

Optimization of *In Vitro* Proliferation Assay for *Haemonchus contortus* Antigen Sensitized Lymphocytes in Goats

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The cell-mediated immune responses generated in the host could be measured by using *in vitro* lymphocyte proliferation assay. Once the lymphocytes are sensitized *in vivo* for a specific antigen, *in vitro* antigenic stimulation causes exponential antigen specific lymphocyte proliferation. *Haemonchus contortus*, an abomasal blood sucking parasite, induces cell-mediated immune responses in goats. Current study was conducted to optimize the methodology of *in vitro* proliferation at different antigen concentrations and to evaluate the cell proliferation using flow cytometer. Blood was collected from four goats (Saanen breed, aged less than 6 months) that were infected with *H. contortus*. Peripheral Blood Mononuclear Cells (PBMC) were isolated using the standard Pancoll-hypaque technique. Cells were cultured in complete RPMI media (RPMI 1640 supplemented with 2.05 mM L-glutamine, 2 g/L sodium bicarbonate, penicillin (100 IU/mL) and streptomycin (100 µg/ml) and 10% FBS) for 3 days at 37°C with 5% CO₂. Well known lymphocyte mitogen, Concanavalin A was used as a positive control at 5, 10 and 20 µg/mL, and *H. contortus* L3 somatic larval antigen was used at 200, 100, 50, 25, 10, 8, 6, 4 and 2 µg/mL concentrations. Concanavalin A at 5 µg/mL and the somatic larval antigen at 25 µg/mL yielded maximum *in vitro* lymphocyte proliferation. The results confirmed the basic requirements essential for goat *in vivo* sensitized lymphocyte proliferation.

Keywords: *Haemonchus contortus*, Lymphocyte proliferation assay, Flow cytometer, Goats

Funding and support provided by International Atomic Energy Agency and Sri Lanka Technological campus is gratefully acknowledged.