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REVIEW ARTICLE

Immunity against Ticks-A Review

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ABSTRACT

Tick and tick borne diseases cause many problems to man and domestic animals world wide. These problems are most closely associated with domestic animals in tropical and subtropical areas around the globe. Currently tick control depends largely on the use of different chemicals. But the development of resistance against commonly available acaricides has created problem in this regard and animal population is becoming susceptible to both the ticks and diseases they transmit, with disastrous outcomes. The ability of manipulating organisms on molecular level and recent advancement in immunological procedures has provided alternatives for tick control. The objective of this review is to update/summarize the recent advances in the development of immunity against tick infestation in animals.

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The investigation of acquired immunity to ticks has a long history. The way in which immunity is expressed varies greatly, depending on the host and tick species concerned. The effects range from simple rejection of the parasite, with little or no damage, to interference with feeding, prolongation of feeding time (Willadsen *et al*., 1989), reduction in engorgement weights, inhibition of egg laying and decreased viability of eggs (Heweston *et al*., 1971), to death of the parasite on the host. For example, the main expression of resistance in *Bos taurus* (European cattle) is the rejection of larvae in the first 24 hours of the parasitic life cycle (Roberts, 1968). The ability to reject ticks is an acquired one and starts to appear about eight days after initial infestation of a previously unexpected animal with the parasite. This response was, therefore, presumed to be immunologically mediated (Roberts, 1968a). Observations of the same parasite on *Bos indicus* (Zebu or Brahman cattle) have given generally similar results (Wagland, 1979) but in this case, a gradual loss of ticks occurred throughout the instars compared with rejection of new instars in 24 hours on *Bos taurus* (Roberts, 1968). The weights of fully engorged female ticks were reduced by 30 per cent on immune *Bos indicus* (Wagland, 1978). There are reports of prolongation of feeding time and reduction of egg laying and egg viability in ticks fed on immune Bos indicus (Hewestson, 1971) but the effects were small on this host species, if they occurred at all (Wagland, 1975) and were apparently absent from those on *Bos taurus* or *Bos taurus* x *Bos indicus* crossbreds (Hewestson and Nolan, 1968; Roberts, 1968a).

Repeated infestations with *I. ricinus* led to pronounce decreased in the percentage of ticks engorging. The engorged weight, the percentage of females laying eggs, the viability of the eggs and the length of feeding was substantially increased (Bowessidjaou *et al*., 1977). Similar effects on the percentage of ticks (adult, nymph and/or larva) engorging and engorgement weights have been observed for *R. evertsi* (Branagan, 1974; Njau and Nyindo, 1987; Clarke *et al*., 1989), *R. sanguineus* (Kohler *et al*., 1967; Garin and Grabarev, 1972), *D. variabilis* (Trager, 1939) and *H. anatolicum excavatum* (Kohler *et al*., 1967). It has been reported that a single infestation of *D. andersoni* on guinea pigs caused reduction in the percentage of larvae engorging to less than 20 per cent and frequently even to zero per cent (Allen, 1973; Wikel and Allen, 1976). There was also a 6-7 fold reduction in the weight of larvae after 5 days of engorgement (Wikel and Allen, 1976). Likewise, a single exposure of guinea pigs to larvae of *I. holocyclus* conferred immunity; the percentage of larvae engorging fell from 40 per cent on a primary infestation to only 1-2 per cent on secondary infestation and the majority of the larvae died on the host (Bagnall, 1975; Bagnall and Rothwell, 1974). Similar results have also been observed in a number of amphibian species which became immune to *A. testudinis*, with the results that engorgement weights, egg numbers and feeding rates were reduced (Schneider *et al*., 1971).

The behavior of *D. andersoni* larvae on normal and tick resistant guinea pigs was studied by Allen (1973). It was observed that the survival rate and mean size of the larvae on resistant hosts was less compared to those on normal hosts and these differences became more obvious as the time progressed. The peristaltic rates (at 32°C) of midgut diverticula were consistently lower in larvae which survived on resistant hosts compared with those on normal hosts. It appeared that most larvae after detachment from the resistant hosts died off between 8 and 26 hours, post infestation. In contrast, these deaths were not observed in the ticks isolated from normal hosts.

Investigations on humoral response provided evidence that antibodies are involved in immunity to ticks. This was proved by transfer of partial immunity against *D. variabilis* (Trager, 1939) and *I. ricinus* in rabbits (Brossard, 1977) and *B. microplus* in cattle (Roberts and Kerr, 1976) by injecting serum from respective immune hosts as indicated by the decreased weight or number of females engorging on treated and untreated hosts. Bowessidjaou *et al*. (1977) also noted that antibody to *I. ricinus* salivary gland appeared towards the end of first infestation and reached high titres on second exposure. The involvement of antibodies in immunity to ticks has also been confirmed by blocking the expression of immunity by giving cyclophosphamide to immune guinea pigs before an infestation with ticks (Wikel and Allen, 1976a). The concentration of cyclophosphamide used was supposed to be just sufficient specifically to block B-cells and although the authors noted some depletion of T cells, the main effect was in fact on B-cells. This evidence of antibodies is supported by the increased concentrations of serum-globulins (Brossard, 1976); presence of antibodies specific to the *B. microplus* (Borssard, 1976; Willadsen *et al*., 1978; Fivaz *et al*., 1984); Njau and Nyindo, 1987; Akhtar *et al*., 2000), salivery gland and gut protein antigen to the *B. annulatus* (Abdel-Wahab, *et al*., 2000), salivary gland/purified tick antigen; IgG to *Ha. Longicornis* (Fujisaki, 1978), precipitating antibodies in amphibia exposed to *A. testudinis* (Schneider *et al*., 1971); and both precipitating and complement fixing antibodies in rabbits with *H. anatolicum excavatum* and *R. sanguineus* (Kohler *et al*., 1967; Weiland and Emokpare, 1968); and precipitating antibodies in buffaloes with *B. microplus* (Akhtar,1995; Akhtar *et al*., 2001).

There are several reports in the literature of delayed hypersensitivity in response to ticks. The reaction at the site of tick feeding varies from cellular infiltration, inflammation and erythema to necrosis. The striking characteristic of the feeding lesion on the guinea pig is the accumulation of basophils, eosinophils, neutrophils and lymphocytes (Allen, 1973; Bagnall, 1975; Brown and Knapp, 1981; Brown, 1988); an increased thickness, induration and necrosis of the skin (Wikel *et al*., 1978). Delayed hypersensitivity in response to tick infestation was confirmed by specific lymphocyte blastogenesis stimulated by the salivary gland antigen from immune hosts compared with no effect on lymphocyte blastogenesis stimulated by the salivary gland antigen from immune hosts compared with no effect on lymphocytes from non-immune donors (Wikel *et al*., 1978); and by moderate suppression of the secondary IgG response to the bites of *I. ricinus* with cyclosporin-A (Immunosuppressive) (Giardin and Brossard, 1989). Cyclosporin-A was found to block the immediate cutaneous (type-I) reaction normally developed in response to the intradermal injection of *I. ricinus* salivary gland antigens. The same method revealed a decreased delayed (type-IV) hypersensitivity to these antigens. It was demonstrated that the cyclosporin-A sensitive cells (mostly T-lymphocytes, mast cells and basophils) played major role in the complex phenomenon of resistance to ticks (Giardin and Brossard, 1989). The involvement of lymphcytes in the resistance response was also indicated by enlargement of local lymph nodes in resistants hosts than those in uninfested and singly infested hosts (Brown and Knapp, 1981).

Detailed sequential quantitative histological analysis of tick feeding sites following primary and tertiary feeding by *H. a. anatolicum* was made by Gill (1984). On primary infestation, the cellular infiltrate at feeding sites in rabbits and cattle was dominated initially by neutrophils then by mononuclear cells. On teritiary infestation, the sites in both hosts were characterized by massive degranulation of mast cells and basophils. Basophils appeared to be the major effectors of resistance at tick feeding sites in both cattle and rabbits.

Immediate hypersensitivity in response to *B. microplus* infestation in cattle has been investigated Riek (1956, 1962). It was reported that cattle exposed to ticks were intensely irritated by larvae. Popular reactions were seen around nymphs and adults on resistant cattle and a transient increase in blood histamine levels was found in exposed cattle during tick infestation. Intradermal injection of extracts of eggs or larvae gave immediate oedematous dermal reactions and Prausnitz-Kustner reactions could be passively transferred locally by serum. Although Riek suggested that these reactions were responsible for resistance but no causal relationship was shown. Riek (1956) stated that there was no correlation between the intradermal test reaction and observed hypersensitivity to ticks. Hence, the importance of immediate hypersensitivity type of response was uncertain, particularly after Tatchell's suggestion that the oedema consequent to an allergic reaction was advantageous to the tick (Tatchell, 1969; Tatchell and Moorhouse, 1968). The results suggest strongly that immediate hypersensitivity is responsible in part for the resistance of cattle to the tick. It has been stated earlier, a major expression of resistance in this tick-host system is the ejection of larvae in the first 24 hours of the life cycle; within this time period they make shorter, repeated attachments on hosts of high resistance compared with less-resistant ones, but if removed from the host they seem to be undamaged; restriction of grooming activity greatly increases the yield of ticks. All these observations suggest a response which is irritating to the host and sufficiently unpleasant to the tick to make it move, without causing it significant damage. Pruett *et al (2006)* reported that *B. microplus* larval antigen of 19.1 kDa protein elicited immediate type hypersensitivity reaction in calves previously exposed to *B. microplus.*

To date, the attempts to immunize artificially against ticks have been partially successful. This lack of success has been a stimulus to the proposals about how one might immunize against ticks and an inducement to enthusiastic

speculation about the potential efficacy of immunization in tick control. There is currently no reason for such optimism, not only because success in laboratory experiments to date has been limited but also because none of the methods of immunization used so far is likely to be acceptable and economically sound in a practical situation. Several attempts have been made to artificially immunize hosts with whole tick extracts, selected organ homogenates, serum and cells from lymph nodes of immune hosts. Immunization by whole tick extracts has been carried out against *D. variabilis* (Trager, 1939a), *H. a. excavatum* (Kohler *et al*., 1967), *A testudinis* (Schneider *et al*., 1971), *I. Holocyclus* (Bagnall, 1975), *A, maculatum* and *A. americanum* (McGown *et al*., 1980, 1981), *B. microplus* (Agbede and Kemp, 1986; Akhtar, 1995), *De. Nuttalli* (Ilchmann and Gossing, 1988) and *R. appendiculatus* (Dhadialla *et al*., 1990). Selected organ homogenates which have been used are the salivary glands of *R. sanguineus* (Garin and Grabarev, 1972), *B. microplus* (Brossard, 1976), *R. appendiculatus* (Nyindo et al., 1989); both salivary glands and digestive system of *H. anatolicum* and *R. sanguineus* (Kohler *et al*., 1967); midgut and reproductive organs; and all internal organs of *D. andersoni* (Allen and Humphreys, 1979); and midgut of *B. microlpus* (Akhtar, 1995). Attempts to transfer immunity by cells have been successful (Bagnall, 1975; Wikel and Allen, 1976) using axillary prescapular and cervical lymph nodes.

The pattern of development of *B. microplus* larvae on immunized cattle was observed by Srivastava *et al*., (1987). They reported that following injections of whole-tick tissue extract (antigen) a series of inflammatory reactions (swelling, redness, painful sensation and significant increase in the thickness of the skin) occurred at the site of inoculation. When larvae were applied to the immunized calves, a protective immunity was stimulated which was demonstrated by a reduced larval attachment rate and reduction in the engorged mean weight of the resultant larvae, nymph and adults. None of the female ticks recovered from immunized calves became fully complete and none of these ticks laid eggs.

Opdebeeck *et al*. (1988) tried vaccines prepared from gut and synganglion tissues; and gut of *B. microplus* and found 87 and 80% protection and ticks produced 95 and 91 per cent fewer eggs, respectively, compared with adjuvant control group in cattle. A vaccine prepared from synganglion alone did not protect cattle. Vaccinated cattle showed 36% protection, seven months after immunization. The membrane component extracted from the midgut of *B. microplus* protected cattle (91%) against challenge with 20,000 larval ticks administered at intervals of seven days but soluble antigens did not protect cattle. Antibody levels measured by ELISA were related to the levels of induced by vaccination. Same authors (Opdebeeck *et al*., 1989) used gel filtration and ion exchange chromatography to fractionate the extract from larvae of *B. microplus.* ELISA was used for immunological evaluation of fractions against sera obtained from vaccinated cattle. Unfractionated, whole larval extracts and selected reactive components were used to vaccinate cattle. The number of ticks dropped was non-significant as compared to control group. Antigen purified by affinity chromatography from crude larval extracts, using Ig ligands from an immuned vaccinated steer, could not protect the cattle. However, more than 80 per cent protection was observed in cattle vaccinated with extracts purified by exposure to the affinity ligands from both larval and adult ticks. In another study, Opdebeeck and Daly (1990) investigated the immune responses of infested and vaccinated Hereford cattle to *B. microplus* antigens. In one experiment, cattle infested with ticks had positive cellular responses to soluble salivary gland extracts (SGS) and significant antibodies against larval membrane (LM), gut membrane (GM), salivary gland membrane (SGM) and SGS. Cellular responses to Con A were not depressed following infestation. Cattle vaccinated with GM, using Quail-A as adjuvant, had positive cellular response to all antigens tested. The antibody levels of vaccinated cattle were significantly higher than the antibody levels of infested cattle. In second experiment, immune responses of cattle infested with ticks were studied during 38 days. Cellular responses in lymphocyte blastogenesis assay to several tick antigens were transiently elevated and significant levels of antibody were measured against LM, GM, SGM, and SGS, day 25 post-vaccination. Infested cattle had positive skin reactions following intradermal injection of larval and adult tick antigens. Jackson and Opdebeeck (1989) reported that even one µg of membrane antigens, extracted from the midgut of semi-engorged *B. microplus*, induced an antibody response in sheep comparable to that induced by 500 µg midgut. Cattle vaccinated with either 500 µg midgut (2 doses) or with 50 or 500 µg midgut (3 doses) had significant antibody responses and was equally protected against challenge. Semi-purified midgut of tick, *A. variegatum*, was also used successfully as a vaccine in rabbits (George *et al*., 1999).

The influence of the bovine lymphocyte antigen MBS on the immune response of cattle to antigens of the tick, *B. microplus*, was examined by Wong and Opdebeeck (1990). Groups of cattle having or lacking the antigen MBS (MBS+ and MB5-, respectively) were vaccinated with either soluble extracts of the gut membrane of adult ticks and adjuvant or injected with adjuvant alone. Cattle vaccinated with soluble gut membrane were significantly protected from two challenges with ticks. Protection against ticks was not significantly affected by the presence or absence of the lymphocyte antigen MBS. Similarly, no differences occurred in antibody levels (measured by ELISA) and cellular responses (measured by lymphoblastogenesis assay) in MBS+ and Mb- group to gut antigens.

Allen and Humpherys (1979) studied *D. andersoni* from the hosts immunized with extracts of midgut and reproductive organs (antigen I) and reported that these ticks produced significantly fewer eggs than those from controls, and larvae were not hatched from these eggs. The effects were more dramatic in the guinea pigs immunized with extracts of all internal organs (antigen II) since the ticks failed to engorge and produced no eggs. The antigen extracts were prepared from female ticks that had been allowed to feed for 5 days on guinea pigs. Extracts from tissues of unfed ticks were ineffective, suggesting that important antigens were produced only during the late development of the tick. Calves immunized with antigen I in the same way gave ticks in which live

weights, egg production and the hatching of larvae were reduced.

The glycoproteins located on the luminal surface of the plasma membrane of tick gut epithelial cells were also capable of stimulating an immune response that protected cattle against subsequent tick infestation (Rand *et al*., 1989). One such tick gut glycoprotein, designed as Bm86 was purified to homogenicity and the amino acid sequences of peptide fragments produced by endoproteinase Lys-C digestion was determined. A cDNA contained a 1982-sp open reading frame and revealed that Bm86 contained 650 amino acids, including a 19-amino acid signal sequence and a 23-aminoacid hydrophobic region adjacent to the carbonyl terminus. The main feature of the protein sequence was the repeated pattern of six cysteine residues, suggesting the presence of several epidermal growth factors like domains. A fusion protein consisting of 599 amino acids of Bm86 and 651 amino acids of B-galactosidase was expressed in *E. coli* as inclusion bodies. Ticks engorging on vaccinated cattle were significantly damaged as a result of the immune response against the cloned antigen.

The mechanism how ticks are killed by immune hosts has been explained by some workers. Agbede and Kemp (1986) conducted the histological examination of *B. microplus* that was fed on cattle vaccinated with extract derived from adult female ticks. They reported that the gut was the primary site of damage. Within 24-48 hours of attachment, digested cells were either sloughed off into the lumen or were completely destroyed, leaving only the basal lamina and muscle layers. Subsequent rupture of the gut allowed host leukocytes to enter the haemocoele and attack other tissues. After six days, the surviving ticks showed a delay in the development of digest cells and gut basophilic cells. Many host leukocytes had escaped into the hemolymph, and these cells destroyed tick muscles and malpighian tubules but not salivary glands. Females which survived to post-engorgement also had damaged gut cells. In males, the histopathology of the gut was similar, but the accessory glands of the reproductive organs were damaged by host leukocytes. These effects on feeding ticks were not found on control cattle and have not been reported on animals with naturally acquired resistance to tick infestations. Numerous components of the immunological responses had been studied in detail using a variety of *in vitro* feeding system (Kemp *et al*., 1986; 1989). These include feeding ticks *in vitro* on host blood or serum which contained Evans blue dye conjugated to bovine serum albumin. Damage to the gut was visualized through leakage of ingested bovine erythrocytes or more readily by the leakage of the albumin dye conjugate into the tick hemolymph. It has been reported by Willadsen and Kemp (1989) that the membrane glycoprotein isolated from the gut of *B. microplus* produced antibodies which bound to the tick gut cells when the ticks were fed on the immunized animals. Hence, gut function in the tick and tick development were thus inhibited. It was also identified that immunoglobulins-G bind to the gut cells /digest cells which cause its damage (Howard and Brownlie, 1979; McGuire *et al*., 1979; Willadsen and Kemp, 1988; Kemp *et al*., 1989). The involvement of complement in enhancing the effect of antibody binding and damage the gut cells have also been proved (Kemp *et al*., 1989; Akhtar, 1995; Jackson and Opdebeeck, 1990). Based on the existing observations, it is suggested that detail studies be carried out to investigate the exact mechanism(s) involved in the gut damage of tick fed on vaccinated animals; although hypothetical mechanism(s) had been illustrated (Akhtar, 1995).

Research has been focused on the number and nature of protective antigens and whether immunity is induce with one instar/antigen and shown to be operative against the other? Fujisaki (1978) found up to four precipitin lines with serum from rabbits infested with *H. longicornis*. It is possible that a tick could secrete an antigen into the host to stimulate an immune reaction. However, an antigen should be characterized not only by its immunological reactivity but also by a biochemical function, for example, as a feeding enzyme. Characterization of tick antigens to which hosts react under natural infestation in terms of their biochemical function has been reported for *B. microplus*. Of the three antigens studied to date in this tick, one is a hydrolytic enzyme, a serine esterase (Willadsen and Williams, 1976) which is present in unfed larvae but disappears within two or three days of attachment on the host and is not detectable in other instars (Willadsen and Riding, 1980). Thus the same antigens need not persist through the life cycle. Partial protection with recombinant Yolk pro-Cathepsin, an aspartic proteinase found in *B. micrplus*, had been obtained (Leal *et al*., 2006).

Virtually little is known about the number and location of antigens within the tick. Almost all the experiments reported to date have used extracts either of whole, macerated ticks or of tick salivary glands as “antigens” and the latter has been the most common source. Although it is reasonable to expect antigens to be in the salivary gland, this might not always be the case, nor need they be confined to this one organ. Analysis of saliva and salivary gland extracts from fed females of *H. a. anatolicum* revealed presence of nine and 17 antigenic glycoproteins, respectively (Gill, 1984). Likewise, a large number of proteins had been identified in extracts of the salivary glands of females of *D. andersoni*. Some proteins produced immunological responses in tick-resistant rabbits and were, therefore, presumed to be secreted in saliva using immunoaffinity chromatography. Antigens were also obtained from the extracts which demonstrated esterase activity (Gordon and Allen, 1987). It has been investigated that antigens from fully engorged (Ag II) or partially fed (Ag I) *B. microplus* adults produce different level of resistance being higher degree in Ag II inoculated rabbits probably due to greater amount of tick tissue extract in Ag II compared with Ag I (Srivastava *et al*., 1987).

Evidence presented that the antigens capable of evoking an effective immune response are present in the cell membrane of the gut cells of *B. microplus* and it is unlikely that these antigens are transmitted to the host during feeding (Kemp *et al*., 1989; Rand *et al*., 1989; Willadsen and Kemp, 1989). Studies on the characterization of such antigens reveal an apparent molecular weight of 89,000 an isoelectric point of 5.1-5.6 and an affinity for wheat germ lectin of Bm 86 antigen of *B. microplus* (Willadsen and Kemp, 1989); molecular

weith of 94,000 and 40,000 daltons of *R. appendiculatus* antigen extracted with Triton X-100 (Dhadialla *et al*., 1990); a range of 2 to 24 proteins subunit with molecular weights from 6000-3, 40,000 daltons in a homogenate of adult male *A. maculatum* (McGown *et al*., 1980); and identification of about 25 proteins from females, seven from males and a number of substances secreted into rabbits by *A. americanum* ticks during feeding (Brown, 1988). However, amongst all the proteins characterized only three of 41, 40 and 39 kb were expected to be crucial to induction of host immunity.

The high level of protection along with higher humoral and cellular responses due to midgut (*B. microplus*) cell culture vaccine in buffalo had been reported (Akhtar, 1995; Akhtar and Hayat, 1998). It was also observed that *in vitro* cultured midgut cells of *B. microplus* showed high protection of membrane associated component compared to soluble component (Akhtar *et al*., 1999). Immunogenic and protective effects of lipopolysaccharide complex and protein subunit components isolated from *in vitro* cultured midgut cells confirmed the role of protein and carbohydrate as a protective antigen or as a factor for specificity of these antigens (Akhtar *et al*., 2001).

Based on the success, increased usage and acceptance of the recombinant Bm86 antigen vaccine in cattle (Fuente *et al*., 1999; de Vos *et al*., 2001), the researchers investigated the potential of a DNA vaccine to induce a protective immune response using a plasmid bearing the full length Bm86 gene. The plasmid was injected either alone or co-administered with a plasmid carrying the ovine genes for cytokines GM-CSF or IL-1. The immunologic responses were measured by antibody production or by antigen-specific stimulation of peripheral blood lymphocytes to incorporate thymidine or release interferon (INF). Results of the DNA vaccination trials demonstrated that none of the treatment groups showed antigen-dependent release of IFN that was greater than the controls, and antibody production by the DNA vaccines was much lower than the higher titres seen in the TickGARD Plus vaccinated animals.

Best response upon challenge with engorging ticks was observed in animals vaccinated with the combination of plasmids encoding for Bm86 and GM-CSF. However, Bm86 DNA vaccine either in combination with the cytokine genes or alone primed significant anamnestic response following a single injection of recombinant Bm86 protein. Further, there was no difference in persistence of antibodies following vaccination regardless the method of primary sensitization (Rose *et al*., 1999). Immunity induced by Bm86 affects the larval stage of *B. annulatus* had also been reported (Pipano *et al*., 2003). Ruiz *et al*. (2007) also suggested the potential of DNA vaccine containing Bm86 gene induced significantly higher cellular and humoral responses against *B. microplus*.

The Gavac, recombinant Bm86 vaccine, proved its efficacy (55-100%) against B. microplus in a number of experimental studies in various countries of the world, especially with acaricides in an integrated manner (Fuente *et al*., 1999; Gracia *et al*., 2000). However, vaccine showed resistant against ticks isolated from Argentina. It also showed variation in susceptibility to Bm86 vaccine as Gavac was less effective. A new Bm95 gene isolated from RNA followed by cloning and expression in the *Pichia pastoris* and the resultant recombinant protein with Montanide 888 adjuvant was highly glycosylated in particulate form. Various *B. microplus* gut antigens have been identified including Bm86, Bm95, Bm PRM that have been used alone or in combination (Fuente and Kocan, 2006; Nuttall *et al*., 2006). The sequence for the *B. microplus* A1 allele of the Bm86 gene was compared with Bm95. Laboratory trials were conducted to demonstrate the efficacy of BM95 against two strains of *B. microplus*. Also field trials in Cuba were carried out to compare its efficacy against the Bm86 based Gavac. This vaccine gave protection against both strains of *B. microplus* (Garcia *et al*., 2000). It was also reported that Bm95 may represent a more universal form of the original antigen isolated from the plasma membranes of tick gut cells. Recently the efficacy of recombinant Bm95 tick antigen was found 81.27% (Kumar *et al*., 2009). It is a matter of great concern how much strain variation in susceptibility to vaccination there may be. However, on the other hand, the cross reactivity between the anti-*Boophilus* vaccine and other genera of ticks is a useful aspect. Truncated constructs of 64P (64TRPs), a cemented protein from salivary glands of *R. appendiculatus*, showed cross-protection against other ticks like *R. sanguineus* and *Ixodes ricinus*. *Amblyomma variegatum* and *B. microplus;* indicating the potential of 64TRPs as a broad-spectrum anti-tick vaccine (Trimnell *et al*., 2005).

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