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Molecular approach and techniques used in the diagnosis of fish parasites

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Abstract

Aquaculture is considered an integral part of achieving food security and poverty reduction by providing nutritional, and livelihood security to millions of people around the world. However, poor health management and a lack of proper diagnostic facilities create problems in the early identification of pathogens leading to mass mortality in fish. However, fish, like any animal, are exposed to and susceptible to a wide range of diseases and parasites. Parasitic diseases of fish are usually encountered more often than microbial diseases. From 30%-50% of the cases received at several fish disease, diagnostic laboratories involve parasites. Some of the parasites could not be differentiated by conventional diagnostic methods from other phenotypically similar pathogens of the same genera. Some attempts have been made using biochemical tests, DNA homology, and protease variability. Moreover, most molecular technologies are mostly lab-based and need time to provide information on parasitic infections. Additionally, such technology couldn't be used in the field, providing direct information on potential parasitic pathogens to adopt immediate management measures and avoid disease outbreaks and production loss. However, in the last fifteen years or so, great advances have taken place in understanding the molecular biology of fish pathogens and their hosts. Among them, vast fields of molecular study like Nucleic acid-based approach, PCR, ITS, RT-PCR, LAMP, Luminex, RAPD, AFLP, RFLP, and microsatellites have been used for parasitic disease diagnosis. Detection of nucleic acid molecules has demonstrated its usefulness for highlighting hardly cultivable, non-cultivable, and even dead microorganisms, generating appropriate novel or replacement technologies. Thus, a better understanding of molecular tools developed for the detection of specific parasites would be helpful to increase diagnostic precision, aid carrier detection, and promote species discoveries in different parasitic groups. Further, studies are still necessary to increase our knowledge about molecular tools in the detection of a diverse group of parasites associated with the disease in fish and also from fish to humans.

Keywords: *Aquaculture, parasitic diseases, diagnostic methods, immunological study, molecular tools, histological, and cellular organization.*

Introduction

Aquaculture is the highest-growing sector in the field of fishery sciences. Fish (both Chondrichthyes and Osteichthyes) that is high in protein is widely consumed for human consumption throughout the Indian subcontinent, which significantly boosts the region's economy. But, nowadays several pathogens like parasites, bacteria, and fungi manifest fishes and adversely affect fish's healthy growth [1-7]. The traditional method for disease diagnosis is carried out through macroscopic as well as microscopic examination for the primary screening procedures of the causative pathogens [8-9]. The conventional diagnostic protocol has been discovered for the same clades of pathogens, and genera [10]. With few diagnostic tools like DNA homology analysis and biochemical assays [11-13], a few consequences

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arise, and previously isolated pathogens are lesser sensitive to these pathogenicity tests. In the last two decades, molecular tools are the detection of fish parasitic pathogens, and their hosts are widely accepted. Molecular-level diagnostic tools are widely accepted methods for a resistor of fish illnesses and their epidemiology of parasitic, bacterial, fungus, and viral diseases [14]. Recognition of nucleic acid assays are also useful for microbes detection [3]. The parasites are differentiated into seven different taxa they are- Subkingdom Protozoa (Phylum Rhizopoda, Phylum Mastigophora, Phylum Ciliophora, Phylum Microspora, Phylum Apicomplexa), Phylum Cnidaria, Phylum Platyhelminthes (Turbellaria, Monogenea, Digenia, Cestoda), Phylum Nematoda, Phylum Acanthocephala, Phylum Annelida and Subkingdom Arthropoda (Branchiura, Copepoda, Malacostraca (order Isopoda) [15]. The fish specimens are highly susceptible to different parasitic diseases, they are economically significant in many tropical and sub-tropical countries. The impact of the different diseases in aquaculture is difficult to analyze by regular examination in case of mortality. The prime parasitic diseases documented in freshwater fishes (salmonids, cyprinids, and eels) are white spot diseases (ich), trichodiniasis, chilodonellosis, gyrodactylosis, dactylogyrosis, argulosis, microsporidiosis [16-17], and several marine fishes (seabass, sea bream, turbot) aretrichodiniasis, costiasis, ceratomyxosis, amyloodiniosis, mycrocotylosis, and sea lice disease [18]. A few parasites are caused severe damage to the pisciculture, like- *Amyloodinium* sp. (Dinoflagellates), Scuticociliatida (Ciliates), *Enteromyxum* sp. (Myxosporea), and Mycrocotylidae (Monogenea). However, their pathological signs should not be negligible and concern the enhancement of cultures and their deleterious effects, which are not the possible cause of high mortality [19].

In the parasitological research field, laboratory tools depend on traditional processes like- light microscopy for the morphological identification in the parasitological study [20]. Molecular biological research are based on the detection of parasites responsible for disease diagnosis [21]. Conventional studies have the merit of being cheaper because they do not require costly chemicals and instruments. Simply completed when a skilled microscopist is able. In another way, molecular techniques opined the incidence of parasitic worms involved in their antigenic determinants or DNA parts [22]. These assessments are not involved in ecological attributes and may often interfere with the possible outcome of a faecal samples test [23].

Recently, the determination and diagnosis of parasitic infestations depend on several laboratory purposes and scientific signs; including medical history, travel history, and geographic coordination of fish species [18]. In another way, new diagnostic kits have shown new ways to the improvement of parasite detection [18]. Firstly, serological analyses that are more precise and highly sensitive such as the luciferase immunoprecipitation system (LIPS), rapid antigen detection system (RDTS), and Dot-ELISA [24]. Secondly, molecular tools like loop-mediated isothermal amplification (LAMP) [25], Luminex, and real-time polymerase chain reaction (RT-PCR) [26] have shown the highest potentiality for use in parasitic determination which enhances specificity and sensitivity. The molecular assays that identify parasites include PCR, RAPD (Dominant marker), AFLP (Dominant marker), RFLP (Co-dominant marker), microsatellite marker method, Luminex xMAP-based technology, LAMP, and the currently added RT-PCR [25, 27-33]. In this review, we mainly focus on different molecular techniques and their uses in the detection of fish parasitic pathogens.

Fish parasitic disease

Importance of disease

Fish diseases indicate poor quality of water and poor management systems. The addition of dissolved oxygen (DO) leads to hypoxic conditions in the aquatic fauna. Fish diseases can be significant since they have the potential to cause mortality. Sublethal illnesses can also result in poor growth, poor flesh quality, and unfavourable alterations. Additionally, some fish infections can spread to humans.

Parasites' major causes of concern

Parasites are living things that eat from and dwell inside of other living things, such as your body. A bug bite, contaminated food or water, or sexual contacts are all ways to contact them. The size of parasites varies, from the microscopic protozoa (one-celled creatures) to the visible worms. The United State is home to some parasite illnesses. Infections with *Giardia* can result from contaminated water sources. Toxoplasmosis, which can be harmful to expectant mothers, can be spread by cats. Others, like malaria, are widespread around the world (NIH 2017). For diseases caused by parasites, there are no

vaccinations. Certain medications can treat parasite infections.

Host-parasite relationship

Host-parasite co-evolution leads to host or parasitic adaptation in spatiotemporally changing situations, e.g. when aggressive species express their variety [34]. A major factor that explains the achievement of these species is the blown of the again-originated populations from the pressure worked out by their original parasites are left behind in the original environment [35-36]. This model states the knowledge that co-evolution primes to widespread resident adaptations, and as such the parasites in a specific area contaminate hosts from the same area more proficiently than they infect hosts from another geographically diverse population [37-38]. In another way, environments are not parasite friendly, host-parasite interactions lead to parasite mal-adaptation, where parasites can show their highest capability in the native (allopatric) hosts than in the sympatric hosts [39-40].

Host defence against the pathogenic which is a well-regulated inflammatory retort signed by leukocyte movements into the site of infection, resolution of inflammation response, and repairing of the tissue architecture [17, 41]. Generally, the association between the host and the parasite governs the consequence of the dirt. Some parasites penetrate the host's immune systems and hide intracellularly, like nematode and cestode species, and others invade the cell immunity including extracellular parasites such as *Argulus* sp., *Ichthyophthirius multifiliis*, and monogeneans species [41].

Many molecules are triggering signalling pathways, where parasite and host cells are critical for parasitic entrance, leading to the parasites' survival [17]. An important advance has been realized in several factors that are key features in parasite virulence and the pathogenesis of the diseases they cause. Among the most extensively studied of these are parasitic conjugate proteases [16]. Parasitic proteases can play a wide role that establishing, maintaining and exacerbating an infection [41]. Parasitic protozoans were attacked and migrated a variation of tissues and organs, regardless of being intracellular or extracellular parasites. The mechanisms for connective tissue distortion might be vital for parasite survival [41].

Fish immune response to parasitic infection

Teleosts show innate and adaptive immunity. Fishes provide an innate immune system, which is the first line of immune defence. Adaptive immunity that highly diverse T and B cell receptors encode recombinant activation genes (RAGs) which caused infections [42-43]. Innate immune response recognition is based on pathogen-associated molecular patterns (PAMPs) by pathogen-recognizing receptors (PRRs). PRRs which are Toll-like receptors (TLRs) (Fig. 1) in nature that helps in molecules like PAMPs are well in the field of fish parasitology.

C-type lectin receptors (CLRs) are useful against host-parasitic interactions. Most of the CLRs are involved in antigen presentation, and capture [44-46]. CLRs guided intracellular internalization motifs, in other ways CLRs consist of ITIM (immunoreceptor tyrosine-based inhibitory motif) or, ITAM (immunoreceptor tyrosine-based activation motif)-like motifs in their cytoplasmic domains, elicit the capability of immune-activation or immune suppressive functions of these receptors. In the general structure of carbohydrate recognition domains (CRDs) they are divided into diverse families including C, F, P, I-type lectins, galectin, and pentraxin etc [47]. However, the mannose receptor (MR), macrophage galactose-type C-type lectin (MGL), dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), DEC205, and Dectin-1 are the most significant molecules from the CLR family [45]. Antigen-presenting cells APCs, such as macrophages or dendritic cells, must first recognise an invading pathogen to determine the type of effector T cell that will then mediate an immune response [48]. Highly specialised receptors, such as a variety of pattern recognition receptors (PRRs), such as C-type receptors (CLRs) and Toll-like receptors, are present in APCs (TLRs). When these receptors bind to conserved pathogen structures known as pathogen-associated molecular patterns, APCs are activated and mature, which is a critical step in the process (PAMPs). CLRs recognise and internalise particular carbohydrate antigens articulated by pathogens and host tissues in a Ca^{2+}-dependent manner, in contrast to TLRs [44, 46-47, 49]. Interactions between proteins and carbohydrates play significant roles in two separate facets of the immune response. Both pathogen recognition and the cellular interactions that result in pathogen neutralisation depend on these interactions [50]. By identifying and attaching particular carbohydrate

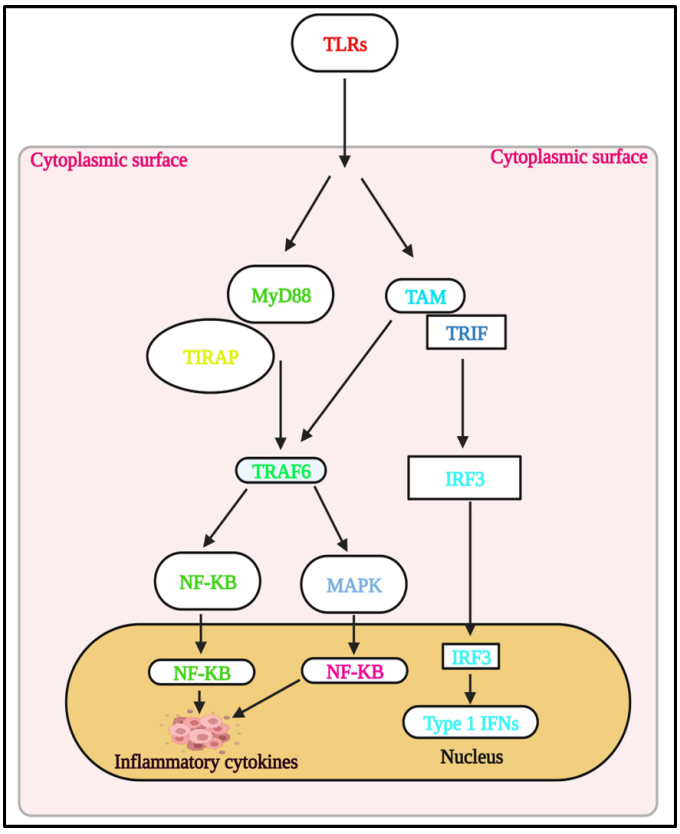

Fig. 1 In this schematic presentation TLR cells MyD88 and TAM are situated in the cytoplasmic surface. Then, TIRAP binds with MyD88 and TRIF binds to several immune cells like TRAF6, IRF3, NF-KB, and MAPK. Finally, NF-KB elicits the secretion of inflammatory cytokines, which fight against inflammation. In another way, IRF3 increases type 1 IFNs production, which enters the nucleus, and further inflammation process is carried forward.

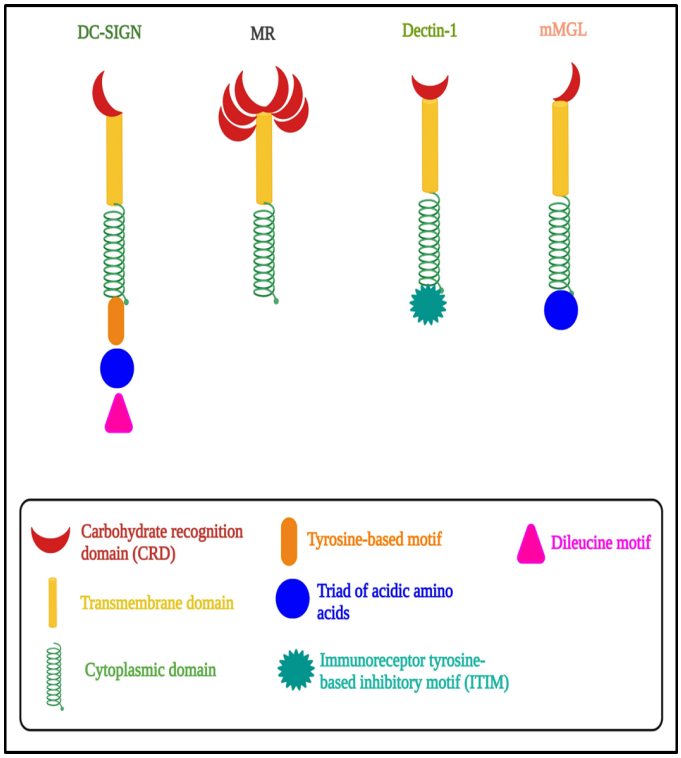

Fig. 2 Structure of membranes of the C-type lectin (DC-SIGN, MR, Dectin-1, and mMGL). These receptors contain one or more carbohydrate-recognition domains (CRDs), transmembrane domains, and a cytoplasmic domain that may contain a tyrosine-based motif, a triad of acidic amino acids, dileucine motif, and an immunoreceptor tyrosine-based inhibitory motif (ITIM) [45].

moieties (typically a nonreducing terminal monosaccharide or oligosaccharide) on the surface of potential pathogens through CRDs, lectin receptors play a significant role in the innate immune response [49]. CRDs can recognise carbohydrate moieties and trigger agglutination, immobilisation, complement-mediated opsonization, and lysis when combined with other domains [46] (Fig. 2).

Inflammatory reactions of phagocytosis and their activity are involved in ciliates, flagellates and finally myxozoans [51-53]. Few humoral parameters like peroxidases, lysozyme and acute-phase proteins are involved in some parasites (Fig. 3).

Ciliates like *Chilodonella*, *Ichthyophthirius*, *Trichodina*, *Uronema*, *Tertrahymena*, and *Epistylus* are obligate parasites and facultative in forms. Some ciliate parasites are highly pathogenic, they are highly prone to mortalities. Due to such harsh conditions, hosts are evolving an effective response to combat parasitic diseases [53]. The flagellate parasitic infection does not depend on the age and size of the fish; it is measured by a dominant gene. The α_2-macroglobulin in the metalloprotease is blood neutralised and secreted by the pathogenic *C. salmositica*. Humoral (e.g. complement-fixing antibodies to lyse the parasite) and cell-mediated (e.g. T-cell cytotoxicity, phagocytosis) are part of the protective mechanism in acquired immunity [51].

Development of a vaccine against parasites of fish

Vaccination is widely useful in aquaculture for a few fish species [54-56]. Molecular techniques against fish vaccines are widely available, but it is most useful for antiviral vaccines [57]. Immunological aspects against *I. multifiliis* is a mostly humoral immune response, it is abundant in parasitic membrane proteins called immobilization antigens (i-antigens) [58-59]. Purified i-antigen confers activity immune system as well [60].

DNA vaccines are lesser proven against *Ichthyophthirius*, due to their enormous potential against a different variety of microbial agents. A similar

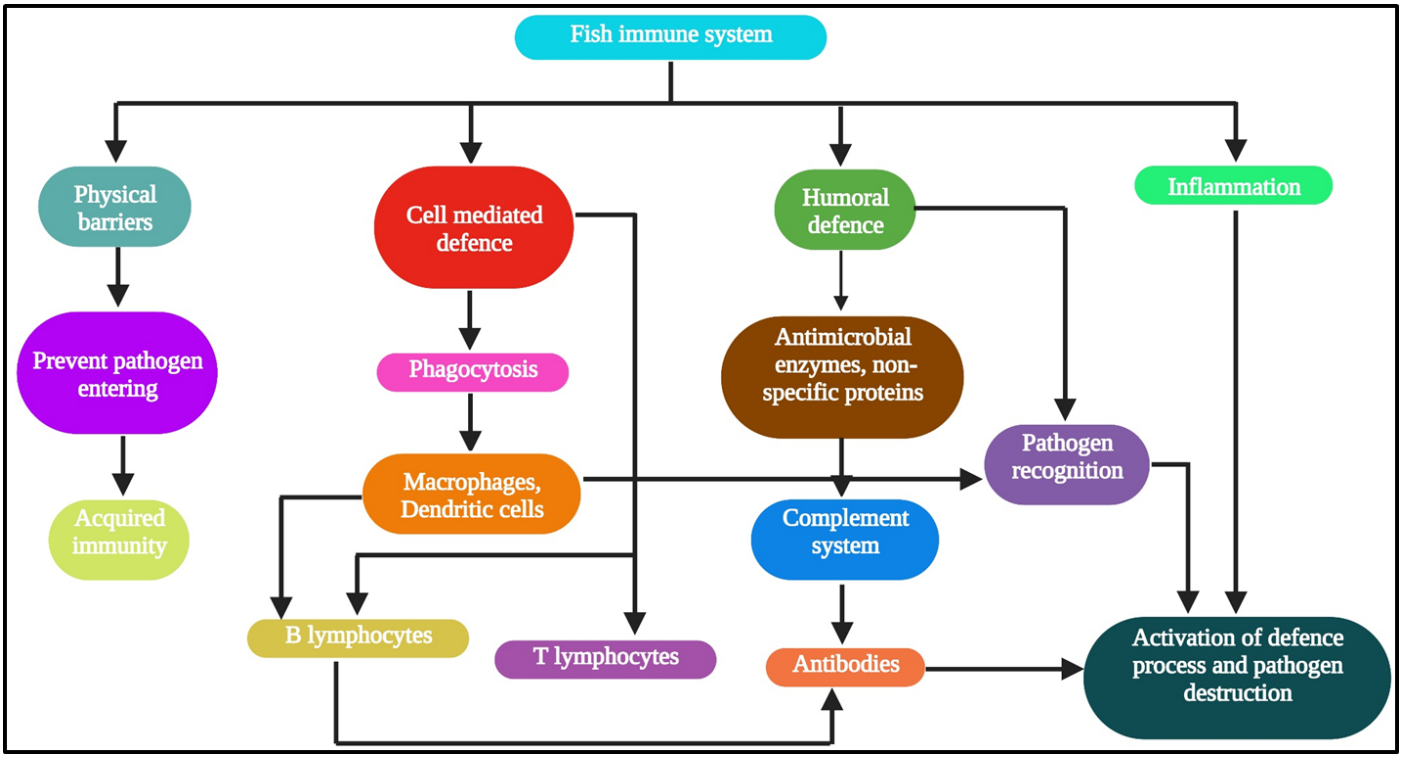

Fig. 3 Physical barriers produce acquired immunity to prevent pathogen entering. The phagocytic process activates during cell-mediated defence mechanism, which turns further activates macrophage and dendritic cells. At last, immunomodulation comes to B and T lymphocytes, B lymphocytes enhance antibody production which creates a defence process to resist pathogen destruction. The humoral defence mechanism elicits antimicrobial enzymes and non-specific proteins which activate the complement system, and finally, antibody shows a defence mechanism against causative pathogens. In, the inflammation process activates the host immune system for pathogenic distribution.

strategy is used against fish parasites, those fishes that have economic importance in the aquaculture sector. *Cryptobia salmositica* is a flagellate parasite, that has protective immunity and vaccination capability [61]. *Lepeophtheirus salmonis* is sea lice, that are an economically important parasite of farmed salmonids. In addition, injection of extracts of the parasitic induction of exact Abs in the same fish [62]. Antibody stimuli are unable to protect against adult and preadult stages. Partial protection against fish louse is not a replication of treatment groups. As per the available study, the vaccination of salmon is useful against this crustacean [63]. Vaccination development is still in a dark position, Abs could target critical host-parasitic interactions that are not classified.

The knowledge gathered from omics data can be quite helpful for creating multivalent vaccines. A multivalent vaccine combines numerous antigens to stimulate the host's immune system in a broad-spectrum protective manner [64]. The same parasite, various parasites, parasite strains, and phases of parasite development can be used to select distinct antigens [65]. A multivalent vaccination would generally be an efficient method of combating aquatic parasites given the myriad of serious issues connected to them, especially the variable antigenic profile of the developmental stages and parasite strains. Although it is hypothesised that fish may be protected from infection by cell-mediated immune responses for some parasites, such as *T. bryosalmonae* [66], the precise protective response is still unknown. In such cases, investigations on the parasite transcriptome, genome, and proteome as well as information on host-parasitic interactions might be used to identify and target many antigenic possibilities [65]. Genomic, transcriptomic, structural proteomic, and immunoproteomic approaches can be employed to find an appropriate antigen while creating a multivalent vaccine. Illustrates a strategy for the assortment of antigenic candidates and the creation of vaccines using omics techniques (Fig. 4). In this method, the targeted parasite or the infected host tissue can be started with data from whole-genome sequencing, RNA sequencing, and proteomics. The data can be further annotated using various bioinformatics tools, including Blast, Blast2GO, and UniProt/Swiss-Prot. After annotation, the function of the gene, RNA transcripts, and proteins, as well as the

many biological pathways, may be examined. Fish can be given vaccine candidates to test the vaccine's potency and effectiveness.

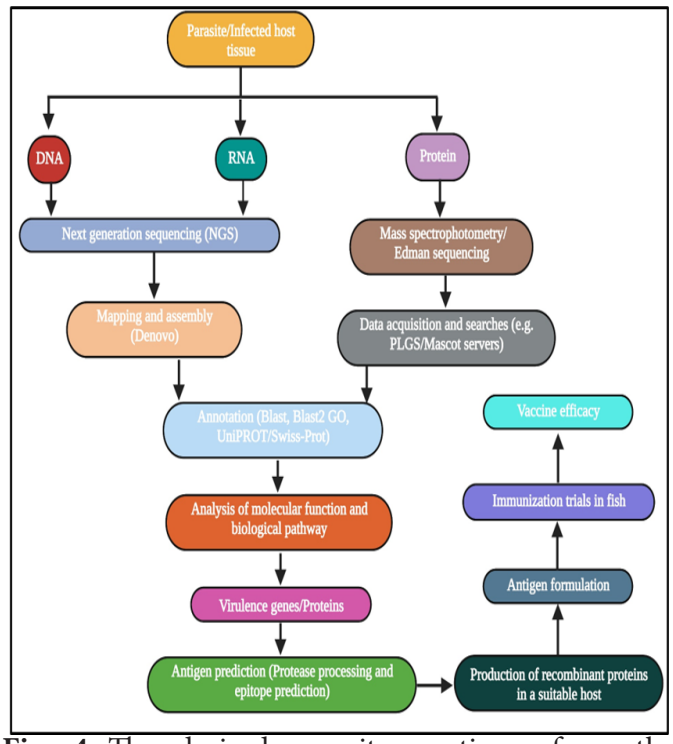

Fig. 4 The desired parasite or tissue from the infected host fish can be used to extract the desired material (DNA, RNA, or protein). The isolated material is used as a sample for Edman sequencing, spectrophotometry, and next-generation sequence analysis (for DNA, RNA and Protein). The immunogenic targets (capable of inducing host immune response) can be chosen and their protective epitopes predicted based on molecular function and biological pathway analysis. Consequently, the vaccine created in this manner may be employed. For the study and functional annotation of parasite compounds, various bioinformatic methods can be applied to the acquired sequences. Following their mass production, these compounds can be coupled with the appropriate adjuvants to create vaccines. Consequently, the vaccine created in this manner may be employed [65].

Economic losses due to parasitic diseases

Parasite infection indicates a population bottleneck in the development of the aquaculture sector [67]. Therefore, disease outbreaks are thought to affect farms' production and financial health by affecting feed conversion, increasing host mortality, and decreasing animal welfare [68]. Clinical disease outbreaks result from a number of cues relating to the manufacturing process, host susceptibility, and the virulence of the specific pathogen [69]. Coincidentally, disease resistance and appropriate measures are crucial for the expansion of the sustainable aquaculture business, and these must be put into practice with the cooperation of farmers and authorities on animal health [67].

Timely diagnosis is of utmost importance

The methods of medical treatment and prevention used in fish are quite specific and frequently dissimilar from those used in warm-blooded species. They necessitate in-depth familiarity with fish environments. A comprehensive understanding of the aetiology of the disease and the biology of the host (fish) forms the basis of the complex combination of therapies that make up preventive plans. It focuses on reducing or eliminating infection (invasion) sources, and the likelihood of further spread, as well as improving fish organism health so that it can tolerate infection (invasion). The primary factor in the elimination of diseases is prevention. For a lot of disorders, no specific treatments have yet been created, hence the use of efficient diagnostic methods is needed.

Methods used for identification of fish parasites

Traditional approaches

Microscopy

Microscopy is a primitive and well-accepted tool for detecting parasites and tissue specimens [70-71]. For the direct microscopy method, observation is very much time taking, laborious which needs a highly specialized laboratory technician. In endemic areas, where resources are concise these were observed difficulties and also misdiagnosed. Still, several helminthic infections are solely dependent on microscopy-based studies and also dependent on molecular studies (Fig. 5).

Histology

The histological study is co-related with microscopic examination like thin or thick tissue staining process and also by using staining for the histopathological study to determine the changes due to pathogens infections. Histological screening is a very common diagnostic tool for aquatic animals [84]. The direct smear method is the first step of the examination for parasite screening, if parasites were observed then

Table. 1 Microscopy-based detection of fish parasite

Sl no.	Name of parasites	Host	Infected organ	Reference
1.	*Amyloodinium* sp.	*Amphiprion percula* (Lacepède, 1802)	Head, and caudal fin	[72].
2.	*Chilodonella* sp.	*Carassius auratus* (Linnaeus, 1758)	Gill, and fin	[73].
3.	*Ichthyophthirius multi-filiis*	*Schizothorax macropogon* (Regan 1905)	Skin, and fin	[74].
4.	*Trichodina* sp.	*Carassius auratus* (Linnaeus, 1758)	Gill	[75].
5.	*Myxobolus* sp.	*Salminus franciscanus* (Agassiz 1829)	Fin	[76].
6.	*Lernaea* sp.	*Rhinogobius similis* (Gill, 1859)	Skin	[76].
7.	*Caligus* sp.	*Lutjanus erythropterus* (Bloch, 1790)	Skin	[77].
8.	*Argulus* sp.	*Schizothorax richardsonii* (Gray, 1832)	Skin	[78].
9.	*Trypanosoma* sp.	*Platydoras armatulus* (Valenciennes, 1840)	Blood	[79].
10.	*Eimeria* sp.	*Chloroceryle americana* (Gmelin, 1788)	Intestine	[80].
11.	*Henneguya* sp.	*Prochilodus lineatus* (Valenciennes, 1837)	Gill tissue	[81].
12.	*Dactylogyrus* sp.	*Osteobrama cotio* (Hamilton, 1822)	Gill	[82].
13.	*Gyrodactylus* sp.	*Oreochromis niloticus* (Bloch 1790)	Fin	[83].

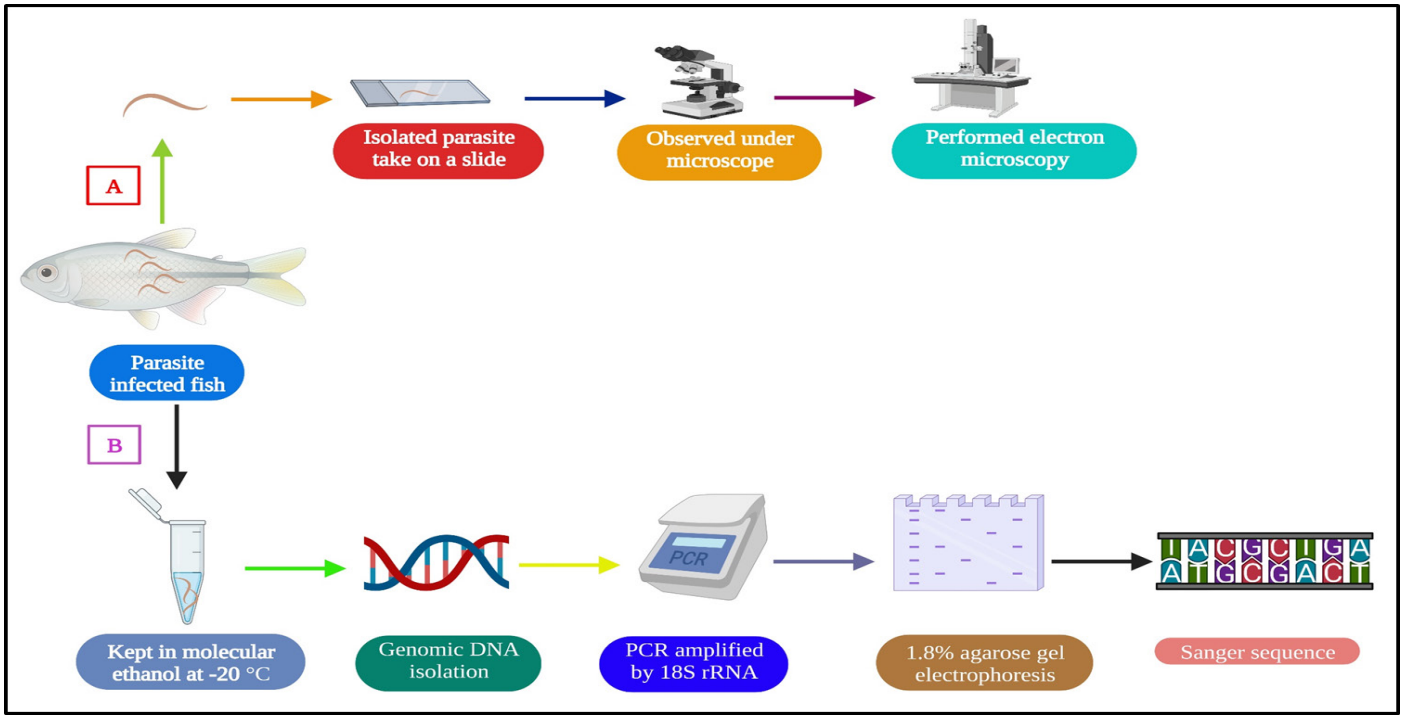

Fig. 5 Microscopical observation of parasite (A) and molecular characterization of the isolated parasites (B).

performed next level analysis fixation and stained for confirmatory diagnosis. Giemsa staining is widely used for many parasite staining [85], and also for blood parasites like *Plasmodium* sp., *Trypanosoma* sp., etc. Tissue stains like haematoxylin and eosin and PAS help in parasite identification and also visualization of cellular architecture [86-87].

In these current conditions, biological specimens or tissue samples are not available. The serological tests can be separated into twice, antigen-detection and antibody-detection tests [18]. In another way, like the hemagglutination (HA) test, direct and indirect immunofluorescent antibody tests, complementation complex, and lastly immunoblotting and rapid diagnostic tests (RDTs) [18]. It becomes very important to identify the parasites from blood smears. Lastly, these serological tests are available for monitoring parasitic diseases for future therapy purposes [18].

Molecular-based approaches

Nucleic Acid-Based Approaches

There will be several limitations to microscopy and serology-based assays predisposed by parasitologists. Besides the PCR, the usage of such new tools as real-time PCR (RT-PCR) for the exposure of numerous parasitic detections. Such new processes as LAMP and Luminex-based assays have also appeared as a newer approach for the identification of parasites. The multiplexed PCR analysis is useful for multiple sequences in the same reaction tube that is needed for several parasitic infections [88]. In the detection of *Cryptosporidium* and *Giardia*, the nucleic acid-based diagnosis was applicable [89].

PCR

The PCR-based process is selective amplification from complex genomes. This happened by denaturing a double-stranded genomic DNA procedure that uses heat. In temperature lowering, primers can anneal to their complementary base sequences. Thus, the extended DNA template trails in both orders from the primer site using enzymatic catalysis with a thermostable DNA polymerase, producing double-stranded products [19, 31].

PCR-based processes are widely used for the diagnosis of diseases in the field of veterinary parasitology [88]. The PCR-based technique is also combined with RFLP or nested PCR for genotyping of organisms. The understanding process in PCR is higher than in light microscopy, and it is also needed for detecting a very low amount of parasites [24]. The PCR-based method is also useful for non-intestinal parasites. Here, we identify species-level analysis [90-98].

There are few consequences, this process is very time-consuming and provides lesser data [99]. In the advanced level study, we need quantitative real-time PCR analysis [27, 99]. In these methods, we easily detect causative pathogens, genetic regulation and expression, allelic function etc. and also get several useful data from these applications [99].

In 18S rRNA-based detection was performed for the diagnosis of *Ichthyobodo* spp., highly variable regions of the 18S rRNA gene were sequenced by a parasitic protozoan of *Cryptocaryon irritans* for their molecular identification. As per available literature [100], monogenean (*Dactylogyrus* spp.) and digenean (*Centrocestus formosanus*) parasites were detected with the help of the 18S rRNA gene.

ITS

The internal transcribed spacer (ITS) is a region of the polycistronic rRNA precursor transcript that is transcribed between the small- and large-subunit ribosomal RNA (rRNA) genes on a chromosome. An earlier study stated an ascaridoid nematode confirmatory test was performed using this method [101].

RT-PCR

This quantitative process requires original template strands by using several fluorescent chemicals like-Sybergreen, Scorpion primers, and fluorescence resonance energy transfer (FRET). The measurement of concentration concluded with a standard curve. At the time of multiplexed RT-PCR, several gene sequences were in one locked tube reaction. Using RT-PCR is useful for quantifying parasitic nucleic acids from different samples, and also estimating the prevalence of parasitic viability [19, 31]. Based on RT-PCR, the identification of *Giardia lamblia*, *Dientamoeba fragilis*, *Blastocystis hominis*, and *Entamoeba histolytica* [102], and the molecular study was performed with the help of RT-PCR detection of canine leishmaniasis [103].

LAMP

Loop-mediated isothermal amplification (LAMP) is a

newer concept for the extension method with highly exact, and sensitive to being able to differentiate between single nucleotide changes [25]. It is identified as six different primers that were famously designed in eight different portions on a specific target gene, and amplification occurs only in specific primers which bind with their products [104]. It is a method that can intensify smaller replicas of genetic elements like 10^9 in less than an hour [104]. LAMP is a DNA amplification-based method and it needs a highly specific and sensitive process. Amplification of DNA is achieved by using a hot water bath for these isothermal conditions [105]. Here, a large amount of whitish magnesium pyrophosphate precipitated from DNA has been observed [106]. The LAMP method can produce 20 µg of DNA for 25 µL of the reaction combination in a single hour [106]. LAMP had more specificity and sensitivity than nested PCR, similar output has been observed but at a faster rate. Their results are static with other demonstrations that rapidly improved specificity and sensitivity observed using the LAMP assay [19]. By using this process, detection of *Toxoplasma gondii* from blood samples of stray dogs, and cats [107]. Cestodan parasite names like *Raillietina echinobothrida*, *Raillietina tetragona*, and *Raillietina cesticillus* were identified by using the LAMP technique [108].

Luminex

Luminex is a new method that used a bead-based xMAP (multi-analyte profiling), that conjugates fluorescent microspheres (beads) lasers, flow cytometry, and digital signal processing, and it instantaneously measures 100 different analytes in only a sample (Luminex Corporation 2014). The microspheres are covalently connected with antigens, antibodies or oligonucleotides which serve as probes in the assay [32]. Several DNA tests have been developed in the field of Luminex platform in the years for the identification and genotyping of bacteria, viruses and fungi such as *Escherichia coli*, *Mycobacterium*, *Trichosporon*, *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Candida* spp. [32, 106, 109-110]. After having been changed for parasitological surveys, recently showed the same reaction using a very minute volume. This technique is useful in the study of antigenic variety for the field of diagnosis of parasitic diseases [110].

An earlier study stated [111] that Luminex beads were useful against the detection of "Antibodies and Proteins" in diagnosing *Plasmodium falciparum*.

RAPD

It is known as AP-PCR (arbitrarily primed PCR), and RAPD has been widely used for the explanation of strains in epidemiological studies. The surveying of parasites of genomes is improved by the benefit of RAPD is an easy, fast, and reasonable procedure that does not entail prime knowledge of the DNA sequence or DNA hybridization [112]. In the case of different genotyping from RAPD makes of random amplification of the genome. The fragments are differentiated by gel electrophoresis and the observed banding pattern is useful in genomic sketching. In this method, primers generally amplify ten bases in a PCR protocol with minute selectivity, primer anneals numerous homologous sites of the genomic portions, and finally generated large portions of DNA fragments by sequential amplification [113].

RAPD is the molecular technique that helps in delineating the strains of microorganisms. It is also useful for parasitic nematodes of plants and humans signifies its high efficacy in the amplification profiles, and is also applicable for the differentiation of polymorphisms between several microorganisms [114-115]. It is mainly used for gene mapping for the depiction of the species, enhances genetic changeability, determines the genetic structure and pinpoints polymorphs in the noncoding genomic parts among the populations of the different microorganisms [116]. By using this process, they identify the genetic structures of *Toxocara* sp. from canine, ruminant, and feline organisms [117].

AFLP

AFLP detects a DNA polymorphism from very low information on the genomic sequences. It is very effective for a large number of band detection and also provides large coverage of the genome [118]. It divides into four steps - DNA ingestion, ligation, amplification, and gel analysis. Polymorphisms are characterized based on the presence or absence of DNA portions in the polyacrylamide gel electrophoresis [29]. The merit of this process is to analyze the entire genome for polymorphisms against parasites which is no prior genetic information [29, 118-119].

RFLP

It is the most widely used molecular method, that helps in the diagnosis of genotypes of the parasites such as *Toxoplasma gondii* [30]. This process is useful to detect different variations at the genomic DNA

Diagnosis of parasitic diseases in fish

Ectoparasites

S no.	Name of parasite	Host	Infected organ	Reference	Treatment	Molecular study
1.	*Neoparamoeba* sp.	Atlantic salmon, seabass, ballan wrasse, and salmon salar	Gill, gill, gill, and gill.	[126-129].	Hydrogen peroxide (H_2O_2) bath [130].	Table. 2
2.	*Amyloodinium* sp.	Sea bass	Blood	[130].	Copper sulphate ($CuSO_4$) treatment; hydrogen peroxide (H_2O_2) [128].	Table. 2
3.	*Chilodonella* sp.	*Carassius auratus* (Linnaeus, 1758), and *Lates calcarifer* (Bloch, 1790)	Gill, gill, and skin	[73].	Formalin baths, potassium permanganate ($KMnO_4$), sodium chloride (NaCl), hydrogen peroxide (H_2O_2), and copper sulphate ($CuSO_4$) [73, 131].	Table. 2
4.	*Ichthyophthirius multifiliis*	*Pangasianodon hypophthalmus* (Sauvage, 1878).	Skin	[1].	Herbal medicine (Turmeric oil) [1].	Table. 2
5.	*Trichodina* sp.		Epithelial cells	[132].	Peracetic acid [133].	Table. 2
6.	*Myxobolus* sp.		Gill lamellae	[134].	Kutuklin, and diflubenzuron [135].	Table. 2
7.	*Lernaea* sp.	Smallmouth bass	Gill	[136].	Emamectin benzoate [137].	Table. 2
8.	*Caligus* sp.	*Trachurus picturatus* (Bowdich, 1825)	Gill lamellae	[136].	Emamectin benzoate [137].	Table. 2
9.	*Argulus* sp.	*Carassius auratus* (Linnaeus, 1758)	Skin	[138].	Extract of *Azadirachta indica* [139].	Table. 2

Endoparasites

S no.	Name of parasite	Host	Infected organ	Reference	Treatment	Molecular study
1.	*Trypanosoma* sp.	Cyprinids (Cruz and Eiras 1997; Poynton and Sterud 2001)	Blood system	[140].	Pentamidine, suramin sulphate, melarsoprol, and nifurtimox [141].	Table. 2
2.	*Eimeria* sp.	Common carp, and crucian carp.	Intestine	[142].	Toltrazuril [142].	Table. 2
3.	*Henneguya* sp.	*Ictalurus punctatus* (Rafinesque, 1818)	Gill lamellae	[143].	Fumagillin and toltrazuril [144].	Table. 2
4.	*Dactylogyrus* sp.	*Carassius auratus* (Linnaeus, 1758)	Gill	[145].	Coumarin extract [145].	Table. 2
5.	*Gyrodactylus* sp.	*Atherina boyeri* (Risso, 1810)	Skin, and gill	[146].	Isoimperatorin [145].	Table. 2

level [120]. This method depends only on the PCR products by restriction enzymes or endonucleases. Due to these enzymatic reactions, DNA gets fragmented into several pieces, which will help at the time of agarose or polyacrylamide gel electrophoresis identification [121]. It is suitable for environmental samples because capable of detecting multiple genotyping in the same genera sample [28].

As per this process utilization, molecular-level identification of *Theileria* sp. diagnosis was performed [122]. In an earlier study [123], *Hymenolepis nana* and *Hymenolepis diminuta* diagnosis were carried out by using this technique.

Microsatellites

Microsatellites are short sequences of DNA (around 300 bps) consisting of tandem repetition of 1-6 nucleotides, and around 100 replicas [124]. Microsatellites are new tools in the field of parasitology. In human and animals parasitic studies it is also applicable [33]. It is predominant in eukaryotic genomes and mutates randomly which loss or gain of function [121]. It is also helpful for parasitic nematodes like *Trichostrongyloid* [33]. With the help of this process, detection and diagnosis

were performed for leishmanial parasites from an asymptomatic canine host [125].

Table. 2 Molecular study for parasite identification

Name of parasites	Molecular tools	Reference
Neoparamoeba sp.	18S rRNA	[147].
Amyloodinium sp.	18S rDNA and 28S rDNA	[148].
Chilodonella sp.	ITS, LSU rDNA gene, and mtSSU	[149-150].
Ichthyophthirius multifiliis	18S rRNA	[1].
Trichodina sp.	18S rDNA, Euk A and Euk B of SSUrRNA	[151-153].
Myxobolus sp.	18S rRNA	[154-156].
Trypanosoma sp.	TBR-1/2, and 18S rRNA	[157-158].
Eimeria sp.	SSU rRNA	[156, 159].
Bucephalus sp.	ITS2, and cox1	[162].
Aporocotylidae (*Seriola dumerili*)	18S, 28S rDNA, and ITS	[163-165].
Atractolytocestus sp.	COX1, and ITS2	[166].
Diphillobothrium sp.	COX1	[167].
Bothriocephalus sp.	28S rDNA, and COX1	[168].
Ligula sp.	COI	[169].
Anisakis sp.	COX1, and ITS-RFLP	[170-172].
Philometroides sp.	18S and 28S rDNA	[173].
Dracunculus sp.	COX1	[174].
Acanthocephala	18S and 28S rRNA	[175-176].
Lernaea sp.	18S rRNA, 28S rRNA, and COX1	[177].
Caligus sp.	18S rRNA, and 18S rDNA	[178].
Argulus sp.	D1-D2 of the 28S rDNA, and 18S rRNA	[179].

Conclusion

The aquatic parasite is comprised of subkingdom protozoa, phylum Cnidaria, phylum Platyhelminthes, phylum Nematoda, phylum Acanthocephala, phylum Annelida, and phylum Arthropoda. Routine parasitological detection is based on light microscopy of parasitic identification. The first step of parasitic identifications are based on wet mount methods that help in the identification of intracellular parasites. Histopathological changes can be diagnosed with the help of microscopy. Molecular biology methods are used to detect parasitic diseases, enhancing the diagnosis of parasitic clarities. The developmental stage is highly-sensitive to parasitic infections useful for monitoring and control for the pathogen in cultured and ornamental fish.

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Conflict of interest

The authors declare that there is no conflict of interest.

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