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Beyond Conventional Technologies: Next-wave Diagnostics for Leptospirosis in India

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ABSTRACT
Leptospirosis is an important, underrecognized disease posing significant consequences for livestock production as well as public health. Among the different livestock species, buffaloes represent a major reservoir host, which in turn directly impacts the dairy as well as beef sectors of India, as buffaloes are the higher contributors in both sectors among all livestock of this country. This disease is caused by pathogenic Leptospira spp., and direct or indirect contact with contaminated urine, water, soil, or infected animals is the main source of its transmission. Infection of this disease is commonly associated with infertility, abortion, stillbirth, and reproductive losses in the animals, and in the case of humans, it may range from nonspecific febrile illness to multiorgan failure, like liver, kidney, lung, and central nervous system failures. In India, due to several reasons, like limited diagnostic infrastructure, nonspecific clinical presentation, and lack of awareness among at-risk populations and health professionals, this disease remains significantly underdiagnosed and underreported, despite its wide distribution in this country. This review presents and summarizes the important diagnostic techniques currently available for leptospirosis and classifies them as conventional, advanced, and cutting-edge. Conventional methods are widely used, such as microscopy, culture, serology, and rapid assays, but due to lower sensitivity, they are not very reliable for early detection of disease. Molecular strategies like conventional PCR and its variants, viz., nested PCR, qPCR, LAMP, and Truenat™, provide improved sensitivity and also offer earlier detection. Most recent and newly developed diagnostic methods, including biosensors, CRISPR-based assays, and next-generation sequencing, further enhance the speed and deliver higher precision in diagnosis. Expanding disease surveillance; strengthening already available diagnostic capacity and infrastructure and integrating with advanced technologies; and improving public awareness are crucial components for reducing disease burden, supporting timely treatment, and protecting both animal productivity and human health in India.

Key Words: Diagnostic Techniques, Human Health, Leptospirosis, Zoonotic Disease

INTRODUCTION

India is a country having a large number of livestock, which are showing a huge diversity, especially for its buffalo population (*Bubalus bubalis bubalis*) which is mainly distributed in the tropical and subtropical regions across Southeast Asia [1-6]. The 49% of the total milk production of India comes from the buffaloes alone, which reflects the significant contribution of buffaloes in the dairy sector. Similarly in meat production, the buffaloes have been shown to make an impactful contribution. Also, these animals have revealed adaptation to varied climatic conditions. Similarly, cattle and other livestock make substantial contributions to the Indian economy and are important for sustaining the livelihoods of farmers and rural agricultural communities [7-13]. Leptospirosis is a zoonotic disease caused by a thin, spiral-shaped, gram-negative bacterium belonging to the genus *Leptospira*. Based upon the structure and composition, 30 serogroups and more than 350 serovars have been reported so far [14]. Pathogenic Leptospira can release biofilm when it comes in contact with the outside environment, which makes its survival favourable in the outside environment or inside the host. Leptospira spread to humans from infected animals through different direct or indirect (through contaminated soil, food, or water) means (Fig. 1).

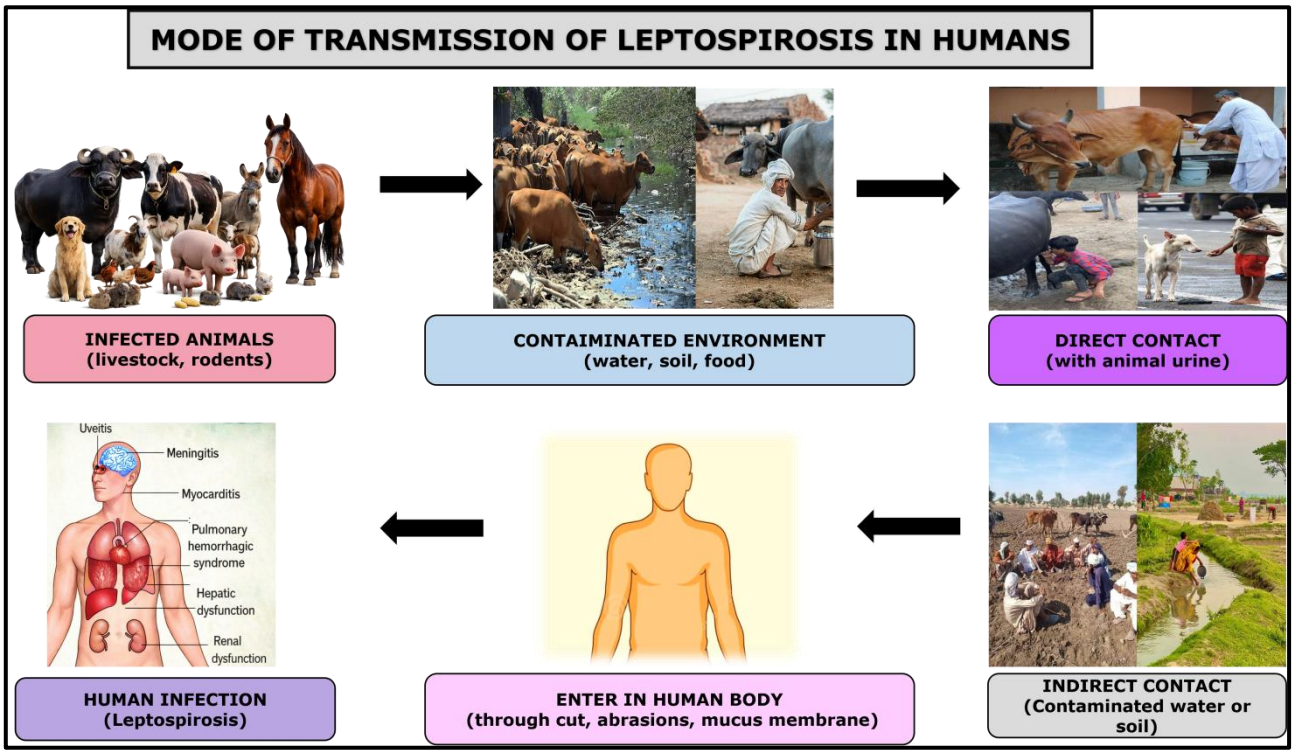

Fig. 1. Mode of transmission of leptospirosis in humans through direct or indirect contact

There are many wild and domestic animals, including major livestock species; buffalo, cattle, goats, equines, pigs, and rodents, that serve as hosts and also as carriers that help in the transmission of these pathogenic bacteria across different species, including humans, and it is among one of the 20 neglected tropical infections listed by the World Health Organization [14,15-17]. Several diseases such as dengue, malaria, melioidosis, and typhus, which are well documented for Leptospirosis co-infection, lead to benefiting the pathogenic bacteria by misdiagnosis and increase the severity of disease [18]. India has the world's largest livestock population, which plays a vital role in the agricultural economy. Infecting bovines leads to the major impact on our agricultural and livestock activities that overall affects our country's economic system. The cattle and buffalos are two major livestock species that live in close contact with humans, majorly in India [19,20]. Bovine leptospirosis in these species is a chronic disease that primarily causes reproductive failure, including abortions, infertility, and stillbirths [21]. The prevalence of leptospirosis in livestock species in India from 2005 to 2023 shows the highest prevalence rate in buffaloes (45.75%), followed by rodents (40.00%), dogs (26.08%), cows (24.26%), goats (19.42%), and pigs (15.80%) and compared to the livestock, prevalence rate of 9.47% has been recorded in humans [22]. Few studies carried out on animal models demonstrated sexual transmission of Leptospira in the case of bovine bulls, and similar findings have been reported in the case of humans [23,24]. The symptoms of this disease appeared within 5-14 days after the exposure, but the clinical illness occurs after an incubation period of 2-30 days [25]. The disease can be divided into two stages of symptoms: mild leptospirosis and severe leptospirosis. A mild leptospirosis, also known as the initial phase (acute phase), starts quickly and lasts 5-7 days, characterized by unspecific symptoms in humans including headache, fever, body or muscle aches, nausea, vomiting, yellow eyes and skin (jaundice), red eyes, diarrhoea, chills, fatigue, stomach pain, and rashes (Fig. 2). The second phase known as severe leptospirosis occurs when antibodies appear in the serum and leptospires are excreted in the urine. The people may suffer more severe conditions in this phase, like multiorgan dysfunction, which includes kidney failure, liver failure, pulmonary haemorrhage, or inflammation of the spinal cord and brain (meningitis) [25,26]. Leptospirosis is widespread globally, and it has been

estimated that it causes more than 1 million infections in humans and is the reason for 60,000 deaths round the year [18,27,28]. In the Southeast Asia region, the incidence of Leptospirosis is estimated to be approx. 2.7 lakhs with 14,000 deaths. In India, there are 100,000-1,000,000 cases expected every year, but less than 10,000 cases are reported. Only 4 states of India reported more than 500 cases per year: Kerala, Gujarat, Tamil Nadu, and Maharashtra, as per the Integrated Disease Surveillance Program. Other states including union territories (UTs) like Andaman, Andhra Pradesh, Assam, Goa, Delhi, Karnataka, Odisha, Puducherry, and Uttar Pradesh also reported cases of this disease [28,29-33]. In India, states and union territories with high to low prevalence of leptospirosis are given in Fig. 3. The spectrum of leptospirosis is extremely complex or wide, which makes its diagnosis difficult [25].

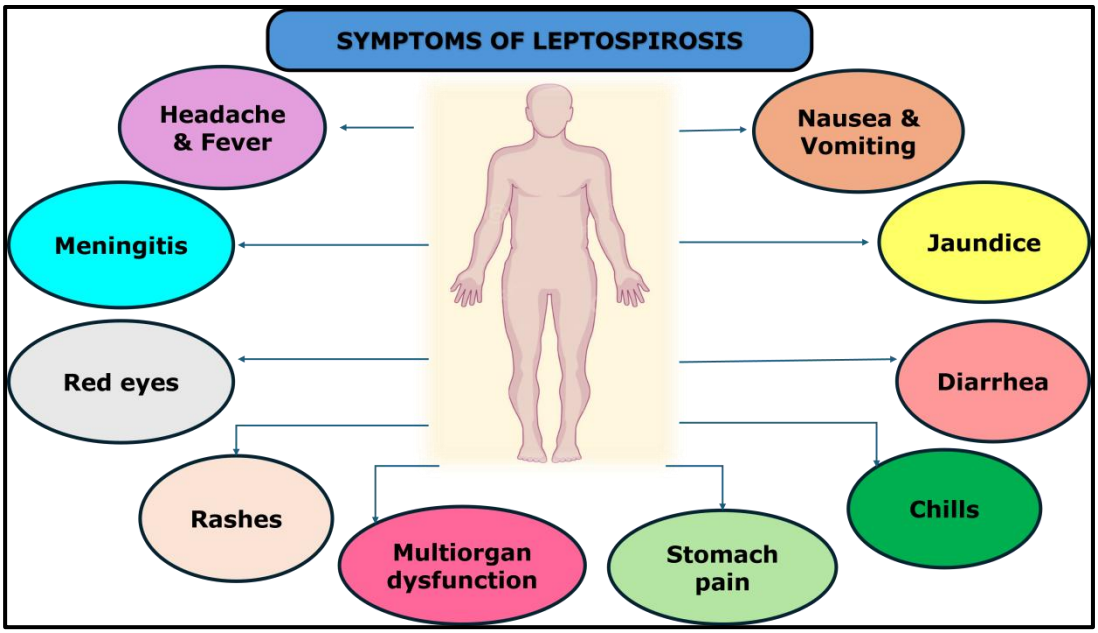

Fig. 2. Major symptoms of leptospirosis in humans

The lack of knowledge and lack of suitable laboratory diagnostic testing in the many regions of the country creates a barrier to identifying the actual burden of this disease. Because of this reason, the disease is under-diagnosed or under-reported in India [34]. The best solution for this issue is to be the need of better monitoring of the disease and co-infections, more awareness among common people, veterinarians, farmers, clinicians and other public health professionals along with the availability of wide laboratory capabilities by exploring more advanced and sensitive diagnostic technologies. Therefore, in this article we are highlighting an update of almost all the diagnostic techniques viz. conventional, advanced, and cutting-edge along with their advantages, limitations, and future challenges which will be utilised to reduce the burden of this disease and improve public health and livestock productivity in India.

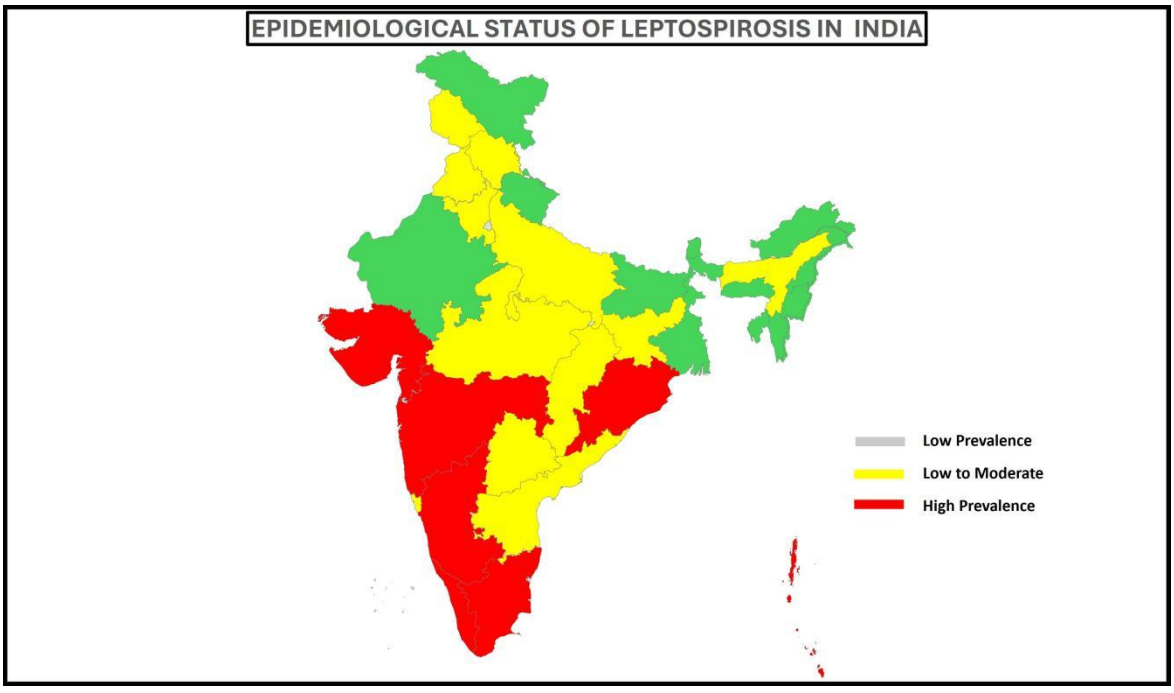

Fig. 3. Epidemiological status of Leptospirosis in Indian

CONVENTIONAL DIAGNOSTIC METHODS

A number of laboratory techniques are available to investigate the diagnosis of Leptospirosis. Different types of microscopies, culture-based techniques, serological tests, and molecular techniques, can be done to diagnose infection by utilizing various animal or human sample sources including blood, urine, and CSF. Conventional diagnostic strategies or methods include Microscopy, Culture Method, Indirect Hemagglutination Assay, Leptocheck-WB, Microscopic Agglutination Test (MAT), Enzyme linked immunosorbent assay (ELISA), and IgM dipstick assay.

Microscopy and Culture method

This is an early and most widely used conventional diagnostic method due to easy availability and its cost-effectiveness. In dark-field microscopy bacteria appear as bright, thin, actively motile rods and spiral-shaped in the sample source. Dark-field microscopy generally requires bacterial concentrations of approximately 10^4–10^5 organisms/mL for reliable visualization. Although microscopy is one of the earliest direct diagnostic methods for testing leptospirosis, it is still not used as a confirmatory test for the identification of leptospirosis because in the acute phase, the load of bacteria in the body is quite low, which makes it difficult to find in the urine, blood, and other samples utilized for the diagnosis [27, 35-36]. Bacterial pathogen of *Leptospira spp*. can be isolated from urine, blood and CSF of infected person. The blood samples from patient should be collected within 5-7 days of the onset of clinical signs for early detection. However, leptospires in blood samples may disappear after 10 days of illness and they may persist in some other organs [37]. Commonly used culture media for leptospires are Ellinghausen-McCullough-Johnson-Harris (EMJH) medium as described by Johnson & Harris and Studdard medium, and Fletcher's medium, or Korthof's medium [38,39]. Leptospira takes time to grow that makes this process time consuming [40].

Microscopy is a direct detection method for visualization of Leptospira bacteria under a dark-field microscope, and there is less need for specialized lab setup; therefore, this method takes less time for delivering the results. Contrarily, the culture method has a strict requirement of specialized media for the observation of the growth of *Leptospira*, and a specialized lab equipped with biosafety cabinets are additional components required for this task. This bacterium takes time to grow, as its doubling time is 6-16 hours, and even under ideal conditions, i.e., 28-30°C, the growth is slow, which makes this process time-consuming [41].

Indirect Hemagglutination Assay, Leptocheck-WB, Microscopic Agglutination Test (MAT), Enzyme linked immunosorbent assay (ELISA) and IgM dipstick assay

The Indirect Hemagglutination Assay (IHA) is a diagnostic test used for screening disease in the acute phase. This test works by detecting total antibodies in the patients' samples [42]. IHA is a sensitive, rapid, and cost-effective serological test that detects genus-specific antibodies against *Leptospira* in the serum sample of patients. Leptocheck-WB, on the other hand, is a rapid card-based qualitative immunodiagnostic tool that is explored for the early detection of IgM antibodies, which signifies a current leptospirosis infection [43]. MAT is considered a gold standard serological test for the detection of leptospirosis. A fourfold increase in the MAT antibody titre provides definite or strong evidence of leptospiral infection, reflecting the high sensitivity of this assay, but this test requires a high enough antibody titre, which means that the MAT may not be advantageous for early diagnosis, as in the early stages of illness since the humoral immune response is delayed and antibodies are usually not found until the first week of illness; therefore, there might be chances of false-negative results during the acute phase [44,45]. This method involves dilution of a patient's sample, which is then mixed with live leptospires in different dilutions to see antigen-antibody reactions via agglutination. The MAT test is read by using the microtiter plate under a dark-field microscope by adding one small drop of reaction mixture on a glass slide [41]. ELISA is a serological assay that generates a visible colour signal that totally depends on specific antigen-antibody interactions coupled with an enzyme-linked reaction to finally verify the status of disease. This assay is primarily used to detect the infection at its early stages by targeting Leptospira-specific IgM antibodies in the host. Furthermore, it targets the IgG antibodies which may be used to assess previous exposure or later stages of infection.

The IgM dipstick assay is a rapid diagnostic technique that is highly sensitive, requires minimal specialized laboratory equipment, and may also provide better results in testing during the acute phase of infection. This technique uses a semiquantitative dot-ELISA format to detect IgM antibodies. In case of acute leptospirosis, this diagnostic method showed a specificity and sensitivity of 90% and 98%, respectively, and was found to be more sensitive than the indirect hemagglutination assay (IHA) and comparable in sensitivity to IgM-ELISA [27,46].

As compared to MAT, IHA shows a specificity and sensitivity of around 95% and 92%, respectively [36]. Even though MAT is considered the gold standard tool for diagnosing Leptospirosis, it is still not found suitable for early detection, while on the other side, tests like IHA and Leptocheck WB are quicker and simpler to perform, making them more practical options for timely diagnosis in acute cases [44,47]. ELISA identifies antibodies against Leptospira in blood and is considered more sensitive than MAT during the acute phase of leptospirosis. Leptospira-specific IgM antibodies are typically identifiable within the initial week of illness; however, their levels may be low or even undetectable during the very early onset of the disease [36,48].

ADVANCE TECHNIQUES USED TO DIAGNOSE LEPTOSPIROSIS

Polymerase Chain reaction (PCR)

PCR is one of the most sensitive and widely used molecular techniques used to identify early stage of infection even when concentration of antibodies is quite low in the body. PCR does not need serum antibodies and is a reliable and rapid diagnostic tool [42,49]. There are number of PCR variants available now a days and having many applications in different fields and one of the best examples of this is Nested PCR. Nested PCR is one of the advanced PCR variants that reduces non-specific binding to increase the specificity and sensitivity of the PCR amplification reaction using different set of primary and secondary PCR primers. This method is mostly employed when the load of the target is low in the sample, and it can substantially improve analytical sensitivity compared with conventional PCR [41]. Research groups used DNA samples isolated from various sources, like urine and serum samples of individuals affected with pathogenic leptospira infection, in order to amplify the *LipL32* gene using PCR technology [50]. Apart from *LipL32* gene, recent studies have reported targeting the s*ecY* gene for the identification of Leptospira DNA in bovine uterine fragments using nested PCR [21]. In addition to that, when compared with another serological tests, the nested PCR may provide 100% specificity (as shown in some studies), and during the acute phase of infection, it has been shown to be a reliable diagnostic tool for leptospirosis [51]. Although nested PCR reflects good specificity & sensitivity, it possesses a risk of contamination, which may result in false positive and false negative findings [50]. Among other fabulous variants, quantitative PCR (qPCR) is one by use of which one could not only know the absence or presence of pathogenic bacteria but also find the load of pathogens in the samples, which can clinically corelate with disease severity, leading to proper treatment and monitoring of disease progression in the body. Upon comparison to conventional PCR, qPCR result delivery rate is fast, as in the case of conventional PCR, amplification of target-specific PCR products should be verified by running amplicons in the agarose gel, while qPCR measures the amplification in real time, and therefore there is no need for post-PCR amplification processes. Additionally, researchers will benefit in the case of qPCR, as this process does not involve the use of carcinogenic dye like Ethidium Bromide (EtBr), used in the case of agarose gel electrophoresis to verify the amplification of specific PCR products amplified by conventional PCR. But both techniques of amplification are expensive and require specialized equipment, and also there is a need for a trained person to handle the machines and other experimental accessories [52-54].

Truenat™ micro–Real-time PCR

Truenat™ PCR is used for a rapid molecular diagnostic technique for Leptospirosis, and this is especially designed for point-of-care use in the areas where there is a shortage of resources, i.e., designed for low-resource settings. The Truenat™ test is done using a collection of plasma samples from a confirmed patient, and then DNA is extracted from the plasma sample of the patient. This test showed results within 1 hour, with high diagnostic accuracy. The Truenat™ micro-RT-PCR showed a sensitivity and specificity of the test of 97.4% and 98.6%, respectively. This diagnostic technique is useful for the detection of pathogens' DNA during the early phase of infection [55].

Loop mediated Isothermal Amplification (LAMP)

Researchers developed a very accurate and rapid DNA amplification technique, which employs four to six primers recognizing six to eight regions of target DNA, guaranteeing high specificity for the test, known as loop-mediated isothermal amplification (LAMP), that enables the amplification of DNA under constant temperature conditions, therefore bypassing the need for a thermal cycler [56-57]. For the diagnosis of leptospirosis, LAMP has been employed by specifically targeting the *secY* and *LipL32* genes. Furthermore, it exhibits multiple advantages over conventional PCR, viz., easier detection, higher sensitivity, and a faster rate of amplification, producing results generally within one hour. Several reports have shown that LAMP diagnoses Leptospira DNA comparable to or greater than conventional PCR [58]. The LAMP technique is suitable for early diagnosis of Leptospirosis because it is simple, quick, and has minimal equipment requirements. It is especially useful in places where resources are very limited and has also proven successful in the field conditions.

16S rRNA sequencing

16S rRNA gene sequencing is a culture-free molecular technique that is being utilized to identify bacteria on the basis of highly conserved sequences of the *16S ribosomal RNA* (*16S rRNA*) gene. It is emerging as a powerful tool for the identification of novel pathogens in patients with suspected bacterial disease. Recently, this technology has been used in reference laboratories for the routine identification of bacterial isolates by targeting the conserved and hypervariable regions of the *16S rRNA* gene with the help of the expand high-fidelity PCR system. Under different geographical and ecological settings, this technique is widely useful for species identification of *Leptospira* from diverse geographical regions [59-62]. Also, in addition to that, 16S rRNA-based nested PCR has been explored for the rapid identification of human Leptospirosis using urine and serum samples [51].

Biosensor based technology

The conventional diagnostic methods are generally time-consuming, which leads to delay in the necessary clinical interventions, and biosensors, therefore, have emerged as a reliable tool and have potential to overcome the limitations faced due to conventional methods.

Initiation of timely and proper medication for Leptospirosis needs early diagnosis of *Leptospira* infection in the body. There are different analytical devices, biosensors, available nowadays that diagnose the pathogen in a suitable time frame, which helps the clinicians to start treatment of the patient as soon as possible. Biosensors are devices that combine the biorecognition elements with the transducer and convert a detectable bio signal to a measurable readout that confirms biological interaction. Among mainly used biosensors, Amperometric biosensors, due to their high sensitivity, might be proven useful tool for the diagnosis of Leptospirosis in future. In addition to that, other sensors based on aptamers have also demonstrated substantial potential for the diagnosis of Leptospirosis due to their high affinity towards different bacterial components as well as their high specificity for their target. Most biosensor platforms remain in experimental or preclinical evaluation. Overall, biosensors might be proven as a user-friendly, cost-effective, and rapid method used to detect microbial infections [63].

CUTTING EDGE TECHNIQUES USED TO DIAGNOSE LEPTOSPIROSIS CRISPR Cas systems

Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein (CRISPR-Cas) systems have recently been used to detect Leptospira DNA by extracting DNA from the whole blood of animals and humans. A Recombinase Polymerase Amplification (RPA)-CRISPR/Cas12a-based fluorescence assay is an ultra-fast and sensitive molecular diagnostic method that shows 85.2% sensitivity, 100% specificity, and 92.7% accuracy for detecting *Leptospira* by targeting the *LipL32* gene of pathogenic *Leptospira spp*. with an exhibit limit of detection of 100 cells/mL. In one way or another, this assay showed higher reliability than the commercially available conventional rapid diagnostic tests (RDT).

Integration of a lateral flow detection assay (LFDA) with RPA-CRISPR/Cas12a enhances the result interpretation as well as makes the test more accessible for field-level applications [64]. Researchers have also developed a dFnCas9 lateral flow immunoassay for the detection of Leptospirosis by targeting the *lip32* gene of *Leptospira*, representing the first report for leptospirosis detection using the dFnCas9 LFIA assay. The CRISPR dFnCas9 LFIA test strip is a novel technology and has potential for point-of-care deployment owing to its portability and ease of use with a detection limit of 1 fg/mL. The clinical samples of both humans and animals may be employed to perform this test. This test may become a cost-effective alternative after large-scale implementation and also might serves as a more reliable tool from a sensitivity point of view for the diagnosis of Leptospirosis [65]. Prior to the implementation in routine clinical practice, regardless, the important advancements, CRISPR-based diagnostic systems, need approval along with necessary and appropriate validation from national regulatory and biosafety authorities.

Next generation sequencing (NGS)

NGS is another cutting-edge molecular technique being employed for diagnosis as well as for characterization of leptospirosis. It is a high-throughput technology that can simultaneously generate millions of DNA sequences reads in a single go. It can provide important diagnostic clues for patients with atypical leptospirosis symptoms and is also advantageous in outbreak tracking and environmental surveillance. This cutting-edge approach of molecular detection does not require the isolation and cultivation of disease-causing microorganisms; rather, it detects pathogens by sequencing microbial nucleic acid fragments in clinical samples [66-68]. This technique is not commonly utilized in the laboratories due to its complexity and high cost. Also, it can help the researchers and clinicians to confirm the diagnosis along with offering important diagnostic clues for patients as well [69].

TREATMENT

The treatment procedure mainly depends on the condition of the patient or severity of the zoonotic disease. Current management of leptospirosis primarily consists of appropriate antibiotic therapy and supportive care. Corticosteroids and other adjunctive therapies have been investigated for selected severe cases, whereas approaches such as bacteriophage therapy and novel natural or synthetic compounds remain experimental [70]. Treatment should be initiated promptly on clinical suspicion, particularly in patients with severe disease, without waiting for laboratory confirmation because serological tests may not yield positive results until approximately one week after the onset of illness, and cultures may generally require a one week to a few weeks to become positive, and that is depending upon the doubling time of bacteria as well as some other factors [71]. During the early phase of infection, antibiotic therapy is most effective and suitable. Penicillin, doxycycline, ampicillin, ceftriaxone, azithromycin, and amoxicillin are commonly used antibiotics to treat mild or severe infections of Leptospirosis [72]. In severe cases of Leptospirosis, especially with pulmonary and renal failure, supportive measures such as haemodialysis and mechanical ventilation therapies may be recommended. In addition to that, third-generation cephalosporins, viz., cefotaxime and ceftriaxone, demonstrated good efficacy against *Leptospira* infection [71]. Serovar-specific vaccines have been developed for humans to deal with this zoonotic disease; Leptospirosis, but they are customized according to specific epidemiological situations, leading to their restricted reach and applicability [73,74].

PREVENTIONS

Leptospira bacteria are commonly found in water and soil contaminated with the urine of infected animals. Therefore, there is a need to avoid direct and indirect contact with animal urine as recommended as a preventive measure. Individuals who are at high occupational risks, such as laborers and farmers working in the flood-prone localities, are advised to equip themselves with all protective gear such as gloves and rubber shoes, and if there is any cut, wound, or abrasion,

properly cover it with an occlusive dressing. Safe drinking water practices along with maintaining appropriate sanitation and hygiene are important components of safety and to mitigating the risk of infection. In high-risk situations such as floods or any outbreak of disease, antibiotics are given under proper medical guidance. Aware people through conducting public awareness and public health programs about the disease and its prevention. Early detection and accurate diagnosis can lead to the initiation of timely treatment and close observation helps to improve the cure and fatality rates [75,76,77]. Vaccination reduces disease severity and urinary shedding therefore the risk of transmission to humans is reduced [72]. There is no widely available universal vaccine for humans, but a number of vaccines are available for animals and humans, viz., attenuated & inactivated vaccines are available against, Leptospirosis and recombinant outer membrane protein (OMP) vaccines, lipopolysaccharide (LPS), DNA vaccines, and mRNA-based vaccines are also under investigation [78,79]. Inactivated vaccines are available for veterinary use in many countries, whereas licensed human vaccines are available only in limited countries. Due to the existence of a wide range of *Leptospira* strains, vaccines are not providing complete protection against the disease [26]**.**

FUTURE PROSPECTIVE OF DIAGNOSIS OF LEPTOSPIROSIS IN INDIA

First of all, there is a strong need to strengthen or expand the already available diagnostic methods present or utilized to date. Conventional and advanced diagnostic methods need to be expanded so that every citizen of the country can access them. PCR allows the detection of *Leptospira* DNA during the early leptospiremia phase when antibodies are not produced. Additionally, PCR offers superior sensitivity during the early leptospiremic phase before antibody production., which can significantly improve clinical management when combined with other serological assays such as MAT and ELISA. On the other hand, the LAMP molecular method has its own applications for the amplification of DNA at a stable, constant temperature without the need for sophisticated thermal cyclers and has enormous potential for its use in rural areas where leptospirosis is common in India. In order to achieve the higher rate of diagnostic accuracy, the use of newly developed technologies should be incorporated into our routine disease diagnosis methodologies, which primarily include next-generation sequencing, biosensor-based technology for disease diagnosis, CRISPR-based systems integrated with lateral flow assays, mass spectrometry, and AI-assisted interpretation of molecular diagnostic data, which will further improve the precision of tests as well as expedition of disease surveillance.

CONCLUSION

Leptospirosis is an underdiagnosed and neglected zoonotic disease in India, mainly transmitted from infected animals to humans through indirect or direct contact. High prevalence of this disease was reported in livestock, primarily in buffaloes, which also act as an important carrier and reservoir host. The disease leads to occupational risk among farmers, dairy workers, veterinarians, and people who are in close contact with animals or ecological conditions. One of the important factors of underreported cases of this disease is lack of infrastructure, limited awareness, and a low rate of access to newly developed advanced diagnostic facilities, especially in rural areas and in the regions where its prevalence is higher. Rapid and precise diagnosis is crucial to getting adequate treatment and prevention from complications, along with reducing the dissemination of disease-causing agents, which leads to control of disease transmission. To date, several diagnostic strategies are available for the detection of Leptospirosis, mainly categorized into conventional, advanced, and cutting-edge methods. Conventional techniques comprise basic culture methods and serological assays, ELISA and MAT. Although these methods are widely employed, they still show some limitations related to specificity and sensitivity and are also time-consuming with great chances of contamination, etc. On the other hand, PCR variants such as qPCR, nested PCR, and LAMP are advanced molecular techniques that can identify cutting-edge technologies, including the most recent diagnostic methods like CRISPR-based diagnostics, biosensors, next-generation sequencing (NGS), and AI-assisted interpretation of molecular diagnostic data approaches, which offer precise, fast, and user-friendly solutions. The goal of this review article is to compile almost each and every possible diagnostic approach used for the diagnosis of Leptospirosis and categorize them as conventional, advanced, and cutting-edge techniques, highlighting their advantages, limitations, and potential applications. Such a detailed approach is necessary for improving diagnostic accuracy, enabling early detection, and strengthening surveillance and control strategies in India.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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