ANALYSIS OF POLYPEPTIDE PROFILES OF STRONGYLD NEMATODES USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS

S. Arunkumar and R. L. Sharma

Department of Veterinary Parasitology, Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600 007

ABSTRACT

Polypeptide profile of soluble extract antigens (SEA) of referral nematodes viz., Haemonchus contortus, Bunostomum trigonocephalum and Oesophagostomum columbianum were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The SEA of H. contortus showed bands at 24.0, 29.0, 35.0, 43.0, 60.0, 94.0 and 106 kDa molecular weight whereas the SEA of B. trigonocephalum showed bands at 21.0, 28.0, 30.0, 47.0, 60.0, 94.0 and 101 kDa size. The SEA of O. columbianum revealed polypeptide bands at 29.0, 39.0, 54.0, 60.0, 94.0 and 112 kDa. It was also revealed that a common polypeptide bands at 29.0, 60.0 and 94.0 kDa were present in all three antigens.

KEYWORDS: Antigenic profiles, gastrointestinal nematodes, SDS-PAGE

INTRODUCTION

Nematode antigens are biochemically complex in nature and are cross-reactive with one another (Meuusen, 1996 and Knox, 2000). Many nematode antigen in particular surface and excreted components are stage specific and provide ample scope for stage specific diagnostics and protections (Raleigh et al., 1996 and Knox, 1998). The surface bound antigenic molecules play a critical role in immune response and host parasite relationship. Serodiagnosis of helminthic infections continues to be a difficult task on account of cross-reactivity and sharing of antigenic molecules amongst various helminths. The most important impediment in the way appears to be that the specificity of antibody detection that greatly restricted by the degree of interspecific cross reactive epitopes, shared between helminths (Cuquerella et al., 1994 and Molina et al., 1999). Therefore, a study of nematode antigen in relation to immune response of host is a prerequisite for understanding immunological mechanisms between parasite and host. Hence, the present study was undertaken to examine the polypeptide profiles of economically important gastrointestinal nematodes viz., H. contortus, B. trigonocephalum and O. columbianum using SDS-PAGE.

MATERIALS AND METHODS

Three species of parasitic nematodes viz., H. contortus, B. trigonocephalum and O. columbianum were collected from a local abattoir of sheep and goats. The parasites were recovered from their respective sites of predilection at necropsy following standard technique (Sahu and Misra, 1986). The parasites were washed repeatedly with distilled water followed by physiological saline and phosphate buffered saline (pH 7.4). Finally, the collected worms were suspended in 0.1 M phosphate buffered saline (PBS pH 7.4). The parasites were identified upto species level using standard keys (Soutsby, 1982).

PREPARATION OF ANTIGENS

Soluble Extract Antigen (SEA)

Soluble extract antigen for each species of the referral nematodes was obtained by processing adult parasites of H. contortus, O. columbianum and B. trigonocephalum separately using standard technique (Klesius et al., 1986).

One gram sample of freshly collected adult nematodes was suspended into homogenizing buffer (0.1 M PBS, pH 7.4 supplemented with 1mM PMSF and 1% Triton X-100). The mixture was subjected to repeated freeze-thawing cycle (approximately 3-10 times). Finally, the worms were homogenized using ground glass homogenizer and the suspension was subjected to high speed centrifugation at 10,000 Xg for 1 hr at 4°C. The supernatant was designated as soluble extract antigen (SEA). It was stored at -20°C until use.