Recent Advances in Diagnosis of Filariasis

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Aftab Shaukat[1,2], Muhammad Tahir Aleem[3,4], Amna Kanwal[5], Azka Kalim[6], Fakiha Kalim[7], Asjad Memoon[1], Mian Hassan Siddique[1], Muhammad Abdul Samad[1], Rizwan Shaukat[8] and Irfan Shaukat[9*]

ABSTRACT

Filariasis is a mosquito-borne parasitic disease caused by filarial nematode worms. Various mosquito genera are responsible for the transmission of this disease condition. Filarial worms have endemic presence in tropical and sub-tropical regions of the world. Filariae exhibit distinctive properties and are regarded as substantially significant in human as well as veterinary medicine as a wide range of filarial worms infest wild and domestic animals around the globe. Precise parasite identification not just helps in clinical settings but also provide significant assistance in research. This chapter highlights the various advancements made regarding the diagnosis of filariasis in the recent years. The traditional methods used for the diagnosis include blood examination, skin biopsy, urine and sputum analysis. However, there are certain barriers that hinder the usage of these traditional methods for the diagnosis of Filariasis. These days, different diagnostic approaches are being used including molecular, serological and imaging techniques. Recently, continuous advancements are being observed regarding the development of better molecular and diagnostic techniques. The significance of genetic and genome-based information is growing on a substantial rate for the detection and characterization of zoonotic parasites. The accurate diagnosis of Filariasis is significant because it will aid the treatment and epidemiological monitoring of disease burdens. Although many recent advances have been made regarding the diagnosis of filariasis still there is room for development of better and more reliable techniques. Development of economical, specific and more reliable techniques can lead to the timely diagnosis of filariasis and thus can be treated more effectively.

Key words: Filariasis, Zoonoses, Diagnosis, mosquito-borne disease, recent advances

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[1]Faculty of Veterinary Science, University of Agriculture Faisalabad, 38000, Pakistan
[2]College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, Guangdong, China
[3]Center for Gene Regulation in Health and Disease, Department of Biological, Geological and Environmental Sciences, College of Sciences and Health Professions, Cleveland State University, Cleveland, OH 44115, USA

[4]MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China
[5]Department of Pathology, Faculty of Veterinary Sciences, University of Agriculture Faisalabad.
[6]Faculty of Medical Sciences, Government College University, Faisalabad, 38000, Pakistan
[7]Department of Parasitology, Faculty of Veterinary Sciences, University of Agriculture Faisalabad, 38000, Pakistan
[8]Faculty of Food, Nutrition & Home Sciences, University of Agriculture, Faisalabad, Pakistan
[9]Department of Biochemistry, University of Narowal, Pakistan
***Corresponding author:** irfan.shaukat@uon.edu.pk

1. INTRODUCTION

Vector-borne diseases (VBDs) are transferred by the bites of hematophagous arthropods like ticks, mosquitoes, blackflies, and sandflies. These VBDs can be bacterial, viral or parasitic (Obradovic et al. 2022). The disease conditions caused by the mosquitoes are among the most crucial public health issues faced globally, causing mortalities in humans, livestock, and wildlife and considerable financial losses (Tolle 2009). Filariasis, one of the mosquito-borne parasitic diseases, is an important disease caused by filarial nematode worms. Different mosquito genera, such as, Anopheles, Culex, Mansonia, Aedes, Ochlerotatus, and Armigeres transmit this disease condition(Foster and Walker 2019). Filarial worms are vector-borne parasitic nematodes, having endemic presence in tropical and sub-tropical regions of the world. In terms of form and structure, the filarial worms are narrow and long having no pharynx and buccal capsule. Esophagus consists of two parts i.e. anteriorly muscular part and posteriorly glandular part. Males possess asymmetrical spicules and are generally smaller in size as compared to the females. Vulva is positioned anteriorly where fully formed larvae are born. These larvae are termed as microfilariae(Simón 2001). Filarial worms have been observed in the central nervous system, subcutaneous tissues, the eye, the heart and lungs, and the lymphatic system (Orihel and Eberhard 1998).Filariae exhibit unique attributes and are regarded as substantially significant in human as well as veterinary medicine (Evans et al. 2022). A wide range of filarial worms infest wild and domestic animals around the globe (Satjawongvanit et al. 2019; Kaikuntod et al. 2020). Moreover, various filarial genera, like Meningonema, Wuchereria, Brugia, Loaina, Dirofilaria, Dipetalonema, and Onchocerca have also been identified in humans. *Wuchereria bancrofti* and *Brugia* spp. cause lymphatic filariasis (elephantiasis)(WHO 2022),*Onchocerca volvulus* causes onchocerciasis (river blindness) (World Health Organization 2019), *Loa loa* causes loiasis (African eye worm) (Tatuene et al. 2014), and *Mansonella* spp. causes mansonellosis (Akue et al. 2011). Many of these neglected tropical diseases (NTDs) are severe public health issues in the endemic regions as these may induce stigmatizing pathologies, exerting socio-economic burden in the people who are affected (Karunakaran et al. 2023). The estimated population that fell a prey to onchocerciasis is estimated to be around 21 million, where 14.6 million is suffering from skin diseases and about 1.15 million with vision dysfunction(WHO 2022).Lymphatic filariasis is the most common type of filariasis and is highly prevalent in Indonesia, Malaysia, India, Pakistan, Nigeria, Philippines, and Bangladesh. Beside these developing countries, it can also be found in developed countries like China, Western Pacific and some areas of America (Abbas et al. 2022). Onchoreasis is common in Africa but it can also be found in Yemen, South and Central America, and Saudi Arabia (Abbas et al. 2022). Fig. 1 shows major types of filariasis and basic difference among them.

As filariasis a mosquito-borne disease so its cycle starts when an infected mosquito bites the skin of the vertebrate host and injects the third stage larvae or L3 larvae into the body of the host. These L3 larvae move towards the lymphatic system where they mature. Maturation of L3 larvae is a slow and gradual process which takes almost 6 to 12 months (Chandy et al. 2011). This

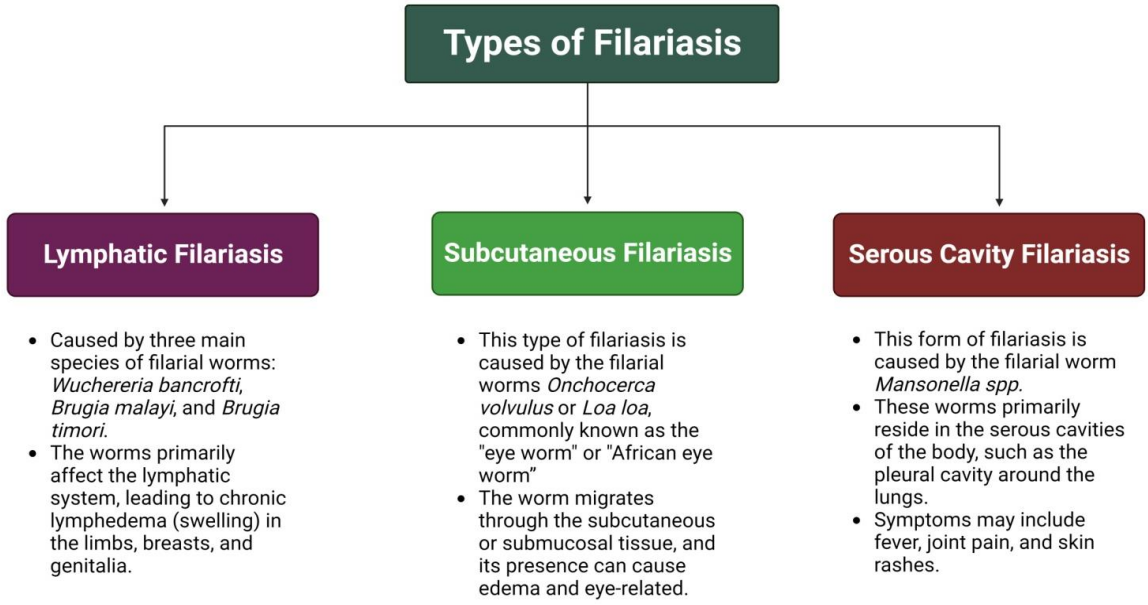

Fig. 1: Common types of filariasis.

maturation leads to the formation of fourth stage larvae or L4 larvae which develop into adult larvae. These adult larvae give birth to the first stage larvae or L1 larvae commonly called microfilarae. These microfilarae flow in the blood stream. The mosquito's uptake the microfilarae where microfilarae develop into infective L3 larvae. L3 larvae move towards their proboscis and penetrate the host's skin when the mosquito bites the host. This continues the cycle. The penetration of larvae triggers the immune responses and symptoms like lymphoedema and hydrocele(Chandy et al. 2011) (Fig. 2).

These parasitic nematodes are generally long-living and hard to identify, often inducing chronic disease conditions spanning over years. Due to these reasons, proficient diagnostic tests are important for their control. Filariae at adult stage are likely to occupy far-off anatomical sites in the hosts. However, microfilariae disseminate profusely in the skin or blood which facilitates transmission and uptake by the blood-feeding arthropods in order to successfully complete their life cycle. The identification of this microscopic life form indicates integral form of test performed for diagnosis. DNA-based methods and immunological diagnostic techniques have since been devised for various filarial worms along with the techniques for the visualization of adult worms in situ (Gruntmeir et al. 2023). All of these approaches have their own pros and cons; therefore, effective diagnosis is mostly the integration of these methods. Precise diagnosis is essential for the detection of lethal infections like canine heartworm and is important for burgeoning zoonoses, such as *Onchocerca lupi*, and potential animal reservoirs, as in the case of *Brugia malayi.* Precise parasite identification not just aids in clinical settings but also provide significant assistance in research (Evans et al. 2022). In this chapter we will delve into the various advancements made regarding the diagnosis of filariasis in the recent years.

2. CONVENTIONAL METHODS USED FOR THE DIAGNOSIS OF FILARIASIS

2.1. DETECTION OF MICROFILARIAE IN BLOOD

Procedures like Knott's technique and membrane filtration technique are generally used in order to detect the presence of microfilariae in the body fluids (e.g. blood etc.) (Garcia and Procop 2016).

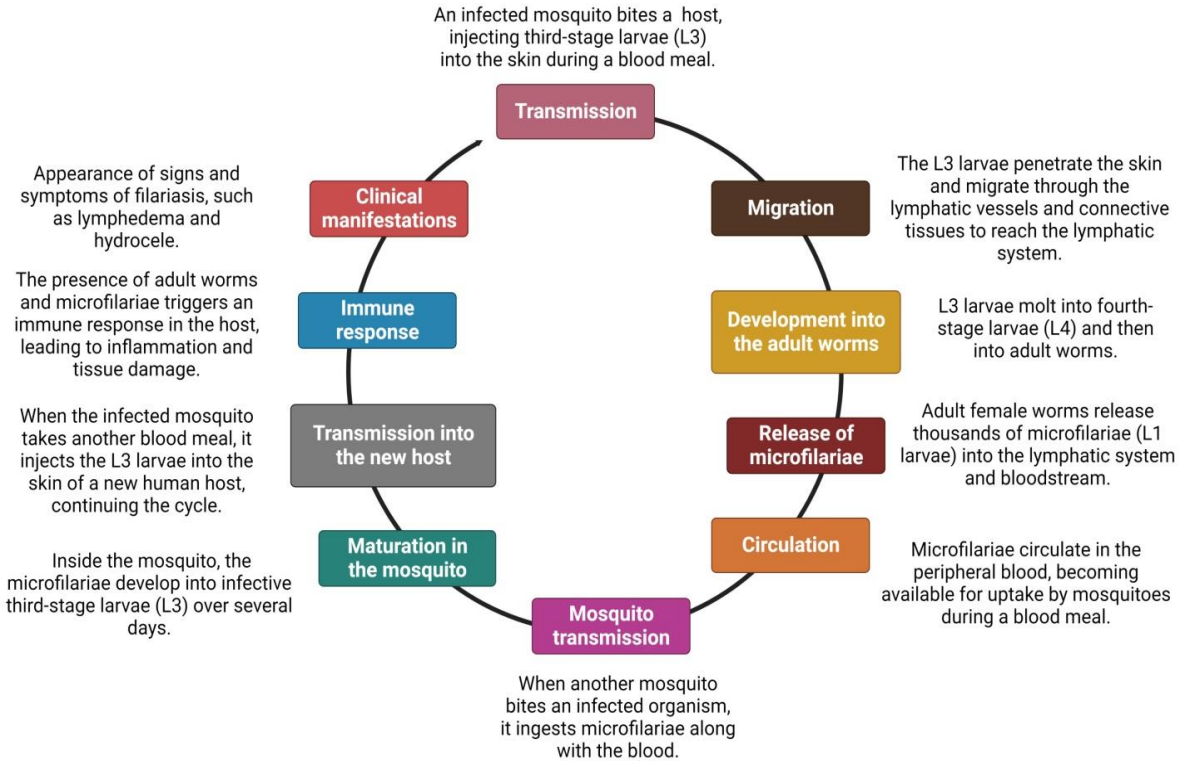

Fig. 2: Pathogenesis and transmission of filarial infection.

2.1.1. KNOTT'S TECHNIQUE

This technique is more commonly used. For this procedure, around 1 milliliter of blood is collected in EDTA (Ethylenediaminetetraacetic acid) or citrate through venepuncture. Alongwith 10 ml of 10% formalin, it is kept in a centrifuge tube. It is shaken in order to facilitate disintegration of red blood cells. This is centrifuged at 300 times *g* for around 120 seconds. The supernatant is removed. A small amount of sediment is observed (World Health Organization 2000; Mathison et al. 2019).

2.1.2. MEMBRANE FILTRATION TECHNIQUE

Fresh blood is collected in EDTA (Ethylenediaminetetraacetic acid) or sodiumcitrate. 10 mL of 10% Teepol saline sol. is combined with around 1 mL of blood. A filter paper is moistened and placed firmly in a filter holder. The Teepol-Blood mixture is poured in a 20 mL syringe and passed though the filter smoothly. Then water is slowly run through filter 2 to 3 times. Around 3 mL of methanol is flushed gently via filter to set microfilariae. Filter is removed and put on a slide. Let the filter to be dried completely. Then the slide is observed when it is entirely dry (World Health Organization 2000; Ash and Orihel 2007).

2.2. SKIN BIOPSY

A skin biopsy is usually carried out for the infections caused by the nematodes inhabiting the tissues. For biopsy, a sterilized needle is used for slightly lifting skin and the lifted skin is shaved off with the aid of sterile blade. The skin sample is placed in a small tube with normal saline for 3 hours. Then the sample is examined to observe the mobile microfilariae (World Health Organization 2000).

2.3. INVESTIGATION OF SPUTUM AND URINE SAMPLES

Microfilariae are generally observed in blood but they can be detected in other body fluids. *Wuchereria bancrofti*have been witnessed in the urine of Chyluria infected organisms (Verma and Vij 2011). Hydrocele fluid, sputum and urine samples are centrifuged and microfilariae can be observed in the sediment (World Health Organization 2000; Garcia and Procop 2016).

2.4. BARRIERS TO THE USE OF TRADITIONAL METHODS

These traditional methods have low sensitivity so their use is not generalized (Eick et al. 2019). Limitations to the investigation of blood specimens are because ofinsufficient saturation of filter-paper, varying results with various filter-papers, and threat to the breaking down of specimens due to raised temperature or humidity(Arkell et al. 2022).

3. MOLECULAR TECHNIQUES FOR THE DIAGNOSIS OF FILARIASIS

Molecular investigations are not generally used. These techniques can be performed in research centers and specialized labs. Polymerase chain reaction (Mendoza et al. 2009) and loop-mediated isothermal amplification are two commonly known molecular techniques (Mathison et al. 2019).

3.1. REAL-TIME PCR

RT– PCRis favourable for labs in the countries that are developed as those countries have proper and developed labs (Mathison et al. 2019). Real-Time PCR is a cost effective, highly sensitive and specific method. It discovers the DNA fragments of the microfilariae in the infected persons as well as animals (McCarthy 2000; Chandy et al. 2011).

3.2. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Loop-mediated isothermal amplification or LAMP is a better choice for the countries being developed because the price of reagents is lower in comparison with that of RT – PCR. It can identify the microfilariae like Wuchereria *bancrofti*, *Brugia* spp., *Onchocerca volvulus*, and *Loa loa* (Drame et al. 2014; Poole et al. 2017; Mathison et al. 2019).

3.3. DNA SEQUENCING

This diagnostic method does not allow the analysis of whole genome. Some microfilariae species can be detected by DNA sequencing approach (Mathison et al. 2019).

4. SEROLOGY

4.1. IMMUNOCHROMATOGRAPHIC TESTS (ICTS)

Immunochromatographic tests are done for the identification of microfilariaeantigens. These tests are rapid, highly specific and sensitive. *Wuchereria bancrofti* can be detected by ICTs (Chandy et al. 2011).

4.2. ANTIBODIES IDENTIFICATION

Antibodies tests are very specific. These tests are usually done against *Brugia malayi* and *Wuchereria bancrofti* (Mathison et al. 2019). For antibodies test, blood specimen is collected in a simple vial and serum is separated. Then the test is done with the help of specialized test kit. *WbSXP-1*, a recombinant antigen, identifies the specialized antifilarial antibodies for *Brugia malayi* and *Wuchereria bancrofti*.

4.3. FILARIASIS TEST STRIP

The Filariasis Test Strip is a *Wuchereria bancrofti* specific RDT (Rapid Diagnostic Test). A Rapid Diagnostic Test or RDT is the one that is fast, simple and accurate. Filariasis Test Strip (FTS) identifies the CFA (Circulating Filarial Antigens) that are usually found in the blood or blood tissues of an individual. It was first used by the company Alere in the year of 2013 (Weil et al. 2013; Chesnais et al. 2016). For the test, the test strips are placed in the trays where we have to work prior to the addition of sample. Then the blood is collected in a micropipette. Blood is gradually added to the strip by pressing the bulb of the micropipette. Results can be read approximately after ten minutes. There are two lines in the test strip (Chesnais et al. 2016). One is test line and the other is control line. If both lines appear pink, then the result is said to be positive. On the other hand, if only control line shows pink colour then the result is said to be negative (Weil et al. 2013). If only test line appears pink or none of the lines show pink colour then the test result in invalid (Fig. 3). Filariasis Test Strip (FTS) is more economical, sensitive and has prolonged product life-span (Weil et al. 2013; Yahathugoda et al. 2015).

*T = Test line

**C = Control line

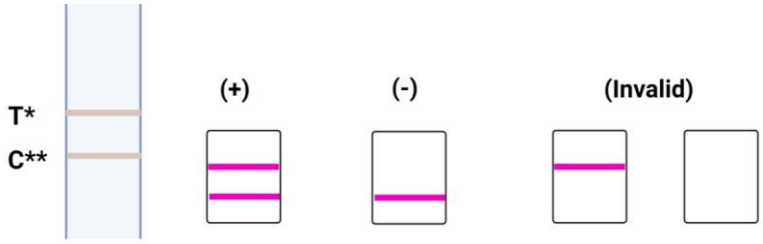

Fig. 3: Possible results for Filariasis Test Strip

5. IMAGING TECHNIQUES

5.1. ULTRASONOGRAPHY

Ultrasonography can help in detection of moving filarial nematodes or the adult filarial nematodes. On examination, movement of filarial nematodes can be seen. This is termed as “Filarial Dance Sign”. This sign can be observed in breast, cords, scrotum, axillary lymphatic, and limbs (Medeiros et al. 2021).

5.2. LYMPHOSCINTIGRAPHY

It is a diagnostic approach for the detection of any abnormality in lymphatic system (Hsueh et al. 2001; Pai 2023). Even in the asymptomatic condition of filariasis, the microfilariae infected organisms may present the abnormalities in the lymphatic system (Chandy et al. 2011).

Table 1 manifests the specific diagnostic approaches used for the diagnosis of certain species of filarial worms.

***Periodicity** =the time of day when the microfilariae circulate substantially in the blood of the vector or host (Aoki et al. 2011).

6. ONE HEALTH APPROACH AND FILARIASIS

Zoonotic filariasis is the filariasis in humans caused due to animal worms and has worldwide occurrence. It was first reported more than a 100 years ago. Since then, the number of reported cases and the parasites involved have gradually increased. Animal filaria require biological vectors like hematophagus insects to infect humans, which fed previously on a diseased animal in a suitable time frame. On the global scale, most people are at some risk but those who are more likely to interact with the vectors can be at higher risk. But there is also a possibility of unrecognized risk factors, as the animal filaria present worldwide distribution (Otranto and Eberhard 2011).There are three forms of filariasis depending upon the worms' predilection sites i.e. serous cavity filariasis, subcutaneous filariasis, and lymphatic filariasis. Lymphatic filariasis is also referred as elephantiasis. It is because, this condition is associated with the blockage of lymphatic system, distention, enlargement of testes, breasts, and limbs (Fassari et al. 2021). Onchocerciasis, also termed as river blindness, is another important disease caused by filarial worms. Black fly of genus Simuliumtransmits the causative agent of this disease i.e., *O. volvulus*. This condition is called river blindness because black flies breed near streams and rivers. It is also one the leading causes of blindness worldwide (Vinkeles Melchers et al. 2021). Dirofilariasis, induced by the filarial worms belonging to genus Dirofilaria, is another zoonotic infection which occurs mostly in canids and cause heart

Table 1: Specie specific diagnosis of filarial nematodes.

Species	Regional prevalence	Shape and size	Periodicity *	Diagnostic approaches	References
Brugia malayi.	It is more prevalent in Southeast Asian countries including Malaysia, South Korea, India, Vietnam, Indonesia and Philippines.	They have nucleate tails and large cephalic space. The length of microfilariae of *B. malayi*is 0.177 to 0.230 mm and the width is 0.005 to 0.006 mm. They give pink colour with Giemsa stain.	Nocturnal	ELISA (Enzyme-Linked Immunosorbent Assay) PCR (Polymerase Chain Reaction) assays	(Lizotte et al. 1994; Fischer et al. 2003; Rao et al. 2006; Ash and Orihel 2007; Hotez 2009; Fox 2018; Mathison et al. 2019; Mulyaningsih et al. 2019)
Brugia timori.	Prevalent in Indonesia especially Lesser Sunda Islands	Microfilariae of B. timori have large cephalic space and nucleated tails. They have an average length of 0.31 mm and the width ranges from 0.006 to 0.007 mm. They do not give pink colourwith Giemsa stain.	Nocturnal	Antigen testing PCR (Polymerase Chain Reaction) assays	(Fischer et al. 2002; Supali et al. 2002; Fischer et al. 2004; Fischer et al. 2005; Ash and Orihel 2007; Mathison et al. 2019)
Loa loa.	Generally affects the organisms of Western and Central Africa	The microfilariae of *L. loa*possess less cephalic space and nuclear tail. They have length of 0.231 to 0.250 mm. They give no colour with Giemsa stain.	Diurnal	Loop-Mediated Isothermal Amplification (LAMP) Immuno-chromatographic card test (ICT)	(Ash and Orihel 2007; Fink et al. 2011; Wanji et al. 2015; Mathison et al. 2019; Campillo et al. 2022)

Mansonella ozzardi	It is commonly found in South and Central America and the Caribbean.	The microfilariae of *M. ozzardi* do not have a sheath but have a slender, nuclear tail. The length of *M. ozzardi*ranges from 0.163 to 0.203 mm.	Aperiodic	RT-PCR (Real-Time Polymerase Chain Reaction)	(Orihel et al. 1982; Ash and Orihel 2007; Tang et al. 2010; Medeiros et al. 2015; Lima et al. 2016; Medeiros et al. 2018; Raccurt 2018; Mathison et al. 2019; Ferreira et al. 2021)
Mansonella perstans	It is more prevalent in Sub-Saharan Africa and some parts of South and Central America.	Microfilariae of *M. perstans*do not have a sheath but have round, nuclear tail. Their length ranges from 0.19 to 0.2 mm.	Aperiodic	Serological assays Loop-Mediated Isothermal Amplification (LAMP)	(Meyers et al. 2000; Ash and Orihel 2007; Downes and Jacobsen 2010; Simonsen et al. 2011; Bassene et al. 2015; da Silva et al. 2017; Mathison et al. 2019; Bobkov et al. 2021)
Mansonella streptocerca	It is more prevalent in Sub-saharaincluding, East Africa, West Africa, Southern Africa and Central Africa.	The microfilariae of *M.streptocerca*do not have a sheath but have a hook-shaped nucleated tail. The length of *M. streptocerca*generally ranges from 0.18 to 0.24 mm.	Aperiodic	Skin biopsy PCR (Polymerase Chain Reaction) assays	(Fischer et al. 1998; Ash and Orihel 2007; Downes and Jacobsen 2010; Fox 2018; Mathison et al. 2019)
Onchocerca volvulus	It is usually found in the Sub-Saharan Africa, South and Central America and Yemen.	Microfilariae of *O. volvulus*has crooked, anuclear tails. They have length of 0.304 to 0.315 mm.	Aperiodic	Antibody tests Skin Biopsy PCR (Polymerase Chain Reaction) Sequencing Luciferase Immunoprecipitation Systems (LIPS)	(Orihel and Ash 1995; Meyers et al. 2000; Lipner et al. 2006; Ash and Orihel 2007; Osei-Atweneboana et al. 2007; Burbelo et al. 2009; BS 2018; Crowe et al. 2018; Mathison et al. 2019; Nyagang et al. 2020; Schmidt et al. 2022)
Wuchereria bancrofti.	Usually found in the Tropic of Cancer in Africa, Asia, the Caribbean, subtropics of South Pacific and South America.	Microfilariae have less cephalic space and an anuclear tail, with the length of 0.244 to 0.296 mm and the width of 0.0075 to 0.01 mm. They are generally colourless with Giemsa stain.	Nocturnal	ELISA (Enzyme-Linked Immunosorbent Assay) RT-PCR (Real-Time Polymerase Chain Reaction)	(Ramzy et al. 1997; Chansiri and Phantana 2002; Fischer et al. 2003; Lammie et al. 2004; Rao et al. 2006; Ash and Orihel 2007; Hotez 2009; Abdel-Shafi et al. 2017; Mathison et al. 2019)

disease in them. *D. repens* and *D. immitis* are the well-known zoonotic species of *Dirofilaria* (Dantas-Torres and Otranto 2020). Out of nine species of Genus Mansonella, *M. streptocerca*, *M. perstans*, and *M. ozzardi* are widely-known zoonotic species. These parasitic worms are transmitted by female *Culicoide* and inhabit the cutaneous membrane of their host (Klion 2013).

Recently, continuous advancements are being observed regarding the development of better molecular and diagnostic techniques. The significance of genetic and genome-based information is growing

substantially for the detection and characterization of zoonotic parasites. A proliferation of cross-host species relationships has lately been found out, which may have significant implications from evolutionary and epidemiological point of view (King et al. 2015; Lamberton et al. 2015). Due to enhancements in the molecular diagnostic techniques and genome sequencing of parasitic organisms, evidence revolving around the intermixing of genetic material is also being gathered (Webster et al. 2016). Precise diagnosis of parasitic infections is significant for the treatment and epidemiological monitoring of disease burdens. Diagnosis comprises of the utilization of clinical history, geography, travel history, and laboratory methods (Medeiros et al. 2021).

7. FUTURE PERSPECTIVES

Filariasis eradicating programs are advancing towards the goals set for the elimination of the filariasis as it is a problem all over world especially the developing countries. Proper diagnostic tools are important for supporting the goals of the programs. Limitations of the current tools used for the diagnosis of filariasis make it tough and challenging to ensure that the objectives of the program are attained. Some diagnostic tools are specific for specific species such as CFA (Circulating Filarial Antigens) tests are usually limited to the detection of*Wuchereria bancrofti*in blood smears. Their results cannot be trusted in case of *Loa loa*. There is an urgent need of discovery of techniques that can diagnose all forms of filariasis at once as it requires more cost and instruments to detect other forms or species regarding filariasis. Although many advances have been made regarding the diagnosis of filariasis still there is room for development of better and more reliable techniques. Loop-Mediated Isothermal Amplification (LAMP) assays are the novel techniques and undoubtedly have many benefits still there are drawbacks in the usage of LAMP assays. The primary drawback is that when there is Primer to Primer interaction or contamination, these LAMP assays give false positive results. So, there is dire need of the development of upgraded and more efficient diagnostic techniques and procedures to surveil and examine different forms of filariasis in humans as well as animals.

8. CONCLUSION

Filariasis is one of the neglected tropical diseases that are affecting the populations all over the world especially the developing countries. Its proper diagnosis and treatment in time is necessary as it can be fatal if not treated. With the development in the medical field, there are also advances in the diagnostic approaches used for the detection of filariasis. DNA sequencing, RT-PCR, LAMP, RDTs (Rapid Diagnostic Tests), FTS (Filarial Test Strip) and lymphoscintigraphy are some of the recent diagnostic methods that are being used for the identification of filariasis in organisms in the present day. Though these methods are better than the traditional methods that were being used in the past still there is need of development of more advanced and trustable diagnostic techniques in order to cope with this NTD (Neglected Tropical Disease). Development of inexpensive, appropriate and more reliable techniques can lead to the timely diagnosis of filariasis and thus can be treated more effectively.

REFERENCES

Abbas et al., 2022. Filariasis: New insights into an old disease. In: Khan A, Abbas RZ, Liu P, Saleemi MK, editors. Animal Health Perspectives: Unique Scientific Publishers; pp: 70-77.

Abdel-Shafi et al., 2017. Molecular identification and phylogenetic analysis of wuchereria bancrofti from human blood samples in egypt. Parasitology Research 116(3): 963-970.

Arkell P et al., 2022. Integrated serological surveillance of acute febrile illness in the context of a lymphatic filariasis survey in Timor-Leste: a pilot study using dried blood spots. Transactions of The Royal Society of Tropical Medicine and Hygiene 116(6): 531-7.

Akue et al., 2011. Epidemiology of concomitant infection due to loa loa and mansonella perstans in gabon. PLOS Neglected Tropical Diseases 5(10): e1329.

Aoki et al., 2011. Basic studies on filaria and filariasis. Tropical Medicine and Health 39(1): 51.

Ash and Orihel, 2007. Atlas de parasitologia humana/atlas of human parasitology, Médica Panamericana.

Bassene H et al., 2015. High prevalence of Mansonella perstans filariasis in rural Senegal. The American Journal of Tropical Medicine and Hygiene 93(3): 601.

Bobkov YV et al., 2021. Altered functional properties of the codling moth Orco mutagenized in the intracellular loop-3. Scientific Reports 11(1): 3893.

BS, 2018. Atlas of fundamental infectious diseases histopathology: A guide for daily practice. College of American Pathologists, Northfield, IL.

Burbelo PD et al., 2009. A four-antigen mixture for rapid assessment of Onchocerca volvulus infection. PLoS Neglected Tropical Diseases 3(5): e438.

Campillo JT et al., 2022. Factors associated with the periodicity of Loa loa microfilaremia in the Republic of the Congo. Parasites & Vectors 15(1): 1-7.

Chandy A et al., 2011. A review of neglected tropical diseases: filariasis. Asian Pacific Journal of Tropical Medicine 4(7): 581-6.

Chansiri K and Phantana S, 2002. A polymerase chain reaction assay for the survey of bancroftian filariasis. Southeast Asian Journal of Tropical Medicine and Public Health 33(3): 504-8.

Chesnais CB et al., 2016. Measurement of circulating filarial antigen levels in human blood with a point-of-care test strip and a portable spectrodensitometer. The American Journal of Tropical Medicine and Hygiene 94(6): 1324.

Crowe A et al., 2018. PCR-coupled sequencing achieves specific diagnosis of onchocerciasis in a challenging clinical case, to underpin effective treatment and clinical management. Infection, Genetics and Evolution 66: 192-4.

da Silva LB et al., 2017. Molecular verification of New World Mansonella perstans parasitemias. Emerging Infectious Diseases 23(3): 545.

Dantas-Torres F and Otranto D, 2020. Overview on Dirofilaria immitis in the Americas, with notes on other filarial worms infecting dogs. Veterinary Parasitology 282: 109113.

Downes B and Jacobsen K, 2010. A systematic review of the epidemiology of mansonelliasis. African Journal of Infectious Diseases 4(1).

Drame et al., 2014. Loop-mediated isothermal amplification for rapid and semiquantitative detection of Loa loa infection. Journal of Clinical Microbiology 52(6): 2071-7.

Eick SM et al., 2019. Seroprevalence of dengue and Zika virus in blood donations: a systematic review. Transfusion Medicine Reviews 33(1): 35-42.

Evans C et al., 2022. Veterinary diagnosis of filarial infection. Human and Animal Filariases: Landscape, Challenges and Control, John Wiley and Sons.

Fassari A et al., 2021. An unusual case of genital filariasis of the testicular tunics in an Italian patient. Annals of Saudi Medicine 41(3): 186-9.

Ferreira MU et al., 2021. *Mansonella ozzardi*. Trends in Parasitology 37(1): 90-1.

Fink DL et al., 2011. Rapid molecular assays for specific detection and quantitation of Loa loa microfilaremia. PLoS Neglected Tropical Diseases 5(8): e1299.

Fischer PU et al., 1998. Detection of the filarial parasite Mansonella streptocerca in skin biopsies by a nested polymerase chain reaction-based assay. American Journal of Tropical Medicine and Hygiene 58(6): 816.

Fischer P et al., 2002. PCR-based detection and identification of the filarial parasite Brugia timori from Alor Island, Indonesia. Annals of Tropical Medicine and Parasitology 96(8): 809-21.

Fischer P et al., 2003. Polymerase chain reaction-based detection of lymphatic filariasis. Medical Microbiology and Immunology 192: 3-7.

Fischer P et al., 2004. Lymphatic filariasis and Brugia timori: prospects for elimination. Trends in Parasitology 20(8): 351-5.

Fischer P et al., 2005. Detection of filaria-specific IgG4 antibodies and filarial DNA, for the screening of blood spots for Brugia timori. Annals of Tropical Medicine & Parasitology 99(1): 53-60.

Foster WA and Walker ED, 2019. Mosquitoes (Culicidae). In: Mullen GR, Durden LA, editors. Medical and veterinary entomology: Academic press; pp: 261-325.

Fox LM, 2018. Blood and tissue nematodes: Filarial worms. In: Long SS, Fischer M, editors.Principles and practice of pediatric infectious diseases: Elsevier; pp: 1388-1394.

Garcia LS and Procop GW, 2016. Diagnostic medical parasitology. Manual of Commercial Methods in Clinical Microbiology: International Edition 2016: 284-308.

Gruntmeir J et al., 2023. Cutaneous filarioid nematodes of dogs in the United States: Are they emerging, neglected, or underdiagnosed parasites? Frontiers in Veterinary Science 10: 1128611.

Hotez PJ, 2009. Parasitic nematode infections. In: Ralph DF, Demmler-Harrison GJ, Kaplan SL, editors. Feigin and cherry's textbook of pediatric infectious diseases (sixth edition): W.B. Saunders; pp: 2981-2996.

Hsueh EC et al., 2001. Lymphoscintigraphy and lymphatic mapping for identification of sentinel lymph nodes. World Journal of Surgery 25: 794-7.

Kaikuntod M et al., 2020. Geographic spatial distribution patterns of Dirofilaria immitis and Brugia pahangi infection in community dogs in Chiang Mai, Thailand. Animals 11(1): 33.

Karunakaran I et al., 2023. Filariasis research–from basic research to drug development and novel diagnostics, over a decade of research at the Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany. Frontiers in Tropical Diseases 4: 1126173.

King KC et al., 2015. Hybridization in parasites: consequences for adaptive evolution, pathogenesis, and public health in a changing world. PLoS Pathogens 11(9): e1005098.

Klion AD, 2013. Miscellaneous filariae. In: MagillD AJ, Solomon RH, Ryan ET, editors. Hunter's tropical medicine and emerging infectious disease (ninth edition): W.B. Saunders, London; pp: 835-839.

Lamberton PH et al., 2015. Modelling the dynamics of mass drug administration selective pressures on schistosome population genetics and genomics. Advanced Parasitology 87: 293-327.

Lammie PJ et al., 2004. Recombinant antigen-based antibody assays for the diagnosis and surveillance of lymphatic filariasis–a multicenter trial. Filaria journal 3: 1-5.

Lima NF et al., 2016. Mansonella ozzardi: a neglected New World filarial nematode. Pathogens and Global Health 110(3): 97-107.

Lipner EM et al, 2006. Field applicability of a rapid-format anti–Ov-16 antibody test for the assessment of onchocerciasis control measures in regions of endemicity. The Journal of Infectious Diseases 194(2): 216-21.

Lizotte MR et al., 1994. A polymerase chain reaction assay for the detection of Brugia malayi in blood. The American Journal of Tropical Medicine and Hygiene 51(3): 314-21.

Mathison BA et al., 2019. Diagnostic identification and differentiation of microfilariae. Journal of Clinical Microbiology 57(10): 10-128.

McCarthy J, 2000. Diagnosis of lymphatic filarial infection. In: Nutman TB, editor. Lymphatic filariasis: Imperial College Press, London; pp: 127-50.

Medeiros JF et al., 2015. A field trial of a PCR-based Mansonella ozzardi diagnosis assay detects high-levels of submicroscopic *M. ozzardi* infections in both venous blood samples and FTA® card dried blood spots. Parasites & Vectors 8: 1-8.

Medeiros JF et al., 2018. Sensitivity of diagnostic methods for Mansonella ozzardi microfilariae detection in the Brazilian Amazon Region. Memórias do Instituto Oswaldo Cruz 113: 173-7.

Medeiros ZM et al., 2021. Lymphatic filariasis: A systematic review on morbidity and its repercussions in countries in the Americas. International Journal of Environmental Research and Public Health 19(1): 316.

Mendoza N et al., 2009. Filariasis: diagnosis and treatment. Dermatologic Therapy 22(6): 475-90.

Meyers WM et al., 2000. Pathology of infectious diseases. Volume 1: helminthiases. Armed Forces Institute of Pathology.

Mulyaningsih B et al., 2019. Study on vector mosquito of zoonotic Brugia malayi in Musi Rawas, South Sumatera, Indonesia. Veterinary World 12(11): 1729.

Nyagang SM et al., 2020. Prevalence of onchocerciasis, attitudes and practices and the treatment coverage after 15 years of mass drug administration with ivermectin in the Tombel Health District, Cameroon. The Pan African Medical Journal 35.

Obradovic Z et al., 2022. A literature review on vector borne diseases. International Journal of Medical Reviews and Case Reports 6(7): 27

Orihel TC and Ash LR, 1995. Parasites in human tissues. In: Orihel C, editor. Parasites in human tissues; pp: 386.

Orihel TC and Eberhard ML, 1998. Zoonotic filariasis. Clinical Microbiology Reviews 11(2): 366-81.

Orihel TC and Eberhard ML, 1982. Mansonella ozzardi: a redescription with comments on its taxonomic relationships. The American Journal of Tropical Medicine and Hygiene 31(6): 1142-7.

Osei-Atweneboana MY et al., 2007. Prevalence and intensity of Onchocerca volvulus infection and efficacy of ivermectin in endemic communities in Ghana: a two-phase epidemiological study. The Lancet 369(9578): 2021-9.

Otranto D and Eberhard ML, 2011. Zoonotic helminths affecting the human eye. Parasites & Vectors 4(1): 1-21.

Poole CB et al., 2017. Colorimetric tests for diagnosis of filarial infection and vector surveillance using non-instrumented nucleic acid loop-mediated isothermal amplification (NINA-LAMP). PloS one 12(2): e0169011.

Raccurt CP, 2018. *Mansonella ozzardi* and its vectors in the New World: an update with emphasis on the current situation in Haiti. Journal of Helminthology 92(6): 655-61.

Ramzy RM et al., 1997. A polymerase chain reaction-based assay for detection of Wuchereria bancrofti in human blood and Culex pipiens. Transactions of the Royal Society of Tropical Medicine and Hygiene 91(2): 156-60.

Rao RU et al., 2006. A real-time PCR-based assay for detection of Wuchereria bancrofti DNA in blood and mosquitoes. The American Journal of Tropical Medicine and Hygiene 74(5): 826.

Rao RU et al., 2006. Detection of Brugia parasite DNA in human blood by real-time PCR. Journal of Clinical Microbiology 44(11): 3887-93.

Satjawongvanit H et al., 2019. Molecular analysis of canine filaria and its Wolbachia endosymbionts in domestic dogs collected from two animal university hospitals in Bangkok metropolitan region, Thailand. Pathogens 8(3): 114.

Schmidt CA et al., 2022. The prevalence of onchocerciasis in Africa and Yemen, 2000–2018: a geospatial analysis. BMC Medicine 20(1): 293.

Simón F, 2001. Heartworm infection in humans and animals. Universidad de Salamanca.

Simonsen PE et al., 2011. Mansonella perstans filariasis in Africa. Acta tropica 120: S109-20.

Supali T et al., 2002. High prevalence of Brugia timori infection in the highland of Alor Island, Indonesia. The American Journal of Tropical Medicine and Hygiene 66(5): 560-5.

Tang TH et al., 2010. Nested PCR to detect and distinguish the sympatric filarial species Onchocerca volvulus, Mansonella ozzardi and Mansonella perstans in the Amazon Region. Memórias do Instituto Oswaldo Cruz 105: 823-8.

Tatuene JK et al., 2014. Epidemiology of Loa loa and Mansonella perstans filariasis in the Akonolinga health district, Centre Region, Cameroon. Health Sciences and Disease 15(1).

Tolle MA, 2009. Mosquito-borne diseases. Current Problems in Pediatric and Adolescent Health Care 39(4): 97-140.

Verma R and Vij M, 2011. Microfilariae of Wuchereria bancrofti in urine: an uncommon finding. Diagnostic Cytopathology 39(11): 847-8.

Vinkeles Melchers NV et al., 2021. How does onchocerciasis-related skin and eye disease in Africa depend on cumulative exposure to infection and mass treatment? PLoS Neglected Tropical Diseases 15(6):e0009489.

Wanji S et al., 2015. Cross-reactivity of filariais ICT cards in areas of contrasting endemicity of Loa loa and Mansonella perstans in Cameroon: implications for shrinking of the lymphatic filariasis map in the Central African Region. PLoS Neglected Tropical Diseases 9(11):e0004184.

Webster JP et al., 2016. One health–an ecological and evolutionary framework for tackling Neglected Zoonotic Diseases. Evolutionary Applications 9(2): 313-33.

Weil GJ et al., 2013. Laboratory and field evaluation of a new rapid test for detecting Wuchereria bancrofti antigen in human blood. The American Journal of Tropical Medicine and Hygiene 89(1): 11.

World Health Organization, 2000 . Bench aids for the diagnosis of malaria infections. World Health Organization

World Health Organization, 2019. Progress in eliminating onchocerciasis in the WHO Region of the Americas: doxycycline treatment as an end-game strategy. Weekly Epidemiological Record 94(37): 415-9.

WHO, 2022. Lymphatic filariasis Available from https://www.who.int/en/news-room/fact-sheets/detail/ lymphatic-filariasis.

WHO, 2022. Onchocerciasis. Available from https://www.who.int/news-room/fact-sheets/detail/ onchocerciasis.

Yahathugoda TC et al., 2015. A comparison of two tests for filarial antigenemia in areas in Sri Lanka and Indonesia with low-level persistence of lymphatic filariasis following mass drug administration. Parasites & Vectors 8(1): 1-7.