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## Development of Subunit Vaccine Containing Aujeszky's Disease Virus Glycoprotein gC as the Main Component

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### A study towards development of a tick vaccine

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Anti-tick vaccines have been considered to be an effective control method against ticks which transmit a variety of animal pathogenic microorganisms as an alternative to the current use of acaricides. This study was aimed at identification and molecular characterization of tick molecules that can be used as antigens against tick infestation using the *Haemaphysalis longicornis*-rabbit model.

Molecules injected into the host by the tick during feeding mostly remain to be characterized. In this study, two of these molecules (p84 and p29) from the *H. longicornis* tick were identified and characterized. The first molecule, is an 84 kDa trypsin-like protein capable of inducing an immediate hypersensitivity reaction in rabbits sensitized against ticks by infestation. The second molecule is an immunodominant 29 kDa extracellular matrix-like protein that was recognized by serum antibodies from tick infested

rabbits, and possibly involved in formation of tick cement. Vaccination of rabbits with recombinant p29 expressed in *Escherichia coli* stimulated a protective anti-tick immune response as indicated by reduced engorgement weights of adult ticks and mortality of up to 40 and 56 % of larval and nymphal ticks that had fed on immunized rabbits.

A candidate tick vaccine antigen can be defined either as "exposed" (tick molecules injected into the host during tick feeding) or "concealed/or novel" (tick molecules not injected into the host during tick feeding). Concealed antigens have generally been advocated over exposed antigens. Based on data from the immunization experiment with rp29 in this study, evidence to show that a well-defined salivary-gland associated antigen delivered at optimal doses in an appropriate adjuvant can confer a significant level of anti-tick immunity has been

provided. Results from the present work and others have shown that anti-tick vaccines based on a single antigen may not be inclusively protective. In addition, immature and mature ticks tend to show different sensitivities to host anti-tick immune responses, and hence a cocktail vaccine that contains multiple antigens will be more protective. Further search for candidate antigens was conducted.

Despite the potential of proteolytic enzymes as possible target molecules in immunological control of ticks, studies on these molecules in ticks have been quite limited. In this study, a generic approach was used to clone and express *in vitro* 2 genes each of serine and cysteine proteinases. The generic approach proposes to use oligonucleotide primers designed from conserved motifs among proteinase families. In other studies, the polymerase chain reaction with these primers was carried out to amplify gene fragments which were used as probes to clone full length genes. In this study, PCR primers designed from the partial gene sequences were used in the 5' and 3' RACE protocols to clone the full length genes. While the primers used in this study have been used by others to clone cysteine proteinase and serine proteinase genes in other

parasite species, the results in the present study were the first reports on molecular cloning and characterization of both cysteine and serine proteinase genes from a hard tick. Two serine proteinase genes, HLSG-1 and -2 encoded polypeptides with molecular masses of 37.7 and 31.2 kDa, respectively. Two cysteine proteinase genes, HLCG-A and -B encoded polypeptides with molecular masses of 33.7 and 37.0 kDa, respectively. Rabbit antisera raised against recombinant product of HLSG- I and -2 expressed in *E. coli* recognized authentic molecules expressed in ticks, which suggested that the genes were expressed in ticks.

In conclusion, this study has provided relevant information towards the development of a vaccine against ticks. Results from the vaccination trial with rp29 in rabbits were very promising and needs further studies to enhance the protective capacity of the vaccine. Data from this work on tick proteolytic enzymes provided fundamental information with respect to obtaining efficacious anti-tick vaccine antigens using generic approaches. Further work is needed for further characterization of the proteolytic enzymes reported in this study.

#### Molecular cloning of genes of *Theileria sergenti* and comparative genome structure analysis of *Theileria* parasites

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*Theileria sergenti* is a tick-borne protozoa of cattle and causes anaemia as an intraerythrocytic piroplasms, which is one of the most economically important disease of grazing cattle in Japan. Cattle which are persistently infected with this

parasite, show elevated parasitaemia, rapid progress of anaemia and mild hyperthermia, which is occasionally fatal, under conditions of stress or coinfection with other pathogenic microorganisms. The methods of controlling this parasite