

IMMUNOLOCALISATION OF MIDGUT ANTIGEN OF *RHIPICEPHALUS HAEMAPHYSALOIDES* BY INDIRECT IMMUNOPEROXIDASE TEST USING METHYLENE GREEN COUNTER STAIN

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ABSTRACT

Immunolocalisation of midgut antigen of Rhipicephalus haemaphysaloides was performed by indirect immunoperoxidase test using methylene green as counter stain. Semi engorged adult female ticks were fixed in bouins fixative, embedded in paraffin wax and sectioned. The tick sections were treated with sera from immunized animals showed positive brown colour reaction on the entire surface of digestive cells and midgut epithelial membrane. The methylene green was used for counter staining which clearly differentiated the midgut from other tick tissues.

Key words: *Rhipicephalus haemaphysaloides*, midgut antigen, indirect immunoperoxidase test, Methylene green.

INTRODUCTION

Haematophagous arthropods are important in the transmission of a variety of pathogens of veterinary significance. Among these, ticks and tick borne diseases in particular, directly and indirectly hamper the growth of the livestock sector (Sansoucy, 1995) and are major constraints in raising a viable livestock industry in tropical and subtropical area.

Immunizations with several antigens derived from various stages of ticks have been studied extensively till date. The importance of concealed antigens has gained momentum after the identification of protective antigen (Bm 86) (Willadsen *et al.*, 1989). The sections of midgut

antigens of four ixodid ticks viz *Haemaphysalis bispinosa*, *Rhipicephalus haemaphysaloides*, *Hyalomma marginatum isaaci*, *Hyalomma anatolicum anatolicum* were probed with hyper immune serum and immunolocalisation of target antibody reactive sites in the sections were observed (Latha *et al.*, 2003). The present study attempted to evaluate the use of counter stain in order to differentiate the reactive sites from other areas of tick tissues.

MATERIALS AND METHODS

Ticks

Rhipicephalus haemaphysaloides ticks were reared on New Zealand white rabbits. Semi-engorged ticks (3-4 days) were collected and fixed in bouins fixative solution for 24 hours and

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embedded in paraffin wax. Each specimen was processed and 4 µm longitudinal sagittal sections were cut and stored unstained for indirect immunoperoxidase test. for indirect test.

Preparation of Midgut Antigen

Midgut antigen was prepared as per the method of Opdebeeck et al. (1988) with minor modifications. The midguts were isolated from semi engorged adult female ticks and placed in 0.15M PBS pH 7.2 and the harvested midguts were mixed with 1 mM disodium EDTA, homogenized @ 1500 rpm for 10 minutes in icebath. The homogenates were sonicated @ 8µ amplitude for 10 minutes in icebath. and centrifuged @ 15,000 g for 30 minutes at 4°C. The supernatant was collected and dialysed against PBS. Phenyl methyl sulfonyl fluoride at 1mM (PMSF) was added per ml of supernatant antigen and used as *R.haemaphysaloides* gut membrane antigen (RhGMAg).

Immunization schedule

For raising hyperimmune serum, 2 New Zealand white rabbits were immunized on 0, 14 and 21 day. The first injection was given intramuscularly with 500µg of *R.haemaphysaloides* gut membrane antigen (RhGMAg) emulsified with Fruends Complete Adjuvant (FCA) per rabbit. The second and third injections were given subcutaneously with 250µg of gut membrane antigen (RhGMAg) with Fruends Incomplete Adjuvant (FIA) per rabbit. Blood was collected on the 28 day for sera separation.

Indirect Immunoperoxidase Test

Immunolocalisation of midgut antigen was carried out as per methodology described by Werner et al. (1996). Indirect immunoperoxidase test (IPT)

was used to demonstrate the immunolocalisation of antigen determinants in midgut. The tick sections were deparaffinised with xylene and rehydrated with grades of ethanol and finally washed with phosphate buffered saline pH 7.2. Sections were treated with 10 per cent goat serum for 30 minutes and washed with PBS. Endogenous peroxidase activity in sections was by incubation with hydrogen peroxydase in methanol for 30 minutes. The tissues were washed several times with PBS, incubated with 1:20 diluted serum collected from immunized and control animals for 1 hour at room temperature. The sections were then washed with PBS and incubated with anti rabbit IgG HRP conjugate diluted at 1:100 for 1 hour at room temperature. After rinsing the slides in PBS, sections were treated with chromogen solution for 10 minutes at 37°C. The reaction was stopped by washing the sections with PBS and then counter stained by methylene green. The sections were dehydrated with graded acetone and cleared with xylene and mounted with DPX mounting medium. The sections were observed under microscope and the results were documented.

RESULTS AND DISCUSSION

Tick sections were treated with sera from immunized animal showed positive brown Colour reaction on the entire surface of digestive cells and midgut caecal epithelial membrane (Fig. 1 and 2). Previously immunolocalisation of midgut antigen of *Haemaphysalis bispinosa*, *Hyalomma anatolicum anatolicum*, *Hyalomma marginatum isaaci* and *Rhipicephalus haemaphysaloides* using indirect immunoperoxidase test, without counterstaining (Latha et al., 2003) has been carried out. The present study thus highlightens the need to use a suitable counter stain such as methylene green to enable easy and better visualization of the reactive sites. This confirms that the antisera raised against midgut antigen target specifically the midgut of ticks.

Fig. 1
Tick Section treated with sera of immunized animal without counter stain

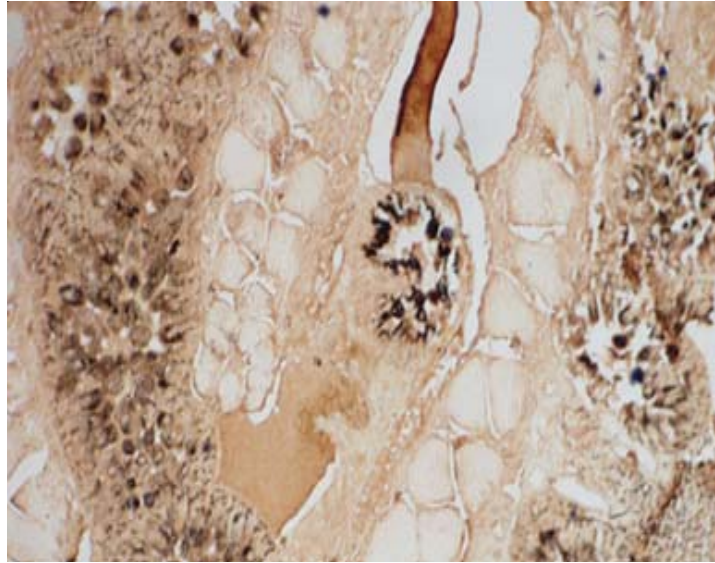
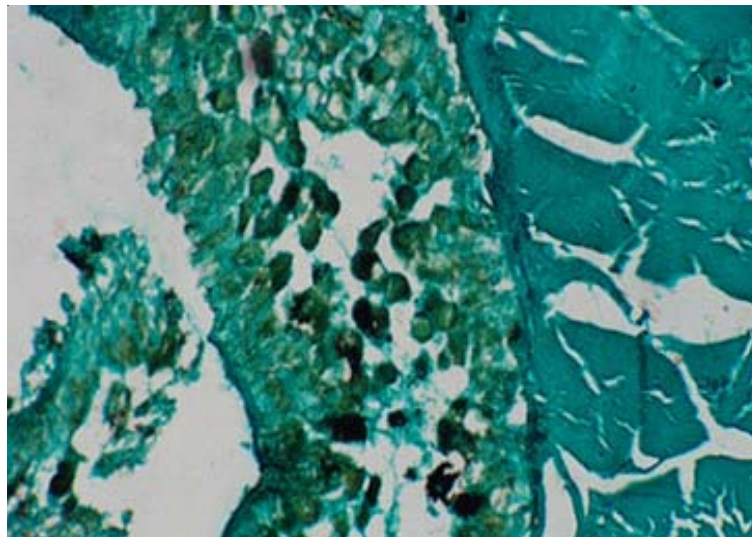


Fig. 2
Tick section treated with sera of immunized animal using counter stain



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