Use of the Miniature Anion Exchange Centrifugation Technique to Isolate *Trypanosoma evansi* from Goats

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ABSTRACT: DEAE (anion exchanger diethylaminoethyl)-cellulose and mini Anion Exchange Centrifugation Technique (mAECT) allow salivarian trypanosomes to be separated from the blood of affected animals. The purpose of this study was to assess the mAECT in goats infected with *T. evansi*. Five adult Canary goats were inoculated intravenously with at least 1×10^5 *T. evansi* isolated from a dromedary camel in the Canary Islands. The goats were monitored for specific antibodies and parasite detection. The inoculated goats became infected and the parasitemia remained very low but was persistent. For mAECT columns, the DEAE gel was equilibrated with phosphate-buffered saline glucose. *T. evansi* was detected by its mobility with a microscope at low magnification (10 × 10). The mAECT proved to be more sensitive than blood smear and buffy coat but less sensitive than mouse inoculation. We conclude that in cases of very low parasitemia in goats, mAECT can be used when other parasite-detection tests have failed.

KEYWORDS: *Trypanosoma evansi*; goat; experimental; miniature anion exchange centrifugation technique (mAECT)

INTRODUCTION

Salivarian trypanosomes can be separated from blood cells and platelets by passing blood from infected mammals through a column with the anion exchanger diethylaminoethyl cellulose (DEAE-cellulose).[1] The separation depends fundamentally on differences in surface charge; the DEAE-cellulose adsorbs the more negatively charged blood components while the less negatively charged flagellates are eluted.[2] An elaboration of the DEAE-cellulose technique, the miniature anion exchange centrifugation technique (mAECT), has been developed for use in humans in the field by Lumsden *et al*.[3] The mAECT allows for the elution of trypanosomes from venous blood and for their concentration at the bottom of a sealed glass tube by low-speed centrifugation (3000 rpm). The trypanosomes may then be detected via low-magni-

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fication microscopy. Given that surface charges differ between species of salivarian trypanosomes, and that the negative charge on erythrocytes also varies with mammalian species,[4] the technique should be adapted to the species of salivarian trypanosome and to the mammalian host. With regard to goats, mAECT has been used previously to isolate *Trypanosoma vivax*, *T. congolense*, and *T. brucei brucei*.[5] The purpose of this study was to assess mAECT in goats infected with *T. evansi*.

MATERIALS AND METHODS

Animals

Five adult female Canary goats were selected for this study. The animals were purchased from a commercial dairy goat farm. The animals, which lacked any antecedents of trypanosomosis, had been recently vaccinated against *Salmonella* spp. and *Chlamydia* spp. and administered ivermectin against external and internal parasites. The animals were housed in an inoculation area of the University of Las Palmas. The animals tested negative in parasitological and serological tests for *T. evansi*. The goats were inoculated intravenously with at least 1×10^5 *T. evansi* isolated from a dromedary camel in the Canaries (Gran Canaria island). The animals were maintained for 8 months. They were examined daily for any clinical evidence of the disease, and they were monitored monthly for specific antibodies and parasite detection.

Parasite Detection Tests

From each animal, blood samples were taken monthly from the jugular vein and collected in tubes containing EDTA and heparin as anticoagulants. The tests used included hematocrit centrifugation, wet film, stained thin smear, and mice inoculation (as described by the Office International des Ëpizooties[6]). The mAECT was also used. The test was provided by the Institute of Tropical Medicine, Antwerpen, Belgium. For goat blood, the DEAE gel was equilibrated with phosphate-buffered saline glucose (Na_2HPO_4 (anhydrous): 8,088 g/L; $Na_2PO_4 \cdot 2H_2O$: 0.468 g/L; NaCl: 2.55 g/L; glucose: 10 g/L). A volume of 300 µL of fresh heparinized blood was eluted on a 2.5-mL DEAE gel bed volume. After centrifugation of the eluate, the presence of *T. evansi* was evaluated by low-magnification microscopy (10×10).

Indirect Hematological Parameters

Packed cell volume (PCV) and total serum proteins were measured to detect effects on host health through the experiment.

RESULTS AND DISCUSSION

The inoculated goats showed a particularly subclinical course of infection. The PCV dropped significantly ($P < 0.05$), and serum proteins also increased significantly ($P < 0.05$) because of globulin increases.

Parasitemias remained very low but were persistent through the experiment. The mAECT proved to be more sensitive than blood smear and buffy coat examination, but it was less sensitive than mouse inoculation. For mAECT, after centrifugation of the eluate, *T. evansi* was detected by its mobility under low magnification (10×10). The mAECT has been used in goats infected with *T. vivax*, *T. congolense*, and *T. brucei*, although the sensitivity obtained was lower.[5] However, similar sensitivities have been shown with *T. evansi* in antelopes[7] and water buffaloes.[8]

We conclude that in cases of very low parasitemia in goats, mAECT can be performed when other parasite detection tests have been ineffective.

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