1. INTRODUCTION

Ticks are obligate haematophagous ectoparasites with worldwide distribution and they have a significant impact on human and animal health. A total of ~850 tick species have been catalogued worldwide. They are excellent vectors for disease transmission second only to mosquitoes as vectors for human and animal diseases (de la Fuente and Kocan, 2003).

Ticks carry and transmit a remarkable array of pathogens which include bacteria, spirocheates, *rickettsiae*, protozoa, viruses, and nematodes. The common diseases caused by tick bites are Lyme disease, human granulocytic and monocytic ehrlichiosis, babesiosis, relapsing fever, Rocky Mountain spotted fever, colorado tick fever, tularemia and tick paralysis (de la Fuente and Kocan, 2003).

1.1. Classification of Ticks

Acarines of veterinary importance which include ticks and mites are classified under the class *Arachnida*, order *Acarina* and families *Ixodidae* and *Argasidae* (de la Fuente and Kocan, 2003). The *Ixodidae* are often called hard ticks because of the presence of rigid chitinous suctum. The three *Ixodid* genera which include *Ixodes*, *Haemaphysalis* and *Dermaentor* show worldwide distribution (Jongejan and Uilenberg, 2004). The *Argasidae* are called soft ticks because they lack the suctum. The two *Argasidae* genera i.e. *Argas* and *Ornithodoros* show worldwide distribution (Jongejan and Uilenberg, 2004).
1.2. Life cycle of Ticks

1.2.1. Life cycle of hard ticks (*Ixodidae*)

Hard ticks use a variety of strategies to optimize their chance of contact with an appropriate host thereby ensuring survival. Some ticks feed on only one host throughout their larval, nymphal and adult stages. These ticks are called “one host ticks”. They remain on one host during the larval and nymphal stages until they become adults. Adult females drop off the host after feeding to lay their batch of eggs (Figure 1) (Walker *et al*., 2003).

![Figure 1.1: Life cycle of one-host tick.](image)

Ticks that feed on two hosts during their lives are called “two host ticks”. These ticks feed and remain on the first host during the larval and nymphal life stages, drop off and attach to a different host as an adult for the final blood meal. The engorged adult female drops off from the host after feeding to lay their batch of eggs (Figure 2) (Walker *et al*., 2003).
Many ticks feed on three hosts, one during each life stage and are appropriately named as three host ticks. These ticks drop off and reattach to a new host during each life stage, until finally the adult female lays its batch of eggs. After laying one batch of eggs the female dies (Figure 3) (Walker et al., 2003).
1.2.2. Life cycle of soft ticks (*Argasidae*)

The life stages of soft ticks which include *Argas*, *Alectrobius*, *Carios*, *Otobius*, *Ornithodoros* are not readily distinguishable. The first life stage to come out of the egg is a six-legged larva. It consumes blood from the host and molts to the first nymphal stage. Unlike hard ticks, many soft ticks go through multiple nymphal stages, gradually increasing in size until they finally molt to the adult stage. Some soft ticks pass through up to seven nymphal molts before they become adults. Soft ticks feed several times during each life stage, and females lay multiple small batches of eggs between blood meals during their lives. The time taken to complete its life cycle is generally much longer than that of hard ticks, sometimes lasting over several years (Walker *et al.*, 2003).

1.3. Ticks and tick borne diseases (TTBDs)

Ticks transmit a number of pathogens like protozoan, bacteria and viruses which are causative agents of many diseases that infect livestock, human and companion animals. The estimation of global costs of TTBDs in cattle is between US$13.9 and US$18.7 billions annually (Minjauw and McLeod, 2003). Some tick borne diseases of pets like canine babesiosis, granulocyte anaplasmosis, canine monocytic ehrlichiosis, and thrombocytic anaplasmosis show a higher incidence in Europe (Beugnet and Marie, 2009). Lyme borreliosis is caused by a group of pathogens which includes *Borrelia burgdorferi*,
*Borrelia afzelii* and *Borrelia garini*. These pathogens are transmitted by the hard ticks like *Ixodes ricinus* and *Ixodes persulactus* (Kempf *et al*., 2009; Tsao JI, 2009). *Ehrlichia chaffeensis* is an obligatory intracellular bacterium transmitted through the *Amblyomma* tick species. This bacterium causes monocytic ehrlichiosis in human (Ganguly and Mukhopadhyay, 2008). Human *Ehrlichia chaffeensis* infections have generally been reported in North America, Asia and Europe (Dawson *et al*., 1996).

### 1.4. TTBD in India

In India alone the cost of TTBDs in animals has been estimated to the tune of US$ 498.7 million (approx. 2000 crores) per annum (Ghosh *et al*., 2007a). The tick borne disease transmission potential has been reported for a number of species which include bovine babesiosis, anaplasmosis, canine babesiosis, ehrlichiosis and equine babesiosis for *Rhipicephalus spp*, theileriosis and babesiosis in sheep and goats for the *Haemaphysalis spp*, bovine tropical theileriosis, equine babesiosis for the *Hyalomma spp*, and aegyptianellosis, spirochaetosis in birds by the *Argas spp* (Ghosh *et al*., 2007b).

### 1.5. Control of TTBDs

The integrated pest management program (IPM) has been accepted as the most suitable option for the control of TTBDs. IPM has been executed in many tropical and subtropical countries where the TTBDs are a major threat for live stock management. Control of
TTBDs can be brought about by 1) acaricidal treatment against ticks, 2) vaccination against tick borne diseases and 3) vaccination against ticks.

1.5.1. Acaricidal treatment for tick control

Acaricides are chemical agents used to kill ticks. Acaricides can be classified as antibiotic acaricides, milbemycin acaricides, bridged diphenyl acaricides, mite growth regulators, avermectin acaricides, carbazate acaricides etc. The predominantly used acaricides for controlling TTBDs are Organophosphates, Carbamates, Pyrethroids, BHC/Cyclodines, Amidines, Macro cyclic lactones, Benzylphenylureas, Avermectins and Organochlorines (Ghosh et al., 2007a). Spraying of chemical acaricides in cattle environment results in the control of tick infestations thereby, reducing the risk of TTBDs in cattle. Some of the commercially available acaricides which are widely used in field conditions include Bifenthrin, Carbaryl, Cyfluthrin, Deltamethrin, Cyhalothrin, Permethrin, Ivermectin and Pyrethrin etc.

The major disadvantages following the usage of acaricides include high cost of use, ticks resistance towards acaricides and environmental pollution. In most cases, the gene that confers the resistance is inherited to the next generations, leading to the complete resistance of ticks to a particular chemical acaricide (de la Fuente and Kocan, 2003). The above mentioned disadvantages made the researchers to concentrate on vaccination against TTBDs.
1.5.2. Vaccination against tick borne diseases

The disadvantages encountered in the use of acaricides forced researchers to explore new frontiers with respect to the use of vaccines towards TTBDs. Early researchers used crude midgut extracts and the whole salivary extracts of tick parasites as vaccine candidates. The advent of newer technologies paved way for the use of conventional/recombinant midgut glycoproteins of ticks as vaccine candidates thus reducing the need for acaricides and paving way for the use of vaccines as a part of Integrated pest management strategies (IPM) (Jonsson, 2004). Extensive research is being carried out worldwide against the tick borne diseases directly with research being directed towards the development of conventional, inactivated or native protein subunit vaccines against the pathogens transmitted by the ticks which are causative agents of babesiosis, anaplasmosis, and theileriosis etc.

1.5.3. Recombinant vaccines against ticks

The usage of live vaccines against tick borne diseases resulted in transmission of other pathogens and dreadful diseases to the animals. This lead to the identification of specific antigen candidates from the midgut and salivary glands of tick parasites that could be used as vaccine candidates. In initial studies, the cattle were immunized with homogenates of whole engorged female ticks which resulted in appreciable level of protection (Johnston et al., 1986). It
was observed that some of the affected ticks suffered from leakage of gut contents into the haemocoel resulting in reduced fecundity. Kemp, (1986) showed through histopathological studies that, tick gut cells were shed into the caecal lumen and the gut epithelium was reduced to thin strips and subsequently ruptured. Following rupture of the gut, host neutrophils and other immune cells invaded the hemocoel to attack muscle and malpighian tubules of ticks. Successive fractionations of the gut lysates helped identify Bm86, a midgut glycoprotein as a candidate antigen for vaccinations. Bm86 is located in the plasma membrane of gut cells and this was confirmed by transmission electron microscopy of immunogold labeled tick gut (Gough and Kemp., 1993). Following vaccination with Bm86 immunity is conferred by the presence of circulating IgG along with complement fixing antibodies (Kemp et al., 1989). Recombinant commercial vaccines such as GAVAC, TickGard, and TickGardPlus incorporating Bm86 antigen have been widely evaluated in field trials and their efficacy has been found to range from 60-70%. This has made them suitable candidates for inclusion in integrated tick control programs against Rhipicephalus microplus.

1.5.3.1. GAVAC

GAVAC is a Bm86 based vaccine that contains the purified recombinant Bm86 protein expressed from Pichia pastoris. The gene was isolated from Camcord strain of Cuba. GAVAC is manufactured and marketed by Heber’s Biotech, Cuba. The efficacy of GAVAC in
reducing *Rhipicephalus microplus* tick infestations has been proven in many efficacy studies that have been conducted in Cuba and South American countries that include Brazil and Argentina.

1.5.3.2. **TickGard**

TickGard incorporates recombinant Bm86 purified from *E.Coli*. The gene was isolated from Yeerongpilly strain of Australia. TickGard is manufactured and marketed by Hoechst Biotech, Australia. A large number of field trials that were conducted demonstrated the significant efficacy of TickGard against tick species in Australia.

1.5.3.3. **TickGard**\(^\text{Plus}\)

TickGard\(^\text{Plus}\) is mixture of recombinant Bm86 and Bm91 (carboxy peptidase protein identified from the salivary glands of *Rhipicephalus microplus* (Yeerongpilly strain), which have shown a significant response in cattle against the tick infestations. This commercial product is owned and marketed by Pfizer, USA.

1.5.3.4. **Efficacy of recombinant vaccines**

The existing commercial Bm86 vaccines consistently showed significant efficacy against *Rhipicephalus microplus* tick infestations in the field ranging from 60-70%. This helped usher in a new era wherein vaccines could be used in combination with acaricides to tackle tick infestations as a part of integrated pest management programs worldwide.
Objectives of the study

- Cloning, expression and purification of Bm95 antigen of Argentinean Strain A in *Pichia pastoris*.

- Determination of the immunogenicity of Bm95 antigen in Rabbits and Cattle.

- Determination of the efficacy of Bm95 antigen against Indian *Rhipicephalus haemaphysaloides* ticks.

- Determination of the efficacy of Bm95 antigen in simulated field conditions against Indian *Rhipicephalus microplus* ticks.