Immune resistance to infestation by an ixodid tick, <u>Rhipicephalus appendiculatus</u>, the vector of the African cattle disease, East Coast Fever was induced in sheep by immunisation with tick extracts. Six groups of four sheep each were allotted randomly to three inoculations of the tick extracts in an attempt to vaccinate them against this tick. Resistance was assessed using various parameters including mean percentage of ticks engorged, tick mortality, mean engorgement weight, mean egg mass laid, mean percentage rate, mean number moulting and mean egg conversion ration. Hosts sera were examined for specific antibodies to tick midgut and female reproductive organs.

Two of the six groups of experimental sheep were controls that is phosphate buffered saline (negative control) (PBS) and tick infestation (positive control). Tick extract immunisation especially solubilised midgut membrane antigens (SMMA) led to a reduction in the viability of eggs laid by ticks feeding on the immunised hosts. This effect was also noted with the soluble female reproductive organ antigens. The animals immunised with solubilised midgut membrane antigens had the highest tick mortality; between 80-90% of tick instars applied were killed compared to the controls which allowed more than 95% of the applied ticks to complete engorgement. Successfully in the laboratory ear challenge. Some ticks which fed on animal's immunised midgut derived antigens appeared engorged with host tissue fluids rather than erythrocytes and others were observed dead while still attracted on the host.

Resistance to infestation by ixodid ticks has previously been reported by others to have a humoral immune component. Therefore, antibodies from resistant host animals were used to detect the tick antigens they recognized as an approach to identification of the target antigen(s) for the above-observed immune responses on feeding ticks. Sheep which were immunised with soluble female reproductive organ derived antigens in Freund's complete and incomplete adjuvants (FCA and FIA) showed high antibody titres. The antibody titres which were detected by Enzyme Linked Immunosorbent Assay (ELISA) reached the highest peak on about day 63 before the laboratory challenge.

However the antigen was only protective at the egg conversion factor and the hatchability level of the female ticks. Immunodiffusion tests showed a minimum of 2 to 3 precipitin lines with the antiserum from sheep immunized with these female reproductive extracts. When the instars were fed on the animals immunised with this antigen, there was no immediate adverse effects observed on the ticks.

Sheep immunised with antigen extract derived from midgut of partially fed female ticks showed dramatic adverse effects on all three instars feeding on them. There were reduced engorgement weights, reduced egg production, egg weights and reduced egg viability. Mortalities were also very high especially from those ticks, which fed on animals immunised with solubilised midgut membrane antigens (SMMA).

Antibodies from resistant animals by immunisation with partially fed tick antigen extract were used to isolate, enumerate as an approach to identification of the target antigen(s) in feeding ticks. Ouchterlony Immunology tests showed 4 precipitin lines. Sera from animals immunised with SMMA detected at least twenty seven antigens. The molecular weights of these antigens as assessed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) using

Immunoblot technique were: 21,000; 22,000; 24,000; 25,000; 28,000; 45,000; 46,000; 48,000; 50,000; 51,000; 52,000; 53,000; 55,000; 57,000; 59,000; 76,000; 78,000; 79,000; 80,000; 82,000; 84,000; 86,000; 88,000; 92,000; 94,000; 105,000KD.

The proteins as have been reported by other workers increased with each day of feeding reaching a maximum by day 6 to 8 and then decreased gradually throughout the post engorgement to preoviposition period. Therefore the rate of synthesis of these antigens appeared to vary with relation to the feeding cycle of tick.

Animals immunised with midgut-derived antigens were further exposed to tick populations in the field situation. The paddocks were seeded with 23 nymphal ticks per square metre. Animals immunised with SMMA managed to reduce tick populations significantly by five times compared to the changeable controls. Following vaccination, serum antibodies to soluble and solubilised extracts of adult ticks were detected by gel diffusion. These antibodies were still evident after 33 weeks which as the experimental period. Therefore immunity produced by vaccination was very effective in controlling populations under experimental conditions. The results reported here show that immunisation against R. appendiculatus, an important economic ecto-parasite of cattle, is feasible.