



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(2): 268-271
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www.thepharmajournal.com
Received: 20-12-2017
Accepted: 21-01-2018

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Hemo-protozoan diseases of livestock: Present challenges in diagnostics, therapeutics and vector control

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Abstract

Blood protozoan diseases have global distribution stretching from the polar circle to equator as their arthropod vectors like ticks and flies have global distribution. The clinical manifestations of the disease vary from anorexia, fever, anemia, ketosis, threatened abortions to death in acute form and carrier stages in chronic form. The conventional diagnostic methods for most of the infections relying on microscopical demonstrations of infective stages in blood or tissue is time taking and detects at chronic stage of disease. The most popular serological tests for detection of antibodies against blood protozoan in both animal and humans are immunofluorescence, ELISA, sib variants and Western Blot but the sensitivity and specificity for detecting specific antibody is the cell culture technique. The long standing use of therapeutic agents against blood protozoan diseases along with their indiscriminate use have developed resistance. Successful control strategies depend on understanding of parasite developmental cycle, biology of tick vectors, immune response of cattle to tick and parasites as well as fulfilling the gap in diagnosis, treatment and prevention to save the life of livestock and huge global economic loss from livestock sector.

Keywords: anaplasmosis, babesiosis, theileriosis, trypanosomosis

Introduction

Blood protozoan diseases have global distribution stretching from the polar circle to equator as their arthropod vectors like ticks and flies have global distribution ^[1, 2]. These have great economic impact on livestock affecting health, productivity, draft power along with food security affecting 80% of world cattle population. The important blood protozoan diseases of livestock are Theileriosis, Trypanosomiasis, Babesiosis and Anaplasmosis ^[3-5]. The clinical manifestations of the disease vary from anorexia, fever, anemia, ketosis, threatened abortions & death in acute form to carrier stages in chronic form. These disease are also have zoonotic importance. The recovered animals remain in a carrier stage for prolonged period and act as an epicenter for infections of other animals.

The conventional diagnostic methods for most of the infections relying on microscopical demonstrations of infective stages in blood or tissue is cumbersome and unable to detect at acute phase of infection. Indirect antibody based serological diagnostic tests though accepted globally has disadvantages due to non availability of non-cross reactive tests. These are also having cross reactivity, low specificity and sensitivity reactions ^[6]. The most popular serological tests for detection of antibodies against blood protozoan in both animal and humans are immunofluorescence, ELISA, sib variants and Western Blot but they do not have 100% sensitivity and 100% specificity. For detecting specific antibody against blood protozoan is the cell culture technique for isolating infective organisms is laborious, lengthy and is prone to failure with specimens from non sterile sites. In the present scenario molecular diagnostic techniques like Polymerase Chain Reaction (PCR), Loop Mediated Isothermal Amplification LAMP are used ^[7]. However the costs of these tests are so high that farmers are unable to bear. Nucleic acid based technique is most advanced to detect latent forms of infection and to bring high success to chemotherapeutic interventions ^[8].

The long standing use of therapeutic agents against blood protozoan diseases along with their indiscriminate use have developed resistance. Frequent use of those drugs has detrimental effect on bone marrow, liver and other vital organs of the host. There is lack of research globally to bring new molecules time to time.

Control of arthropod vectors mostly done through application of acaricides through dipping, spray, pour-on and hand dressing but these are highly toxic,

expensive, provides a soft span of protection, ticks easily develop resistance as well as detrimental to environment ^[9]. Anti-tick vaccines for animals using whole somatic antigen of ticks and proteins of salivary gland is need of the hour ^[10].

Control of infection basically depends on identification of carrier animals and vaccination of animals against specific blood protozoa. The vaccines are not available against all species as well as unable to protect against persistent infection so its use do not bring 100% protection ^[11].

The non availability of easier and accurate diagnostic technique both in latent and acute phase of infection, newer and safe therapeutic agents, vaccines against all species of blood protozoa and arthropod vectors responsible for transmission and detection of carrier animals are global challenges for control of blood protozoan diseases in livestock.

Theileriosis

Bovine tropical Theileriosis is caused by *Theileria annulata*, an intracellular protozoan parasite transmitted by Ixodid ticks of species *Hyalomma anatolicum anatolicum*, *H. marginatum* and *H. excavatum*. It is a lymph proliferative disease with high morbidity and mortality in cattle. The other species like *Theileria parva*/ *mutans*/ *sergenti* / *velifera*/ *buffeli*/ *orientalis*/ *tarurotragi* are of less important as they cause mild form of infection.

Sub-clinical infection in cattle with *T. annulata* produces chronic carrier animals which act as epicenter of infection for ticks and those are important for epidemiological aspect ^[12].

In India about 10 million cattle are at risk for tropical theileriosis with annual economic loss of USD 800 million ^[13] and it imposes about USD 384.3 million annual loss to Indian livestock sector ^[14].

The affected cattle shows clinical signs like fever, anorexia, enlargement of lymph nodes preferably prescapular lymph nodes. Leukopenia, lymphocytosis, diarrhoea, marked anemia, conjunctival petechiation, cough, abortion, respiratory distress and death ^[15-16].

Diagnosis can be made by clinical signs, detection of piroplasm in RBC and schizonts in Lymph node smear during acute stage but this gives false negative results in most of the cases. In sub-clinical and chronic cases microscopical examinations fails to detect the protozoa. The serological test like ELISA (Enzyme Linked Immune Sorbent Assay) using recombinant proteins of *T. annulata* Surface Protein (TaSP) can detect antibodies in infected animals and this did not show any cross reaction in lateral flow devices ^[17]. PCR particularly Nested and Real Time PCR are more sensitive and specific than other tests for determining piroplasm-carrier animals ^[18-19]. RLB micro assay is a recently developed technique that uses oligonucleotide probe to detect and identify *Theileria* and *Babesia* simultaneously by specifically amplifying rRNA gene of V4 hyper variable region ^[20].

The successful treatment comes through deep intramuscular injection of Buparvaquone (2.5 mg/kg bwt) without any side effects against *T. annulata*. Halofuginone a coccidiostat orally can treat clinical *T. annulata* & *T. parva*. Chemotherapy gives protection for a short period and develops carrier states in their host ^[21]. Oxytetracycline @ 20mg/ kg bwt has therapeutic effect only at the time of infection and not in clinical cases. The newer chemotherapeutic agents providing long term protection is need of the hour.

Vaccination with *T. annulata* schizont tissue culture vaccine can protect for three years against the same species and with

sporozoite *T. parva* vaccines with long acting Tetracycline can give lifetime immunity.

Control of ticks through acaricidal drugs is ineffective. Dipping, spraying, pour-on etc cannot able to control rather ticks develop resistance and there occurs environmental and food contamination ^[9]. An anti-tick vaccination using whole somatic antigen of tick and protein of salivary gland is need of the hour ^[10].

Trypanosomosis

Trypanosomosis is commonly known as SURRA is a most important blood protozoan disease of livestock having zoonotic importance. It is caused by *T. evansi* in bovines and clinically characterized by fever, anorexia, circular movement, maniacal behavior, profuse lacrimation with eye ball discoloration and death. Hypoglycemia is a major biochemical alteration. It causes multi organ dysfunction in affected animals so called surra.

There is a constraint in diagnosis due to lack of diagnostic techniques with high sensitivity and specificity. The microscopic detection and animal inoculation techniques requires parasitemia at a level of 1,00,000 parasites /ml of wet blood film. It gives false negative result many a time. The mni-anion-exchange centrifugation technique detect at low parasitemia @ 50 parasites/ml ^[22]. The Indirect Fluorescent Antibody Test (IFAT) is of high specificity and sensitivity for detecting antibody. A monoclonal antibody based latex agglutination test is more effective in diagnosis at field condition as well as very simple and cost effective ^[23]. The other tests like Nested and Real Time PCR, LAMP, nucleic Acid Sequence based amplification (NASBA) have recently developed for diagnosis.

The common chemotherapeutic agents used for treatment are isometamidium chloride @ 0.5 mg/kg bdwt and @ 1 mg/kg bdwt intra muscularly as curative and preventive purpose respectively. Diminazine acetate @ 3.5-7 mg/kg bwt intra muscularly as curative purpose. Quinopyramin Sulphate @ 5 mg/kg bwt subcutaneously for curative purpose and a combination of Quinopyramine Sulphate & Chloride @ 7.5 mg/kg bwt subcutaneously as preventive purpose which can give protection for 4-6 months. The latest drug Melarsomin dihydrochloride (Cymerlasan) as trypanoscidal drug used @ 0.25, 0.25-0.5, 0.5 and 0.75 mg/kg bdwt in camel, horse, cattle and buffaloes respectively through deep intramuscular route.

Prevention is difficult as development of vaccine cannot be done due to variation of glycoprotein between and within trypanosome species which is a major stumbling block. The world wide research is looking after development of vaccine after identifying non variant regions such as PFR protein of kinoplastid flagellum ^[24]. The newer area of research is to develop a live attenuated vaccine using ionizing radiation may be feasible. Tick control is also a major problem as they are frequently developing resistance against acaricides.

Babesiosis

Bovine babesiosis commonly known as Red Water fever, Tick fever or Piroplasmosis is prevalent worldwide as because the vector tick *Boophilus microplus* is wide spread in both tropics and sub tropics and concerned to be an economic important disease of Cattle, Horse and Dog. It is caused by *Babesia bigemina*, *B. bovis* in cattle and clinically characterized as fever, hemolytic anemia followed by hemoglobinuria, jaundice, icterus, abortions, increased rate

and depth of respiration, anorexia and death. It is of zoonotic importance.

The infection reaches peak in summer depending on vector population. The infective stage of babesia sporozoite enters into blood stream when tick bites which develop into piroplasm inside RBC then causes its lysis & infects other RBCs. It causes intravascular and extravascular hemolysis with circulatory disturbance. It has both acute and persistent subclinical disease in cattle. *B. bovis* causes adhering of erythrocytes to capillaries of brain causing neurological signs like nystagmus, circling movement, hyperesthesia, head pressing, convulsions, ataxia, teeth grinding and muscular tremors [10].

It can be diagnosed through the microscopic examination detecting intra erythrocytic babesia parasites only in acute cases and not in sub clinical infections [25] most of the times it gives false negative results after stained with giemsa stain. It can be diagnosed from brain smear collected from grey matter of cerebral cortex by detecting brain capillaries filled with infected erythrocytes [26]. Diagnosis through microscopic examination is a gold standard test but it has poor sensitivity. Serological tests like Indirect Fluorescent Antibody Technique (IFAT) has better sensitivity but cross reaction among different genus and species is its drawback. Other molecular diagnostic techniques like PCR have more sensitivity and specificity as it diagnoses at very early stage of infection. Detection of Nucleic acid is also having high sensitivity and specificity for disease diagnosis [25]. In recent years the 18s rDNA gene encoding rRNA of small ribosomal unit of Babesia is frequently used as molecular marker for diagnosis of Babesiosis. The determination of species, sub species & genotype is most important for treatment and control.

Indigenous cattle living long time with local ticks & tick borne diseases have developed innate resistance or innate ability to develop good immune response for protection. There is an inverse age resistance in cattle which may be from passive immunity through colostrums [10].

Early diagnosis and prompt treatment gives high success rate. Imidocarb @ 1.2 mg/kg bwt subcutaneously as therapeutic purpose and 3 mg/kg bwt as prophylactic purpose gives protection for 4-8 weeks and also it eliminates carriers. Diminazine aceturate @ 3.5 mg/kg bwt intra muscularly gives good results and protects for 2-4 weeks. Newer drugs like Quinuronium sulphate & Amicarbalideiso-thionate has better efficacy. The ancillary treatments as per clinical signs and condition of the animals are very important. Prevention and control comes through immunization. Chemoprophylaxis and vector control.

Anaplasmosis

Bovine anaplasmosis is also known as gall sickness is a rickettsial disease caused by *Anaplasma marginale* and *A. centrale*. It is transmitted by numerous species of ticks, biting flies and blood contaminated fomites so occurs globally. Females are more susceptible than males due to hormonal disturbances and production stress [27]. Recovered animals from primary attack remain as lifelong carrier [28]. The wild cervids are important reservoirs. It is clinically characterized by fever, anorexia, dullness, rapid deterioration of body condition, muscle tremor, labored breathing and death.

Diagnosis can be made from clinical signs and detection of *Anaplasma* inclusions in Giemsa stained RBC. The ELISA and Card Agglutination Tests mostly used for detection of

Rickettsia in Low Parasitized Carrier Animals. But they have low specificity. Nucleic Acid based diagnostic methods have high sensitivity and specificity.

It can be treated with Tetracycline @ 8-10 mg/kg bwt intramuscularly thrice a day during early stage of infection. Long acting Tetracycline @ 20 mg/kg bwt intramuscularly two injections at 3 day interval eliminates chronic infection. Imidocarb @ 1.2 mg/kg bwt is also effective. Symptomatic treatment as per condition of patient like blood transfusions, lyophilized liver extracts etc are required.

Both live and killed vaccines against Anaplasmosis are available which gives protective immunity and fails to protect cattle persistently infected with *A. marginale*. Iatrogenic transmission should be prevented. Eradication of Anaplasmosis is not practicable due to wide range of carrier insects, long period of infectivity in carrier animals, wild reservoir and stress. It brings high economic loss to dairy farmers.

Conclusion

Blood protozoan diseases are the major bottleneck of Livestock development in tropics particularly in developing countries. These bring huge economic losses and impairs export and import trade of live animals and animal products. Control of vectors such as tick is very difficult as they develop resistance against acaricides and vaccines against them is not available. The wild animals remain as a reservoir hosts. The affected animals after recovery remain as carrier. Tests which have high sensitivity and specificity without cross reaction among the species for detection of protozoa in both carrier and affected animals is not available. Chemotherapeutic agents for complete elimination of organisms with long term protection is not available. Vaccines against all species of protozoa giving solid immunity is not available. Successful control strategies depend on understanding of parasite developmental cycle, biology of tick vectors, immune response of cattle to tick and parasites as well as fulfilling the gap in diagnosis, treatment and prevention to save the life of livestock and huge global economic loss from livestock sector.

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