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Original Research

Development of Lateral Flow Assay for Point-of-Care Diagnosis of Trypanosomosis in Equines

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ABSTRACT

Trypanosomosis, commonly known as "Surra" in Indian subcontinent caused by *Trypanosoma evansi*, is a worldwide parasitic disease of wild and domestic animals. The disease is transmitted mechanically by biting and interrupted feeding of infected animals by hematophagous flies. The diagnostic methods available for this disease require high-end instruments and skilled manpower and are time-consuming. In the present investigation, we attempted an immunoassay based on gold nanoparticles for diagnosis of *T. evansi* infection in equines. Antibodies to horse IgG were raised in rabbits and conjugated to gold nanoparticles after purification with the protein-A column. The soluble whole cell lysate antigen was prepared from purified *T. evansi* parasites and coated as test line on nitrocellulose membrane laminates, along with control line to detect anti-*Trypanosoma* antibodies. The experimental and field serum samples were tested and evaluated to determine the sensitivity and specificity of the assay. The antigen-specific antibodies were detected in experimental serum samples between 10 and 21 days post infection by the lateral flow assay (LFA). The LFA results showed 96.3% sensitivity and 93.9% specificity comparable with enzyme-linked immunosorbent assay. The study suggests that the LFA can be used as a point-of-care diagnostic method in the field, which does not require any specialized instrument for the detection of *T. evansi* infection in animals.

1. Introduction

Trypanosomosis is a widely distributed parasitic disease of domestic as well as wild animals and has significant economic importance. The disease caused by a protozoan parasite, belonging to genus *Trypanosoma* spp., has a worldwide distribution in different species of animals. In India, *Trypanosoma evansi* is responsible for the disease called "Surra" in a number of domestic animals such as horses, camels, donkeys, mules, cattle, buffaloes, dogs, pigs, sheep, and goats [1]. *T. evansi* infections are generally found in animals, but some reports suggested its ability to jump the species barrier and infect humans as well [2]. It is transmitted mechanically by the bite of hematophagous flies viz. species of *Tabanus* and *Stomoxys*. Interrupted feeding behavior of *Tabanus* fly has played a significant role in the transmission of the disease.

The diagnosis of trypanosomosis is being carried out by various methods including wet blood film examination, stained thick and thin blood smear examination, animal inoculation, biochemical tests, enzyme-linked immunosorbent assay (ELISA), card agglutination test, latex agglutination test, DNA-based techniques (PCR), etc. [3]. Although these detection methods help in the accurate and sensitive diagnosis of trypanosomosis in animals, these detection methods require skilled manpower and high-end instruments and are time-consuming. Early detection of the disease is necessary to reduce the mortality in animals and economic losses. Therefore,

Animal welfare/ethical statement: The animal experimentation was carried out according to rules and regulations set forth by the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA) Animal Welfare Division, Ministry of Environment, and Government of India. The research protocol for experimentation was duly approved by the "Institute Animal Ethics Committee" of the National Research Center on Equines, Hisar, Haryana, India.

Conflict of interest statement: All authors declare that they have no conflict of interest with the contents of this article in any respect.

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there is a need for a simple, fast, reliable, sensitive, and point-of-care test to detect the disease at an earlier stage for the prompt treatment.

In last few years, immunochromatographic or lateral flow assays (LFAs) based on gold nanoparticles (NPs) have been developed against several pathogens, which played an important role in fast and accurate detection of various diseases and microorganisms, e.g., *Plasmodium falciparum* [4], *Staphylococcus aureus* [5], and *Cryptosporidium parvum* [6], detection of Leptospira in urine [7], rapid detection of *Salmonella typhi* in human serum [8], and rapid screening of atrazine (one of the most widely used herbicides) in water [9]. Such LFAs are being widely used in clinical practices as these provide short analysis time and simple detection procedure without the need of skilled personnel and expensive equipment. In the present investigation, we have developed the LFA for detection of trypanosomosis in equines using reference serum samples and also validated test from field seropositive samples.

2. Materials and Methods

2.1. Materials

Nitrocellulose membrane laminates, along with conjugate pads, sample pads, and absorbent pads, were obtained from Advanced Micro Devices (Ambala, India). Gold tetrachloride and sodium citrate were purchased from Sisco Research Laboratories, India. Bovine serum albumin (BSA) and IgG purification kit were purchased from HiMedia, India. Protein-A was purchased from Sigma-Aldrich, India.

2.2. Test Samples

The serum samples of six ponies (previously infected experimentally with *T. evansi*) were used as reference positive for preliminary evaluation, whereas sera of two healthy ponies were used as uninfected controls [10]. The serum samples of these ponies were collected at different time intervals (0, 3, 5, 7, 10, 14 DPI [days post infection] and thereafter weekly up to 56 days) post infection after the onset of the experiment along with uninfected controls and stored at −40°C. Apart from the experimentally infected samples, a total of 88 equine serum samples collected from the field during 2013–2015 pretested by antibody ELISA (n = 55 seropositive & n = 33 seronegative) were taken from the repository of Parasitology Laboratory, National Research Center on Equines (NRCE), for evaluation of sensitivity and specificity of the assay.

Cross-reactivity studies were also performed with positive serum samples of *Theileria equi*, equine herpes virus 1 (EHV-1), equine influenza, and *Burkholderia mallei* in LFAs against *T. evansi* antigen. The 10 serum samples each, positive against these pathogens, but negative for *T. evansi*, were selected from the repository of disease investigation laboratory of NRCE and subjected to LFAs.

2.3. Antigen Preparation

The horse isolate of *T. evansi* (isolated from the organized equine farm of NRCE in Hisar, Haryana) was maintained in vivo in Swiss albino mice. At peak parasitemia, parasites were purified from mice blood by DEAE-cellulose chromatography, followed by centrifugation and two washings of parasites with PBS (pH 7.2). The whole cell lysate (WCL) antigen was prepared by the ultrasonication method [11] with some modifications. The protein concentration of the antigen was determined by Bradford method [12], and antigen thus prepared was stored at −40°C until further use.

2.4. Raising and Purification of Polyclonal Antibodies in Rabbits

The polyclonal antibodies (anti-horse immunoglobulins) were raised in New Zealand white rabbits according to the method of

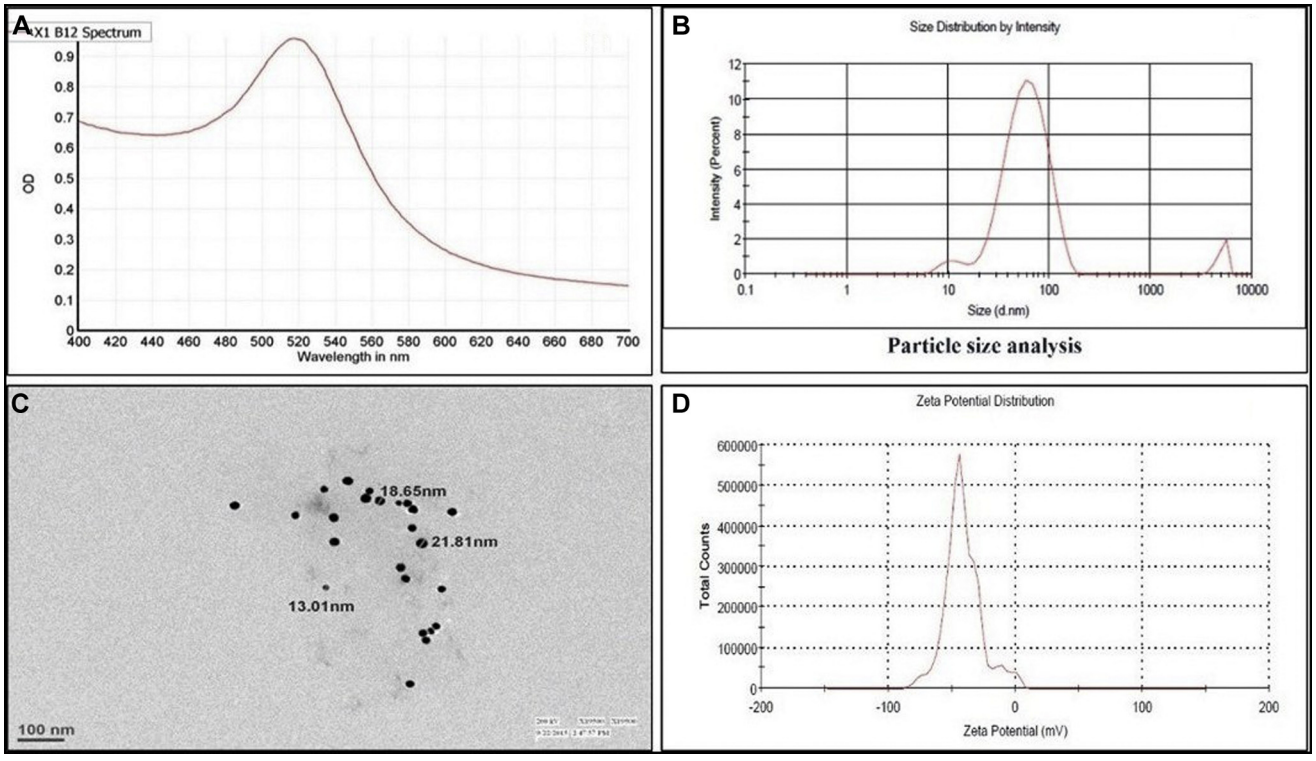

Fig. 1. Characterization of gold nanoparticles: (A) UV-visible spectrum, (B) particle size analysis, (C) transmission electron micrograph, and (D) zeta potential. OD, optical density.