CHARACTERIZATION OF EXCRETOARY/SECRETORY ANTIGEN OF BUNOSTOMUM TRIGONOCEPHALUM IN SHEEP

*S. Arunkumar

Department of Veterinary Parasitology, Madras Veterinary College
Tamilnadu veterinary and animal sciences university, Chennai-600007, India

ABSTRACT

In the present study, E/S antigen from Bunostomum trigonocephalum was prepared by in vitro culture method and hyper immune serum was raised against this antigen in rabbits. On SDS-PAGE analysis, the E/S antigen revealed six polypeptides at 21.0, 29.0, 47.0, 50.0, 94.0 and 101.0 kDa. Further, three immuno-reactive polypeptides at 29.0, 47.0 and 60.0 kDa were detected in Western blotting. This antigenic characterization study will be helpful in developing suitable sero diagnostic in bunostomosis in sheep.

Key Words: Bunostomum trigonocephalum, E/S antigen, Western Blotting

INTRODUCTION

Bunostomum trigonocephalum is an important hookworm in small ruminants in tropical areas. They inhabit in small intestine and cause progressive anaemia with associated changes in blood picture, hydramia, oedema and stunted growth. Host immune response to helminthes is generally hampered by two main factors namely the complexity of antigenic profiles and the presence of cross-reactive determinants on antigens. To identify specific antigens, excretory/secretory antigens have received increasing attention recently. This is due to the fact that E/S antigens relatively display less complexity compared to somatic antigens. Further, the work on antigenic characterization of E/S antigen of B. trigonocephalum is scanty in India. Hence, the present investigation was undertaken to analyze the polypeptide profiles of E/S antigen of B. trigonocephalum for its use in serodiagnosis.

MATERIALS AND METHODS

Preparation of Antigen

Adult live Bunostomum trigonocephalum were collected from the small intestine of sheep slaughtered at corporation slaughter house, Chennai. The collected worms were washed five times in normal saline and subsequently washed five times in Phosphate buffered saline (PBS, pH 7.4), containing penicillin (500 IU/ml) and streptomycin (5 mg/ml) and nystatin (1 mg/100ml). Then, the worms were identified based on morphological features using standard keys (Soulsby, 1982). The fresh and highly motile worms were transferred to RPMI 1640 medium containing penicillin (100 IU/100 ml) and streptomycin (1 mg/100ml) and cultured at a concentration of approximately 400 worms per 20 ml in a culture flask at 5 per cent CO₂ atmosphere at 37°C for 24 hours. The medium was changed every 6 hours after incubation and fresh medium was added with 2 per cent glucose throughout incubation. Worm viability was monitored throughout this period on the basis of motility, integrity of the worms. Moreover, random samples of the culture fluid obtained during and directly after the incubation period were plated out on agar in order to exclude bacterial contaminations. After the incubation period, the culture medium was collected by decantation and filtered through a 0.22 μm filter (Millipore). Then, the culture medium was centrifuged at 10,000 rpm for 30 minutes at 4°C and the supernatant was labelled as excretory/secretory (E/S) antigen. This culture procedure was repeated several times in order to obtain sufficient quantity of antigen. Finally, the antigen obtained was concentrated by dialysis (membrane cut off, 12 kDa) against polyethylene glycol (PEG 6000 – SRL, India) over a period of 6 hours. The