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Significance of Diagnosis in Efficient Disease Control in Aquaculture and Fisheries

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ABSTRACT

Aquaculture is an important and rapidly growing component of agriculture worldwide and contributing 44 % of total fish production globally. Diagnosis is an indicator or marker given for a disease condition to identify by its signs, symptoms, and from the various diagnostic procedures. The correct identification will further aid in accurate disease diagnostics, proper treatment, and medication. As also performed in human diseases, the diagnosis information and case history, clinical signs should be carefully used while probing samples for the diagnosis can also be useful in aquaculture systems. The most significant approach to decrease or prevent the losses due to diseases is early disease diagnosis, monitoring at regular intervals, and proper action at the first sign at any unfavorable condition. The diagnostic tools and techniques are gaining greater importance in aquaculture diseases due to their specificity, sensitivity, speed and correctness. Effective disease control is the outcome of early diagnosis; afterwards the control and management will be implemented. This strategic manner will be more effective to prevent spread of individual disease to become a disease outbreak.

Keywords: *Aquaculture, Fisheries, Microscopy, Molecular diagnostic, Diseases diagnosis*

1. INTRODUCTION

Aquaculture and fisheries is an important and rapidly growing component of agriculture worldwide and contributing 44 % of total fish production globally (Assefa, & Abunna, 2018). In spite of many difficulties and challenges, the increased growth of production is achieved in aquaculture. Among many challenges, infectious diseases act as a major barrier to increased production and are responsible for major losses annually (Reid, et al., 2019). To minimize aquatic diseases, early disease diagnoses are important with scientifically proven and recommended ways (Bondad-Reantaso et al 2005). Diagnosis is an indicator or marker given for a disease condition to identify by its signs, symptoms, and from the various diagnostic procedures. Infectious diseases are one of the greatest threats to the success of aquaculture and fisheries, with reduced yield quality and quantity due to disease increasing production costs for farmers. With the intensification of aquaculture systems, disease becomes a major threat, ultimately reducing production (Diana et. al., 2013). Diagnosing fish diseases is a key task in smart aquaculture, playing a key role in detecting fish diseases, understanding disease signs, and improving fish welfare and health (Li, Daoliang et. al., 2022). Early and accurate diagnosis of infectious diseases is very important for the following reasons: Diagnosis improves the effectiveness of treatment and avoids long-term complications for infected people. It is directly related to the total production of the aquaculture and fisheries sectors.

The external signs and symptoms are helpful in defining the diagnostic criteria of diseases. Disease diagnosis is referred to as the set of procedures and techniques for indicating the nature of the disease and helps to exactly isolate the primary and secondary pathogens responsible for a particular disease. The correct identification will further aid in accurate disease diagnostics, proper treatment, and medication. (Noga, 2010). As also performed in human diseases, the diagnosis information, case history, and clinical signs should be carefully used while probing samples for the diagnosis can also be useful in aquaculture systems (Cunningham, 2002). This paper briefly describes the significance of early and effective diseases diagnosis in the field of aquaculture and fisheries.

2. LITERATURE REVIEW

Molecular techniques for detection of fish pathogens are reviewed by Altinok and Kurt, 2003 and the potential for their application are discussed in this review paper they only focused on polymerase chain reaction (PCR), restriction enzyme digestion, probe hybridization, in situ hybridization, and microarray but other diagnosis methods were not discussed in this review paper. MacAulay et. al., 2022 reviewed on moving towards improved surveillance and earlier diagnosis of aquatic pathogens: From traditional methods to emerging technologies. They farmed their review on comparison of current surveillance and diagnostic technological development for use in the aquatic environment, against three gold standard ideals of high sensitivity, specificity, rapid diagnosis, and cost-effectiveness but he did not reviewed all the existing technologies used worldwide in aquaculture and fisheries.

Khati et. al., 2015 focuses on factors that cause disease to the aquatic environment and the recent trends and techniques of disease surveillance, monitoring, treatment and control in fishes which will help the fish farmers in reducing risks arising due to occurrence of disease and maintaining disease free aquaculture and sustainable production but they did not explained all the techniques used in aquaculture and fisheries rather than only focused on fish diseases.

Shefat and Karim, 2018 conducted a review on the significance, underlying causes and negative effects of nutritional diseases of fish on aquaculture production and health safety. This review will be helpful to provide basic knowledge on nutritional diseases management in aquaculture and to raise awareness among the farmers fisheries management. However they did not focused on other areas of diseases diagnosis rather than only focused on nutritional diseases of fishes. Assefa and Abunna, 2018 had been reviewed on the maintenance of fish Health in aquaculture although rather than trying to treat every disease case, this review advised to follow a preventive approach before the event of any disease outbreaks.

The significance and application of new techniques as a routine tool in a diagnostic laboratory and awareness of these tools among aquaculturist and local fish farmers is an area where relevant literature is scarce and this may contribute to restrain to the farmers to adopt these methods. This review will give a better understanding about the significance of diagnosis in efficient disease control in aquaculture and fisheries.

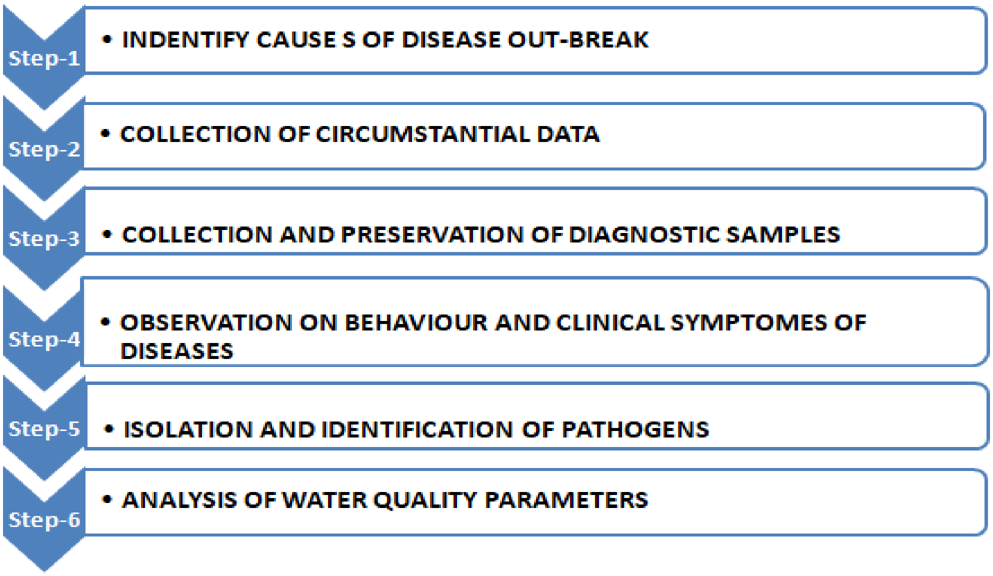

Figure 1: Steps of Diseases Diagnosis in Aquaculture and Fisheries

3. STEPS OF DISEASE DIAGNOSIS IN AQUACULTURE AND FISHERIES (Figure 1): Aquaculture diseases are one of the most important factors for sustainable aquaculture management. The diseases both infectious and noninfectious are common in aquaculture systems (Raja Et al., 2015). To control these diseases it is specifically required to do stepwise diagnosis and further control and management of both types of diseases. There are some basic steps to be followed in aquaculture system such as causes of diseases outbreak; circumstantial data which is required to collect information regarding affected area, Collection and preservation of diagnostic samples (bacteria, Virus, fungus, parasites and histopathological observations etc.) (Table 1); observations on behavioral and clinical symptoms of diseased sample which includes patho-morphological examinations; Patho-anatomical examinations and hematological examinations (Table 2); isolation and identification of pathogens which may include bacteriological examination, virological, parasitological, fungal pathogens and histopathological examination (Table 3); when the diseases outbreak is caused in ponds and tanks or any other systems like RAS and Biofloc so it is necessary to analyze water sample and the value of its parameters i.e. CO_2 , pH, alkalinity, turbidity, ammonia & nitrate etc. and examine whether these values are within the permissible limits or not (Anderson et, al., 1991; Ozbay et. al., 2014; Noga, IES

2010; Abdelsalam et. al., 2023).

Table 1: Different Pathogens and their preservation methods

Pathogen Type	Preservation methods
1. BACTERIA, VIRUS AND FUNGUS	Chilling or Freezing
2. PARASITE	10% formalin
3. FOR HISTOPATHOLOGIAL STUDIES	Proper fixative like 10% neutral buffered formalin

Table 2: Types of examination and organs examined

Types of Examinations	Organs Examined
1. PATHOMORPHOLOGICAL EXAMINATION	▪Physical Appearance: healthy, stunted, emaciated or sick ▪Visible Signs on Body, Pigmentation, visible signs on the body like hacmorrhages, patches etc ▪Gills, skin, fins examined foe presence of pathogen and errosion/patches/haemorrhages etc.
2. PATHOANATOMICAL EXAMINATION	▪ Viscera should be examined i.e. organs like liver, spleen, kidney, gut, pancreases, air bladder examined for any abnormality in their colour and consisting the presence of any haemorrhages, lessions/ inflamations etc. ▪ Squash preparation of these organs for the presence of parasites/bacteria/fungus etc.
3. HAEMATOLOGICAL EXAMINATION	Haematological parameter from live specimens such as haematocrit values differencial count, haemoglobin, total RBC and WBC count, ESR etc.

Table 3: Types of samples isolated and methods of isolation of different samples

Isolated SampleType	Method of isolation
1. BACTERIOLOGICAL	▪Swabs from different organs should be inoculated in suitable media under aseptic condition.. ▪Later isolation, identification and characterization of the bacterial agents involved
2. VIROLOGICAL	▪ Sample of kidney, liver, spleen and gonads from diseased fishes cell line and their cytopathic effect CPE etc. ▪ Detection of virus can be made by electron microscopy of the infected tissue
3. PARASITOLOGICAL STUDIES	▪ Sample was collected from diseased fish and should be preserved in 10% formalin. ▪ Sample for parasites should be taken from the live fish only, because many parasites leave the host where they die.
4. FUNGAL PATHOGENES	▪ The disease specimen and should be cultured and isolated in pure form in suitable media. ▪ If immediate examination is not possible then they should be preserved in proper fixative 10% formalin
5.HISTOPATHOLOGICAL EXAMINATION	▪3-4mm thick sections diseased fishes organs are collected and fix in 10% neutral buffered formalin for 18-24hrs. ▪Treatments through grades of alcohol cleaning by xylol ▪Wax impregnated tissue are then cut into thin sections (5-7 meu)

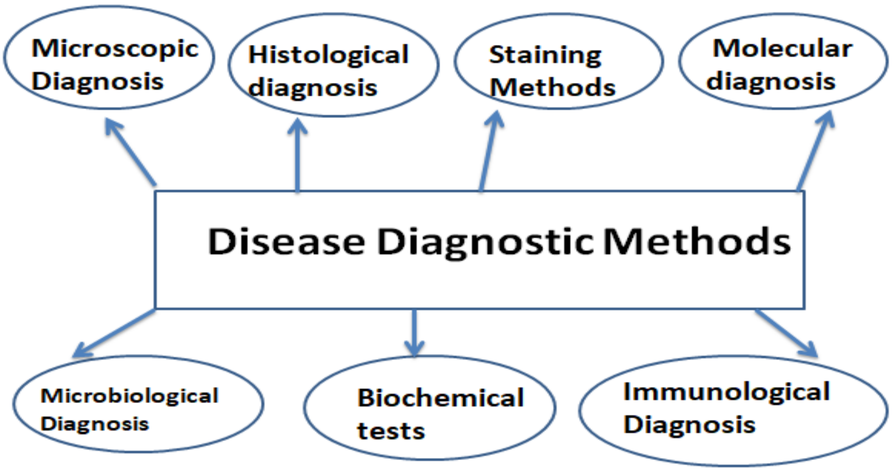

Figure2: Various Diseases Diagnostic Methods in Aquaculture and Fisheries

4. CLASSIFICATION OF DISEASE DIAGNOSTICS METHODS (FIGURE 2):

4.1. Microscopic Diagnosis: Microscopes are very important, quickest and a basic diagnostic key for aquaculture to distinguish between a water quality and a parasite problem (Li et al 2002). But the accuracy varies according to the handling of equipment and experience of the microscopist.(Rurangwa et al 2004). Stains used in sample preparation make the process easy and little simple to diagnose (Frickmann et al 2017).

Generally light microscopy is used to diagnose histological samples, blood samples, and squash of organ samples and stained microorganism (Kent et al 2020). Electron microscopy is another very efficient and reliable tool for diagnosis of diseases sample for observing the changes in the tissues with the help of transmission electron microscopy and surface level changes of tissues by scanning electron microscopy at higher magnifications (Nishiyama et al 2010).

4.2. Histological diagnosis: To analyze the structure and function of stained tissue sections histopatholgical examination is used **to** be done (Belsare et al 2012). It also helps in analysis of the alteration in the tissues due to the influence of diseases and pathogens invasion (Locke et al 2014). Histology plays an important role in diseases diagnosis. Stained smears of tissues are examined under light microscope and changes noted during the examination will aided as a helping tool to segregate, diagnosed and described infectious and non-infectious diseases in aquaculture (Soto et al 2019).

4.3. Staining Methods: Staining **of** Histological tissue components are clearer to examine and further diagnose (Wolf et al 2018). Haematoxylin and eosin (H&E) are most commonly used for wide ranging histological staining (Bancroft et al 2012). Gram staining is another staining method used to stain microorganisms. Immuno histo chemistry methods have been developed for diagnosis of viral diseases like pancreatic necrosis virus (IPNV), infectious salmon anaemia virus (ISAV) in paraffin embedded tissue sections (Olsen et al 2015). Histological techniques contribute towards the diagnosis of tissue pathology and emphasized the sequential changes and progressive stages of diseases development of infectious and noninfectious diseases (Roberts 2012). With the help of examining stained sections, bacteria, viruses, fungi and parasites, one can be easily diagnose diseases (Lakshman 2019). The improved technique of image analysis tools by FRS allows quantitative data generation to supplement diseases diagnosis (Bruno et al 2006).

4.4. Microbiological Diagnosis: The microbiological techniques especially bacterial diseases can be identified by various microbial diseases (Frickmann et al 2017). The samples are taken under aseptic condition and grown in selective media. Various microbiological diagnostic methods are used to identify in microbial disease diagnosis like staining methods, motility test, culture in selective media and biochemical tests (Markey et al 2013).

The staining methods used in bacterial pathogens diagnosis are gram stain, acid fast stains, fluorescent stains, colloidal carbon stains, Wrights and Giemsa stains, trichrome stain (Gomori-Wheatley stain) and iron hematoxylin stain. Motility test is used to diagnose the motility of bacteria and they are mainly of three types hanging drop method, semi solid agar method and three coverslip method (Yaashikaa et al 2016). Culture of bacteria in selective media is used to identify particular kind of bacterial diseases (Declercq et al 2013).

4.5. Biochemical tests: Various biochemical tests are used for the identification of bacteria based on the character of particular bacteria to give its positive and negative result for a particular biochemical test (Gram et al 2002). There are number of bacterial test used in diagnosis of aquatic organism diseases such as Indola test, Methyl Red and Voges-Proskauer 4

Tests, Catalase Test, The Citrate Test, Oxidase Test, **Nitrate Reduction Test,** Urease test, Phenol Red Broth, Casease Test, Gelatinase Test, Lipase Test, Starch Hydrolysis, Triple Sugar Iron Agar, Decarboxylation Test, Coagulase Test (Velmurugan et al 2015).

4.6. Immunological Diagnosis: These tests are helpful in farm level management without the presence of instruments instead of microscopic techniques (Francis-Floyd 2011). It helps in the qualitative and quantitative estimation of pathogens. The morphological and visual estimation require more experience as the morphology of the animals change according to their growth and development (Dingemanse et al 2009). Hence the antibody based immunodiagnostic techniques plays a major role in aquaculture (Adams 2009).

There are number of advantages of this method of diagnostic over other traditional methods that it can easily indentify suppressed and sub clinical stages and can able to recognize antigen mediated differences (Adams, & Thompson, 2011). They give rapid results with exact diagnosis. Moreover conventional immunodiagnostic methods will also result in the growth of monoclonal antibodies based assays, it will useful to diagnose pathogenesis of diseases with more accuracy (Mialhe et al 1995). There are series of monoclonal antibody-mediated methods which are useful in the diagnosis of various pathogens responsible for spread of disease in aquatic organism (Trust, 1986). There are various tests with specific characteristics of diagnosis such as Agar Gel precipitation test, Agglutination test, ELISA, Dot ELISA, Latex Agglutination Test and Fluorescent (El Deen et al 2018).

4.7. Molecular diagnosis: Molecular diagnosis tools are very significant in correct diagnosis of fish diseases (Toranzo et al 2005). Complete genome sequencing of aquatic pathogens has increasingly gaining great advances and aids as a relevant tool to control pathogens and also increase chances of correct diagnosis (Waiho et al 2021). Relationships between genotypic and phenotypic sequences, offers to diagnose changes in nucleic acid sequences, polymorphism, specific, sensitive and fastest analysis (Altinok & Kurt 2003). The advancement in this technique will further help in diagnose exact epidemiological causes of diseases outbreaks or presence of a specific diseases or pathogen (Georgiadis, et al 2001). The numbers of methods are contributed in molecular diagnosis of diseases like Polymerase Chain Reaction (PCR), Multiplex PCR, labeling and detection of nucleic acids, Restricted enzyme digestion, Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), In Situ Hybridization, DNA microarray, Loop Mediated Isothermal Amplification (LAMP) (Bej, et al 1991). Molecular methods are more reliable and faster than serological, cultural, histological methods for identifying fish diseases (Cunningham 2002). The major advantages of molecular methods are identification of noncultural pathogenic agents, amplification of DNA, to diagnose latent infection, can differentiate anti-genetically similar pathogen and also to handle smaller amount of specimen (Lunger, & Clark, 1978). There are some disadvantages of this method which are higher cost in comparison to other methods and also have difficulty in diagnosing new pathogens due to deficient molecular data (Stefani, 2009).

4.8 *Advantages of early diagnosis of various aquaculture and fisheries diseases*

Aquatic animals belong to complex and dynamic environmental conditions so they require specific attention in order to disease diagnostic and health monitoring (Raja & Jithendran 2015). Their feeding habits and the death rate is also not clear due to underwater state (Bondad-Reantaso et al. 2005). The problems and consequences faced by aquatic animals are also based on individual species and their particular system-specific (Huntingford, et al 2006). The major problem related to disease diagnostic is the wide range of diseases found in aquaculture because aquaculture diseases are directly related to the host, the pathogen, and the environment along with its components such as oxygen, pH, temperature, toxins, wastes, and the quality of management such as handling, drug, treatments, transport, procedures, etc. (Mishra, 2017; Snieszko 1974).

Diseases may be occurred due to a single or multiple factors and broadly classifies into infectious such as virus, bacteria, fungi, parasites and non-infectious diseases generally caused due to environmental stresses, genetic factors, and nutritional deficiencies (Saravanan, et al 2013). The most significant approach to decrease or prevent the losses due to diseases is early disease diagnosis, monitoring at regular intervals, and proper action at the first sign at any unfavorable condition (Wise, et al 2004).

4.9 *Importance of case history in aquaculture and fisheries disease diagnosis*

Case history detailed information is very significant and vital for correct disease diagnosis and for proper remedial measures (Plumb, & Hanson, 2010). At the time of diseases outbreak, the cautions and inspection of the case history will help in correct detection and pinpointing the conditions under which the disease has developed (Noga 2010). The case histories related to water qualities, feeding habits and conditions, feed intake ratio, fertilization rate and schedule, time last microbial attack, liming schedule, treatment and medication details and sources of seed and stocking densities information and record are some helpful and significant past information which may lead to a better future and also help in early disease detection (Rath 2018).

4.10 *Importance of surveillance in aquaculture and fisheries diseases diagnosis*

The aquatic diseases diagnosis and aquatic organism health plan and policy making is not easy due to deficient health data and surveillance system (Bondad-Reantaso et. al., 2005). This data will further be use in diseases diagnosis, diseases control, quarantine of organism and health certification. Surveillance of diseases will ensure the undiagnosed disease incidences and further implement in controlling strategies before the diseases will become widespread among aquatic organism (Sofos, 2008). There are two types of surveillance which are widely used named as active surveillance which require surveys and passive surveillance which require using data collected for other various purposes from laboratories, field visits, research projects, farmers and from aqua-culturists (Hadorn & STäRk, 2008).

4.11. *Necessity of new approaches in rapid diagnosis and disease control*

A unanimous and single approach in diseases diagnosis, prevention and control is not successful rather than combining multiple approaches of different strategies are more appealing in the aquaculture diseases diagnosis (Wall, 2019). The information and diagnostic methods including various general and specific disease diagnostic methods and tests will be valuable toolkit for rapid and accurate diagnosis (Johansen et. al., 2010). There is also requirement to frame a better methodology for day to day data collection and exposure of the various diagnosis incidences and its improved impact on socio -economic aspect of various associated population.

4.12. *Challenges in aquaculture and fisheries diseases diagnosis*

Diseases diagnosis in aquaculture is more complex than terrestrial animal disease control due to aquatic environmental conditions that accelerate rapid diseases transmission (Harvell et. al., 1999). In the terrestrial animals diagnosis of diseases is simple due to the individual animal will be depicted and diagnosed but in aquaculture it becomes difficult due to nature of water as it transmit diseases faster from one to another individual (Cameron, 2002). It is the matter of hours to days the aquatic diseases spread from one to another organism. Moreover for diagnosis samples will be collected not only from the aquatic organism but also from the water bodies in which the organism live to determine the important water characteristics like pH, turbidity etc (Stumm and Morgan, 201

4.13. *Current approach and development in aquaculture and fisheries diseases diagnosis and control*

A detailed understanding of the diseases triangle that clearly depicts the association between host, pathogen and environment is very important to diagnose aquaculture diseases (Johnson and Paull, 2011). Since the aquaculture diseases is the outcome of a long chain of incidences so the control methods should go beyond the boundaries of individual pathogen (Flegel, 2019). Conventional and traditional approaches have gain less success to prevent aquatic diseases. Recent approaches trying to control diseases outbreak very prominently reveal the importance of linkage and diseases triangle factors (Host, Pathogen and Environment) with various components of the production system (Oleckno, 2008). Above and beyond the ecosystem management approaches to prevent against the entry of pathogens and farm level deterioration System Level Approach (SMA) is needed for aquatic animals health management (Subasinghe et. al., 1998). Large level acceptance of such advances between the farmers and aqua-culturist will be better sustainable solution for coming days rather than using drugs and other chemicals for control of diseases as they will kill the pathogen but will not work on the root cause of diseases (Mishra et. al., 2017).

The cooperation and collaboration between developing and developed countries over the years played a major role in combating aquaculture diseases and its spread. But the scope for further improvement is also there for developing new, innovative and cost effective tools to diagnose and control aquatic diseases (Stickney, 2016; Picardeau et. al., 2014). Some efforts like quality control and cost effective approach towards feed, seed and water, to provide good nutrition, to recognizing host specific and non-host specific immunity and defense system for disease control, development of affordable and effective vaccines to prevent outbreaks, use of immune stimulants and non-specific immune enhancers to reduce diseases venerability, use of probiotics to improve aquactic environmental quality (Naiel,2022). The prospective of commercial and cost-effective and universally acceptable, however effective and approachable and rapid diagnostic tools for developing countries should be increased by the joint ventures between developed and developing countries for early and rapid diseases diagnosis in aquaculture sector (Raffle et. al., 2019).

5. FUTURE PROSPECTS

Monitoring of syndromic aquatic diseases by farmers presents a real opportunity to overcome the hurdles associated with traditional laboratory-based monitoring in aquaculture. The use of unmanned autonomous drones and robots (e.g. SeaDrone, Shoal, OpenROV, OceanOne, Deep Trekker, Aquabotix, Apium Swarm Robotics, Blueeye Pioneer, PowerRay) for underwater exploration/operations and integration with data systems have demonstrated the potential for surveillance ecosystems and intensive cage surveillance. Similarly, integrating augmented reality into data mining and machine learning frameworks via wearable devices (such as Google Glass and Microsoft HoloLens), data collection suits, and virtual workspaces (such as PondVis) can improve data collection, analysis, and task performance. Finally, stakeholder engagement, long-term goals and roadmaps for implementation, and interdisciplinary collaboration among engineers, fish biologists, oceanographers, and data scientists are essential to develop reliable data mining and machine learning frameworks that support sustainable growth in supporting global seafood production. Infectious diseases make up the largest share of these challenges, causing billions of dollars in losses each year. Therefore, problem-planning prevention and control strategies based on globally accepted principles and locally applicable strategies are recommended. These strategies should focus on preventing the development of infectious diseases rather than treating diseased swarms.

6. CONCLUSION

Daily screening of aquatic organism behavior and feeding motions allocate rapid diagnosis of diseases. The early diagnosis will prevent most of the population to become diseased and also help sick organism to be diagnosed and treat quickly. The organism will be recovered early if the diagnosis and treatment will be successful and early implemented. The diagnosis of aquaculture diseases is equally valued as the planning and control strategies and broad level acceptance of principles and local practices followed after correct diagnosis. Diagnostic methods in aquaculture systems became advanced right from microscopic examination and morphological characterization to molecular analysis and probe-based diagnosis. Molecular markers are gaining greater significance in aquaculture diseases along with complete genome sequencing of pathogens which are helpful in improving diagnosis and further management and control of pathogens. The diagnostic tools and techniques are gaining greater importance in aquaculture disease diagnosis due to their specificity, sensitivity, speed and correctness. Effective disease control is the outcome of early diagnosis; afterwards the control and management will be implemented. The lack of knowledge about diseases and its early signs and symptoms among the farmers will converted into greater losses in aquaculture and fisheries sectors and rapidly reduce overall production in this sector. This problem will effectively be overcome by early diseases diagnosis and after treatment. Preventing and controlling measure both play equal role in efficient controlling of aquatic diseases. Although human medicine receives more funding for research into new diagnostic methods, there is always an opportunity to transfer these methods to the aquatic environment if industry and researchers take the time to adapt.

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