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Advance Serological and Molecular Techniques for Diagnosis of Toxoplasmosis

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ABSTRACT

Toxoplasmosis is a zoonotic disease which is prevalent globally. The causative agent of it is *Toxoplasma gondii* which is an intracellular protozoan parasite. This parasite is important in both medical and veterinary fields. Main route of transmission of disease is eating undercooked meat that contains viable tissues or drinking water and food that has been contaminated by oocysts.

Keywords: Toxoplasmosis, Transmission, Diagnostic Tools

Introduction

Toxoplasma gondii is intracellular epicomplexan parasite that affects all warm blooded vertebrates including humans. Felids are definitive host for this parasite to transmit the disease by excreting oocycts. Cats that fed on cooked feed, can't play role in transmission of infection Transmission occurs through consumption of raw meat or ingesting contaminated food, soil and water or by accidently ingesting of oocyst by environment [1]. Person to person transmission is not possible except it can transmit from mother to child. This intracellular parasite is isolated from all regions globally except Antarctica [2]. It is present in both low income and developed countries. Pregnant women and immune-compromised groups are at greater risk. Fever, cough, diarrhea, dyspnea, icterus, abortion and still birth are common clinical signs in animals [3]. In humans fever, muscle aches, swollen lymph node especially around neck, abortion and still birth problem [4]. In past diagnosis was done by etiology, clinical signs and location of infection. Now, serological and molecular techniques are used to identify the infection.

Advances in Serological assays:

Mainly Immunological tests are used for *T.gondii* identification. Measurement of different antibodies (IgM, IgG, IgA, IgE) done, whose pattern of rise and fall change during infection [5]. In past, many immunological tests including Sabin Feldman test, IFAT, LAT, IHT, ELISA, Modified Agglutination Test (MAT), WB, and IgG Avidity test were all used to find out if someone had T. gondoii. Toxoplasmosis can be diagnosed through a variety of methods, however, the most widely employed in clinical laboratories is ELISA. Conventional indirect ELISA assays typically tested for crude tachyeite antigen, which was found to be highly sensitive and specific. [6].

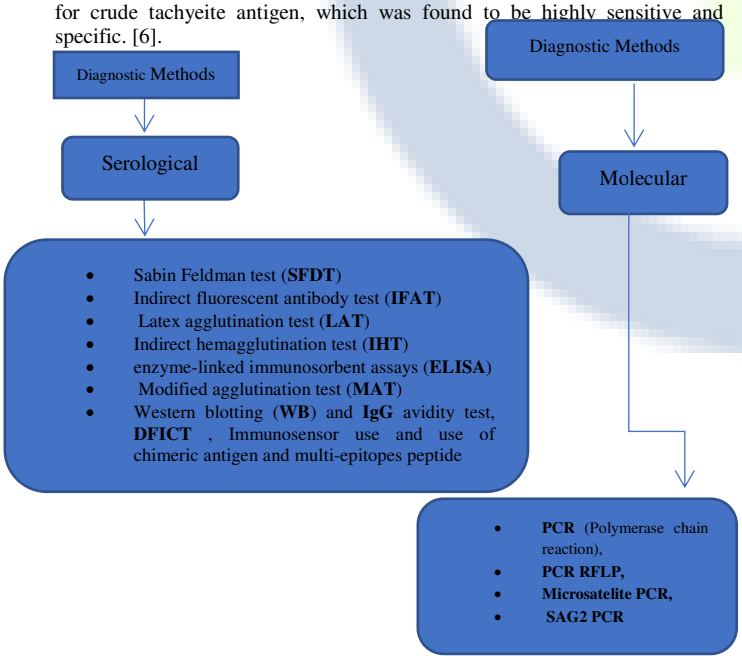

Fig.1: Diagnostic methods of Toxoplasmosis

Use of chimeric antigen and multi-epitopes peptide of *T gondii*:

Chimeric antigens, also known as synthetic peptides, are the most commonly used antigens in modern medicine. These antigens contain multiple immune reactive epitopes derived from distinct *T. gongii* antigens, which have been selectively selected. In recent years, novel methods have been developed to predict the presence and localization of specific epitopes on the antigenic surface of *T. gonadii* antigens. These epitopes, which are known to be hydrophobic, are often well-preserved on the surface of these antigens. The methods used for this analysis range from peptide-based microarray analysis with bioinformative methods to epitope-based mapping, cDNA library phage display, and antibody-reactivity. [7].

Dynamic flow immune-chromatographic test (DFICT):

The Dynamic Flow Immune-Chromatographic Test (DFICT) is a modified version of the Inter-systemic Catheterization Test (ICT) that utilizes immune-chromatography and fluid dynamics to detect Toxoplasma infections in cats and dogs. The detection of antibodies in the DFICT was demonstrated to be highly sensitive, even at 1:320 serum dilution, and did not cross-react with antibodies from other associated canine pathogens such as Distemper virus, Parvovirus, Coronavirus, Leishmania, Neospora Caninum, or feline pathogens such as Panleukopenia Virus, Calicivirus, or N. Caninum. Storage at 4 °C for several months did not alter the sensitivity or specificity of the DFICT. To test the sensitivity of the DFICT, a volume of 5 microliters of serum was added to the hole sample, and 100 microliters of liquid gold-SPA was added to the reagent hole. Visualization of the result can be completed in 5 minutes [8].

Immuno-sensor:

A biosensor is a diagnostic instrument that converts biological responses into quantifiable signals. It is composed of two main components: the bioreceptor, which detects the target, and the transducer, which converts this recognition into electrical signals. There are four types of biosensors, which are classified according to the signal transduction method used. The four types are electrochemical biosensors, optical biosensors, piezo-electrical biosensors, and thermal biosensors. For the detection of serum biomarkers, the most sought-after type is the chip-based immunosensor, which is available in a variety of sizes and shapes. These chips are available in portable and compact forms, as well as in self-contained forms for use in diagnostic laboratories [9].

Molecular test:

Molecular techniques can be employed to detect the presence of *Toxoplasma gondii* in biological samples. PCR technology can be employed to amplify a specific part of the genome, which can then be visualized by staining on agaroses and polyacrylades, or by laser detection on automated sequencers. Additionally, PCR techniques can be used to directly amplify the result of the staining, allowing for a more precise detection of the bacterium. The sensitivity of the PCR-based techniques is dependent on the appropriate method of isolating the genetic material from the samples, the characteristics of the DNA sequences to be amplified, and the parameters of the amplification reaction [10]. PCR can be used to differentiate between different strains of Toxoplasma gondii. There are several PCR-based methods that have been developed for genotyping and subtyping of T. gondii strains, including multilocus PCR-RFLP, microsatellite PCR, and SAG2 PCR. These methods can help identify the genetic diversity of T. gondii strains and provide valuable information for epidemiological and clinical studies.

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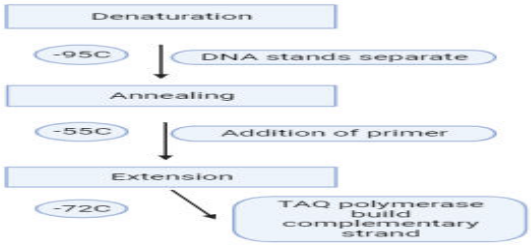

Fig.2: Steps of polymerase chain reaction

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