International Journal of Agriculture, Environment and Bioresearch

Vol. 06, No. 06; 2021

ISSN: 2456-8643

MOLECULAR METHODS FOR DIAGNOSIS OF HELMINTH PARASITES OF FRESHWATER FISHES IN NIGERIA: A REVIEW

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https://doi.org/10.35410/IJAEB.2021.5681

ABSTRACT

Molecular techniques are potentially faster and more sensitive than culture, serology, and histology methods that are traditionally used to identify fish parasites. Parasitic disease reduces fish production by affecting the normal physiology of the fish and if left uncontrolled, could result to mass mortalities or in some cases could serve as a source of infection for human and other vertebrates that consume it. These parasites could be Ecto-parasites or Endo-parasites. The protozoans and helminthes are reported to be the major group of parasites of fish involved in parasitism in Nigeria. These techniques include polymerase chain reaction (PCR), multiplex PCR, restriction enzyme digestion, DNA micro-arrays, Loop mediated isothermal amplification (LAMP), and Nested PCR. Helminth parasites can be detected from asymptomatic fish by molecular diagnosis techniques, so disease outbreak could be prevented. In this paper, molecular techniques for the detection of fish parasites are reviewed and the potentials for its application are as well discussed. Thus, the application of new techniques as a routine tool in a diagnostic laboratory in areas where relevant literatures are scarce, will contribute to the reticence of adoption of these methods. It is hoped that knowing more about the genome of helminthes could provide useful insight into their biology. Knowing how they function and cause disease is expected to lead to new, more effective ways of treating these infections.

Keywords: Molecular characterization, helminthes, molecular diagnosis, Clarias gariepinus, fish diseases.

1. INTRODUCTION

The African catfish (*Clarias gariepinus*) which belongs to the family *"Clariidae"* is the most farmed fish species in Nigeria. This is attributed to several advantageous characteristics exhibited by these species such as its ability to tolerate varying range of environmental conditions, fast growth rate, high stocking densities under culture conditions, high disease resistance, acceptability of formulated feed, high fecundity, ease of artificial breeding, and high market value (Eyo *et al.*, 2014).

Fishes are host to taxonomically diverse parasites and infections can significantly affect fish production through direct mortality, nutrient devaluation, alteration of biology and behaviour, lowering of immune capability, growth and fecundity reduction and sometimes mechanical injuries which depends on the species of parasites, intensity of infestation and depth of parasite penetration with the host tissue (Morenikeji *et al*., 2009; Lagrue *et al*., 2011 and Nimbalkar *et al*., 2015). Parasitic community, diversity and burden may also be determined by seasonal fluctuation, geographic location, feeding habit, host age, size and sex (Goselle *et al*., 2008 and Nimbalkar *et al*., 2015).

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One of the major problems of the fishery sector which remains to be addressed as an important constraint in improving the productivity of the sector in both wild and cultured population are parasite and disease associated with fish (Subashinge *et al*., 2002). Parasites of fish are a great concern since they often produce a weakening of the host's immune system, thereby increasing their susceptibility to secondary infections that often result in the nutritive devaluation of fish and subsequent economic losses (Onyedineke *et al*., 2010).

Parasitic disease reduces fish production by affecting the normal physiology of the fish and if left uncontrolled, could result to mass mortalities or in some cases could serve as a source of infection for human and other vertebrates that consume it (Ayotunde *et al*., 2007). These parasites could be Ecto-parasites or Endo-parasites. Protozoans and helminthes have been reported to be the major group of parasites of fish in Nigeria (Adikwu *et al*., 2004, and Omeji, 2011).

1.1 Description of helminthes

Helminthes are worm-like parasites that survive by feeding on a living host to gain nourishment and protection, which sometimes resulting in illness of the hist. there are variety of different helminthes, ranging from the very large to the microscopic ones (yourgenome.org).

"Helminth" is a general term meaning "worm". All helminthes are invertebrates with long, flat or round bodies. There are many different kinds of helminthes, ranging in length from less than one millimeter to over one metre. Helminthes infect a range of hosts, including man. Their effects inside their host also vary, causing a wide spectrum of diseases, from mild to potentially deadly (yourgenome.org).

1.1.1 Why study the genetics of worms?

Caenorhabditis elegans, a round worm (nematode) with around 1, 000 cells, was the first animal to have its genome sequenced. Although *C. elegans* is a very simple organism, sequencing its genome paved the way to a comprehensive view of its development and behaviour (yourgenome.org). It is hoped that knowing more about the genome of other helminthes could provide useful insight into their biology. Knowing how they function and causes disease is expected to lead a new, more effective ways of treating these infections (yourgenome.org).

1.1.2 Impact of disease to aquaculture

Despite some reports of disease outbreaks in farmed fish, the level of awareness of the impact of disease to aquaculture is lacking as revealed by numerous personal interactions and the reports of Kolndadacha *et al*., (2007). Parasites play an important role in the ecology of aquatic ecosystems as well as in the aqua and mariculture. Hence, fishermen or consumers often observe parasites in fish only when they are so obvious as lead to rejection of fish (Martens and Moens, 1995).

Consequently, disease is one of the major problems of the fishery sector which remains to be addressed as an important constraint in improving the productivity of the sector in both wild and cultured population are parasite and disease associated with fish (Subasinghe *et al*., 2002). Parasites of fish are a concern since they often produce a weakening of the host's immune system, thereby increasing their susceptibility to secondary infections that often result in the nutritive devaluation of fish and subsequent economic losses (Onyedineke *et al*., 2010).

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1.2 Related literatures on helminth parasites

The emanating need to culture fishes for protein consumption for the teeming rapidly growing populations in the developing countries have made it necessary to intensify studies on the parasite fauna of the African freshwater fishes (Akinsanya *et al*., 2007). There is appreciable documentation of parasite fauna of *Clarias gariepinus* in Nigeria. Similar works have been done by Oniye *et al*., (2004) in Zaria, Yakubu *et al*., (2002) in Plateau State, and Ibiwoye *et al*., (2004) in Bida, Akinsanya and Otubanjo, (2006), reported that fish from African freshwater were infected by a variety of adult helminth parasites ranging from monogenean, digenean, cestodes, nematode, acanthocephalans, and aspidogastrean.

Paperna, (1996) reported different helminth parasite had varying degrees of been pathogenic. For example, *Spirocamallanus spiralis*, a common nematode parasite in the stomach of catfish, has been reported to be non-pathogenic in spite of the form of attachment by their buccal capsule to the stomach mucosa of infected fish. Meanwhile, white species of *Philometra* and *Acanthocephalans* caused mild to severe pathology in fish. Parasites of fish could also constitute health hazards to humans who ingest poorly cooked fish (Ibiwoye *et al.*, 2004).

Akinsanya and Otubanjo, (2006) reported that fish from African freshwater were infected by a variety of adult helminth parasites ranging from monogenean, cestodes, nematodes, acanthocephalans and aspidogastrean. Paperna, (1996) reported different helminth parasite had varying degrees of been pathogenic. For example; *Spirocamallanus spiralis*, a common nematode parasite in the stomach of catfish which was reported to be non-pathogenic in spite of the form of attachment by their buccal capsule to the stomach mucosa of infected fish (Paperna, 1996), while species of Philometra and Acanthocephalans caused mild to severe pathology in fish. Parasites of fish could also constitute health hazards to humans when ingested with poorly cooked fish (Ibiwoye *et al*., 2004).

Documentation of parasite fauna of fishes, especially used in aquaculture is on the increase. In northern Nigeria, a number of gastrointestinal helminth worms have been documented in *C. gariepinus* by Oniye *et al*., (2004) in Zaria and Yakubu *et al*., (2002) in Plateau state. Oniye *et al*., reported the occurrence of cestodes, *Anomotaenia* sp. (2.5%), *Monobothrium* sp. (13.33%) and *Polyonchobothrium clarias* (1.67%); the nematode, *Procamallanus laevionchus* and an acanthocephalan, *Neoechinorhynchus rutli* (0.83%).

Therefore, the morphological simplicity and similarity of some fish helminth parasites makes the identification of closely relate species extremely difficult and requires skill and experience. Consequently, any method which could quantify and qualify easily identifiable parameters would represent a great advance in fish helminth taxonomy and would permit differentiation by laboratory technicians (Zhu *et al*., 1998).

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However, molecular classification protocols are generally tested and applied successfully to separate and distinguish several helminth species as identification and differentiation of species based on their morphological characters is not always feasible or reliable (Garcia-Varela *et al*., 2000). Recently, various studies have been demonstrated that the internal transcribed spacers (ITS) and 5.8 ribosomal DNA (rDNA) sequence provide distinguishing genetic markers in fish parasitic nematodes (Shih, 2004) and acanthocephalans (Garcia-Varela *et al*., 2005; Shih *et al*., 2010).

Hence, several marker technologies have been employed in fish diversity studies which include morphometric, isozymes, cytologic and recently molecular markers (DNA markers) Ferguson and Dangamam, (1998). Of the molecular markers used, Random Amplified Polymorphic DNA (RAPD) has been applied for the study of genetic diversity in selected organisms including fish (Cagigas *et al*., 1999; Bartish *et al*., 2000; Lumeret *et al*., 2000; Mohd-Azmi *et al*., 2000; Hwang *et al*., 2001). This marker is a dominant marker, and according to Theodorakis and Bickham, (2004), it can sample a large number of loci and does not require the prior DNA sequence information to perform the assay.

1.3 Parasitological analysis of helminth parasites

In parasitology, routine laboratory diagnosis involves conventional methods, such as optical microscopy, used for the morphological identification of parasites (Kompalic-Cristo *et al*., 2005). However, the occasional difficulty of identifying these parasite structures mat decrease the sensitivity of such methods. Currently, because of these difficulties, molecular biology has been employed to detect parasites responsible for parasitic diseases (Jardim *et al*.,2006).

However, traditional parasitological analysis has the advantage of being less costly without requiring expensive reagents and equipment. Additionally, such analysis can be easily performed when a trained microscopist is available. On the other hand, molecular technology demonstrates the presence of parasites based on their antigenic components or DNA segments (Scott, J. A, 1995). These tests are not influenced by environmental factors that usually can interfere with the results of a sample, thus ensuring highly reliable results (Portela *et al*., 2003).

Current laboratory diagnostic methods for the identification of parasites include: polymerase chain reaction (PCR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), microsatellite marker method, Luminex Xmap-based technology (areas of multi-analyte profiling), loop-mediated iso-thermal amplification (LAMP), and the recently added real-time PCR (Guy *et al*., 2004).

However, there is paucity of information on the molecular characterization of helminth parasites affecting *Clarias gariepinus* in Sokoto, possibly due to limited studies on fish diseases. Akoll and Mwanya, (2012) attributed this to lack of diagnostic tools and high cost of identifying and characterizing such parasites in subsistence aquaculture. Since, it requires a cost-effective, sensitive and specific system of surveillance and monitoring of fish populations to detect pathogen carrier fish. However, since molecular diagnostic techniques are faster and more sensitive than conventional diagnostic techniques, pathogens can be detected from asymptomatic fish, so disease outbreak could be prevented. Thus; this review aims at:

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- evaluating molecular techniques for the detection and identification helminth parasites in fish.
- explain constraints to molecular diagnosis of helminth parasites.

2. DIAGNOSIS OF HELMINTH PARASITES

Economic impediment of fish parasites comes not only from mortality, but also from 9treatment expenses and growth reduction during and after an outbreak of disease which militates against the expansion of aquaculture. Therefore, an understanding of the etiology of fish parasites to its genus which is generally sufficient to implement an effective therapeutic or prophylactic strategy for combating the disease (Paperna, 1996).

Helminthes are worm-like parasites that survive by feeding on a living host to gain nourishment and protection, sometimes resulting in illness of the host. Their effects inside their host also vary, causing a wide spectrum of diseases, from mild to potentially deadly (Lucy, 2015). Although helminth is a simple organism, sequencing its genome will pave the way to a comprehensive view of its development and behaviour (Lucy, 2015). Therefore, it is hoped that knowing more about the genomes of other helminthes could provide useful insights into their biology, knowing how they function and causes of disease is expected to lead to new, more effective ways of treating these infections.

The occasional difficulty of identifying these parasites to the specie level has led to the evolution of molecular biology, which has been employed to detect parasites responsible for parasitic diseases (Jardim *et at*., 2006). Molecular technology has helped to demonstrate the presence of parasites based on their antigenic components or DNA segments (Scott, J. A., 1995). Thus, great advances have taken place in understanding the molecular biology of fish pathogens and their hosts, and molecular biology has become a routine tool in the search for improved methods of diagnosis and control of fish diseases and the epidemiology of parasitic diseases (Ilhan, A., and Ilknur, K., 2003).

Molecular techniques can be used to increase sensitivity and specificity of pathogen detection. These techniques include polymerase chain reaction (PCR), restriction enzyme digestion, probe hybridization, in situ hybridization and microarray. Since molecular diagnostic techniques are faster and more sensitive than conventional diagnostic techniques, pathogens can be detected from asymptomatic fish, so disease outbreak could be prevented. Thus, antibiotic treatment can be reduced, therefore; creation of antibiotic resistant helminth may be eliminated (Ilhan, A., and Ilknur, K., 2003).

2.1 Molecular techniques for the study and diagnosis of parasite infection

The utilization of molecular biology techniques brought new views to different biomedical areas. The molecular helminthology laboratory specializes in the use of molecular biology tools for investigations on the field of host-parasite interactions (Beech *et al.,* 1994; Coles *et al*., 2006; Humbert *et al*., 2001 and Jacobs *et al*., 2006). The other aspects of the use of helminthology are the characterization and determination of species, sub-species and strain or generated mutations during treatment of animals with anthelmintics (Humbert *et al*., 2001; Kaplan 2004 and Le Jambre *et al*., 1999).

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For any biological sample, the efficiency of the DNA extraction methods is determined through DNA quality and recovery rate. The extraction of DNA with highly quality and quantity is a key step in the genetic analysis. Numerous direct DNA extraction methods have been used in the preparation of DNA from various organisms. The isolation of genomic DNA from small amounts of biological materials such as blood smears (Shayan, P., and Rahbari, S., 2005) or small single worms, is not always applicable using the traditional DNA isolation method based on the phenol/ chloroform/ isoamyl alcohol, which is the most used technique for DNA extraction in developing countries.

In contrast, the most commonly used DNA extraction method in industrial countries based on the selective binding of the nucleic acids on the silica based carriers (Coles *et al*., 2006; Shayan, P and Rahbari, S., 2005 and Jiang *et al*., 2005). The advantage of the latter mentioned compared to the traditional method can be summarized in the better recovery, purity, safety and speed. The only disadvantage of this method is that it is costly. Since all DNA extraction kits are based on the selective binding of the DNA to the carrier, and are produced in industrial countries, thus, are so expensive to obtain.

2.2 Molecular characterization of helminth parasites in fish

These techniques are faster and much more sensitive than other methods used in the diagnosis of fish infections. In these techniques, DNA is obtained from the sample, examined, analyzed and hybridized in the restriction fragment length polymorphism (RFLP), and amplified by polymerase chain reaction (PCR) using specific primers for diagnostic sequences. Thus, using the PCR-restriction fragment length polymorphism (RFLP) method, a number of related nematodes can be differentiated on their banding pattern in agarose gel (MacKezie, K. and Abaunza, P., (2013). The techniques for molecular diagnosis of parasitic fish infections are:

2.2.1 Polymerase chain reaction (PCR)

Polymer as a chain reaction is a technique to amplify a specific region of DNA, defined by a set of two "primers" at which DNA synthesis is initiated by a thermostable DNA polymerase. Usually, at least, a million-fold increases of a specific part of a DNA molecule can be realized and the PCR product can be found by gel electrophoresis (Radonic *et al*., 2004). Primers are important to get the best sensitivity and specificity (Timi, J., and Mackenzie, K. 2015). Agarose gel electrophoresis shows the results of *Gyrodactylus anguillae*, using the PCR products and M, 100bp molecular weight marker (bp) (Elgendy *et al*., 2016). In recent research, the improvement of novel sciences, enabling the detection of PCR products on a constant premise has prompted far reaching appropriation of ongoing RT-PCR as a method for decision and quantitative changes in quality expression (El-Raziky, 2016).

2.2.2 Multiplex PCR

These techniques make it possible to produce considerable savings of time and effort in the laboratory without compromising test. Since its introduction, it has been applied successfully in many parts of the nucleic acid diagnostics, including analysis of gene deletion, quantitative and RNA detection (Suszkiw, 2011). In the field of parasitic fish diseases, the strategy has been seen to be a significant technique for recognizing the infections, microscopic organisms, growths of parasites at the same time (Cunningham, 2002). In fisheries, *Cryptocaryon irritatans* parasites

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has been discovered using the Multiplex PCR. The amplified PCR product size of both DNA samples A and B were of 200 basic pairs (Chen, *et al*., 2008). Adult specimens of *Diplectanum* species were isolated from the skin of sea bass for standardization of the PCR technique 3, 4 and 5 at 650bp. Lane 1 represents the 100bp (El-Raziky, 2016).

2.2.3 Restriction enzyme digestion

Restriction enzymes is a technique used to create "fingerprint" of a particular DNA molecule. These enzymes are capable of cutting DNA into discrete fragments that can be portioned by gel electrophoresis because of the sequence specificity of restriction enzymes. This model of DNA fragments makes a "DNA fingerprint", and each DNA molecule has its own specific fingerprint. Other restriction enzymes can be used in more characterization of a particular DNA molecule. The location of these restriction enzyme is placed on the DNA molecules, which can be compiled to create a restriction enzyme map, PCR-amplified internal transcribed spacer region of ribosomal RNA genes for identifying freshwater mussels (Unionoida) and parasitic glochidia larvae from the host fish gills (Alexandra *et al*., 2012). Molecular systems generally are more valuable for parasitological analysis than the immunodiagnostic methods (El-Raziky, 2016 and Holzer, *et al*., 2007).

2.2.4 DNA micro-arrays

These is a technique that uses DNA micro-arrays to detect the unique DNA sequences. DNA sample that hybridizes to a given place on the micro-array can be detected by fluorescent array detection and the data analyzed by computer programs (Velkova-Jordanoska, 2006). Thus tool is matured in density, sensitivity, lower cost, rapid detection, automation, and low background levels. Micro-arrays may provide a better choice for vast scale diagnostic testing and can survey a sample for a multitude of sequences simultaneously (Suszkiw J., 2011).

DNA micro-arrays are suitable for the simultaneous detection of 15 fish pathogens based on 16S ribosomal RNA polymorphisms (Warsen *et al*., 2004). The amplified products of *Mayxobolus sp*. Sample A was likely by Mayxoboluscerebralis 18S ribosomal RNA gene's partial and complete sequence and amplified product of *Mayxobolus sp*. (Conraths, F., and Schares, G., 2014). Micro-array analysis of the expression of genes in Japanese flounder, *Paralichthys olivaceus* leucocytes to *Neo-heterobothrium hirame* infection from monogene of a parasite were done by (Matsuyama *et al*., 2007). Furthermore, the time of analysis is short. DNA micro-array technology can be used in the future to diagnose fish diseases specially during the symptomatic period of diseases.

2.2.5 Loop mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a new nucleic acid amplification method that amplifies DNA with high efficiency, specificity and rapidity under isothermal conditions (Notomi *et al*., 2000 and Shen *et al*., 2015). This technique was applied to parasitic pathogens, such as *myxozoan* parasite of Salmonid fish *tetra-capsuloides bryosolmonae* (El-Matbouli, M., and Soliman, H., 2005).

2.2.6 Nested PCR

In this method, two pairs of primers were used in two frequent runs of polymerase chain reaction. This protocol was 100 times at least more sensitive than serological methods,

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depending on the magnetic bead enzyme immunoassay. For instance, the detection of *Maxybolus cerebralis* from rainbow trout (*Oncorhynchus myknis)* by (Skirpstunas *et al*., 2006).

2.3 The advantages of molecular methods

Molecular techniques permit great advances to improve diagnosis and control of pathogens in aquaculture with more specificity, speed and sensitivity of diagnosis. Therefore, molecular tools should be configured as a routine technique in aquaculture and laboratories for improved methods of diagnosis and control of infectious fish diseases in laboratories (Ahmed I., Noor El Deen, Mona S. Zaki, Olfat, M. Fawzi., 2018).

2.3.1 The disadvantages of molecular methods

Molecular techniques procedures are of high cost, and cannot detect unsuspected pathogen specially, new pathogens which will be hardly detected by molecular methods (Ahmed I. E., Noor El Deen, Mona S. Zaki, Olfat, M. Fawzi., 2018).

2.4 The traditional methods of controlling fish parasites

Classical methods for parasitic diseases treatment depend on anti-parasitic chemical drugs with their drawbacks (Forwood *et al*., 2013). In addition, some considerations should be taken such as LD_{50} of drug of choice, method of application, sensitivity of host to chemotherapy (Noga, 2012). A few chemicals such as malachite green are carcinogenic on fish and man, with long withdrawal time and remaining viability on fish substance. They are accumulated as hurtful residues in the fleshy fish and environments (Zhan, T., and Braunbeck, T., 1995). Some chemicals need low water temperature such as formalin (Fajer-A'vila *et al.,* 2003), and the organic matters decrease the effect of potassium permanganate (Noga, 2010).

3. CONSTRAINTS TO MOLECULAR DIAGNOSIS OF HELMINTH PARASITES

According to Wilson, (1997); the choice of tissue sampled may have significant effects on the PCR results. Substances like haemoglobin, bacterial constituents, and high concentrations of no target DNA is capable of inhibiting amplification. These high concentrations of DNA from the host and high numbers of fish cells as well inhibited the amplification of *A. salmonicida* DNA (Hoie *et al*., 1997) and are likely to inhibit other amplifications. However, the impact of PCR inhibitors such as selective components and fish tissues can be minimized using a high quality nucleic acid extraction system.

Conversely, to eliminate contamination during necropsy and PCR, new disposable dissecting tools should be used for each fish. Equipment disinfection should include both manual cleaning of equipment to remove all tissue from crevices of dissecting tools and the use of chemical reagents such as bleach, followed by a series of rinse in water. Sterilization of materials should be carried out according to the protocol outlined in Obiajuru and Ozumba, (2009).

Alcohol and flaming are not satisfactory for this method because, they are not reliable to destroy DNA. Samples that needs to be homogenized, to avoid cross contamination between samples, the use of stomacher should be employed, rather than use polytron because alcohol treatment of polytron will not destroy the parasites, especially, enveloped ones. However, to avoid these, a separate room should be used for the steps outlined below:

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- Necropsy
- DNA extraction
- PCR master mix preparation
- DNA quantification and the addition of DNA to PCR mixture
- Thermocycler and electrophoresis

Consequently, laboratory coats, gloves, and pipettes should not be taken from one room to another. Uracil-DNA glycosidase (UNG), should be used with dUTP to eliminate PCR contamination with the reaction product. The UNG will degrade uracil-containing DNA at 200c and is inactivated at elevated temperatures (Grizzle *et al*., 2003). In order to check the efficiency and the sensitivity of the PCR, it is important to apply standard molecules as indicators of the procedures. It is also important to detect false negative results due to pipetting errors or the inhibition of the amplification, often observed when PCR is performed with complex samples such as blood and tissues (Grizzle *et al*., 2003). To eliminate false negative PCR results, internal control such as β -actin gene primers should be used to amplify β -actin gene. The size difference will allow easy discrimination between their PCR products. Finally, the use of the internal control will undoubtedly facilitate the interpretation of negative PCR results and it will be easy to identify samples which were inhibiting the amplification (Grizzle *et al*., 2003).

4. CONCLUSION

In conclusion, molecular techniques, typically, the DNA is extracted from the sample of interest which can be probed by DNA hybridization and analyzed by restriction fragment length polymorphism (RFLP). Conversely, DNA is amplified by the polymerase chain reaction (PCR), using specific primers for diagnostic sequences. This may be followed by RFLP, PCR linked to hybridization with specific oligoprobes or, non-specific primers used to produce random amplified polymorphic DNA (RAPD).

Thus, the sequencing of the complete genomes of pathogens is allowing great advances in studying the biology, and improving diagnosis and control of pathogens. Hence, the use of nucleic acid as targets, and new methods of analyzing polymorphism in this nucleic acid, can improve specificity, sensitivity, and speed of diagnosis and offer the means to examine the relationship between genotype and phenotype of various pathogens.

Consequently, progress in techniques will aid epidemiological studies as well as identifying some causes of disease outbreaks or the presence of pathogens. Therefore, molecular biology will serve as a routine tool in the quest for improved methods of diagnosis and control of fish pathogens and the epidemiology of infectious fish diseases. However, in Sokoto State, Nigeria, there is paucity of information on the application of these techniques on a routine basis. Hence, the time is now for their application in the diagnosis of diseases in aquaculture.

5. RECOMMENDATION

Molecular tools and scanning electron microscopes are considered an excellent new trend in the diagnosis of the parasitic infections specially in imported fishes. Hence, these conventional methods for diagnosis of parasitic infections are considered the corner stone for identification of

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parasites. Therefore, proper management and nutrition should be kept in mind when managing parasitic disorders. However, early diagnosis of parasitic freshwater fish infections is recommended as a way of preventing freshwater parasitic fish disease.

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