Diagnosis of leishmaniasis has always been a major challenge as its clinical features resemble some other commonly occurring diseases such as tuberculosis, typhoid, and malaria. Reliable laboratory methods become important for differential diagnosis. Demonstration of the parasites in stained preparations of bone marrow and splenic aspirates being risky and invasive is still the gold standard for diagnosis. Serological tests utilizing rapid immunochromatographic formats or rK39 in enzyme linked immune sorbent assay, immunoblotting, direct agglutination test have complications related to high proportions of positive asymptomatic individuals and the inability to diagnose a relapse. Among the molecular techniques, polymerase chain reaction is the most commonly used technique that is successfully implied for diagnosis. This review provides updated information on the recent developments in the field of diagnosis in leishmaniasis, various methods utilized with their advantages and limitations.

Keywords Leishmania · Diagnosis · Parasitological · Immunological · Molecular

Introduction

The parasitic protozoan of genus Leishmania and family Trypanosomatidae are responsible for causing leishmaniasis. They are characterized by possessing kinetoplast, a distinctive form of mitochondrial DNA (Sharma and Singh 2008). Leishmaniasis is distributed in subtropical and tropical regions affecting 97 countries in America, Africa, Asia and Europe (Steverding 2017). It can occur in three main forms viz., Visceral Leishmaniasis (VL), Cutaneous Leishmaniasis (CL) and Mucocutaneous Leishmaniasis (MCL). The numbers of cases show changes or variations with time and are challenging to estimate. For VL, approximated cases per year may have been reduced to < 100,000, but earlier estimations ranged up to 400,000 or more. For CL, the number of cases has ranged from 700,000 to 1.2 million or more (CDC 2018). The disease is prevalent on every continent except Antarctica and Australia. In the eastern hemisphere, leishmaniasis is found in the Middle East, Africa, Asia, and Southern Europe. In the western region, it is prevalent in Central and South America and Mexico (Chhabra and Singla 2014). It is not seen in Uruguay and Chile. Infrequent cases of CL have been reported in Oklahoma and Texas (WHO 2016). VL moved from Southern India and became endemic in Eastern states of Assam, Bihar, and Bengal until the late 1990s (Singh 2014). More recently, epidemiology of VL was seen mainly in eastern states of the country, including West Bengal, Jharkhand, Uttar Pradesh, and Bihar (Thakur et al. 2018). Bihar is the most distressed state with 90% of the caseload, although the VL elimination target has been successful in eliminating 366 out of 456 blocks in Bihar (Olliaro et al. 2017). Also, VL cases reported in India have declined from 32,803 in 2005 to 6231 in 2016. Besides the states mentioned above with high caseloads, reports on re-emergence of the disease in the states of Assam, Tamil Nadu, and Gujarat along with the finding of newer distressed regions have been outlined during the last decade (Dhiman 2014). Although there is an overall reduction in VL incidence in India as a consequence of the VL
elimination program in the highly endemic areas, the new cases from mostly non-endemic regions appear to be increasing, and this is of utmost importance to avoid reemergence and to achieve sustainable elimination of disease from the country (Thakur et al. 2018).

*Leishmania* comprises of two developmental stages amastigotes and promastigotes. The amastigotes are non-flagellated spherical cells ranging in size from 2 to 4 μm in diameter. Promastigote forms are thin elongate cells with an emergent flagellum and anterior kinetoplast. They are usually lance-like in shape and range in size from 5 to 14 μm in length by 1.5–3.5 μm in width. Different parasite species are usually not differentiated by morphological differences, but rather on the basis of geographical, biological and clinical features (Chhabra and Singla 2014). Both vertebrates and invertebrates are their hosts, the mammal being the final host and sandfly (*Phlebotomus* in the Old World and *Lutzomyia* in the New World) (Sharma and Singh 2008), the intermediate host. Promastigotes being propagative forms occur in the female sandfly lumen, and amastigotes are found inside phagolysosomes in different mammalian hosts. The transmission begins when the infected phlebotomine sandfly carrying *Leishmania* parasite bites its mammalian host (human) (Teixeira et al. 2013).

The disease encounters humans when the reservoir host and flies share the same environment (Lemma et al. 2017). The first form is anthroponotic VL, with transmission of infection from humans to humans and the second form is zoonotic VL, with transmission between animals to humans. Epidemiological studies of VL worldwide have incriminated several animal species as reservoirs for zoonotic VL, including dogs, jackals, rodents, and foxes (Mukhtar et al. 2000; Reithinger et al. 2002; Chappuis et al. 2007). Transmission of disease begins when infected sandfly bites its mammalian host (Fig. 1) (Ready 2013). However, the infection can rarely be transmitted by other means such as needle sharing, blood transfusion, or from mother to child during pregnancy (Alemayehu and Alemayehu 2017). Clinical symptoms of leishmaniasis rely on interactions between immune responses of host and aspects of *Leishmania*; this results in a spectrum of diseases from localized skin lesions to involvement of reticuloendothelial system (Sharma and Singh 2008).

**Co-infections**

Co-infection of leishmaniasis and Human immunodeficiency virus (HIV) has important diagnostic, clinical and epidemiological implications. HIV is one of the significant health problems worldwide, and about 369 lakh people are living with HIV and 20 lakh new infections are surveyed every year. Overlapping between transmission areas of leishmaniasis and HIV co-infection is very commonly observed (Lindoso et al. 2016). The presence of parasites in peripheral blood of HIV infected patients makes them a source of infection for the vectors (Chappuis et al. 2007). Both diseases target the same immune cells, thus exerting a cumulative effect on the immune system (Pintado et al. 2001). In co-infected patients, HIV modifies the presentation of *Leishmania*. VL increases the rate of onset of AIDS and decreases the lifetime of HIV infected patients. Also, HIV upregulates the rate of VL to a great extent. This dual response leads to insufficiency in CD4+ T cell immune response, enhancing the severity of disease (Singh 2014).

Intestinal parasitic infections might also affect the disease severely by changing cell-mediated immunity and by damaging effects of malnutrition (Diro et al. 2015). A strong Th2 response, a characteristic feature of helminthic infection (Maizels et al. 2012) is seen to suppress the...
protective Th1 response in patients with VL contributing to immunosuppression that is characteristic to VL patients (Nylén and Sacks 2007) (Fig. 2).

CL and malaria are the two most severe vector-borne parasitic diseases (Alvar et al. 2012) affecting millions of people. Overlapping geographical distribution of *Leishmania* and malaria depicts that susceptibility and severity to both conditions are elevated during co-infection. Since the two parasites do not compete for the same host cells, the interaction between them is not direct and is dependent upon host immune response induced by the pathogen (Coleman et al. 1998).

Co-infection of VL and pulmonary tuberculosis are increasing problems of public health in developing countries. Infection of leishmaniasis changes the protective immune response to BCG vaccine against tuberculosis (Li and Zhou 2013). Although the transmission mechanism and etiology of both diseases are different, yet they share several features. Tuberculosis is an immunosuppressive condition that provokes the progression of latent leishmaniasis to clinical leishmaniasis, and VL can provoke latent tuberculosis (Shweta et al. 2014).

**Immune response to Leishmania**

The life cycle of *Leishmania* begins when the infected sandfly carrying the metacyclic promastigote bites the host during its blood meal. Within few minutes the promastigotes are taken up by the phagocytic cells such as macrophages and neutrophils (Antoine et al. 1998). After internalization inside phagolysosomes of macrophages, promastigotes begin to differentiate into small, non-motile amastigote forms, that divide multiple times by binary fission, ultimately rupturing the macrophages to infect surrounding macrophages (Liu and Uzonna 2012).

Once the initial infection starts, monocytes are recruited to infected tissue and begin to differentiate into macrophages (Yang et al. 2014). T cells in lymphoid tissues and spleen are activated both by dendritic cells and the macrophages. The naïve T cells get activated into Th0 cells (intermediate stage) which then travel to the liver and get triggered as Th1 cells on coming in contact with dendritic cells and macrophages in IL-12 environment (Siewe et al. 2016). During this attachment, CD4+ T cells recognize the antigens which are bound to Major Histocompatibility Complex (MHC) (Dhanji and Teh 2003). CD4+ T cells then produce IL-12, which in turn triggers CD8+ T cells and enhance CD4+ T cell multiplication. Both CD8+ T cells and CD4+ T cells yield IFN-γ, which in turn activates the macrophages to wipe out the parasites (Siewe et al. 2016). The advancement of the disease is mainly related to Th2 type of immune response along with an increase in levels of interleukin-10, IL-5, IL-4 and transforming growth factor (Torres-Guerrero et al. 2017).

**Treatment**

Treatment remains an overwhelming question as different species of *Leishmania* show various manifestations, which makes the treatment even more complicated (Kotb Elmahallawy and Agil 2015). Despite the availability of different treatment approaches to treat leishmaniasis, therapeutic tools are not adequate to eradicate this infection. These compounds face high cost, drug resistance, toxicity and some other side effects (Ghorbani and Farhoudi 2018). Pentavalent antimoniate was first introduced 60 years ago, and it was the most effective drug with good
results (Torres-Guerrero et al. 2017). Pentavalent antimonial such as sodium stibogluconate is given at a dose of 20 mg/kg body weight for a month, and these drugs can be used both in combination or alone (Akbari et al. 2017). Treatment with antimonials is affected due to adverse effects such as cardiac arrhythmia, pancreatitis, hepatotoxicity and nephrotoxicity (Freitas-Junior et al. 2012). These are also not recommended for pregnant women due to several limitations like parenteral administration (Akbari et al. 2017). Amphotericin B is an anti-fungal drug that is used as a second line treatment (Sundar et al. 2000). Liposomal formulation of this drug is more useful for the treatment of VL (Copeland and Aronson 2015). Miltefosine is the first oral drug for the treatment of VL. It is given for a month at a dose of 50–100 mg/day. Despite its numerous advantages, it has certain side effects such as nephrotoxicity, hepatotoxicity and being teratogenic (Sundar and Singh 2016). The available treatment for leishmaniasis is overwhelmed with resistance to some of the currently available drugs. Mechanism of drug resistance is often related to lower drug uptake, increased efflux, rapid rate of drug metabolism, modifications of drug targets and over expression of drug transporters. The widespread presence of leishmaniasis and appearance of drug resistance reveals the urge to develop and explore novel, less toxic and more promising therapeutic approaches (Yasinzai et al. 2013). Combination drugs for the treatment of VL can be utilized to combat the drug resistance developed by monotherapy. It can broaden the spectrum, enhance the activity of the drug by additive or synergistic action, decrease the duration and dosage, reduce the side effects, the cost of treatment and the emergence of drug resistance (Musa et al. 2012).

**Vaccines**

Patients once cured of leishmaniasis, are generally immune to second attack of the disease (Gupta et al. 2013). This fact leads to the development of vaccines to immunize the individuals which are at risk of infection (Reithinger et al. 2007). No vaccines are available for humans against leishmaniasis. However, vaccines such as genetically attenuated live parasites; freeze-thawed/heat-killed parasites, recombinant proteins and DNA vaccines are in the process of development (Thomaz-Soccol et al. 2018). The live attenuated *Leishmania* parasites can be used to develop a successful vaccine (Ismail et al. 2017). However, chances of reversion of attenuated form of *Leishmania* to virulent form and possibility of attenuated form, causing the disease in immunocompromised individuals is a major concern (Palatnik-de-Sousa 2008). Vaccines with a dead parasite or with fragments are called "first generation vaccines" against the *Leishmania* parasites (Thomaz-Soccol et al. 2018). Other vaccine candidates like recombinant proteins or subunit vaccines are weak immunogens that fail to mount an adequate immune response (Dunning 2009).

Vaccines made from DNA have many advantages over other vaccine (first and second generation vaccines) strategies as they are cheap, fast, and simpler to produce on a large scale, they do not require low temperature for transport and storage and can give protection against more than one species of *Leishmania* and for a long time (Donnelly et al. 2000) But the major concern with DNA vaccine is the risk of integrating parasite DNA into the mammalian genome, which can sometimes result in the induction of an autoimmune disease or cancer (Dunning 2009). However, in light of its many advantages over other *Leishmania* vaccine strategies, DNA vaccination could prove to be the best approach for use in therapy and prophylaxis against human leishmaniasis (Dunning 2009).

**Diagnosis**

The first sign of infection is small erythema at the site of bite. After this erythema formation, the infection begins, and parasites cause an inflammatory reaction due to which erythema develops into an open ulcer or visceralize to organs like spleen and liver. These inflammatory reactions of parasites depend upon the species of parasite or its host immunity, strain and several other unknown factors (Reithinger and Dujardin 2007). Thus, the early diagnosis of leishmaniasis is of great significance to prevent the development of severe clinical manifestations and mortality in patients with VL. Conventional diagnosis depends upon the microscopical examination of amastigotes in the aspirates from tissues of different organs like spleen, lymph nodes, liver, skin, and can also be done by parasite culturing from these sites. However, this process of aspirate examination is not comfortable for the patients, and the method of isolating the parasite from culture is time-consuming, expensive and difficult to perform (Mugasa et al. 2010).

A number of diagnostic methods have been produced with considerable variations in accuracy of diagnosis, including the parasitological examination (histopathology, microscopy and parasite culture), serological technique and molecular diagnostics (Fig. 3) (Goto and Lindoso 2010). Thus, this review aims to highlight the recent progress in the diagnosis of leishmaniasis and its limitation with focus on what can be the most successful methods for developing fast, economic, specific and sensitive diagnostic tests.

**Culture techniques** Parasite culturing enhances the detection sensitivity, although it is seldom used for routine clinical practices. Isolating and culturing the parasites gives better diagnostic sensitivity and organisms can also
be identified to genotype and species level. Parasite species characterization and identification can be done by combination of culture and multilocus enzyme electrophoresis (Reithinger et al. 2007). Solid NNN (Novy-MacNeal-Nicolle) medium containing 20–30% rabbit blood or liquid Schneider’s insect culture medium can be used for isolating and culturing the promastigote forms (Singh 2006). Some new culture methods which can enhance the sensitivity are micro-culture method (MCM), recent modifications of MCM includes usage of peripheral blood mononuclear cells (PBMC) and buffy coat (WBC rich layer) (Maurya et al. 2010). Mini and microculture techniques are advantageous being cheaper as compared to other cultures because they need small volume of culture medium and are also easy to handle even if the parasite burden is low (Boggild et al. 2008).

**Lateral flow biosensors (LFB)** LFBs are the devices based on single use paper carrier materials, where dry reagents are activated by putting fluid sample. LFBs are significant for the diagnosis as they are sensitive, specific, affordable, rapid, robust and equipment free (Parolo and Merkoçi 2013). Immunochromatography based assays are easy to use and provide rapid qualitative results (Mettler et al. 2005; Pattabhi et al. 2010). Lateral flow strips for rK39 antigens are available commercially for detection of visceral leishmaniasis, but their performance is still not optimal (Welch et al. 2008; Solano-Gallego et al. 2011). Alternative methodologies include using interdigitated electrodes for detection of antibodies, implementation of paper biosensors, or utilizing nanoparticles for detecting Leishmania antigens and unamplified DNA (Andreadou et al. 2012, 2014). Several lateral flow assays have been proposed for detecting PCR amplified Leishmania specific DNA, including OligoC-TestT PCR-oligochromatographic test; an OligoC-TestT variation for Leishmania typing; a generic lateral flow oligochromatographic dipstick paper (Rivas et al. 2015).
Parasitological methods of diagnosis

Parasitological methods of diagnosis remain the gold standard for diagnosing leishmaniasis (de Vries et al. 2015) and are very crucial in eco-epidemiological studies (Shirian et al. 2014). Diagnosis in the laboratory can be achieved by direct examination of amastigotes in giemsa-stained lesion smears of scrapping, biopsies, or impression smears (Boelaert et al. 2007). The most frequently used samples are bone marrow or splenic aspirates. However, amastigotes can also be identified in other samples like buffy coat of peripheral blood, lymph nodes, and liver biopsies (Elmahallawy et al. 2014). Of all the samples, the highest sensitivity was observed with splenic aspirates (93–99%) (Srivastava et al. 2011). Splenic aspirates are an abundant source of Leishmania parasites, but their examination corresponds to a high risk of hemorrhage in unskilled hands (Barrett and Croft 2012). In a study conducted in India, it was observed that fatal bleeding appeared in two out of 9612 splenic aspirate procedures in a duration of 10 years (Sundar and Rai 2002). Also, one death was reported out of 671 patients that were administered with splenic aspiration in Kenya for a period of 10 years, and three deaths were reported in India out of 3000 (Boelaert et al. 2007). Giemsa-stained bone marrow showed a sensitivity of about 60–80%, but the sensitivity of peripheral blood smears was low particularly in patients with low parasitemia (Elmahallawy et al. 2014) and higher in HIV-coinfected individuals (Cota et al. 2013). Although liver biopsies have little risk of rupture and bleeding but identifying the amastigotes is possible only in case of heavy infections (Barrett and Croft 2012). Lymph node aspiration technique can be used when an increase in the size of a lymph node is seen, such as in VL patients and the sensitivity of this method range from 52 to 58% (Boelaert et al. 2007). However, in vitro, cultures of blood cells and tissue aspirates have shown 100% sensitivity (Hide et al. 2007). Cultures prepared from bone marrow and splenic aspirates show high specificity (Srividya et al. 2012) and the highest sensitivity is observed with splenic aspirates (93–99%).

Being very tedious, exorbitant, and time-consuming, it is confined to research laboratories only (Singh and Sundar 2015). In a study, 155 suspicious patients with CL in Mashhad City were investigated by three diagnostic methods, and the results so obtained were compared. Results showed a sensitivity of 44.5% by microscopic investigation of smears and 48.5% with cultures (Shahbazi et al. 2008). In another study, 62 CL samples were diagnosed using kinetoplastid DNA PCR, microscopic evaluations and parasite culture in Diagnosis and Treatment Centre in Turkey. Results indicated a sensitivity of 54.3% with culture and 71.4% by microscopy. However, microscopy and culture technique when combined together, sensitivity was increased to 88.6% (Zeyrek et al. 2018). Parasites can also be inoculated in the laboratory animals, like mice, guinea pigs, hamsters or rodents (Ready 2014), but this method is not considered as a first diagnostic procedure as several months are required to demonstrate the parasites in these animals (Sundar and Rai 2002). Parasitological methods are most favored and first line diagnostics for determining the disease. However, drawback of the parasitological approaches is low sensitivity, requirement of technical expertise for carrying out the procedure and further risks associated with the tests (Reithinger 2008).

Immunological methods of diagnosis

To overcome the limitations of parasitological methods of diagnosis, immunological methods were developed (Singh and Sundar 2015). These methods are based on the presence of specific humoral responses (Elmahallawy et al. 2014). The trademark of mucocutaneous and CL is that the humoral immune response is very low (Singh et al. 2005). Thus, immunological tests are not frequently utilized in areas where CL prevails as circulating antibodies are very low and in regions with cross-reacting parasites like Trypanosoma cruzi exist specificity can be fluctuating (Reithinger and Dujardin 2007). However, in VL, hyperimmunoglobulinemia is observed. Utilizing this interaction between hosts and parasites, many antibody detection methods have been developed for the diagnosis of leishmaniasis (Boelaert et al. 2004). Some of these diagnostic methods are enzyme-linked immunosorbent assay (ELISA), western blot, indirect fluorescent antibody and direct agglutination (Goto and Lindoso 2010). The sensitivity of these immunological tests depends mainly on the assay and its methodology, while specificity depends on the antigen rather than the serological format used (Elmahallawy et al. 2014).

Fluorescent antibody test

In this test, a fluorescent marker is attached to an antibody, which results in a reporter molecule that is rapid, easy to measure and bind to a target molecule with high specificity. This test can be both direct, where the labeled antibody binds the antigen and indirect in which secondary polyclonal antibody binds patient that bind prepared antigen. This test is amongst one of the frequently performed tests for detecting the anti-leishmanial antibodies by using the promastigotes. However, cross-reactions with trypanosomal sera have been observed (Boelaert et al. 2004). To minimize the cross-reactions with trypanosomal sera, promastigote is used as antigens (Elmahallawy et al. 2014; Rezvan and Hamoon Navard 2017). A study conducted in
Iran to estimate the performance of enzyme-linked immunosorbent assay (ELISA) for detection of total IgG and IgM, showed sensitivity of 83.6% and 84.7% and specificity of 62.7% and 54.6%, respectively. Sensitivity and specificity of ELISA for detecting IgG1 and IgG4 were 64%, 75% and 85%, 49%, respectively. However, immunodiagnosis of CL showed 91.6% sensitivity and 81% specificity with IFA (Indirect Fluorescent Antibody) (Sarkari et al. 2014). Similarly, in another experiment 81% specificity with IFA (Indirect Fluorescent Antibody) immunodiagnosis of CL showed 91.6% sensitivity and 64%, 75% and 85%, 49%, respectively. However, specificity of ELISA for detecting IgG1 and IgG4 was 62.7% and 54.6%, respectively. Sensitivity and specificity of direct agglutination test (DAT) was 70.5% and 100%, and ELISA was 83.6% and 90.5% (Mikaeili et al. 2007).

Direct agglutination test (DAT)

DAT is a simple, reliable, cost-effective, and semi-quantitative test (Srividya et al. 2012). DAT has been approved in various countries including India, Brazil, Nepal, Sudan, Bangladesh, Kenya, and Ethiopia. This test is dependent on the agglutination of promastigotes of *Leishmania* that react with the anti-leishmanial antibodies leading to promastigote agglutination (Elmahallawy et al. 2014). The test is economical and easy to perform; thus, it can be used both in laboratory and fields. The sensitivity and specificity of the direct agglutination test as found by various studies ranges from 70.5–100% and 53–100% (S. Mondal et al. 2010). During a study conducted in Spain, the sensitivity of DAT was observed to be 86.4% for suspected VL patients and for individuals underlying HIV infection, increase in sensitivity of 91.3% was seen (Bangert et al. 2018). DAT can be performed on serum, plasma and whole blood (Moody and Chiodini 2000). But, the main drawback of direct agglutination test is that it doesn’t have prognostic value for calculating the cure of disease, as after several years of treatment they remain positive.

Moreover, the long incubation period of 18 h and requirement of serial dilution of serum or blood makes it disadvantageous (Singh 2006). In order to overcome these problems, promastigotes are used as freeze-dried or suspension (liquid) form. The freeze-dried forms can tolerate heat, thus being more heat stable makes the use of new formulated DAT in fields (Abdallah et al. 2004). However, for quick identification (less than 3 h) of antibodies against *Leishmania* in blood collected on filter paper and in the serum samples, FAST (Fast Agglutination Screening Test) has been developed (Schoone et al. 2001). The sensitivity and specificity with FAST were reported to be 91.1– 95.4% and 70.5%–88.5%, respectively (Hailu et al. 2006).

Enzyme-linked immunosorbent assay (ELISA)

This test is amongst one of the most sensitive serodiagnostic methods for VL. However, the sensitivity of the test relies on the antigen used (Table 1) (Singh and Sundar 2015). Different antigens used in ELISA are:

1. The crude soluble antigen of promastigote achieved by freeze-thawing the live promastigotes. Ryan et al., in 2002, developed ELISA that can detect IgG and IgM antibodies in CL and VL patients and the overall sensitivity is shown by this ELISA was 95.1%.

2. Promastigote of *L. donovani* liberates excretory, secretory and metabolic antigens within protein-free medium (Martin et al. 1998). Sensitivity and specificity shown by these antigens is 100%, but besides this, retrospective and further multisite interpretations are needed. Romero et al. (2004) described that ELISA showed a sensitivity of 89% using an antigen of *Leishmania mexicana* and 71% with the antigen of *Leishmania braziliensis*. In another study, using a sequence-specific peptide antigen in 33 patients infected with CL of *L. major* observed sensitivity of 67% (Jensen et al. 1999).

3. In a study conducted by Kaul et al. (2000) for evaluation of diagnostic efficacy of 200kDA amastigote antigen of *L. donovani*, positive antibody response against this antigen and LASA (*Leishmania* amastigote soluble antigen) was seen in 96.6% and 100% of VL patients respectively.

4. Kaur and Kaur (2013) demonstrated the serodiagnostic potential of heat shock proteins Hsp 70 and Hsp 83 in combination through ELISA and western blotting. Occurrence of both Hsp 70 and Hsp 83 suggested that these antigens can be useful for serodiagnosis of VL.

5. Recombinant antigens like rk39, rGBP, Ld-RGBP, rORFF, etc. have been developed. The surface of amastigotes and promastigotes of *L. major* express a hydrophilic protein (gene B protein, rGBP) encoded by gene B. *L. donovani* gene B homolog (Ld-rGBP) encodes a protein that comprises of up to 22 replicas of repetitive element in which 14, 9 are conserved within 2 species (Singh 2006). One more recombinant protein of *L. infantum*, rORFF has been created for the diagnosis of VL in India (Raj et al. 1999). ELISA utilizing this antigen is reported to be significantly specific and sensitive (Singh 2006). A recombinant antigen, rk39 is demonstrated to be accurate against the antibody that emerges during VL caused by *L. donovani*. Utilizing this antigen, specificity and sensitivity was noted to be 99% in immunocompetent patients with VL (Singh et al. 2010) and also rk39 ELISA has anticipating values for interpreting VL in
co-infected individuals like AIDS (Singh et al. 2005). The rk39 antigen is available commercially now as nitrocellulose papers strip impregnated with antigens and is suitable for using in field conditions also.

6. Carvalho et al. (2018) in their study, evaluated new antigens that were identified by immunogenic screening for serological diagnosis of human VL. It was found that different antigenic L. infantum peptides, based on linear B cell epitopes, had potential for the development of an immunoassay obtained from isolated or multi antigens with ability to improve the sensitivity and specificity values for the VL serodiagnosis.

7. Also, two more recombinant proteins rK9 and rK26 apart from rK39 have been generated from the kinesin gene of L. chagasi. (Singh et al. 2005; Mohapatra et al. 2010). An experiment conducted for comparative evaluation of rK9 and rK26 with rK39 and crude soluble antigen observed sensitivity of 78%, 38%, 100%, and 80% respectively while the specificity of rK9, rK26, rK39 and Crude Soluble Antigen (CSA) was 84%, 80%, 96%, and 72% respectively.

Thus, it was concluded from the study that rK39 was most appropriate antigen as compared to rK26 and rK9. (Mohapatra et al. 2010). Apart from these, recombinant antigen KE16 has been cloned from Indian L. donovani, and this antigen is found to be 100% specific and sensitive (Sivakumar et al. 2006).

The specificity and sensitivity of ELISA using recombinant HSP83 displayed better results in comparison to using crude L. major antigens for diagnosing a cutaneous form of the disease, next to mucocutaneous and VL (Al-Salem et al. 2014). Although ELISA is a technique with enhanced specificity and sensitivity but the need of expert people, sophisticated technologies and requirement of electricity restricts its use to advanced laboratories only (Elmahallawy et al. 2014) (Table 2).

**Immunochromatographic test (ICT) or immunochromatographic assay (IC)**

IC is an accurate, rapid and straightforward immunochromatographic test based on rK39 antigen (Sundar and Rai 2002). The sensitivity and specificity of rK39 in VL cases was found to be different among varying populations. Sensitivity and specificity were 90% and 100% in Brazil (Carvalho et al. 2003) and 100% and 93–98% in India, respectively (Sundar et al. 2002a, b). A study conducted in Mediterranean region on large sets of patients from rK39 showed the sensitivity of 78% in all VL patients. However, the sensitivity dropped to 67.3% in patients with HIV co-infection (Bangert et al. 2018). These variations in sensitivity were due to the presence of different antibody responses in different groups. This test can be used in fields as well because it is quick, economical and gives positive results (Elmahallawy et al. 2014). Vink et al. (2018) in their experiment evaluated Loopamp™ *Leishmania* Detection Kit (Loopamp) and CL Detect™ Rapid Test (CL Table 1: Sensitivity of various tissues examined parasitologically

| S. no. | Tissue                      | Sensitivity | References                        |
|-------|-----------------------------|-------------|-----------------------------------|
| 1.    | Splenic aspirate            | 93–99%      | Srivastava et al. (2011), Bhargava and Singh (2012) |
| 2.    | Bone marrow aspirate        | 60–80%      | Srivastava et al. (2011), Elmahallawy et al. (2014) |
| 3.    | Lymph node                  | 52–58%      | Srivastava et al. (2011), Zijlstra et al. 1992, Boelaert et al. (2007) |
| 4.    | Blood buffy coat            | 53%         | Singh (2006)                      |
Detect), for Cutaneous Leishmaniasis (CL) diagnosis. It was observed that out of 274 CL suspects included in the study, CL Detect test showed 65.4% sensitivity and 100% specificity, while it was 87.6% and 70.6% for Loopamp kit respectively.

**Leishmanin skin test (LST)**

One of the significant attributes of cutaneous form of leishmaniasis is an occurrence of delayed-type hypersensitivity (DTH), and this can be estimated by leishmanin skin test which is also called Montenegro reaction (Stockdale and Newton 2013). Delayed-type hypersensitivity skin reactions are recognized as positive, when LST > 5 mm and negative, when LST < 5 mm (Passos et al. 2000). After asymptomatic infection and in a period of weeks to months after treatment against VL, results of the test become positive, showing a healing or protective response (Khalil et al. 2005). Thus, this experiment fails to discriminate between the past or present infection. Furthermore, active PKDL, VL, and DCL are signalized by a negative skin test (Neogy et al. 1990; Rezvan and Hamoon Navard 2017). However, in areas endemic to VL, the sensitivity of this test in asymptomatic infections is similar or even higher than that of other serologic tests (Gadisa et al. 2012). LST shows a higher sensitivity of 86.4–100% and is simple to use (Antonio et al. 2014). Thus, LST is becoming a valuable method for identifying the exposure to *Leishmania* parasites and distinguishing the asymptomatic cases in epidemiologic surveys (Riera et al. 2008). LST is frequently employed as a measure of the occurrence of MCL and CL in animal and human populations and successful cure of VL, as it prevails negative during active VL and will be transformed to positive after treatment. Despite its benefits, this test is not helpful in PKDL patients as the results are not related to the presence of the infection (Zijlstra et al. 2000).

**Latex agglutination test (LAT)**

This test is amongst one of the latest developed tests for quick detection of anti-leishmanial antibody against A2 antigens obtained from the amastigote form and crude antigens from promastigote forms. When compared with DAT, the sensitivity was 88.4%, and specificity was 93.5% (Akhoundi et al. 2013). In a study designed to evaluate the use of kala-azar latex agglutination test (Katex), the sensitivity of Katex was observed to be moderate, i.e. 75%, but specificity was 100%. This reduced sensitivity restricts the use of Katex in resource-limited settings (Salam et al. 2012).

Immunological methods are a landmark in the diagnosis of leishmaniasis. Although many different methods have been developed, yet all are met with few limitations. Moreover, these are too costly to be used in developing countries. The requirement of very advanced apparatus limits their use in field conditions. Some commercially available kits have different sensitivity and are sometimes unable to detect the parasite infections in case of *Leishmania*-HIV co-infections (Schallig et al. 2004) (Table 3).

**Xenodiagnosis**

In this method of diagnosis, the infected lesion or tissues are exposed to the phlebotomine vector, and the gut of vector is examined later for the existence of flagellates of *Leishmania* (Sadlova et al. 2015). Sadlova et al. (2015) conducted an experiment in which they inoculated BALB/c mice intradermally in the ear pinna with *L. donovani*. This study showed that even small number parasites in mice can cause massive infection in *Phlebotomus orientalis* (vector) and thus constituted a suitable laboratory animal for xenodiagnosis. Xenodiagnosis shows high sensitivity and is comparatively more straightforward than other methods, but it cannot differentiate between species of *Leishmania*. Furthermore, it is a time-consuming process that is not possible without the insect/animal (Akhoundi et al. 2017).

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**Table 2** Sensitivity and Specificity of ELISA using different antigens

| S. no. | Antigens                                                                 | Sensitivity | Specificity | References                                |
|-------|---------------------------------------------------------------------------|-------------|-------------|-------------------------------------------|
| 1.    | Crude soluble antigen                                                     | 80–100%     | 84–94%      | Ryan et al. (2002)                        |
| 2.    | Secretary, metabolic and excretory, antigens liberated by promastigote of *L. donovani* | 71–89%     |             | Romero et al. (2004)                      |
| 3.    | rK39 as antigen                                                          | 75–98%     | 79–89%      | Elmahallawy et al. (2014), Chappuis et al. (2007) |
Molecular methods

The conventional parasitological and serological techniques exhibit certain constraints to the diagnosis of leishmaniasis (de Paiva-Cavalcanti et al. 2015) and these limitations have led to the discovery of molecular methods (Tlamcani 2016). In contrast to regular diagnosis, molecular methods are recommended more for CL because of their excellent accuracy and fast speed (Azizi et al. 2012). Molecular techniques serve as a supplement as well as an alternative method of diagnosis. The main reason for approval of molecular techniques in the routine laboratories worldwide is the feasibility, safety, and reliable molecular tools.

Furthermore, numerous applications and the encouraging outcomes have led to continued approval of these methods (de Paiva-Cavalcanti et al. 2015). Though various molecular methods of diagnosis have been developed viz., multilocus enzyme electrophoresis and pulse-field gel electrophoresis yet, assays based on polymerase chain reactions presently constitute the main way of molecular diagnosis for health professionals and researchers. Information and knowledge of DNA sequencing have been much used for establishing the PCR (polymerase chain reaction) based assay for different utilizations in interpreting the parasite and disease (Singh et al. 2005). In a study conducted to differentiate *Leishmania* species based on internal transcribed spacers (ITS)-PCR, in contrast to parasitological methods of diagnosing CL, it was observed that ITS-PCR is not only valid for identification of species but also showed a high sensitivity and specificity (98.8% and 100%) as compared to microscopy and culture methods (79.6% and 86.2% sensitivity respectively) (Shahbazi et al. 2008). PCR and its modifications such as quantitative-PCR, semi-nested-PCR, and nested-PCR have significantly been used for diagnostic assays using various samples and target regions (da Silva Solca et al. 2012; Reis et al. 2013). Initially, the qPCR technique had been used to discern DNA from the causative agent in different samples of human and animals to study host-parasite interactions and to quantitatively estimate parasitic load in infected patients (de Paiva-Cavalcanti et al. 2015).

Moreover, *Leishmania* species characterization is also a vital function of the PCR (Mohammadiha et al. 2013). Pairing PCR with different methodologies such as Restriction Fragment Length Polymorphism (RFLP) analysis and gene sequencing have helped in the confirmation of different species in epidemiological researches (Wang et al. 2011). With the emergence of recombinant technology and nucleic acid engineering, a variety of schemes have been developed for constructing the recombinant protein for the diagnostic purposes (Singh et al. 2005). Table 2 shows the advantages and disadvantages of various molecular methods for the detection of *Leishmania*.

### Conventional PCR

It facilitates the amplification of RNA or DNA through continually repeated cycles. The elucidation of some DNA fragments that are species-specific made this procedure feasible for diagnosing leishmaniasis (Smyth et al. 1992). Also, PCR techniques are more appropriate than parasitological methods of diagnosis (culturing and microscopy) especially for samples that have lower parasite load (Antinori et al. 2007) thus beneficial in observing the disease progression, estimating the result of anti-leishmanial therapy, describing the species discrimination and drug resistance (Schönian et al. 2011). Moreira et al. (2007) conducted a comparative study of different diagnostic methods and observed sensitivity of 100%, 96%, and 95.65% respectively for symptomatic, oligosymptomatic and asymptomatic groups and specificity of 100%. Similarly, in another study, PCR was targeted against kinetoplastid DNA (kDNA) using Uni21/Lmj4 primers, and the results were compared with parasitological methods, cultures, and smears. The results showed that kDNA PCR was most sensitive, i.e. 100%; however, microscopy and cultures were only 71.4% and 54.3% sensitive respectively (Zeyrek et al. 2018). Abd El-Salam et al. (2014), conducted an experiment in which *Leishmania tropica* was extracted from CL patients of Kohat and analyzed by PCR, microscopy and culture techniques. Of the 113 samples, PCR, microscopy, and culture showed 87.61%, 53.98%, and 46.90% sensitivity. Although conventional PCR shows
enhanced sensitivity when compared to the parasitological method of diagnosis, but an essential drawback of this method is their inability to discriminate between clinically active VL and asymptomatic infections (Sudarshan and Sundar 2014). Also, conventional PCR have a high risk of contamination, which takes a longer time to give the results.

**Nested and semi-nested PCR**

This form of PCR is significant in differentiating the species (Akhoundi et al. 2017). In this technique, two sets of primers are utilized in two consecutive cycles. The second set of primers amplifies the secondary target in the first product. This step makes sure that the outcomes of the 2nd PCR have very less impurity due to primer dimers, alternative primer targets and hairpins (Akhavan et al. 2010). Shirian et al. (2014) executed this method by using scraped off slides from twenty suspected individuals with MCL and got positive results in 18 individuals (90%). However, through direct microscopy, only eight cases were found positive (44.4%). In another study, a combination of the parasitological, immunological and molecular technique was used to discern the course of infection in dogs after sporadic exposure to L. infantum and nested PCR which showed 100% sensitivity as compared to all other methods (Oliva et al. 2006). Although this method is highly sensitive, yet it has some limitations. After achieving the first stage, the product should be transferred to a new tube or material of the second stage are added to it. This step increases the chances of contamination.

**Multiplex PCR**

In this method, different DNA targets can be amplified simultaneously, and different PCR reactions are performed in a single experiment. Amplicons of specific DNA targets having varying sizes can be produced by using numerous primers set in one PCR mixture only. The annealing temperature of all sets of primers can be developed to perform accurately in one reaction tube and size of amplicons should be distinct enough to be anticipated by gel electrophoresis. Different types of markers like kDNA mini-circle (de Pita-Pereira et al. 2008) and multicopy SL RNAs (Spliced Leader RNAs) (Jorquera et al. 2005) have been utilized in this method for diagnosis of leishmaniasis. This method can be used for clinical laboratories, but a relative decline in sensitivity is one the drawbacks of this method as compared to single PCR method. Utilizing this technique for the detection of infectious agents need accurate primer design and extensive optimization of the test.

**Real-time PCR (quantitative PCR)**

This method depends upon analysis by fluorescent signals that are generated during amplification. Fluorescent dye and fluorescent tubes are used to create the fluorescence (Galluzzi et al. 2018). RT-PCR is fast and has an extensive dynamic range as there is no requirement of opening the reaction tube; also, cross-contamination is decreased. RT-PCR is convenient for diagnosis of PKDL (Ghosh et al. 2018) and canine leishmaniasis where it helps in monitoring parasitic load in different tissues throughout and after the treatment (Manna et al. 2008; Pennisi et al. 2005). In an experiment, 91 PKDL patients from Bangladesh went for skin biopsy, and both real-time PCR and microscopy was performed. RT-PCR showed the sensitivity of 91.2% and microscopy showed 50.6% (Ghosh et al. 2018).

Similarly, another study was conducted to detect and quantify L. donovani for diagnosis of VL and monitoring its response to treatment. RT-PCR showed a sensitivity of 93.33% and specificity of 100% (Hossain et al. 2017). Quantitative estimation of products, reduced contamination, and high speed are among the significant advantages of this method but high cost and need of an expert skilled person to deduce the results are its current significant drawbacks.

**Nucleic acid sequence-based amplification (NASBA)**

This method is a transcription-based amplification and isothermal complex constructed to discern the RNA target (van der Meide et al. 2005). NASBA has its different modifications such as quantitative-NASBA (QT-NASBA) and paired with oligochromatography (NASBA-OC). In an experiment conducted on 50 samples from healthy control and 30 samples from VL, sensitivity and specificity of NASBA-OC was 93.3% and 100% respectively (Mugasa et al. 2010). Similarly, in another study, QT-NASBA method was used to evaluate Leishmania parasites in samples of a skin biopsy from CL patients. This study demonstrated that QT-NASBA could detect parasite level 100 fold lower than detected by conventional PCR. Sensitivity and specificity observed, in this case, was 97.5% and 100%, respectively (van der Meide et al. 2005). NASBA is the only isothermal amplification method that utilizes RNA as starting material, but the main limitation of this method is that they are prone to contamination of ribonuclease, which can degrade the target RNA (Zanoli and Spoto 2013).

**Oligochromatography-PCR (OC-PCR)**

It is a rapid and straightforward scheme for detecting nucleic acid-based amplification (NASBA) or PCR.
outcomes anticipated on dipstick by hybridization with a gold-conjugated probe that allows the detection of sequence-specific products. Although this method is greatly sensitive, yet it does not provide discrimination between different species of *Leishmania* (Mugasa et al. 2010; Saad et al. 2010). *Leishmania* OligoC-Test and NASBA, coupled to oligochromatography, are used as low tech molecular diagnostic methods (Mugasa et al. 2010). In a study conducted to calculate specificity and sensitivity of NASBA-OC and OligoC-TestT from blood specimen of 84 VL patients. NASBA-OC showed specificity and sensitivity of 100% and 79.8% while *Leishmania* OligoC-TestT showed specificity and sensitivity of 88.8% and 96.4% respectively (Basiye et al. 2010). Saad et al. (2010) experimented with checking the diagnostic accuracy of *Leishmania* OligoC-TestT and NASBA-OC for diagnosis of leishmaniasis in Sudan and found the sensitivity of OligoC-TestT and NASBA-OC on lymph node to be 96.8%, blood 96.2% and on the bone marrow to be 96.9%. Despite its high sensitivity, Oligochromatography-PCR is not an option for routine diagnosis in primary health centers due to the requirements of basic molecular laboratories.

**Randomly amplified polymorphic DNA (RAPD)**

In this method, DNA amplification by PCR is performed using a short primer that is randomly determined. This short primer can be thus utilized for any organism even without early knowledge of the target sequence. RAPD (Randomly amplified polymorphic DNA) can be performed in combination with some other techniques or used alone to check genetic intra-specific and inter-specific differences in species of *Leishmania* (Botilde et al. 2006). In a study conducted for identification of *Leishmania* species causing CL in Kharve, Iran, RAPD-PCR was found successful in identifying the causative species, i.e. *L. tropica*. However, its use is limited due to its demand for pure leishmanial DNA and accurate polymerase chain reactions conditions that assure the specificity (Akhoundi et al. 2017).

**Amplified fragment length polymorphism (AFLP)**

This is a very convenient method for quick visualization of polymorphic DNA fragment from organisms with no previous information of the sequence. Amplified fragments so obtained are isolated and then seen on PAGE, and recorded an occurrence or lack of the polymorphism (Kumar et al. 2010). It is a reproducible technique as it combines the sensitivity of PCR reaction with the specificity of RFLP. AFLP is greatly used in ecology, biology, genetics, and phylogenetics of many organisms (Bensch and Akesson 2005). AFLP is a fast and compelling technique for the identification of marker and identifying closely linked species of *Leishmania* and genetic variations in strains (Kumar et al. 2010). Kumar et al. (2010) used the test to differentiate the species of *Leishmania* causing CL and VL, and showed clear cut variation in genetic distance between *L. major* and *L. tropica*. Similarly, Restrepo et al. (2013) also used AFLP to characterize genetic variability of *Leishmania* parasites isolated from Panamanian CL patients. The technique was found to be successful in clearly separating some groups of *L. panamensis* and highly related species like *L. panamensis* and *L. guyanensis.*

**Loop-mediated isothermal amplification (LAMP)**

LAMP is a parasite species-specific amplification of DNA, rapid and needs only basic laboratory equipment (Kothalawala and Karunaweera 2016). LAMP is a helpful tool for diagnosis is sometimes used as a substitute technique for PCR because it is an economical, more sensitive and faster method. As the reaction is isothermal in nature, there is no requirement of a thermal cycler, except for heat block and water bath (Khan et al. 2012). All these features of LAMP make it suitable to use under field conditions. In this method, each initial single stranded DNA product supplements subsidiary template for chain-reaction utilizing 2nd outer or inner primer with the help of stem-loop intermediate structure. This assay was developed for detection of *L. donovani* in patients with PKDL and VL. The sensitivity and specificity were found to be good in both the cases reaching a sensitivity of 96.4–98.5% in VL blood samples, 96.8–98.5% in samples of tissue biopsy and 96.8% for PKDL cases (Verma et al. 2013). In a study conducted on 31 CL patients, LAMP was found to be positive for 19 out of 23 microscopically positive patients, yielding a sensitivity of 82.6%. And specificity 100% (Kothalawala and Karunaweera 2016). In another experiment conducted for evaluation of VL in Sudan, Loopamp (*Leishmania* detection kit) showed a specificity of 99.01% and sensitivity of 100% (Mukhtar et al. 2018). In another study, LAMP assay was designed for CL on 105 patients in South-West Colombia. Sensitivity and specificity shown by LAMP was 95%, and 86% for the diagnosis of CL and for VL sensitivity and specificity was 92% and 100% (Adams et al. 2018). Despite high sensitivity and specificity, the major disadvantage of LAMP is need of non-extreme GC strand, the possibility of the presence of secondary structures and required temperature range. (Akhoundi et al. 2017).

**Multilocus enzyme electrophoresis (MLEE)**

This method allows identification of microorganisms by electrophoretic mobility of various intracellular enzymes.
Isoenzymes play a significant role in metabolic processes in different cellular components and are produced by multiple genes. The typical kind or strain-specific mobility pattern can be confirmed by using the defined set of isoenzymes. MLEE is regarded as a reference standard for identifying species of Leishmania and can also discriminate between species of Old World and new world. However, MLEE cannot differentiate between populations because of lower marker number, homoplasy or destined gene heterozygosity that cause more than one mobility scheme (Jamjoom et al. 2004). MLEE is not suggested because of the requirement of specialized equipment, laborious parasite culturing and due to lack of power to discriminate between some species and populations (e.g., L. infantum MON-1) (Schöni et al. 2011) (Table 4).

Diagnosis of leishmania-hiv co-infection

In AIDS patients, VL is an opportunistic infection, and unusual pathological presentations of VL in HIV-infected patients create a challenge for diagnosis (Srivastava et al. 2011). However, regardless of the status of HIV, parasitological mode of diagnosis is the gold standard because of its high specificity (Singh 2014). For HIV-infected and non-infected individuals, spleen tissues have the best sensitivity, followed by bone marrow and lymph nodes. However, bone marrow aspiration is most used because of its excellent sensitivity (67–94%) and reduced complication than splenic aspiration (Alvar et al. 2008; Lima et al. 2013). Also, culture can improve sensitivity, but it requires a particular medium and is generally not available in endemic areas. Cota et al. (2013) observed specificity and sensitivity of parasitological methods to be 100% and 93.2%. Concerning immunological diagnosis of HIV-infected cases, there are few pieces of evidence of the accomplished tests with significant variety of studies (Cota et al. 2012). Serological tests, in this case, are less reliable. Also, there is confusion about which technique is better than other (Lindoso et al. 2014). As far as a demonstration of antibodies are concerned immunoblotting, enzyme immunoassays, direct agglutination tests (DAT) (Singh

| S. no. | Assay Types of leishmaniasis | Specificity | Sensitivity | Advantage | Disadvantage | References |
|-------|-----------------------------|-------------|-------------|-----------|--------------|------------|
| 1.    | Conventional PCR Cutaneous leishmaniasis (CL) | 87.61–100% | 100% | Precise results, high specificity, and sensitivity. Uncomplicated Diagnostic interpretations | Time consuming and incompetent to evaluate the destined DNA. Qualitative Approach. Restricted detection range | Moreira et al. (2007), Zeyrek et al. (2018), Abd El-Salam et al. (2014), De Paiva-Cavalcanti et al. (2015) |
| 2.    | Nested PCR Cutaneous Leishmaniasis (CL), Visceral Leishmaniasis (VL) | 90–100% | – | Shows higher sensitivity and specificity. A convenient method for investigating the molecular epidemiology in the field | Qualitative test. Incompetent to evaluate the target DNA requires prolonged time and is expensive | Shirian et al. (2014), Oliva et al. (2006), De Paiva-Cavalcanti et al. (2015) |
| 3.    | Real Time-PCR PKDL, VL | 91.2–93.33% | 100% | Elevated specificity and sensitivity. Numerical potential and rapid results. Differentiation of species can be achieved by melting Temperature | Complexity in elucidating the outcomes, Requires a skilled operator Presence of thermocycler makes it costly | Hossain et al. (2017), Ghosh et al. (2018), De Paiva-Cavalcanti et al. (2015) |
| 4.    | NASBA VL | 93.3–97.5% | 100% | NASBA is the only isothermal amplification method that utilizes RNA as starting material. There is no requirement of the complicated laboratory structure. Shows higher specificity and rapid results | Prone to contamination of ribonuclease, which can degrade the target RNA | van der Meide et al. (2005), Mugasa et al. (2010), Zanoli and Spoto (2015), De Paiva-Cavalcanti et al. (2015) |
Three major diagnostic methods have been discussed in this review with variations in accuracy of diagnosis; including the parasitological examination (histopathology, microscopy and parasite culture), serology and molecular diagnostics. In parasitological methods, the amastigote stage is demonstrated in spleen, liver or lymph node aspirates. Although, parasitological methods of diagnosis remain the best method for leishmaniasis diagnosis but the presence of inevitable disadvantages delays this diagnosis in the field. The serological methods are sensitive, specific and economical. Diagnosis based on antibodies, like rK39 strip test is used in affected countries over the world despite their limitation of remaining positive in healthy individuals for very long periods even after cure. Molecular methods are even more sensitive and are a powerful technique that provides early detection of the parasites. Many molecular methods have been successfully implemented for leishmaniasis diagnosis, but the technique is used mostly in research laboratories, and its application in clinical practices and health facilities requires skilled persons who are generally not available in poor and developing countries. Compared to other diagnostic techniques, molecular approaches remain expensive and require technical expertise. However, efforts should be made to make diagnosis more user-friendly and cost-effective, especially in the remote areas where leishmaniasis is endemic. Leishmaniasis could be prevented by reducing human contact with infected phlebotomine sandflies (the vector), or by reducing the number of infected animals (the reservoir). Although no published studies on the effectiveness of diagnosis for prevention and management of the diseases are present but early diagnosis allow fast treatment and could contribute to the therapeutic success. This can be achieved by training the physicians working in primary health centres for proper identification, diagnosis and treatment of leishmaniasis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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