

Immunodiagnosis Tools for Parasitic Diseases

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Abstract

Regarding harmful agents in livestock, parasites play an important role moreover it also effect 1/4th of the human populace by various zoonotic diseases (e.g. Toxoplasma, Cryptosporidium, Trypanosomiasis, etc.). In livestock the parasites successful invade host immune responses, so early diagnosis of parasitic agents by various immunodiagnostic techniques is very important. Numerous immunological/serological techniques have been emerged such as the complement fixation test (CFT), immunodiffusion (ID), indirect haemagglutination (IHA), indirect immunofluorescent antibody test (IFA), various forms of enzyme-linked immunosorbent assay (ELISA) [Sandwich ELISA, indirect and direct ELISA, competitive ELISA, falcon assay screening test ELISA (FAST-ELISA), dot-ELISA, rapid antigen detection system (RDTs), and luciferase immune precipitation system (LIPS)] and radioimmunoassay (RIA). They targets different components of parasite, moreover they can detect disease before the emergence of clinical sign. These tests are used for many important parasitic diseases like *Echinococcus multilocularis*, *Wuchereria bancrofti*, *Taenia solium* and parasite causing Babesiosis, Toxoplasmosis, Visceral leishmaniasis, Human African Trypanosomiasis in both man and animals. Moreover now a day's nano and biosensor technology has also been used for improvising the diagnostic aspect. This current mini-review is an attempt to consolidate some information of different serological based test for early diagnosis of main parasitic disease.

Keywords: Parasite; Immunodiagnostic techniques; Immune system; Zoonotic disease

Introduction

According to World Health Organization (WHO) population of world will reach about 9.7 billion by 2050. So along with agricultural food, animal food is also required as alternate food resource. Health along with production of animals is affected by various parasitic diseases. It was estimated that only with a 6% reduction in animal disease could provide food for an additional 250 million people [1]. Parasite are divided mainly into different groups i.e. trematodes (flukes), cestode (flat worm), nematodes (round worm), arthropods and protozoan. Most of the parasites have a 2 host, prey predator life cycle, one act as intermediate host while other is a definitive host. All cause some of the most devastating and prevalent diseases in both humans and animals. So there timely detection is very important aspect to reduce the loss of livestock's health and production [1]. Simple microscopic detection has many limitations so immunological detection plays a vital role as it is based on antibodies detection. Numbers of immunological/serological techniques have been emerged such as the complement fixation test (CFT), immunodiffusion (ID), indirect haemagglutination (IHA), indirect immunofluorescent antibody test (IFA), various forms of enzyme-linked immunosorbent assay (ELISA) [Sandwich ELISA, indirect and direct ELISA, competitive ELISA, falcon assay screening test ELISA (FAST-ELISA), dot-ELISA, rapid antigen detection system (RDTs), and luciferase immunoprecipitation system (LIPS)] and radioimmunoassay (RIA). In RIA basic immunoassay principles were established some 30 years ago in studies on binding of radiolabelled insulin to antibodies to insulin (anti-insulin antibodies) [2]. Less frequently used tests include card agglutination, latex agglutination and capillary agglutination. Almost all of these tests are based on the reaction of antibodies with antigenic parasite components (whole or soluble) resulting in antigen-antibody complexes. The complexes thus formed are detected by the addition of antiglobulins coupled to fluorescein and rhodamine dyes, radioisotopes or enzymes.

Some tests which are routinely used for parasite detection

- Complement fixation test (CFT): Trypanosomiasis, Helminthiasis, Anaplasmosis, Babesiosis, Toxoplasmosis.

- Rapid card agglutination test (CAT): Anaplasmosis, Babesiosis, Trypanosomiasis.
- Indirect fluorescent antibody test (IFA): Theileriosis, Helminthiasis, Anaplasmosis, Besnoitiosis, Ehrlichiosis/Cowdriosis, Malaria, Babesiosis, Trypanosomiasis, Toxoplasmosis.
- Radioimmunoassay (RIA): Babesiosis.
- ELISA: Babesiosis, Besnoitiosis, Helminthiasis, Toxoplasmosis, Trypanosomiasis Anaplasmosis, Ehrlichiosis/Cowdriosis [3].

Different parasite targeted by immunodiagnosis techniques

***Echinococcus multilocularis*:** Cystic echinococcosis (CE) and Alveolar echinococcosis (AE) is a severe zoonotic disease caused by the larval stage (metacestode) of the helminth *Echinococcus multilocularis*. By affinity chromatography a native Em2 antigen (also termed Em2a) was purified originally from *E. multilocularis* metacestode tissue extracts [4]. By immunoprecipitation, western blot and ELISA it was demonstrated that it was an *E. multilocularis*-specific antigen with apparent mass of 54 kDa. Both *in vivo* and *in vitro* studies revealed that Em2 is a structural component found only in the metacestode laminated layer, and not in freshly hatched oncospheres, protoscolices or adult stages [5]. Moreover a glycosylated antigen Em2 (G11) has been found to be the major antigenic component of Em2 [5]. Walker et al. identified an Em492 in *E. multilocularis* metacestode [6]. Both

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the antigens are related immunologically because both share the galactose-a (1, 5)-galactose epitope. In protoscolices of *Echinococcus granulosus* homologous component of Em492, termed E4+, had also been identified. E4+ has been involved in the modulation of the cellular immune response in experimental mice infections by stimulating interleukin (IL)-10 secretion and subsequent induction of the type 2 cytokine response. Other *E. granulosus* antigens with well-characterised glycosylate moieties include the carcinoma associated Tn antigen, hydatid cyst fluid antigen 5 and the blood group P1 epitope [6,7]. However, to our knowledge, none of these components has been described in *E. multilocularis*.

In alveolar echinococcosis infection the test based on sero-diagnosis mainly are based on native and recombinant antigens of the *Echinococcus multilocularis* metacestode such as Em2, Em2plus, Em2plus ELISA, CH-10, N3C, pAP, Em70, Em90, rII/3, rII/3-10 and rEM10. From protoscolex a crude *E. multilocularis* antigen was extracted and a native EM2 protein was identified as a 70 kDa. Two new polypeptides of the *E. multilocularis* metacestode, referred as to Em70 and Em90, have also been identified as potential antigens for the serodiagnosis of AE. For the sero-diagnosis of AE now a day's test mainly based on native and recombinant antigens of the *Echinococcus multilocularis* protoscolex and/or adults such as rEM4, EM13, Em18,16, Em18, rEm18, rEm18-1, rEm18-2 are used [8]. For effective discrimination between AE and CE patients the first echinococcus component used was Em2. Now a day's at least one western blot kit (Echinococcus Western Blot IgG; LDBIO Diagnostics, Lyon, France) is available commercially for routine serological diagnosis and differentiation of *Echinococcus* spp. This test, based on the detection of specific IgG directed against *E. multilocularis* whole larval antigen (both Em16 and Em18 antigens) allows correct differentiation between AE and CE patients in 76% of cases, thereby achieving similar diagnostic sensitivities to those reported previously for the Em2plus ELISA assay.

Main antigen targeted for serological tests differentiation between alveolar and cystic echinococcosis are Em1+Em2, Em18+Ag B, 16, 18, 27b kD, 18 kD, Em18, EmII/3, rEM10+rEG55, rEm18 [8]. Main copro-antigen targeted for sero-testing characteristics for *Echinococcus multilocularis* infection in definitive hosts are Anti-E.g (*E. granulosus*). ESP Ad (excretory secretory product of adults), Anti-E.m (*Echinococcus multilocularis*). S. Ad. Anti-E.m. S (sensitivity). Ad. mAb (monoclonal antibodies) Em9, Anti-E.m. S. Ad, Chekit Echino test [8].

Wuchereria bancrofti: Bancroftian filariasis is an infectious disease produced in man by the filarial parasite, i.e., *Wuchereria bancrofti*. In bancroftian filariasis serum immunoglobulins IgE and IgG were found to be at elevated level compared to controls. By solid phase radioimmunoassay the specific IgE antibody levels were found to be lowered in chronic filariasis and ELISA using *Brugia malayi* and *W. bancrofti* antigens respectively. Homologous and Heterologous antigens are also been used for its effective immunodiagnosis. Research targeting the utility of *W. bancrofti* microfilarial antigens in gel diffusion (GD), indirect haemagglutination test (IHAT), counter immuno electrophoresis (CIE), indirect fluorescent antibody test (IFAT) and ELISA for detection of filarial antibody in filarial sera is going on. For the detection of antibody GD was found to be least sensitive but on the other hand ELISA was observed to be highly sensitive. Using soluble *W. bancrofti* microfilarial antigen, the efficiency of three tests (IHAT, IFAT and ELISA) was compared for the detection of antibody. Filarial antibody could be detected in 93%, 100%, 81% of the microfilaraemics, 75%, 90%, 100% of chronic pathology and none of the non-endemic sera by IHAT, IFAT and ELISA respectively.

Excretory-secretory (ES) antigens: By maintaining *W. bancrofti* microfilariae (mf) in medium 199 (3–4 thousand mf/ml of medium) supplemented with organic acids and sugars of Grace's medium but without serum, the *W. bancrofti* microfilarial (mf) excretory-secretory (ES) antigens were obtained. Utility of *W. bancrofti* ES antigen has also been explored in IHAT, Penicillinase ELISA and CNBr-Sepharose IFAT for detection of filarial antibody. By fractionation of ES antigens with the help of membrane filtration an ES4 antigen fraction (which was found to be a glycoprotein in nature and was highly reactive) microfilaraemia sera was obtained. *W. bancrofti* mf ES antigen specific IgE antibody was detected in filariasis and tropical eosinophilia by immunofluorescence assay and ELISA [8]. These studies showed the detection of ES antigen specific IgG+A+M antibodies. Circulating filarial antigen was concentrated from microfilaraemia plasma by salt precipitation and was identified as an antigen of microfilarial origin using anti rabbit mf sera in CIE [9]. Under field condition for the detection of monoclonal antibody of the circulating antigen of *W. bancrofti* adult worm ICT Filariasis, a rapid card test format was also evaluated [10]. Results concluded that it has high sensitivity, lack of cross-reactions, single reagent and rapidity of the test so, ICT can be recommended for screening of Bancroftian filariasis. In Egypt for lymphatic filariasis AMRAD-ICT-Fil was evaluated in 1813 endemic and 102 non endemic participants [10]. The infection rates detected were 9% by AMRAD-ICT-Fil, 8.8% by ELISA, 3.5% by membrane filtration and 2.8% by thick smear. In Sri Lanka the sensitivity, specificity and cost effectiveness of the same test card was estimated against thick blood film (TBF) and nuclepore membrane filtration (NMF). Results depicted that it is the most effective (both sensitive and specific) its cost is very high [10].

Babesiosis: To differentiate *B. equi*-infected horses from *B. caballi*-infected and uninfected horses Babesia Immuno Chromatic test (ICT) for the rapid detection of antibodies for *B. equi* was developed. Using a recombinant *B. caballi* 48 kDa rhostry protein (rBc48) and a recombinant truncated *B. equi* merozoite antigen 2 (rEMA-2t) for the simultaneous detection of *Babesia caballi*- and *B. equi*-specific antibodies (BceICT) was developed by same investigators in 2006 [10]. In dogs for the detection of antibodies to *B. gibsoni*, ICT was developed and evaluated with sera using recombinant truncated P50 (P50t). Kim and his co-workers developed two ICTs with nitrocellulose membrane based immunoassays for rapid serodiagnosis of bovine babesiosis caused by *B. bovis* (BoICT) and *B. bigemina* (BiICT) [10].

Toxoplasmosis: In cats and mice for detection of antibodies against *Toxoplasma gondii* ICT was applied using recombinant truncated surface antigen- 2. Under field condition this test is simple, rapid, accurate and relatively inexpensive [9,10].

Visceral leishmaniasis: For visceral leishmaniasis (VL) under field conditions an ICT targeting leishmanial antigen K39 by antigen-impregnated nitrocellulose paper strips was adapted [10]. On strip a drop of serum and/or peripheral blood is applied followed by buffer resulted the formation of two visible bands indicating the presence of anti-k39 IgG. Moreover it was reported that ICT K26 was equally specific but less sensitive than ICT K39. These two antigens increased the overall sensitivity of the test by complementing each other [10]. In India, Bangladesh and Brazil the usefulness of ICT rK39 was assessed in diagnosis of VL. Moreover in post Kala-azar dermal leishmaniasis (PKDL) sera ICTrk39 was also evaluated. For PKDL the strip test depicted 91% sensitivity and 100% specificity [10]. In India anti-K39 ICT (IT-Leish; DiaMed AG) was proved to be highly sensitive and specific for the sero-testing of VL. Later on it was concluded that ICT strip test is easy, quick, requires no technical facilities with higher

sensitivity and specificity entailing it to be the ideal test for the diagnosis of kala-azar in field level [10].

Regarding some other important haemo-protozoan disease ELISA has been used as superior test in South American trypanosomiasis filariasis and schistosomiasis [11-13]. ELISA is not yet as specific or sensitive as immunofluorescent methods in malarial antibody tests probably because the antigen preparations are still crude. In some other parasitic disease it was seen that purification of antigens remains an obstacle for serodiagnosis in all these conditions but is rapidly progressing; for instance, the purification of schistosoma-egg antigen should further increase the test's specificity and sensitivity in this disease [11,14]. Evaluation of the ELISA enzyme-linked procedure is not yet complete in some other protozoal infections, but current methods such as counter electrophoresis in occult amoebic abscess or immunofluorescent methods in giardiasis yield acceptable results [15,16].

Human African trypanosomiasis: In region like sub-Saharan Africa a vector-borne parasitic disease Human African Trypanosomiasis (HAT), or sleeping sickness, is endemic [17]. As we all know this disease is caused by an extracellular parasite called *Trypanosoma* (genus). Many serological tests has been used for its diagnosis like-

- a. CATT (Card-Agglutination Trypanosomiasis Test)- CATT is an antigen-antibody based test, useful for initial population screening to identify suspected cases. The test was developed in the late 1970s. It can be carried out on blood, capillary blood obtained from a finger prick, or blood from impregnated filter papers [18]. Antigen used for the test CATT is complete bloodstream forms of *Trypanosoma bruce gambiense* variable antigen type LiTat 1.3 and for *T. evansi* it is RoTat 1.2. The sensitivity of CATT on blood is about 91%, with a range of 78–99.8%, and negative predictive values as high as 99-100% has been reported in mass population screening [19,20].
- b. Cytokines and Chemokines- The level of cytokines and chemokines has been measured for the investigation of their diagnostic potential both in *T. b. gambiense* and *T. b. rhodesiense*. The most interesting cytokines and chemokines used for staging sleeping sickness are IL-10, IL-6, IL-1 β , CCL-3, CXCL-8, SLPI, Lipocalin 2, ICAM-1, VCAM, MMP-9, MMP-2, CXCL-10 and CXCL-13. A recent study initially evaluated the most promising molecules such as CXL-10, CXCL-13, ICAM- 1, VCAM-1, IgM, MMP-9 and B2MG and confirmed their capacity to act as accurate staging markers [21-23].
- c. Proteomics- Another approach currently under investigation is the evaluation of the changes in protein expression between pathological and healthy conditions. Only a few studies have established first and second stage HAT disease CSF protein profiles. Two of these proteins, osteopontin and beta-2-microglobulin, were confirmed to be accurate markers of first and second stage patients [24]. It is important to research and study new protein biomarkers, particularly for discriminating stage 2 and stage 1 of the disease, and this is possible thanks to progress in matters of protein and peptide analysis with the evolution of mass spectrometry [25].
- d. Polysomnography- In recent years, research has been conducted on the most typical clinical manifestation of HAT: the alteration of the normal sleep-wake cycle [26]. Studies show a high number of Sleep Onset Rapid Eye Movement Periods (SOREMP) in stage 2 patients during their sleep, not only restricted to nighttime, but

also during daytime sleep too. Treatment with Melarsoprol seems to reduce the appearance of SOREMPs.

Regarding chagas disease the immunoblot assays have been also conducted; among the various immunological techniques the most important is TESAblot that consists of detection of antibodies against the antigens TESA (trypomastigote excreted-secreted antigen). This assay is currently commercialized and has been widely used because of its high sensitivity and specificity and ability to resolve doubtful serology and cross-antigenicity issues with related protozoan parasites in regions where the disease is endemic [27-31]. The TESA antigen assay also has been used for other tests like ELISA [32].

Neurocysticercosis (NCC): Immunodiagnosis of NCC can be done by two ways: by identifying antibodies against cysticercal antigens or by identifying parasite's antigens directly. The source of antigens for immunodiagnosis has been commonly from *Taenia solium* (the parasite responsible for NCC) but related species as *Taenia crassiceps*, *Taenia saginata* or *Taenia taeniformis* have also been used as antigen sources. Now a days multiple antigens have been used and among them, main are low molecular mass (LMM) antigens, excretory/secretory (ES), crude soluble extract (CSE), total saline extract, antigen B, lentil lectin glycoproteins (LLGPs), vesicular fluid (VF), membrane and scolex extracts, somatic antigens, recombinant proteins, and synthetic peptides. Multiple methods used to date for the immunodiagnosis of NCC like complement fixation, agglutination, radio immune assays, ELISA and Western Blot (WB) can be counted [33]. LLGP-WB has a sensitivity of >90% and a specificity of 100%. In Indian patients almost two thirds of the NCC patients have an SCG; LLGPs have shown to be less sensitive than for multiple cysticerci. Antigen for immunodiagnosis from the members of the 8 kDa family, Ts18var1 has been produced in insect cells as well as TsRs1, Ts18var1, and Ts18 Var3; the 14 and 18 kDa proteins produced by recombination; Ts14, Ts18var1, TSRS1 and TSRS2var1 by chemical synthesis, and full-length Ts18 and Ts14 by chemical ligation, Ag1V1/Ag2 by recombination as well as Ts8B1, Ts8B2, Ts8B3, Ts14 and a 10 kDa protein, GP50, which is not a member of the 8 kDa family but it is part of the LLGPs, was produced by recombination in bacteria and in a baculovirus expression system [33-39]. Outside from LLGPs other proteins that have also been produced or synthesized include T24 (integral membrane protein that does not bind to lentil lectin) produced in a drosophila cell line; HP6-Tsag (oncospherical adhesion protein of *Taenia saginata*) in bacteria and baculovirus systems with similar specificities between the systems (93–95%), but higher sensitivity for the inactive cases by the baculovirus protein (48–64%); peptide NC-1 selected by phage-display; peptides KETc12, 410 and 413 synthesized from a cDNA library of *T. crassiceps* and recombinant TS24 and Es33. The methods of production are varied, as well as the results and the ways to evaluate the produced protein, some giving very good sensitivities but in other cases, the native protein is much better than the produced one [33].

Use of nanotechnology for the effective diagnosis of parasitic disease: Nanotechnology exactly means any technology performed on a nano-scale that has practical applications to our every-day occurring daily activities. This can offer an improvement in imaging and diagnosis of the fatal parasitic diseases as well, slipping away the limitations of some biological barriers. In the parasitology field the use nanotech have only a few applications and the research have been reported on *Leishmania* sp. and *Plasmodium* sp. [40,41]. Nano-particles have also shown improvement in the bioavailability and drug selectivity, even in very complicated treatments like cerebral malaria by *Plasmodium falciparum* infection [41]. Some nano-partial like Pegylated or polyplex offer fantastic options to increase the biodistribution of a

compound. In veterinary medicine it may also help in mass treatments of ectoparasites, facilitating the drug-parasite interaction. Moreover the effectiveness of phytotherapeutic products can be improved, like the recent treatment of antiparasitic silver nano-particles using *Cissus quadrangularis* against *Hippobosca aculata* and *Rhipicephalus (Boophilus) microplus* [42]. Nano-devices also help veterinarians to suggest a correct health management [43]. In Cuba and Brazil some project are currently running using nano-peptides as a preliminary test against ruminant helminths (*Haemonchus contortus* and *Fasciola hepatica*). This technology can also allow the development of adjuvants for veterinary parasite vaccines, boosting their present low efficacy. Newly, a nano-microparticulated malaria vaccine elicited long-lasting protective antibody titers with only a single dose [44].

Biosensor: A biosensor is an analytical device that converts molecular recognition of a target analyte into a measurable signal via a transducer. Common to all such instruments is a support material, on which one of two affinity-pairing partners (the recognition element) is immobilized. The partners may be an enzyme and its substrate, an antibody/antigen pair, a receptor and its specific ligand, or even living cells and an analyte that binds specifically to them. Bioreceptor types used in biosensors are as follows: Nucleic acid, Enzyme, Whole-cell, Antibody and receptor, Micro and nano and biomimetic [45,46].

For parasitic diseases, biosensors offer the possibility of an easy-to-use, sensitive and inexpensive technology platform that can identify parasite rapidly and predict effective treatment [47,48]. It has many advantages which include small fluid volume manipulation (less reagent and lower cost), short assay time, low energy consumption, high portability, high through put and multiplexing ability [49,50]. For the detection of malaria various biomarkers have been used among them the plasmodial lactate dehydrogenase and histidine-rich protein II (HRP II) has received increasing attention [51]. The widely used rapid detection tests (RDTs) for malaria succumb to many drawbacks which promotes exploration of more efficient economical detection techniques. A low-cost biosensor system was also made with nanostructured films containing specific *Leishmania amazonensis* and *T. cruzi* antigens and employing impedance spectroscopy as the detection method [52,53].

Conclusion

Concise information provided in this mini-review regarding the progress in the field of diagnostic test in various parasitic diseases of livestock and humans may be a guideline for veterinarian clinician and academician.

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