*Int.J.Curr.Microbiol.App.Sci* (2018) *7*(7): 3261-3277

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 7 Number 07 (2018)**
Journal homepage: http://www.ijcmas.com

**Review Article** https://doi.org/10.20546/ijcmas.2018.707.380

# Advances in Diagnostics of Parasitic Diseases: Current Trends and Future Prospects

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**K e y w o r d s**

Parasitology, genomics, Serological, Molecular techniques, OMICS technologies

**Article Info**

***Accepted:***
24 June 2018
***Available Online:***
10 July 2018

**A B S T R A C T**

Parasitic diseases constitute a major group of chronic infectious diseases in livestock and jeopardize animal health results in poor production. However, treatment and control of diseases are largely dependent on timely diagnosis. Usually, the diagnosis of parasitic infections relies on testing for the presence of parasites through direct faecal examination, blood smear, lymph node biopsy etc, but clinically, it is often difficult to elucidate the entire offending organism. Accurate diagnoses of parasitic infections are always a prerequisite for successful treatment and control of animal diseases. Besides, the rapid development of drug resistance against anti-parasitic drugs urges the need for the development of the alternative, early diagnostic techniques. In modern years, research has been focused towards alternative methods to improve the diagnosis of parasitic diseases. In this paper, we reviewed the application of various diagnostic techniques for the detection of parasitic infections currently in use and future developments.

## Introduction

Livestock sector plays a pivotal role in improving the socio-economic conditions of developing countries. In India, livestock sector contributes 4.11% of GDP and more than one fourth (25.6%) total output of the agricultural sector GDP (Livestock census, 2012). Among infectious diseases, parasites are a major cause of production loss in terms of morbidity and mortality, results in significant economic losses and its impact directly on the livelihood of farmers. The global loss due to ticks and tick-borne diseases (TTBDs) was estimated to be between the US $ 13.9 and 18.7 billion annually while in India the cost of controlling TTBDs has been estimated at the US $ 498.7 million/annum (De Castro, 1997; Minjauw and McLeod, 2003). In India, tick-borne diseases in animals, like theileriosis and

babesiosis causes economic loss to the tune of US $ 800 million and the US $ 67.2 million, respectively, per annum (Devendra, 1995; Montenegro *et al.,* 1998). So, to formulate effective treatment and control strategies against parasitic diseases, specific diagnosis of parasites is essential to know the true status of parasitic diseases in animals of a particular region. Since the introduction of light microscopy, morphological identification of parasites has been the cornerstone of routine laboratory diagnosis in Parasitology. However, the sensitivity of identifying parasites to occult or acute infection is less. Further, serology based diagnosis is not specific in all the cases. So, currently, to address these issues, nucleic-acid based methods have been employed to detect parasites responsible for parasitic diseases. In the present review, we addressed different serological and molecular techniques employed for diagnosis of different parasitic diseases of animals.

## Microscopy-based method

Microscopy-based detection methods are economically cheaper and considered the gold standard for diagnosis of parasitic infections. However, due to limitations such as technical expertise, occult/ acute infection status of animal etc. may reduce the sensitivity of this test.

## Serology based methods

Serology tests are considered as the gold standard when biologic samples or tissue specimens are not available for diagnosis. It can be divided into two categories: antigen-detection and antibody-detection assays. Serology based method requires considerable skill, time-consuming and labour-intensive in nature. Some tests which are routinely used for parasite detection are addressed (Table no 1).

## Complement fixation test

The Complement fixation test is one of the most widely applicable serologic techniques. Once the required reagents, antigen, complement, sheep erythrocytes and antibody against erythrocytes are prepared and standardized, the complement fixation test used for detection of trypanosomosis, helminthosis, anaplasmosis, babesiosis and toxoplasmosis (Ndao, 2009; Deepak and Singla, 2016). Based on this test, a commercial kit (COFEB Kit) has been developed for the diagnosis of equine piroplasmosis (Sengupta, 2004). Complement fixation test screens a large number of samples at a time and can be automated with relatively simple and inexpensive equipment. It shows increased specificity with a reproducible result. Limitation of this test is not much sensitive and cannot be used for immunity screening, time consuming and labour intensive assay. Non-specific binding of complement may produce false positive results.

## Latex agglutination test

Latex agglutination is observed when a sample containing the specific antigen (or antibody) is mixed with an antibody (or antigen) which is coated on the surface of latex particles. This test has been used for diagnosis of *Fasciola* spp. *Trichinella spiralis*, *Babesia bigemina*, and *Toxoplasma gondii* (Ndao, 2009; Deepak and Singla, 2016). Card agglutination for trypanosomosis tests (CATT) was originally developed for the diagnosis of *Trypanosoma gambiense gambiense* later on for *T. evansi* (Surratex based on *trypanosome*- antigen detection in blood or serum) infection in livestock using latex beads coated with native RoTat 1.2 (Songa and Hamers, 1988). Recently, the N-terminal fragment of VSG RoTat 1.2 has been expressed as a recombinant protein in the yeast *Pichia*

*pastoris* and incorporated in a latex agglutination test, the rLATEX/*T. evansi* (Roge *et al.,* 2014).

## Indirect fluorescent antibody test

Indirect fluorescent antibody test may be used for the detection of antibodies in serum or for the demonstration and identification of antigens in tissues or cell cultures. This test has been applied to the detection of theileriosis, helminthosis, anaplasmosis, besnoitiosis, ehrlichiosis/ malaria, babesiosis, trypanosomosis, toxoplasmosis (Ndao, 2009; Deepak and Singla, 2016). This test is fast, relatively cheap, easy to detect and highly sensitive and specific. This test used on pathogens that can't be easily cultured and allows viewing of labeled cells in a natural environment. The disadvantage of this test is the potential for cross-reactivity and the need to find primary antibodies that are not raised in same species or different isotypes.

## Radioimmunoassay

In radioimmunoassay, radioisotopes are used to measure the immune complex formed by the combination of antigen and antibody. This test used for detection of *Babesia bovis* and *Trypanosoma congolense (*Ricciardi and Ndao, 2015; Ranjan *et al.,* 2015). This test is highly specific and sensitivity. Radiolabeled reagents produce severe radiation hazards. The demerits of the test are requires special laboratory, trained staff to handle radioactive material and requires special arrangements for storage and disposal of radioactive material.

## Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a method of quantifying an antigen immobilized on a solid surface. In this test uses a specific antibody with a covalently coupled enzyme. ELISA test has been applied for the detection of babesiosis, besnoitiosis, helminthosis, toxoplasmosis, trypanosomosis anaplasmosis, and ehrlichiosis (Ricciardi and Ndao, 2015; Ranjan *et al.,* 2015). The first commercial ELISA kit for the diagnosis of *Theileria annulata* infection in cattle based on a recombinant protein known as *T. annulata* surface protein (TaSp-1) and named as SVANOVIR (Al-Hosary *et al.*, 2015).

## Dot-ELISA

In the dot ELISA, the plastic plate is replaced by a nitrocellulose or other paper membrane onto which a small amount of sample volume is applied. The dotted membrane is incubated first with an antigen-specific antibody followed by an enzyme-conjugated anti-antibody. A coloured dot is formed on the membrane on the addition of chromogenic substrate. In the last few years, published studies have demonstrated the use of the dot-ELISA for the detection of *Fasciola gigantica*, *Haemonchus contortus, Theileria equi, Trypanosoma cruzi,* and *Trypanosoma brucei* (Ranjan *et al.,* 2015; Deepak and Singla, 2016).

## Luciferase Immunoprecipitation System (LIPS)

LIPS is a modified ELISA-based assay in which serum containing antigen-specific antibodies can be identified by measuring light production. Currently, this test has been successfully applied for the identification of sera samples infected with *Strongyloides stercoralis* (using a Ruc-NIE fusion) and Loa loa (using a Ruc-LlSXP-1 fusion) (Ramanathan *et al.,* 2008; Burbelo *et al.,* 2008).

## Immunochromatographic assays

Immunochromatography is a combination of chromatography (separation of components of a sample based on differences in their

movement through a sorbent) and immunochemical reactions. The principle of immunochromatography is the same as ELISA sandwich method, the only difference is that immunological reaction is carried out on the chromatographic paper by capillary action. For this system, two kinds of specific antibodies against antigen are used. One of the antibodies is immobilized on the chromatographic paper and the other is labeled with colloidal gold and infiltrated into sample pad. An immunochromatographic unit is completed by attaching the sample pad at the end of the membrane. In the last decade, many immunochromatography tests have been developed using recombinant antigens such as rEMA2 and recombinant *Babesia caballi* 48-kDa rhoptry protein ((rBc48) for *T. equi* and *B. caballi* infections in equine, respectively (Huang *et al.,* 2004; Cruz-Flores *et al.,* 2010). In cattle, some immunochromatography tests, developed using recombinant antigens are recombinant merozoite surface antigen-2 (rMSA-2), spherical body protein-4 (SBP-4), rhoptry-associated protein 1 (RAP-1) and *Theileria annulata* (TaSP-1) antigen for *Babesia bovis, Babesia bigemina* and *T. annulata* infections, respectively (Kim *et al.,* 2008; Guswanto *et al.,* 2017). In dog P50 antigen and BgSA1 are for *Babesia gibsoni* infections (Verdida *et al.,* 2005; Jia *et al.,* 2007).

Surra Sero K-SeT test developed for detection of *Trypanosoma evansi* infection in domestic animals and the test is based on recombinant variant surface glycoprotein (rVSG) RoTat 1.2, produced in the yeast *P. pastoris*. The overall sensitivity of the Surra Sero K-SeT was higher when compared with CATT/*T. evansi*. Hence this may become an alternative for the CATT/*T. evansi* for sensitive detection of antibodies against *T. evansi* in domestic animals (Birhanu *et al.,* 2015). Currently, lateral flow test (LFA) has been used for the identification of sera sample infected with *T. evansi* in equine. The test was compared with ELISA; it was observed that 93.31% sensitive and 100% specific, as none of the negative field sample, was found positive in LFA (Yadav, 2018).

## Molecular-based methods

The use of DNA/RNA based methods derives from the premise that each species of parasite carries unique DNA or RNA sequences that differentiate it from other parasites. The molecular technique with the widest variety and application in Parasitology diagnostics is PCR. Besides the conventional PCR, including nested and multiplexed PCR, we have seen the implementation of the real-time PCR for the detection of several parasitic infections. Newer technologies such as random amplified polymorphic DNA (RAPD), microsatellite marker, loop-mediated isothermal amplification, Luminex based assays, nanotechnology, and biosensor have also emerged as possible new approaches for the diagnosis of parasitic diseases.

## Polymerase Chain Reaction (PCR)

PCR, providing exquisite sensitivity and specificity for the detection of nucleic acid targets, has become one of the most important tools in parasite diagnostics (Gasser *et al.,* 2006). The PCR is a tool for the accurate identification of parasites and their genetic characterization, the diagnosis of infections, the isolation and characterization of expressed genes, the detection of anthelmintic resistance, and mutation scanning approaches for the high-resolution analysis of PCR products. Exploration of genetic markers like 18 sRNA, ITS-1 and ITS-2 helps in the species identification of Amphistomes (Lofty *et al.,* 2010), *Fasciola* (Alba *et al.,* 2015) and coccidia (Gadelhaq *et al.,* 2015). *Eimeria ninakohlyakimovae* and *E. christenseni* infections in Indian goats using 18S rRNA and

ITS-1 genes have been genetically characterized using PCR based molecular techniques (Verma *et al.,* 2017).

## Real-Time Polymerase Chain Reaction (RT-PCR)

RT- PCR is the latest improvement in the standard PCR technique to be implemented in Parasitology laboratories. The fluorescence readings are plotted by computer software and results can be transmitted electronically, eliminating the need for post-PCR reaction analysis by electrophoresis.

The Real-time PCR assay provides quantification of the sample using several fluorescent agents such as TaqMan probes, SYBR Green dye and Scorpion primers (Ricciardi and Ndao, 2015). Several studies have been conducted on the application of SYBR Green I RT-PCR to protozoans *viz.*, *Cryptosporidium, Leishmania, Trypanosoma, Giardia* and *T. gondii* (Tavares *et al.,* 2011).

## Nucleic acid sequence-based amplification (NASBA)

NASBA is a promising gene amplification method. This isothermal technique is comprised of a two-step process whereby there is an initial enzymatic amplification of the nucleic acid targets followed by detection of the generated amplicons. The entire NASBA process is conducted at a single temperature, thereby eliminating the need for a thermocycler.

Recently, NASBA has been used for diagnosis of *Babesia* and *Theileria* using RNA as an initial template (Skotarczak and Sawczuk, 2008) and also used in combination with gold nanorods to develop a colorimetric assay targeting the 18S rRNA of *Leishmania* (Niazi *et al.,* 2013).

## Loop-Mediated Isothermal Amplification (LAMP)

LAMP is a simple, rapid, highly specific and cost-effective single tube technique for the amplification of DNA. Amplification and detection of gene can be completed in a single step using LAMP that take just 15-60 minutes, by incubating the mixture of samples, primers, *Bst* DNA polymerase with strand displacement activity and substrates at a constant temperature (about 60-65°C). LAMP is an isothermal nucleic acid amplification technique so it does not require expensive thermal cyclers. In-tube detection of DNA amplification is possible hence there is no need to run gel electrophoresis after amplification. Recently, parasitologists have adapted the LAMP technique to detect several parasitic diseases, viz., *Cryptosporidium* spp *E. histolytica*, *Plasmodium spp*, *Trypanosoma spp*, *Taenia spp*, *Schistosoma spp*, *Fasciola hepatica*, *F. gigantica*, *T. gondii Theileria*, *Babesia* and *Eimeria* (Alhassan *et al.,* 2007; Guan *et al.,* 2008;; Ranjan *et al.,* 2015; Barkway *et al.,* 2015). It can also be used for identification of parasites in their vectors such as *Dirofilaria immitis* has been detected in mosquitos using this technique (Aonuma *et al.,* 2009). Also, this technique could detect the miracidium after the first day of exposure in snails, the intermediate hosts of *Schistosoma* (Abbasi *et al.,* 2010).

## Luminex xMAP technology

Luminex is a bead-based xMAP technology (multianalyte profiling), a system that combines flow cytometry, fluorescent microspheres (beads), lasers and digital signal processing, and is capable of simultaneously measuring up to 100 different analytes in a single sample. In diagnostic Parasitology, this technology is still new, but it has been used to diagnose *E. histolytica, Giardia, Cryptosporidium, Ascaris, Necator,*

*Ancylostoma, Strongyloides, T. gondii, Toxocara canis, T. cati,* and *T. spiralis* (Ndao, 2009; Ranjan *et al.,* 2015; Reslova *et al.,* 2017).

## Random Amplified Polymorphic DNA (RAPD)

This technique is also known as arbitrarily primed PCR. Test is based on amplification of genomic DNA with a single primer selected from an arbitrary nucleotide sequence. RAPD has been extensively used for description of strains in epidemiological studies. The surveying of genomes of parasites is enhanced by the advantage that RAPD is a very simple, fast and inexpensive technique that does not require either prior knowledge of the DNA sequence or DNA hybridization. Several studies shows that, method can be used for to differentiate species of *Leishmania*, in addition to polymorphisms studies of parasites such as *Plasmodium, Trypanosoma, E. granulosus* and *T. solium* and *W. bancrofti* (Tavares *et al.,* 2011; Ranjan *et al.,* 2015).

## Amplified Fragment Length Polymorphism (AFLP)

AFLP is the selective amplification of restriction fragments from a digest of total genomic DNA using the polymerase chain reaction (PCR). AFLP has been successfully applied to differentiate isolates of *C. parvum* into two distinct genotypes, as well as strains of *Leishmania* belonging to cutaneous leishmaniosis and visceral leishmaniosis (Blears *et al.,* 2000; Kumar *et al.,* 2010).

## Restriction Fragment Length Polymorphism (RFLP)

RFLP is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another. The RFLP technique is currently one of the most commonly used molecular method for diagnosis of species and genotypes of parasites such as *T. gondii, Cryptosporidium* spp. and *Theileria* spp. (Quan *et al.,* 2008; Molloy *et al.,* 2010; Zaeemi *et al.,* 2011). Recently, semi-nested PCR-RFLP was used for detection of persistent anaplasmosis (Jaswal *et al.,* 2014).

## Microarray technology

Microarray is one of the most recent tests being used in veterinary research. Originally developed for the mapping of genes, it is being used to detect a wide variety of veterinary pathogens. An array is an orderly arrangement of samples where matching of known and unknown DNA samples is done based on base pairing rules. This is a combination of DNA amplification with subsequent hybridization to oligonucleotide probes specific for multiple target sequences. It allows analysis of a larger number of genetic features in a single trial. It has been used in detection and genotyping of *Plasmodium, Toxoplasma* and *Trypanosoma* (Duncan *et al.,* 2004).

## Microsatellites

Microsatellites are the short DNA sequences consist of tandem repeats of one to six nucleotides with approximately one hundred repeats. These are utilized because of the frequent polymorphism, co-dominant inheritance, high reproducibility and high resolution of the genes. Microsatellites have been described in Parasitology and used in some parasites of both humans and animals.

Despite their potential usefulness, microsatellite markers were developed only for some parasites such as species of *Trichostrongyloid nematodes* and *T.gondii* (Temperley *et al.*, 2009; Ajzenberg *et al.*, 2010).

## Nanotechnology

Nanotechnology is the study of extremely small structures, having size of 0.1 to 100 nm. With the help of nanomedicine early detection and prevention, improved diagnosis, proper treatment and follow up of diseases are possible. Certain nanoscale particles are used as tags and labels, biological can be performed quickly, the testing has become more sensitive and more flexible. A small number of parasites have been the target for nano-technology, focusing primarily in *Leishmania* sp. and *Plasmodium* sp. (De Carvalho *et al.*, 2013; Waknine-Grinberg *et al.*, 2013). Currently, researches are going on using nanopeptides against *Haemonchus contortus* and *Fasciola hepatica* in Cuba and Brazil.

**Table.1** OIE recommended test for the international trade of animal and its products (OIE, 2008)

| S.No. | Disease name | Prescribed tests* | Alternative tests |
|---|---|---|---|
| **1.** | Trichinellosis | Agent identification | Enzyme-linked immunosorbent assay |
| **2.** | Trichomonosis | Agent identification | Mucus agglutination test |
| **3.** | Dourine | Complement fixation | Enzyme-linked immunosorbent assay, Indirect fluorescent antibody |
| **4.** | Equine piroplasmosis | Enzyme-linked immunosorbent assay, Indirect fluorescent antibody | Complement fixation |
| **5.** | Theileriosis | Agent identification, Indirect fluorescent antibody | - |
| **6.** | *Trypanosoma evansi* infection | Card agglutination tests | - |
| **7.** | Bovine anaplasmosis | - | Card agglutination test, Complement fixation |
| **8.** | Bovine babesiosis | - | Enzyme-linked immunosorbent assay, Indirect fluorescent antibody, Complement fixation |
| **9.** | Bovine anaplasmosis | - | Card agglutination test, Complement fixation |
| **10.** | Bovine babesiosis | - | Enzyme-linked immunosorbent assay, Indirect fluorescent antibody, Complement fixation |
| **11.** | Trypanosomosis (Tsetse-transmitted) | - | Indirect fluorescent antibody |
| **12.** | Mange | - | Agent identification |

## Biosensing technology

A biosensor consists of two components: a bioreceptor and a transducer. The bioreceptor is a biomolecule that recognizes the target analyte whereas the transducer converts the recognition event into a measurable signal. In parasitological point of view, a low-cost biosensor system was made with nanostructured films containing specific *L. amazonensis* and *T. cruzi* antigens and employing impedance spectroscopy as the detection method (Perinoto *et al.,* 2010) Over the long term, we believe that biosensor technology combining nanotechnologies, advances nucleic acid amplification methods and next-generation sequencing analysis will be a powerful systemic tool for pathogens detection and surveillance system to control animal disease outbreaks and prevention (Wang, 2005; Vidic *et al.,* 2017).

## Application of high throughput 'omics' technologies in veterinary parasitology

The advent and integration of high-throughput '-omics' technologies (e.g. genomics, transcriptomics, proteomics, metabolomics, glycomics and lipidomics) are revolutionizing the way biology is done, allowing the systems biology of organisms to be explored. These technologies are now providing unique opportunities for molecular, genetic host parasitic interaction, diagnosis, development of drugs and vaccines against parasitic diseases (Cantacessi *et al.,* 2012; Cantacessi *et al.,* 2012)**.**

## High throughput sequencing (HTS)

Whole genome sequencing started with the sequencing of a bacteriophage in 1977 using the Sanger sequencing technique. In the last few years, it has become possible to sequence the whole genome of key parasites and related organisms, such as *Caenorhabditis elegans* (Brenner, 1974). In fact, the genome of this nematode was the first completed genome for any multicellular organism. It represents a tremendous resource for research on helminths. The ongoing development of HTS technologies has revolutionized biomedical research. These breakthrough platforms have rapidly evolved from next-generation sequencing (NGS) or second-generation platforms [454 / Roche sequencing, Illumina (Solexa) sequencing, SOLiD systems and Ion Torrent sequencing)] to third-generation [PacBio RS II (Pacific Biosciences) and Heliscope sequencer (Helicos BioScience)] and fourth-generation sequencing machines [MinION (Oxford Nanopore)]. HTS technologies are now providing the opportunity to detection, identification, characterization of previously unidentified parasites, molecular marker profiles, whole genome sequencing and pathotyping or resistance typing information. Sequencing, mapping and comparing the genomes of cells in healthy and disease states, cheaply, rapidly, and accurately can alter the way clinicians think about how to treat patients shifting from traditional medicine to a genome based era of preventive and therapeutic decisions (Ku and Roukos, 2013; Belák *et al.,* 2013). In the last years, numerous studies have demonstrated the utility of NGS technologies for investigating, for instance, aspects of the systematics, population genetics and molecular biology of parasites including strongylid nematodes, whitefly, ticks, *Giardia intestinalis, Trichomonas vaginalis*, *Cryptosporidium* and *Toxocara* (Chen *et al.,* 2009; Wang *et al.,* 2010; Cantacessi *et al.,* 2012; Gasser, 2013; Qablan *et al.,* 2014; Zahedi *et al.,* 2017).

## Bioinformatics

Bioinformatics comprises mathematical approaches and algorithms applied to biology and medicine using Information Technology tools, e.g. databases and mining software.

Analysis of omics data typically follows four steps: (1) data processing and identification of molecules, (2) statistical data analysis, (3) pathway and network analysis, and (4) system modelling. Examples include *de novo* genome assembly, genome annotation, identification of co- or differentially expressed genes at the level of transcripts or proteins and the inference of protein– protein interaction networks (Ballereau *et al.,* 2013). Recent studies have utilized bioinformatic platforms to explore the complement of molecules transcribed in different developmental stages and both sexes of key parasitic nematodes, including *Trichostrongylus columbriformis* (Cantacessi *et al.,* 2010) *Haemonchus contortus* (Cantacessi *et al.,* 2010), *Necator americanums* (Cantacessi *et al.,* 2010) and *Oesophagostomum dentatum*; (Lin *et al.,* 2012). Accurate bioinformatic analyses of transcriptomic and genomic data are crucial for the provision of meaningful biological information on parasites. Until recently, detailed bioinformatic analyses have been restricted largely to specialized laboratories with substantial computer and software capacities. However, the introduction of new integrated bioinformatic systems, such as Bio-cloud (http://cloud.genomics.cn) and Artemis (http://www. sanger.ac.uk/resources/software/ artemis/), for the *de novo* assembly and annotation of NGS sequence data could represent a turning point for 'omic' research (Santhoshkumar *et al.,* 2012; Cantacessi *et al.,* 2012). The annotation of proteins inferred from the genomic and transcriptomic datasets is usually performed by assigning predicted biological function ⁄ s based on comparison with existing information available for *C. elegans* and for other organisms in public databases (e.g. WormBase, http://www.wormbase.org; InterPro, http:// www.ebi.ac.uk/interpro/; Gene Ontology, http://www. geneontology.org/; OrthoMCL, http://www.orthomcl.org/; BRENDA, http://www.brenda-enzymes.org/). Using this approach, predictions for key groups of molecules, such as those linked to the physiology of the nervous system, the formation of the cuticle, proteases and protease inhibitors, and protein kinases and phosphatases, have been made in relation to their function and essential roles in biological processes (Cantacessi *et al.,* 2012; Cantacessi *et al.,* 2012; Ballereau *et al.,* 2013).

## Transcriptomics

Transcriptomics is the genome-wide identification and quantification of RNA species such as mRNAs, non-coding RNAs and small RNAs, in health and disease, and in response to external stimuli. High-throughput sequencing of RNA has become the standard assay for measuring gene expression, and numerous studies conducting "RNA-Seq" experiments in parasites have now been performed and deposited in the sequence archives. Investigations of the transcriptome of parasites using different approaches is gradually leading to a better understanding of the biochemical and molecular processes involved in parasite development, reproduction and interactions with their host/s (Cantacessi *et al.,* 2012; Cantacessi *et al.,* 2012). In NGS, particular the 454 platform was used recently for the *de novo* sequencing of the transcriptomes of important parasites such as trematodes *Clonorchis sinensis* (Young *et al.,* 2010), *Fasciola hepatica* (Young *et al.,* 2010), *Fasciola gigantica* (Zhang *et al.,* 2017), *Paramphistomum cervi* (Choudhary *et al.,* 2015), *Trichostrongylus colubriformis*, (Ku and Roukos, 2013), *Ixodes ricinus* (Schwarz *et al.,* 2013), *Haemaphysalis flava* (Xu *et al.,* 2015), *Rhipicephalus appendiculatus* (De Castro *et al.,* 2016), *Dermanyssus gallinae* (Schicht *et al.,* 2014), *Tritrichomonas foetus* (Morin-Adeline *et al.,* 2015) and *Neospora caninum* (Ramaprasad *et al.,* 2015).

## Proteomics

Proteomics allows the study of proteins present in a given tissue or fluid (the proteome). It is of significant importance to numerous scientific areas, including animal and veterinary sciences. In the last few years, there has been an awakening interest in using proteomics and the complementary, essential advances in bioinformatics, to address problems of veterinary pathogenesis. Mass spectrometry is widely used to proteomics and currently a most important tool for identification and diagnosis of parasitic infections. Mass spectrometry (MS) relies on the deflection of charged atoms by magnetic fields in a vacuum to measure their mass/charge (m/z) ratio. A typical experiment follows five steps: (1) introduction of the sample, (2) ionisation of its particles, (3) acceleration, (4) deflection proportional to the mass and charge of the ion, and (5) detection, recorded as a spectrum showing peaks on a plot of relative quantity as a function of the m/z ratio. A mass spectrometer consists of an ion source, a mass analyser that measures the mass-to-charge ratio (m/z) of the ionized analytes, and a detector that registers the number of ions at each m/z value. The mass analyser is, literally and figuratively, central to the technology. There are four basic types of mass analyser currently used in proteomic research. These are the ion trap, time-of-flight (TOF), quadrupole and Fourier transform ion cyclotron analysers, and they are very different in design and performance, each with its own strength and weakness (Aebersold and Mann, 2003). In recent years, the identification of novel biomarkers in parasite diagnostics has relied on the use of mass spectrometry (MS) platforms. Such instruments include matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), surface-enhanced laser desorption ionization time of flight mass spectrometry (SELDI-TOF MS), liquid chromatography combined with MS (LC–MS–MS), isotope-coded affinity tags (ICAT), and isotope tags for relative and absolute quantification (iTRAQ) (Ndao, 2009). Most studies published on parasitic diseases have all focused on the use of MALDI-TOF MS and SELDI-TOF MS. Pathogenesis of gastrointestinal nematode infection was recently studied by quantitatively investigating the expression of proteins by abomasal mucosa of resistant and susceptible sheep breed after experimental *Haemonchus contortus* infection (Nagaraj *et al.,* 2012).

## MALDI-TOF MS

MALDI is soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules such as DNA, protein, peptides and sugar or polymers. It is three steps method; first, the sample is mixed with suitable matrix and applied to a metal plate. Second, a pulsed laser irradiates a sample triggering desorption of matrix material and third ionization of analyte molecules. The typical detector used with MALDI is the time of flight mass detector (TOF-MS) TOF is a method where the ions are accelerated by an electric field, resulting in ions of the same strength to have the same kinetic energy. The time it takes for each ion to traverse the flight tube and arrive at the detector is based on its mass-to-charge ratio; therefore the heavier ions have shorter arrival times compared to lighter ions (Hillenkamp *et al.,* 1991; Lewis *et al.,* 2000). MALDI-TOF MS has emerged as an alternative technique for the identification of a number of arthropods such as *Culicoides* (Kaufmann *et al.,* 2012), mosquitoes (Suarez *et al.,* 2011) and ticks (Karger *et al.,* 2012).

## SELDI-TOF MS

SELDI-TOF can be described as a type of MALDI-TOF mass spectrometry where the sample matrix, known as Protein Chip, has an

active role in sample purification as well as the desorption/ionization step. This technology is based on the separation of proteins using their chemical and physical characteristics (i.e., hydrophobic, hydrophilic, acidic, basic, metal affinity) by performing a chromatographic separation of the sample to be analyzed. Three major components constitute SELDI-TOF: the Protein Chip arrays, the mass analyzer, and the data analysis software (Merchant, 2000; Tarawneh and Bencharit, 2009). SELDI technique has been applied to the study of serum biomarkers of parasitic diseases such as human African trypanosomosis (Agranoff *et al.*, 2005), fasciolosis (Rioux *et al.*, 2008) and cysticercosis (Deckers *et al.*, 2008).

In conclusion, present communication gives the information of detection and diagnostic test in various parasitic infections of livestock and humans may be a guideline for veterinarian clinician and academician.

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**How to cite this article:**

Rupesh Verma, G. Das, H.V. Manjunathachar and Nirmala Muwel. 2018. Advances in Diagnostics of Parasitic Diseases: Current Trends and Future Prospects. *Int.J.Curr.Microbiol.App.Sci.* 7(07): 3261-3277. doi: https://doi.org/10.20546/ijcmas.2018.707.380