Diagnostic Tools for Zoonotic Infections

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ABSTRACT

The recent growth of infectious diseases stemming from zoonotic origins has placed a significant global burden on both morbidity and mortality. Additionally it has generated substantial economic challenges. The complexity and dynamism of the resurgence and epidemiology of zoonoses are shaped by diverse factors which can be broadly classified into human-related, pathogen-related and climate related parameters. The diagnosis of animal diseases serves a dual role as both the origin and solution to various ailments. It is instrumental in managing and preventing diseases, playing a crucial part in overall disease control. Therefore, the imperative for the development of veterinary diagnostic tools becomes apparent, ensuring comprehensive animal welfare and effective monitoring of disease spread. Here we discussed various molecular and non-molecular diagnostic methods for zoonotic infections. These diverse approaches include viral metagenomics, clinical recognition, standard labortary assessments, and labortary tests. By examining recent advancements in diagnostic methodologies, this chapter aims to underscore the importance of ongoing research and innovation in the field of zoonotic disease diagnostics for enhanced public health preparedness and intervention strategies.

Key words: Disease management, Diagnostic, Zoonotic, Viral metagenomics, Clinical tests, PCR, DNA fingerprinting, DNA based composition, Radioimunoassay.

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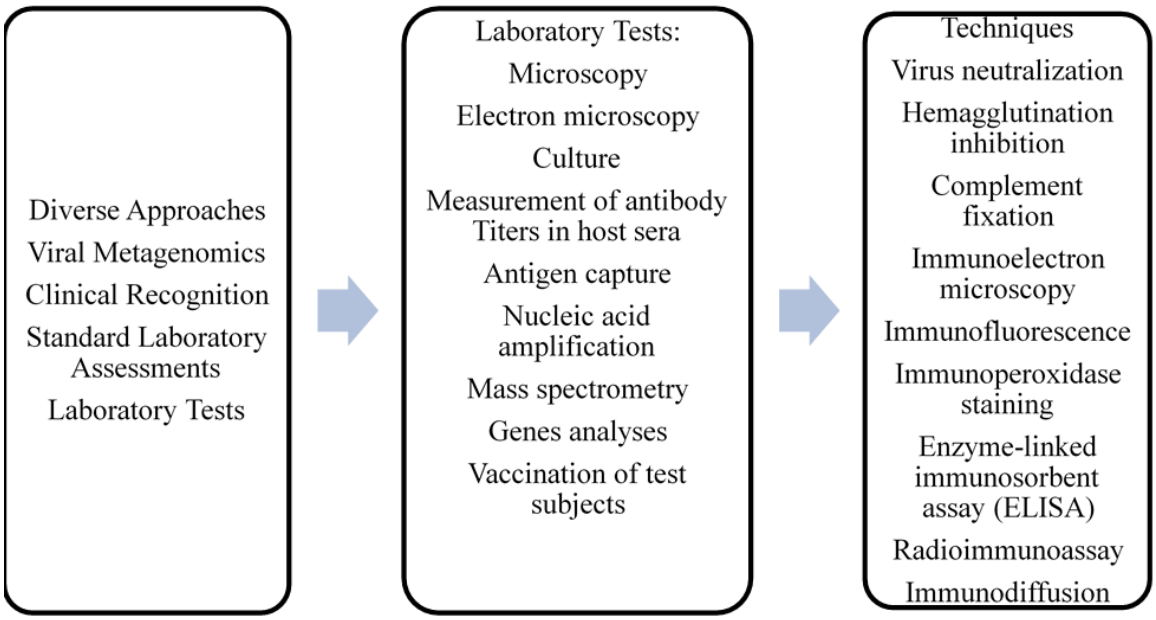
1. INTRODUCTION

Some unidentified and mysterious illnesses with no proper treatment have been observed all over the globe (He et al. 2020; Smolarz et al. 2021; Shafaati and Zandi 2022). Table 1 shows rapid diagnosis for molecular and non-molecular zoonotic infections.

Furthermore, such diseases are unique to specific regions of the world and have spread to new ones (Malki et al. 2020). Therefore, we need to be able to spot emerging diseases and determine its cause (Connolly et al. 2021).

Table 1: Rapid diagnosis for molecular and non-molecular zoonotic infections.

Molecular diagnostic methods	Non-molecular diagnostic methods
• Polymerase chain reaction (PCR) • Hybridization • Metagenomics • Gene probing • DNA Fingerprinting • rRNA sequencing • DNA Base composition • Electron Microscope • Mass Spectrometry • Restriction Endonuclease and Oligonucleotide Fingerprinting	• Cultures • Beta-Glucan Assay • CAGTA Assay • Histopathology • Glutomannan assay • Serological Tests (Antigen tests, Antibody tests) • Optical microscope



2. DIVERSE APPROACHES

The technique that incorporates simultaneous and diverse approaches is the most promising for evaluating novel diseases (Basith et al. 2020).

2.1. VIRAL METAGENOMICS

A culture-independent method called viral metagenomics is used to examine all viral genetic populations in biological material (Sommers et al. 2021). This methodology becomes a powerful tool for identifying

new and emerging viruses, considering that animals remain a reservoir for the virus that can cause zoonosis. Less than 1% of bacterial hosts have been cultivated, so it is difficult to identify and measure the dynamics of the viral population in the environment (Zhang et al. 2022). On the whole, these restrictions can be overcome by metagenomics investigation of uncultured viral communities, which can also offer insights into the makeup and organization of environmental viral communities.

2.2. CLINICAL RECOGNITION

An immediate individual or group recognizes a disorder that does not share traits with any other recognized condition (Shah et al. 2020). It could be an illness with a recently identified or previously documented ailment with an atypical presentation. As a result, it is advantageous to alert hospital infectious disease specialists, emergency room physicians, and other combatants to the potential for a novel disease or one that has not previously been observed in the region (Sami et al. 2021).

2.3. STANDARD LABORATORY ASSESSMENTS

It could be an illness with a recently identified or previously documented ailment with an atypical presentation. Therefore, it is advantageous to alert hospitals for novel disease specialists, emergency room physicians, and other combatants to the potential for a novel disease or one not previously observed in the region to encourage such recognition.

2.4. EPIDEMIOLOGY STUDIES

The epidemiological field is a scientific, systematic, and analytical study of the frequency, distribution, and origins of medical symptoms and events in particular groups (such as communities, schools, cities, regions, nations, and the world) (Luca et al. 2022).
The investigations may shed light on the new condition's distribution, the race, age, and sex of vulnerable people, the immune response of those who are resilient and those who are potential animal vectors or recipients, the lubricating period, and the mode of transmission (Belbasis and Bellou 2018).

2.5. LABORATORY TESTS

All conceivable laboratory tests need to be performed when an indication of a new and significant disease is indicated. These exams comprise of:

- Microscopy
- Electron microscopy
- Culture
- Measurement of antibodies serum
- Antigen capture assays
- Nucleic acid amplifications
- Mass spectrometer
- Genes Measurements
- Vaccination of test subjects

2.6. MICROSCOPY

A microscope can be used for a variety of things, depending on its type. While some are utilized in instructional settings, others are appropriate for biological purposes (DeVree et al. 2021).

Light microscopy can be performed quickly, but precision depends on the equipment's quality and the microscopist's competence. The ability of doctors to employ microscopy for diagnosis outside of an accredited laboratory is frequently constrained by regulations. To differentiate between invasive illness and surface colonization—a distinction that is difficult to make using culture methods—microscopic inspection of tissue may be necessary (Richert-Poggeler et al. 2019).

3. MICROSCOPY USING ELECTRONS AND IMMUNOELECTRON MICROSCOPY

These techniques are best suited for quickly detecting viral isolates from cell cultures and actual specimens (Madanayake et al. 2023). Electron microscopy only allows for family-level identification, whereas immunoelectron microscopy with a suitable, particular antibody may allow for the creation of finer differences (Gulati et al. 2019).

4. CULTURE

Culture is the development of microorganisms located in a nutrient-rich either liquid or solid medium; more organisms make identification easier. Antimicrobial susceptibility testing is also made more accessible by culture (Namdari et al. 2019).

5. IMMUNOLOGIC TESTS

The antigens are used to search for pathogen-specific antibodies in the patient's samples. Detection antibody for a pathogen's antigen in the patient's samples. Although there are different protocols for handling specimens, they should normally be chilled or frozen if testing needs to be postponed to avoid bacterial contamination proliferation (Normann et al. 2020). There are numerous techniques used or put forth for the cultivation-independent characterization of infectious pathogens (Gilboa et al. 2020; Preena et al. 2020). Through the use of molecular pathogen discovery techniques, new pathogens connected to both acute and chronic illnesses have been discovered during the past ten years (Belizario et al. 2021).

6. LIMITATIONS

6.1. THE PATHOGEN'S HOST RANGE

A pathogen once exclusive to dogs, swine, or cats now threatens human life (Tomori and Oluwayelu 2023).

6.2. MODIFICATION OF TISSUE TROPISM

A virus that in the past only occasionally caused moderate enteritis now frequently causes severe myocarditis and severe encephalitis (Shieh 2022).

6.3. IMMUNE EVASION

Large DNA viruses with many genes that help them escape or manipulate the host's immunity include orthopox viruses (Verdonck et al. 2022). These genes could undergo mutations that alter the virus's host range. Mutations can cause viruses of all sizes, including RNA viruses, to switch species. Additionally, some

RNA fragments are capable of recombining to create new variations that are immune-evading for both humans and the animals that serve as their reservoirs (Ma et al. 2019).

6.4. ENVIRONMENTAL EFFECTS

Human beings' activities include constructing routes across the bush and moving animals or vectors (Mishra et al. 2021).

6.5. ZOONOSES WITHOUT A VECTOR

Pathologists can now identify the root cause of dying more quickly than ever before and frequently when it would have been impossible in the past, using techniques like polymerase chain reaction (PCR) testing, in situ hybridization, and immunohistochemistry (Sledzinskaet al. 2021).

Table 2: Main categories of tests used to determine an accurate diagnosis of a viral outbreak in an animal

Taxonomy	Techniques	Examples	References
Family (genus)	• Complement Fixation • Electron Microscopy • Cytopathology	GBV-A, a virus from the family Flaviviridae, is found in wild monkeys, whereas GBV-B, from the genus Hepacivirus, is found in infected tamarins. Pegivirus is the name of the fourth genus in the Flaviviridae GBV-D and GBV-C family observed in bats, rodents, horses, and pigs	(Koonin et al. 2021)
Species (types)	Neutralization	Brown bats' Eptesicus fuscus and Myotis lucifugus were examined for the presence of the rabies virus and antibodies that can neutralize it.	(Gold et al. 2020)
Subtype	Kinetic neutralization Monoclonal antibody serology	Tembusu virus (TMUV), a new flavivirus that is developing in ducks, was neutralized by an antibody response in 2010. Influenza-neutralizing antibodies A virus and the effectiveness of their preventative measures in mice	(Lv et al. 2019)
Variant	• Restriction Endonuclease • Oligonucleotide fingerprint • Nucleic acid hybridization	Dromedaries with MERS-CoV Infection variant detection are detected and in pregnant white-tailed deer, SARS-CoV-2 and its alpha form were found and also in Norway rats. Endonuclease fragments were used to find the *bla*CTX-M-15 and *bla*CTX-M-1 (CTX-M ESBL gene variants) in Escherichia coli isolates and animal faeces	(Lado et al. 2021; Cool et al. 2022; Higgins et al. 2023)
Mutant (including by point mutation)	Nucleic acid sequencing	Asiatic lions in India were infected with the SARS-CoV-II Delta mutation	(Karikalan et al. 2022)

7. VIRAL DIAGNOSIS

There are three main categories of tests used to determine an accurate diagnosis of a viral outbreak in an animal:

1. Characterization and isolation of the causative virus.
2. Measurement and detection of antibodies.
3. Direct demonstration of viral nucleic acids, viral antigens, virions, or in tissues, secretions and excretions.

There are numerous interesting diagnostic tests employing nanomaterials that are focused on the chemicals that cause animal diseases, but many of them have not yet reached a balance between specificity, sensitivity, cost-effectiveness, and repeatability. (Ramakrishnan et al. 2021). Table 2 shows main categories of tests used to determine an accurate diagnosis of a viral outbreak.

7.1. SPECIMEN PREPARATION FOR INOCULATION

Once the material has arrived at the laboratory, it should be handled and immunized as soon as feasible. If delays of more than a day are anticipated, the specimen may be frozen. Swabs are treated by rotating them in the vessel of transport and applying significant pressure to the container's side to release the substance (MacDonald et al. 2022). The excrement is dispersed using a vortex mixer. Tissue samples are meticulously cut with scissors and homogenized in a glass or mechanical homogenizer.

Table 3: The main serological methods in diagnostic virology.

Techniques of choice	Working Principle	Examples	References
Virus Neutralization	Antibody prevents cytopathology, protects animals, or decreases plaques by neutralizing the virion's infectivity.	Antibodies against the West Nile virus were discovered in wild birds from Southern Spain. Virus-neutralizing antibodies and related variables against rabies in the immunized domestic dogs of Kathmandu Valley, Nepal.	(Ferraguti et al. 2016) (Rimal et al. 2020)
Hemagglutination Inhibition	Antibody suppresses the hemagglutination of viruses.	In Kerman, Iran's southeast, dogs were used in an experiment to test for the avian H_9N_2 influenza virus's ability to hemagglutinate.	(Saberi et al. 2019)
Complement Fixation	Complement is bound by the antigen-antibody complex, rendering it effective to lyse hemolysis-sensitive sheep red blood cells.	Complement fixation test for detection of specific antibodies against bovine brucellosis in chosen peasant association in Guto Gida district, East Wollega Zone, Oromia Ethiopia	(Tosisa et al. 2020).
Immunoelectron Microscopy	Electron microscopy can reveal virions that have aggregated into antibodies.	After a single dose of immunization, immunoelectron microscopy-based immunogenicity of a recombinant VSV-vectored SARS-CoV vaccine generated strong protection in rhesus monkeys.	(Shan et al. 2022)
Immunofluorescence	The fluorescent antibody binds to subcellular antigens and fluoresces under ultraviolet light microscopy.	To investigate the seroprevalence of *Bartonella henselae* in Egyptian cats and people, an immunofluorescent test was performed	(Sayed et al. 2022)
Immunoperoxidase Staining	The intracellular antigen is recognized by the peroxidase-labeled antibody, and upon the addition of substrate, colored precipitate results.	*Brucella melitensis* in experimentally infected foetuses is diagnosed by immuno-peroxidase staining in the liver of the foetus.	(Mazlan et al. 2021)
ELISA (Enzyme-linked immunosorbent assay)	The reaction causes the substrate to change colour when an antibody or antigen that has been enzyme-labeled binds to it.	To find antibodies against *Toxoplasma gondii* in a cohort of hunting dogs, researchers used an ELISA with the modified agglutination test (MAT)	(Bellatreche et al. 2022)
Radioimmunoassay	An antigen or antibody that has been radiolabeled binds to it, such as when it is connected to the solid phase.	The ELISA test was developed based on radioimmunoassay to test samples from potentially infected animals.	(Broussard 2020)
Immunodiffusion	In a gel, soluble antigens and antibodies precipitate in clear lines.	Agar gel immunodiffusion assay for the diagnosis of canine brucellosis using Brucella ovis antigen	(Bolotin et al. 2022)

Membrane filters with an average pore diameter of 0.45m filter out contaminating microorganisms before inoculation. Then mycoplasmas can pass through these kinds of filters. The suspension needs to be refiltered using 0.22m filters to get rid of mycoplasmas after the virus has been successfully isolated and grown to a significant titer. Faeces and tissue homogenates should be diluted by at least a factor of 10 before being centrifuged at 1000g for 15 minutes to produce a filterable supernate. The virus concentration is believed to be very low, so a high dosage of antibiotics may be chosen for filtration (Diaz-Linan et al. 2021).

7.2. UTILIZING SEROLOGY TO IDENTIFY VIRAL ISOLATES

The main serological methods used to identify various viruses have been enlisted in Table 3.

7.3. COMPLEMENT FIXATION

Indirect diagnostic tests with great sensitivity like the complement fixation test (CFT) have a lot of false-positive outcomes (Elschner et al. 2021).
Inactivated serum samples are diluted twice in Complement fixation test (CFT) buffer and combined with CFT antigen and complement hemolytic units. Sera, complement, and antigen are combined in the plates and left to interact overnight at 4°C. This allows the complement to be fixed.
The plates are then incubated for 45 minutes at 37°C with a 2% suspension, then centrifuged for 5 minutes at 600g, and then the process is repeated. Samples exhibiting 25-75% hemolysis are regarded as suspicious, samples showing 100% hemolysis in a dilution are classified as negative. The classification of all suspicious test results is affirmative (Elschner et al. 2019).
Complement fixation can be utilized to classify an isolate and perform initial screening on it. Essentially a slightly streamlined version of the complement fixation of complement fixation test, the immune-adherence hemagglutination test is currently employed more commonly to identify antibodies than antigens (Coombs 2012).

7.4. HEMAGGLUTINATION AND INHIBITION OF HEMAGGLUTINATION

Numerous viral families' virions attach to red blood cells to hemagglutinate them. Hemagglutination is suppressed if antibodies and viruses are combined before the inclusion of red blood cells (Cho et al. 2022). Dissociating the virions using detergents can boost the hemagglutination level of some viruses, such as the canine distemper virus (Suarez et al. 2020). Antisera may need to be prepared to get rid of hemagglutination inhibitors that aren't specific. Test for determining the titer of antibodies to viral hemagglutinin by inhibiting hemagglutination. In titers, dilutions are stated as reciprocals (Spackman and Sitaras 2020).

8. METHODOLOGY

1. Periodate is applied to the sera, which is subsequently subjected to heat at 56°C for a half-hour to inactivate non-specific hemagglutination inhibitors.
2. After that, the sera are diluted in microtiter wells. The appropriate viral strain is then injected with hemagglutinating units into each well. Each well receives 0.05 ml of red blood cells following a 30-minute room temperature incubation period (Chivukula et al. 2021; Zhang et al. 2022).
3. Hemagglutination has been prevented where there is enough antibody to coat the virions, and as a result, at the bottom of the well, the erythrocytes condense to form a button (Saegerman et al. 2021).

Erythrocytes get agglutinated by the virus when there is insufficient antibody present. For example, the horse lacked any antibodies that could have blocked the hemagglutinin-inducing effects of this particular influenza virus strain. The first dose of the vaccine resulted in some antibody production, and the second dose induced a booster response (Drozdz et al. 2020).

8.1. NEUTRALIZATION OF VIRUS

A unique antibody may neutralize a virus's infectiousness through several different processes. To get rid of general viral inhibitors. The serum first needs to be inactivated by being heated at 56°C for 30 minutes (Cuevas et al. 2022). Suitable cell cultures are infected with serum-virus combinations. Afterward, they are allowed to develop until the virus-only controls show cytopathic effects by reducing a virus's ability to infect, an antibody shields cells against viral annihilation (Amanat et al. 2020).

8.2. RESTRICTION ENDONUCLEASE AND OLIGONUCLEOTIDE FINGERPRINTING

For the majority of routine diagnostic purposes, even to the level just mentioned, it is often not necessary to isolate antigenically. However, there are circumstances in which going further to identify differences between variants or subtypes within a particular serotype could provide viral epidemiological data. Similarly, viral DNA can be isolated from infected cells or virions, and the fragments can then be separated using agarose gel electrophoresis after being cut with properly chosen restriction endonucleases (Guo et al. 2022).

Ethidium bromide per silver staining is required to obtain restriction endonuclease fragment patterns, which are frequently referred to as fingerprints. All dsDNA virus families have found a use for methodology, particularly referred to as fingerprints. All dsDNA virus families have found use for the methodology, particularly in epidemiology research but also in pathogenesis research. These methods' resolution allows us to distinguish different isolates of the same viral species, even if they did not all come from the same epizootic, depending on the viral family. Minor amounts of genetic drift, which are typically not reflected in serological differences, can occasionally be detected using this technique (Laudermilch and Chandran 2021).

8.3. ELECTRON MICROSCOPY FOR THE DIRECT DETECTION OF VIRIONS

Electron microscopes are used for quick viral diagnosis. Negative staining techniques and understanding the concentration of virions are crucial for accuracy. Technology in the medical field is constantly advancing. This method can diagnose viral skin disease using vesicular fluid, scrapings, or scabs. It can also be used for accurate diagnosis in cell culture (Hopfer et al. 2021).

8.4. IMMUNOELECTRON MICROSCOPY

It is a technique that uses immune serum to increase the susceptibility of electron microscopic techniques. After mixing the sample with the antibody for an overnight period, the sample is typically cleared by low-speed centrifugation.

The immune complexes are subsequently centrifuged at a low speed to form pellets, which are then negatively stained. The antibody used could be a mixture of these antibodies, serum from an old animal that is hyperimmune to many viruses, or type-specific monoclonal or polyclonal antibodies (Zhang et al. 2022).

8.5. DIRECT IDENTIFICATION OF VIRAL ANTIGENS

These techniques rely on the direct interaction of viral particles, or antigens, with specific antibodies that have been prelabeled in some way to quickly identify the interaction, in situ in tissues, excretions, or secretions. The labeling techniques used—are immunoperoxidase staining and radioimmunoassay.
The labeling methods are divided into four categories: radioimmunoassay, immunoperoxidase staining, immunofluorescence, and ELISA. Viral antigens can also be identified using two proven serological techniques, precipitation and complement fixation (Bassani-Sternberg et al. 2016).

8.6. IMMUNOFLUORESCENCE

Immunofluorescence is a method of unique significance in the quick identification of viral infections due to its specificity, speed, relative simplicity, and sensitivity (Zhang et al. 2022).
The classic instance of immunofluorescence is the diagnosis of rabies, for which immunofluorescence has been recognized as diagnostic for more than 20 years (Chiebao et al. 2019).

9. STAINING WITH IMMUNOPEROXIDASE

An alternate approach involves using an enzyme-labeled antibody to locate and detect viral antigens in infected cells. The process results in a pore-durable, non-fading, and anatomically clearer preparation than immunofluorescence, and it uses less expensive equipment. Similar steps and ideas apply to immunofluorescence (Burrell et al. 2017).
The conjugated antibody, bound to the antigen through a direct or indirect procedure, is detected by adding a substrate suited for the specific enzyme. In the case of peroxidase, this is H_2O_2 coupled with a benzidine derivative. Endogenous peroxidase, which is found in many tissue cells, especially leukocytes, causes false positive results, which is a drawback of the method. By using a diligent approach and proper controls, this issue can be avoided (Arshad et al. 2022).

9.1. RADIOIMMUNOASSAY

A radioactive element, most frequently 125I, serves as the label in radioimmunoassay. The technique is incredibly sensitive, allowing the detection of viral antigens at low concentrations ranging from 10–12M. There are numerous radioimmunoassay techniques available. The principles are the same for both direct and indirect approaches as for immunofluorescent staining.
In the most basic configuration, a sample that might contain a virus or viral antigen is allowed to attach to the bound antibody, washed, and then an antiviral antibody is measured in a gamma counter after additional washing (Burrell et al. 2017).
A more popular approach is indirect radio-immunoassays, which include a second layer of 125I-labelled anti-IgG as an indicator antibody in place of the detection antibody. Different animal species must be used to generate the antiviral antibodies that make up the capture and detection antibodies (Inoue et al. 2010).

9.2. DIRECT ISOLATION OF LEPTOSPIRES FROM CLINICAL SAMPLES

A common method for identifying Leptospira directly from clinical samples is dark-field microscopy (DFM). Early detection of leptospires in bacterial infections is crucial. The direct fluorescent antibody (DFM) is

highly sensitive for detecting leptospires in both blood and CSF, with a sensitivity rate of 64.7%. DFM is a good way to diagnose the early stages of an illness, with high sensitivity in identifying leptospires in blood and CSF. A skilled specialist is needed and 100 fields on each slide must be studied to deem it negative. As the illness progresses, DFM's sensitivity may decrease.

9.3. LEPTOSPIRES' CULTURE

A Bunsen burner will be used to aseptically add blood samples dropwise to the semi-solid medium that has been prepared. Within 24 hours, the sample-containing medium will be switched over to the liquid media. Every day for three months, each tube will be examined for the development of leptospires, and after every 2-3 weeks, they will be routinely switched to a new medium. The media developed by Stuart, Korthoff, and Fletcher is also utilized for culture. Although cultivation is the most common method of detection, its application for routine isolation is debatable because it requires a longer period for development, with a gestation period of 6–20 hours (Hornsby et al. 2020).

9.4. SEROLOGICAL METHODS

The serum of people who have contracted Leptospira can be tested using a variety of serological assays for antibodies against the parasite. We can detect IgG and IgM antibodies with MAT and ELISA- based test kits, and a wide variety of commercial fast diagnostic card tests that are already on the market (Trozzi et al. 2023).

9.5. MOLECULAR TECHNIQUES

Molecular methods can quickly and accurately diagnose infections, including leptospires, in medical or environmental samples (Girones et al. 2010).

9.6. POLYMERASE CHAIN REACTION (PCR)

Due to its specificity, sensitivity, and capacity to identify even the smallest amounts of nucleic acid particles. PCR is the best way to identify infections. Quantitative PCR is better than MAT or culture for quick and reliable results (Wood et al. 2019). This finding has important implications for medical and epidemiological analyses of this worldwide neglected disease. Leptospira is also detected using a variety of PCR techniques, including nested PCR, randomly primed PCR, etc. The drawbacks of PCR are similar to those of other diagnostic techniques and include complex lab requirements, degradation, false-positive results, initial validation, etc. These problems necessitate highly competent and trained personnel as well as a significant financial commitment (Hoorfar et al. 2004).

9.7. QUANTITATIVE PCR

PCR techniques detect Leptospira, but have limitations such as specialized equipment, sample degradation, and false positives. qPCR (real-time PCR) is preferred for accurate results in a short time by monitoring the DNA amplification rate. To evaluate disease severity, bacterial DNA amount and density must be assessed, often through targeting the LipL32 gene. qPCR is a reliable method for the early detection of Leptospirosis but is Challenging to implement in low-resource labs due to the required skilled personnel and expensive equipment (Ruijter et al. 2021).

9.8. IMMUNOCAPTURE-POLYMERASE CHAIN REACTION

New techniques have been developed to quickly identify leptospires from clinical samples, including the immunocapture-polymerase chain reaction methods created in 2018 using ELISA and PCR. A combination of molecular and serological methods is important for an accurate diagnosis of leptospires. The IC-PCR technique is a powerful tool that rapidly identifies leptospires and provides crucial information about specific serovars or serogroups present. This study shows it to be more accurate than traditional PCR methods (Jian et al. 2020).

9.9. OTHER MOLECULAR METHODS

Other molecular methods such as RAPD (random amplified polymorphic DNA), nucleic acid probe techniques, DNA-DNA hybridization, REA (restriction enzyme analysis), fingerprinting, PFGE (pulsed-field gel electrophoresis), etc., are available in highly developed laboratory settings. They support research into and comprehension of genetic diversity and genomic diversity profiles.
Several diagnostic techniques are available to detect and diagnose Leptospirosis, including ELA (IgM-Enzyme Immunoassay), MCAT (microcapsule agglutination test, LEPTO Dipstick, macroscopic SAT, IHA (indirect haemagglutination assay), and LEPTO Dri-dot. However, these rapid tests are less sensitive and specific than IgM rapid and immunochromatography techniques, despite the availability of several serological rapid tests.

CONCLUSION

Our ability to diagnose the cause of the disease is greatly improved by following all the stages consistently and sequentially. This method not only increases the scientific knowledge base but also helps zoo employees conduct retrospective studies and aids other researchers with the new database. It also enhances the ability of the zoological community to recognize disease trends and enables researchers to identify diseases that pose a threat to captive and wild animals.

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