes place horizontally, vertically, by biological vectors such as fish parasites (e.g. *Piscicola salmositica*, *Salmonicola* sp.) and by carrier fish [127]. IHNV is released in faeces, urine and external mucus. In persistently infected rainbow trout, truncated virion particles were detected; those were proved to be mediators of persistence [128]. Several vaccine trials have been performed [129], as IHNV rapidly alter its virulence by evolution under selection pressure [122,130].

**Viral hemorrhagic septicemia (VHS)**

Causative agent is viral hemorrhagic septicemia virus (VHSV) which was initially assumed to be endemic in Europe in rainbow trout but further studies indicated the larger host and geographical range of this virus [131]. It is an enveloped ssRNA virus belonging to *Novirhabdovirus* genus of *Rhabdoviridae* family [132]. Its genome (11 kb) has been sequenced [133] and comprises six open reading frames (3’-N-P-M-G-NV-L-5’) encoding N-, P-, M-, G-, NV- and L- proteins [134]. Many finfish species are susceptible to VHSV like species of family Clupidae, Pleuronectidae, Salmonidae, Esocidae and Gadidae [15]. Multiplication of the virus takes place in endothelial cells of blood capillaries (leading to hemorrhagic lesions in internal organs and musculature), leucocytes, hematopoietic tissues and nephron cells. Clinical symptoms in affected fish are hemorrhages in muscles and liver. Externally, fish may show hemorrhage on skin, around eye orbit or exophthalmia in eye. Cytopathic effects of VHSV have been assessed on macrophage cell line [135]. VHSV is very diverse in virulence & pathogenicity [136]. Till now four of strains (I-IV) have been identified in VHSV with several sub strains [137] and most strains limited to northern hemisphere. Strain I comprises European isolates; strain II include isolates from the Baltic Sea; strain III consists of North Atlantic Ocean isolates; strain IV involve Japanese/ Korean (IVa) and North American (IVb) isolates. In 1998, thousands of pacific herring, hakes and walley pollock were found dead in Alaska due to American strain of VHS [138]. Pathogenicity in pacific herrings and Atlantic salmon [139] and phylogenetic diversity & biogeography of VHSV has been assessed using Bayesian approach [140]. A few rapid and accurate methods to detect VHSV are available [141]. In developed countries like UK, US, Norway, Denmark; effective control has been achieved but due to dynamic pathogenicity of the virus permanent eradication is still awaited [142].

**Spring viraemia of carp (SVC)**

Spring viraemia carp virus (SVCV) causes SVC in carps (*Cyprinus carpio*). Outbreaks usually occur in the spring time, when water temperature ranges between 10-17 °C [143]. SVCV belongs
to genus *Vesiculovirus* and it lacks the nonvirion gene [144]. Its genome consists of one molecule of ssRNA that has been completely sequenced [145] and molecular analysis & virulence diversity have been addressed of the virus [146]. Virion particle has an inner nucleocapsid that is surrounded by a lipid containing envelope with spikes. Viral RNA encodes five structural proteins; L-, G-, P-, N- and M-proteins [145]. SVCV isolates are classified in 4 genogroups; Ia, Ib, Ic and Id [147]. Strain Ia occurs in US and England, Ib in Ukraine [148] and Moldova [149], strain Ic in Russia [150] and Id in UK. SVCV is mainly associated with losses in common carp and koi carp in spring season and can infect many fish species [15]. Important disease symptoms include exophthalmia, abdominal swelling, hemorrhages in the skin, gills & eyes and darkening of the skin [150]. Pathogenesis of SVCV infection and related proteins expression was assessed on epithelioma papulosum cyprini cells [151]. For diagnostics; antibody detection and PCR methods are available for epidemic alert [152]. SVCV shed in faeces, urine and sexual fluids and transmit via contact and animate vectors such as *Argulus foliaceus, Piscicola geometra* [144]. Attempts for DNA vaccines were made and permanent vaccine development is however still in progress [153].

**Infectious salmon anemia (ISA)**

Infectious salmon anemia virus (ISAV) is the infectious agent for ISA in salmons (*Salmo salar*). ISA was first recognized in Atlantic salmons from Norway [154]. In 1996, ISA cases were reported from Canada [155]. There ISAV was found to be associated with hemorrhagic kidney syndrome (HKS) in Atlantic salmon [156]. Thereafter in 1998, ISA was detected in Scotland [157]. Subsequent reports came from USA [158], Chile [159], and Faroe Islands [160]. The virus was then isolated from salmons and rainbow trout on SHK-1 cell line and well characterized [161]. ISAV belongs to the family *Orthomyxoviridae* and genus *Isavirus* [162]. It is an enveloped pleomorphic virus with eight segments of –ve sense ssRNA as genome (14.5 kb) which encodes hemagglutinin-esterase (HE) [163], a surface glycoprotein, responsible for strain virulence [164]. ISAV produce serious losses in aquaculture especially in salmons [161]. Systemic infection is characterized by anemia, ascites, congestion and enlargement of the liver and spleen [159]. ISAV is divided in two groups on the basis of HE gene; the European group and North American group [164]. Molecular studies revealed high polymorphism regions (HPR) in the virus [162]. Pathogenesis and cell tropism in atlantic salmon gill epithelial cells has been demonstrated on SHK-1, ASK-II and TO cells using IFAT [165]. Clinical diagnostic and laboratory identification methods (*in situ* hybridization, RT-PCR, IFAT, cell lines) are available for virus [166]. The virus transmit horizontally, vertically, by sea lice (*Caligus elongates, Lepeoptheirus salmonis*) or via coprophagy [167]. For disease control several vaccination field trials were performed and effective vaccines are available in sensitive zones; North America, Faroe Islands and Norway [168]. Alphavirus-based replicon expression vectors are gaining very well acceptance for recombinant vaccine development [169]. HE gene has been confirmed as potential candidate gene for vaccination and a HE based alphavirus-replicon expression system has been developed against ISAV [170].

**Epizootic hematopoietic necrosis (EHN)**

Epizootic hematopoietic necrosis virus (EHNV) is the causal agent of this necrosis and was initially identified in red fin perch (*Perca fluviatilis*) and rainbow trouts in Australia [171]. EHNV is a member of *Iridoviridae* family and placed in the genus *Ranavirus* [172]. Virion particles are icosahedral and retain dsDNA as genome [173]. It mostly infects red fin perch [174]. Some other...
species like *Esox lucius*, *Sander lucioperca* and *Ameiurus melus* are also susceptible to EHNV infection [175]. Susceptibility of some other native and non-native species of southeast Australia towards EHNV has also been confirmed [176]. EHNV causes severe necrosis in hematopoietic tissues in perch and rainbow trout and create great loss in aquaculture production. Histopathology of infection involves necrosis in liver, spleen & renal hematopoietic tissues [177] and ulcerative dermatitis and swim bladder edema are observed additionally in rainbow trout [172]. Immunodiagnostic and PCR methods have been developed and validated for EHNV [178,179]. EHNV spread via spill over of virus from endemic reservoir [172]. To date no effective biosecurity measures are available for EHN control, a permanent vaccine development against EHN is still awaited.

**Mortalities due to Red sea bream iridovirus (RSIV)**

RSIV was first identified in red sea bream (*Pagrus major*) of Japan [180] and thereafter mortality outbreaks were reported worldwide [181]. It was isolated from kidneys and spleen of infected fish using cell cultures and well characterized [182]. It is a member of genus *Megalocytivirus* within *Iridoviridae* family [183]. Other megalocytiviruses were also reported time to time in severe outbreaks with necrosis; infectious spleen and kidney necrosis iridovirus in mandarin fish [184], sea bass iridovirus, rockbream iridovirus [185] and orange spotted grouper iridovirus [186]. Complete genome of RSIV has been sequenced and phylogenetic analysis was performed using major capsid protein and ATPase genes [187]. This virus affects especially juveniles of red sea bream. Infected fish manifest anemia, petechia of the gills and enlargement of the spleen [188]. Pathogenicity of the RSIV was demonstrated in Japanese amberjack (*Seriola quinqueradiata*) [189]. Histopathology of affected tissues showed development of inclusion body bearing cells that are specific characteristic of RSIV infection [190]. Antigenic and molecular studies revealed three lineages in megalocytiviruses [172]. Clinical diagnostics and detection strategies (e.g. serological analysis, RT-PCR, cell culture, immuno-fluorescence) have been established for RSIV [191]. Experimental efforts for DNA vaccines were attempted time to time and a formalin inactivated vaccine is commercialized against RSIV [192]. A recombinant vaccine (351R-GADPH fusion protein) has found to be an effective immunogenic intervention for RSIV [193].

**Viral nervous necrosis (VNN)**

Nervous necrosis virus (NNV) causes severe nervous necrosis in wide range of economically important species [194]. Although initially described in Australia in barramundi [195], this disease was subsequently identified in turbot in Norway [196], sea bass in Martinique [197] and parrotfish & red spotted grouper in Japan [198]. Thereafter the virus was isolated by cell culture on SSN-1 cell line [199] and genetic studies well characterized as member of genus *Betanodavirus* within *Nodaviridae* family [200]. Virion particles are spherical, non-enveloped and retain two +ve sense ssRNA (RNA1 and RNA2) molecules [201]; among which non-structural proteins are the product of RNA1 and coat proteins are encoded by RNA2 [202]. VNN create serious economic loss in aquaculture worldwide especially in larvae and juveniles [203]. This RNA virus causes encephalopathy and retinopathy in marine fishes such as sea bass, halibut, striped jack and grouper [204]. Nervous necrosis is characterized by vacoulation of neural cells of brain, retina and spinal cord [205] due to which affected fish lost its balance and reflects muscular and visual dysfunction.
Histopathology and virulence diversity of NNV has been demonstrated in larvae of humpback grouper (*Cromileptes altivelis*) [206]. Betanodaviruses affect wide range of host species [207] and to date reported from south and Mediterranean Europe and Tunisia, Norway, East Asia, Oceania, UK, and North America. Molecular phylogenetics revealed four clades in the virus as striped jack NNV, tiger puffer NNV, bar fin flounder NNV and red grouper NNV [208]. Phylogenetic studies have also been performed in larva of clownfish *Amphiprion sebae* Bleecker [209]. Genome sequencing coupled with transcriptome analysis (using microarray) reveal some candidate immune genes during viral infection for vaccine development [210]. Transcriptome profiling of viral infection has also been recently demonstrated in Atlantic cod brain [211]. Rapid and convenient RT-PCR methods have been developed for diagnosis and epidemic alert [212]. NNV infect actually larval stage, in which vaccination is quite difficult however potentiality of some peptide vaccine (virus like particles; VLP) and live vaccines have been investigated [213,214]. VLPs are proved to be effective vaccine candidates as they elicit efficient antibody response [215]. A recombinant RGNNV capsid VLP has been recently purified [216].

**Infectious pancreatic necrosis (IPN)**

IPN was first recognized in brook trout (*Salvelinus fontinalis*) at West Virginia, USA [217] and was the first fish virus to be isolated *via* cell culture approach [218]. In the following years IPNV strains were recognized in many countries around the world [219]. This virus can transmit by faeces, urine and sexual products of infected fish and it easily spread by shipment of contaminated fish eggs from one country to another [220]. It may also spread by faeces of piscivorous birds, viz, heron, crow, grackle, kingfisher, sparrows, mallards, egrets and ospreys [221]. Now IPNV is probably present in all major trout farming countries and mainly infect fry and juveniles of rainbow trout, brook trout and atlantic salmon. It is an icosahedral, non enveloped aquabirnavirus [217] and very diverse in host range affecting fishes from freshwater to mariculture [222]. To date 4 serogroups (A, B, C, D), and 9 serotypes (A1-A9) has been identified within serogroup A which are antigenically very diverse [223]. A1 serotype exists in USA; A2, A3, A4, A5 & A10 in Europe; and A6-A9 are reported from Canada. Serotypes A1, A2 and A3 have been reported from Asia also [224]. Virion particles retain two segments (A and B) of dsRNA which encode 5 viral proteins. Segment B encodes VP1 protein (RNA polymerase) and segment A encodes VP2, VP3, VP4 and VP5 proteins. Among these VP2 is expressed as epitope that determine serotype specificity [225]. VP3 is an inner structural protein that bound to RNA and create ribonucleoprotein core of the virus [226]. VP5 protein regulates apoptosis programming of host cell. Virulence diversity (in VP2) especially serotyping and host range among them hindered the progress of control & biosecurity measures [227]. Recently stress induced virulence dynamics in IPNV has been studied in atlantic salmon naive fry [228]. IPNV has been demonstrated to induce apoptosis preceding necrosis in fish cell lines by down regulating the survival factor Mcl-1, a Bcl-2 homologue [229]. The virus can be identified by laboratory procedures like RT-PCR, flow cytometry, cell culture and histopathology [230]; and immunohistochemistry of viral infection has been demonstrated in cultured rainbow trout [231]. IPNV reside in leucocytes and reduce immunity component secretion from host cell. Innate immune components after viral infection are well described in salmonid fish [232]. Several kinds of prevention & disease control methods are available for the pathogen and vaccines have been commercialized against the virus [233]. Immunogenicity and antibody components evoke against viral infection in Atlantic salmon, has been described in detail by Munangandu and coworkers [234,235].
Losses due to salmon alphaviruses

Some alphaviruses also pose serious threats to aquaculture [236]. Alphaviruses belongs to Togaviridae family and cause sleeping disease and pancreas disease in rainbow trouts and Atlantic salmon respectively. Salmon alpha virus (SAV) was identified as aetiological agent for outbreaks in Ireland [237], Norway [238] and Scotland [239]. Sequence analyses of SAV revealed six subtypes (SAV1-6) in the pathogen based on E2 and nsP3 genes [240]. These subtypes are reported from Europe [241], Norway [242], Ireland, England, Germany, France, Italy, Spain and Scotland [240]. Polyprotein sequences of Scottish and Irish isolate was compared recently in which Irish isolate showed high viral load and cytopathic effects in cell culture [243]. Clinical signs of this disease are cardiac myopathy and degeneration of exocrine pancreas [244]. Alphaviruses transmit by arthropods and insects (principally mosquitoes) [245]. Virion particles are spherical, enveloped and retain 12 kb long +ve sense ssRNA [246]. Full genome has been sequenced of some subtypes [247]. Recently some Norwegian SAV3 strain’s genomes have been sequenced that revealed defective viruses with some genome deletions [248]. Host pathogen interactions and innate & adaptive immune responses against SAV-3 Norwegian strain has been well characterized in Atlantic salmon [249]. Subunit vaccines (of spike proteins E1, E2), DNA vaccine and inactivated whole virus vaccines have been developed for SAV control [250]. And, inactivated whole virus vaccine has been proved better immunogenic when compared to spike protein vaccine or DNA vaccine [251].

Diseases due to herpesviruses

Herpesviruses also produce a great loss in aquaculture production. Important members include oncorhynchus masou virus (OMV), icthalurid herpes virus (IcHV) and koi herpes virus. OMV is also known as salmonid herpesvirus (SaHV) and belongs to the Herpesviridae family. This family includes enveloped quasispherical viruses and retains a linear dsDNA as genome. OMV was isolated in Japan from ovarian fluid of masou salmon (Oncorhynchus masou). Virus multiplies in endothelial cells of blood capillaries, hematopoietic tissues and hepatocytes; and produce epithelioma around upper and lower jaw. Intestinal hemorrhage and white spot on liver are additional internal signs. It is reported to be an oncogenic virus for fishes and produce skin ulcerative condition in affected salmon. The DNA of SaHV was shown to molecular weight of about 100×10^6 Da [252]. SaHV shed by horizontal and vertical transmission [253]. In the survivors of acute infection, epidermal papillomas are developed [254]. This virus can be identified by direct methods as neutralization tests IFAT or ELISA [15]. Formalin-inactivated OMV vaccine is recommended for prevention [255].

KHV belongs to Cyprinivirus genus within family Alloherpesviridae [256] and is responsible for explosive losses in common carp and koi carp [257]. It was first recognized in UK [258] and then reported worldwide in carp industry [259]. Recently, KHV has been detected in South Korea also [260]. KHV is an enveloped dsDNA virus [261] with genome size 295 kb [259] and persists initially in latent or carrier phase in infected fish without exhibiting any clinical signs [262]. Clinical symptoms may include gill mottling with red and white patches, sunken eye, pale patches on skin and notched nose [263]. Affected fish may exhibit hyper secretion of mucus in severe stage of infection. Detection methods for KHV like PCR, ELISA are available for diagnosis [264]. A safe and effective vaccine is currently not available for KHV. Recently, some anti-KHV bacteria
have been isolated from carp intestine and their habitat water [265]. A DNA vaccine comprising ORF25 fragment has been constructed and evaluated for immunogenicity [266].

IcHV is also known as channel catfish herpesvirus (CCHV). It is responsible for economic losses of channel catfish (Ictalurus punctatus), blue catfish (Ictalurus furcatus) and white catfish (Ictalurus catus) fry and fingerlings in North America, Russia and Honduras [267]. IcHV genome is 137.2 kbp long and has 77 ORFs [268]. The envelope glycoprotein is encoded by ORF 59 [269]. From all of the ORFs, ORF 50 encodes a secreted, mucin like glycoproteins (gp250) due to which there are different isolates of IcHV [270]. A thymidine kinase (TK) is encoded by ORF 5 [271]. It was demonstrated that IcHV virulence is reduced at temperature below 20 °C. Diagnostic and identification can be done by IFAT, neutralization test, ELISA [272] or by PCR [273]. Recombinant TK gene deletion mutants of IcHV were constructed and exposure of TK- mutants to fish induces protective immunity against challenge [274].

Table 2: Viruses in shrimps and their geographical distribution

<table>
<thead>
<tr>
<th>Virus</th>
<th>Genotype</th>
<th>Family</th>
<th>Geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taura syndrome virus (TSV)</td>
<td>(+) ssRNA</td>
<td>Dicistroviridae</td>
<td>Asia, Americas</td>
</tr>
<tr>
<td>Yellow head virus (YHV)</td>
<td>(+) ssRNA</td>
<td>Roniviridae</td>
<td>Asia, Mexico</td>
</tr>
<tr>
<td>Macrobrachium rosenbergii nodavirus (MrNV)</td>
<td>(+) ssRNA</td>
<td>Nodaviridae</td>
<td>Asia, Australia</td>
</tr>
<tr>
<td>Infectious myonecrosis virus (IMNV)</td>
<td>(+) ssRNA</td>
<td>Totiviridae</td>
<td>Indonesia, china, brazil, Thailand</td>
</tr>
<tr>
<td>Infectious hypodermal and hematopoietic necrosis virus (IHHNV)</td>
<td>ssDNA</td>
<td>Paroviridae</td>
<td>Asia, Africa, Americas</td>
</tr>
<tr>
<td>Hepatopancreatic parovirus (HPV)</td>
<td>ssDNA</td>
<td>Paroviridae</td>
<td>Asia, America, Africa, Madagascar</td>
</tr>
<tr>
<td>White spot syndrome virus (WSSV)</td>
<td>dsDNA</td>
<td>Nimaviridae</td>
<td>Asia, America</td>
</tr>
<tr>
<td>Baculoviruses</td>
<td>dsDNA</td>
<td>Baculoviridae</td>
<td>Asia, Africa, America, Australia</td>
</tr>
</tbody>
</table>

Global impacts of fish diseases and pathogens

Emerging diseases in aquaculture have crucial effect on country economy especially of coastal nations (Table 3). Like in 1996, 40% of total shrimp production in US was affected due to viral diseases that were estimated up to US $ 3 billion [275]. Major significant production loss was always immediate after any pathogen has emerged and has pronounced impact till on fish/shrimp industry till any biosecurity measure developed. Poor, uneducated, low income rural farmers are critically affected. WSSV has been estimated the worst shrimp pathogen as produced US $ 4-6 billion loss in Asia till ten years after its emergence [276]. The loss to fish based industry because of diseases caused by IHHNV and TSV is estimated to be approximately US $ 2 billion [277]. ISAV produce 30 million dollars loss in Scotland and approximate 20 million in Norway and Canada within one year after its emergence. In 2010, ISA hit Chilean salmon industry by decreasing their production from 600,000 (in 2008) to 100,000 metric tonnes [278]. Another example of ISA loss was of British Columbia in 2011 [279]. KHV produced critical loss in Indonesia [280]. These pathogens account for severe impact on susceptible species and their biodiversity by increasing host range, changing genetic diversity and affecting predator/prey animals [281]. For farming, aquatic animals are transferred actually to stress environment in which they are exposed to artificial feeding, poor water quality and high stocking density. These factors facilitate pathogen to compromise host defense system, replicate in the susceptible hosts and thus
contribute to disease transmission [282]. Expanding world trade of aquatic animals contributes to increase in geographical range for pathogens [283].

Table 3: Geographic prevalence of notifiable viruses in fishes and vaccines developed against them [15].

<table>
<thead>
<tr>
<th>Virus</th>
<th>Vaccine(s) and manufacturer(s)</th>
<th>Targeted antigen(s)</th>
<th>Geographic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHSV</td>
<td>-----------</td>
<td>-----------</td>
<td>Europe, North America, Asia</td>
</tr>
<tr>
<td>IHNV</td>
<td>Aqua Health Ltd. Novartis Canada; Microtex International Inc. British Columbia Canada</td>
<td>G protein</td>
<td>Europe, North America, Asia</td>
</tr>
<tr>
<td>SVCV</td>
<td>Bivota Czech Republic</td>
<td>Inactivated virus</td>
<td>Americas, Europe, Asia</td>
</tr>
<tr>
<td>EHNV</td>
<td>-----------</td>
<td>-----------</td>
<td>Australia</td>
</tr>
<tr>
<td>ISAV</td>
<td>FORTE VI Aqua Health Ltd. Novartis Canada; Microtex International Inc. British Columbia Canada</td>
<td>Inactivated virus</td>
<td>Norway, Canada, USA, Chile, UK, Faroe islands</td>
</tr>
<tr>
<td>NNV</td>
<td>-----------</td>
<td>-----------</td>
<td>North America, Europe, Asia, Australia, Africa</td>
</tr>
<tr>
<td>RSIV</td>
<td>-----------</td>
<td>-----------</td>
<td>Asia</td>
</tr>
<tr>
<td>IPNV</td>
<td>AquaVac IPN Oral Intervet International BV Netherland</td>
<td>VP2 and VP3</td>
<td>Americas, Europe, Asia</td>
</tr>
<tr>
<td>KHV</td>
<td>-----------</td>
<td>-----------</td>
<td>Asia, Africa, Europe, north America, Israel</td>
</tr>
<tr>
<td>SAV</td>
<td>PD, Pharmaq AS Norway; Norvax Intervet International BV Netherland</td>
<td>Inactivated virus</td>
<td>Europe, Norway, Ireland, England, Germany, France, Italy, Spain, Scotland</td>
</tr>
<tr>
<td>OMV</td>
<td>-----------</td>
<td>-----------</td>
<td>Japan</td>
</tr>
</tbody>
</table>

Diseases in fish or shrimps are caused by natural infection or spill-over from carrier reservoir [284]. Pathogens of these diseases especially the viral ones, have emerged in new geographical region due to their global trade [285]. IHHNV spreads throughout Americas by translocation of infected *P. monodon* from Philippines [286]. ISAV emerged in farmed Atlantic salmons worldwide from wild species of Norway and Canada [287]. IHNV, VHSV, SVCV and KHV spread Europe, Asia and Americas as result of prolific international trade. Similarly, there is wider transmission of many viruses like WSSV, TSV and IMNV which as devastating pathogens for shrimps [276,288]. So the present scenario demands effective vaccines against the mentioned pathogens for sustained aquaculture. To date vaccines have been developed for some economic bacterial & viral pathogens only [289] and no permanent biosecurity procedure is available for fungal & parasitic diseases. Vaccines can be typed as inactivated, attenuated and recombinant subunit & DNA vaccines. An ideal vaccine must be multivalent, orally deliverable and must provoke strong long lasting immune response. Recombinant subunit & DNA vaccines are potential in this regard. Most of the vaccines developed yet are delivered by intraperitoneal injection but it is an expensive, labor intensive and time consuming process. So efforts are in progress to develop orally deliverable vaccines via feeding route. Factors like increasing demand for seafood, global population and limited production from fisheries enhancing the global trade of aquaculture to complete this demand; that eventually led to emergence of above mentioned and new diseases in finfish & shrimp aquaculture. Development of cell lines resulted in enhanced surveillance of aquatic virosphere. Recombinant
technologies and analytic advanced techniques may play a promising role to overcome aquaculture economic losses. Genome sequences of the fish pathogens provide us information about protein expression in host which may represent key virulent proteins as suitable target(s) for vaccine development. Understanding of molecular and metabolic mechanisms will indeed improve our knowledge in vaccine development against these pathogens.

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