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Developments in tick vaccines–An update

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ABSTRACT

Ticks are the obligate haematophagous and economically important ectoparasites parasitizing various domestic and wild animals, especially amphibians, reptiles, birds and mammals. They are second to mosquitoes in terms of being competent and versatile vectors of many bacterial, viral, protozoan and rickettsial diseases. They are responsible for causing direct and indirect losses to livestock industry. Current control methods are primarily based on use of acaricides. Due to the development of acaricide resistance, environment contamination and residues in meat and milk, control of ticks through immunization appears to be most feasible, cost-effective and environment friendly method. Identification of protective tick antigen is the main limiting step in vaccine development. Tick antigen should have critical function in tick, so that if the function is disrupted, it would lead to death or reduce the fecundity at the levels that will impact the tick population. Various protective, exposed and concealed candidate antigens have been identified and characterized by adopting different strategies like immune-mapping, expression library immunization (EST), RNA interference and bioinformatics. In this review, an attempt has been made to present a comprehensive account on vaccine development in ticks.

Keywords: Antigen, Development, Livestock, Ticks,Vaccines

Ectoparasites in general and ticks in particular are responsible for causing and transmitting important diseases in humans and livestock. About 899 species of ticks parasitize various animals especially amphibians, reptiles, birds and mammals (Ghosh and Nagar 2014). Ticks cause direct and indirect losses to livestock industry all over the world. Direct losses include anemia, tick paralysis (Acute ascending flaccid motor paralysis), tick toxicosis and hide damage. Indirectly, they act as the vectors of many bacterial, viral, protozoan and rickettsial diseases (Table 1). Ticks are the most competent and versatile vectors of many diseases second to mosquitoes (de la Fuente *et al*. 2016). They have the wide host range and have tendency to feed on several hosts. Their high reproductive capacity, hardiness and longevity enables them to survive under unfavourable conditions which ensure ample opportunity to transmit pathogens (Ghosh and Nagar 2014). Brazil and American economies suffer annual losses of USD $2 billion and AUD $170 million, respectively due to *Rhipicephalus microplus* alone (Grisi *et al.* 2002, Playford 2005). India is predominantly an agricultural country with 70% of its population dependent on income from agriculture. In India, cost of TTBDs has been estimated to be US $787.63 million per annum (Singh *et al*. 2022). Thus, the ticks are responsible for huge economic losses to farmers and pose a global threat to livestock as well as human health (Tramboo *et al.* 2021).

Need for vaccines for control of tick infestation

Control of ticks based on the use of acaricides has some drawbacks like selection of acaricide resistant ticks, environment contamination and residues in meat and milk (Graf *et al.* 2004, Ghosh *et al.* 2007). Use of biological agents like entomophagic fungi against wide range of ticks (*R. appendiculatus, Ixodes scapularis* and *Amblyomma variegatum*) though safe, but haven't been successfully implemented yet due to environmental instability and damage to non-target species (Merino *et al*. 2013). Control of ticks by vaccination are cost-effective, would prevent environmental contamination and selection of acaricide resistant ticks. Control by immunization has been well-documented and this technology has been adopted by many countries, but the identification of protective tick antigens is the limiting step for the development of effective tick vaccines (de la Fuente and Kocon 2003, Sparagano *et al*. 2022).

Main difficulties in immunodiagnosis and immunoprophylaxis of parasitic infections in general

- Lack of knowledge about antigens that induce protective immunity.
- Lack of knowledge about mechanisms that induce the protective immunity.

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Table 1. List of important tick borne diseases

Tick borne pathogen	Host	Tick borne disease	Tick species involved
Ehrlichia ruminantum	Farm animals	Heart water	*A. hebraeum, A. variegatum*
Anaplasma platys	Companion animals	Canine cyclic thrombocytopenia	*R. sanguineus*
Anaplasma phagocytophilum	Humans and farm animals	Human granulocytic anaplasmosis	*Ixodes ricinus*
Rickettsia ricketsi	Humans	Rocky mountain spotted fever	*D. variabilis, D. andersoni, R. sanguineus, A. americanum*
Anaplasma marginale	Farm animals	Bovine anaplasmosis	*R. microplus, D. andersoni*
Borrelia burgdorferi	Humans and companion animals	Lyme disease	*I. scapularis, I. pacificus*
Tick borne encephalitis virus	Humans	Tick borne encephalitis	*I. ricinus, I. ovatus, I. persulcatus*
Thelaria annulata and *T. parva*	Farm animals	Bovine thelariosis	*Hyalomma anatolicum anatolicum, H.a.excavatum*
Babesia divergens and *B. mircoti*	Humans	Human babesiosis	*persulatus*
B. canis, *B. vogeli*	Canines	Canine babesiosis	*R. sanguineus, D. reticulatus, Haemaphysalis leachi.*
B. divergans, B. bovis, *B. bigemina, B. ovata*	Farm animals	Bovine babesiosis	*R. microplus, R. annulatus, I. ricinus*
Louping ill virus	Farm animals	Louping ill	*I. ricinus*
KFD virus	Rodents, shrews and monkeys	Kyasanur forest disease	*Haemaphysalis spinigera* and *H. turturis*
Borrelia miyamotoi	Rodents	Tick borne relapsing fever	*Ornithodoros* spp.

Source: Bhowmick and Han (2020).

- Inability to obtain parasitic antigens in bulk because most of metazoan parasites cannot be cultured in laboratory.
- Parasites have the ability of evading host's immune response.
- Extreme antigenic complexity and antigenic variation of the parasites.
- Antibody detection sero tests do not differentiate between present and past infections (Shahardar 2021).

Immune response in ticks

While sucking blood, ticks inject saliva into the wound bed. Tick saliva contain various digestive enzymes which assist them in obtaining blood meals. The saliva of ticks also contain some compounds which minimize the host immune response. Kinases and histamine binding proteins present in tick saliva destroys bradykinin which mediates pain and itch. Saliva of some ticks like *Ixodes scapularis* contain the complement binding proteins that inhibit the generation of C3 due to which scratching and grooming responses are reduced. Due to immune suppression and reduced inflammation, the arthropods are able to feed effectively and also benefit pathogenic organism injected in saliva.

Host response to injected saliva are of three types (Shahardar 2021). Some salivary components of low molecular weight bind to skin proteins such as collagen, thus act as haptens stimulating Th1 response. Subsequent exposure to these haptens induce delayed hypersensitivity reaction; Some salivary antigens bind to langerhans cells inducing cutaneous basophil hypersensitivity, a Th1 response associated with the production of IgG and basophil infiltration; Third response to salivary antigen is Th2 response with the production of IgE and immediate hypersensitivity reaction). This response may induce several local inflammation in the skin leading to pain or purities.

Development of tick vaccines

The concept of tick vaccines was first demonstrated by Targer in 1939, who observed that repeated tick larval infestation triggered acquired immune response against ixodid ticks in guinea pigs and rabbits. Similar phenomenon was observed when guinea pigs were inoculated with native protein tissue extract from *Dermacentor variabilis* tick (Targer 1939). Acquired immune response was determined to be based on the reduction in number of engorged ticks, reduced blood feeding, reduced weight, production and viability of eggs (Ndawula and Tabor 2020). Immunization of host with tick antigen was demonstrated following the work of Allen and Humphreys (de la Fuente and Kocan 2003).

Strategies adopted for identification of protective tick proteins/antigens: Varius strategies adopted are:- Fractionation of crude tick extract and evaluation of fractionated protein by immunization and challenge experiment; immunomapping of tick antigens that elicit an antibody response in infected host; identification and characterization of protective antigen using monoclonal antibodies; expression library immunization and RNA interference; and Bioinformatics for analysing information

regarding DNA or amino acid sequences or annotations about the sequences.

Attributes in selecting candidate antigen for the development of anti-tick vaccines: These include- Target antigen must be easily assessable to the host immune effector cells injected during blood feeding; Antigen should have critical function in the tick biology and when it is disrupted, it should lead to death or reduce fecundity at the levels that impact the tick population; and Antigens should share the conserved epitopes among several tick species to protect against multiple vector infestation (Diaz-Martin *et al*. 2015).

Two sources of candidate antigens have been identified:

a. Exposed antigens are those antigens that are secreted in tick saliva during attachment and feeding on a host, are usually peptides synthesized in salivary gland, e.g. P29. 64TRP, Hl 34, etc.
b. Concealed antigens are those antigens that are normally not exposed to host's immune system and are usually present in tick gut wall and interact with immunoglobulin's taken during blood meal. These antigens are effective against both immature and adult stages and also show transmission blocking and protective activity against tick borne pathogens, e.g. Bm86 based antigens (Nuttall *et al*. 2006).

Targets used for vaccine development

Some of the antigens which have been exploited for vaccine development include: (a) Crude extracts (GUTS (Ground up tick suspension)/Salivary gland extract); (b) Specific salivary gland antigens (P29, Hl34, RIM36, 64P, metalloprotease, cystatins, Salp15, Salp25D, tHRF); (c) Gut antigens (Bm86 based antigens, Bm86 homologos and orthologs, Acid peptidases, ferritins, aquaporins, serpins); (d) Transmission blocking antigens (64P, TROSPA, Salp15); (e) Antigens directed against multiple tick species (subolesin); (f) DNA based vaccines; and (g) Miscellaneous (voraxin, glutathion-s-peroxidase, vitellogen).

Vaccination trials with Crude antigens

Monohar and Banergee, (1992) observed that immunization of rabbits with whole tick extracts of *Hyalomma anatolicum anatolicum* resulted in alteration of tick biology, besides providing cross immunity to *H. marginatum isaaci.* The antigen preparation was found more effective than salivary gland extracts. The animals were found with higher level of antibodies and lower lymphocyte stimulation index, which was not correlated with protection obtained. In another trial, immunization of rabbits with mid gut extracts of *H. a. anatolicum* along with saponin and FCA resulted in reduced reproductive capacity in ticks (Thakur *et al*.1992). Similarly, immunization of rabbits with mid gut extracts of *H. dromedarii* enhanced immunity and provided cross protection against *Hyalomma anatolicum anatolicum* (Kumar and Kumar 1996).

Allen and Humphery in 1979 immunized guinea pigs against *Dermacentor andersoni* with extracts of mid gut and reproductive organs (antigen I) and all internal organs (antigen II) which resulted in reduction in number and production of eggs, larval hatching and failure of ticks to engorge, respectively.

Immunization with whole salivary gland extract of *Hyalomma anatolicum anatolicum* along with *Ascaris* extract as immunomodulator conferred high level of immunity in calves (Sran *et al*. 1996). Immunization with whole nymphal extracts of *H. a. anatolicum* were found more suitable than soluble and membrane antigens in protecting cattle (Sangwan *et al*. 1998).

Crude extracts of partially fed adults and unfed larva of *R. microplus* was used to immunize calves and it was found that both were able to induce protection. Proteins of 105.4 and 92.2 kDa were found to be immunodominant (Ghosh and Khan 1996, 1997).

Drawback: Vaccination with crude tick extracts and tick homogenates have produced inconsistent results and the results obtained in preliminary study have not been confirmed under field conditions. This ultimately resulted in identifying, purifying and testing new vaccine candidates.

Salivary gland antigens

Cement cone antigens: Screening of a cDNA expression library from *Haemaphysalis longicornis* (Hl) females, rabbit immune serum raised against tick saliva, resulted in the identification of a collagen-like protein P29 and an unannotated protein Hl 34 (that is rich in tyrosine and proline residue repeats), acting as cement cone antigens (Tsuda *et al.* 2001). Immunization of rabbits with the recombinant P29 resulted in mortality of *H. longicornis* larvae and nymphs (40% and 56% respectively) (Mulenga *et al.* 1999). Similarly immunization with recHl 34 reduced survival of both immature and adult *H. longicornis* stages and caused 17% reduction in engorgement weight

RIM 36 and 64P are the two cement cone antigens of *R. appendiculatus* involved in feeding and attachment of ticks. RIM 36 is highly immunogenic glycine rich protein marker for detection of cattle that has been exposed to feeding ticks. Secreted 15 kDa protein 64P similar to mammalian skin keratin and collagen when expressed in recombinant form (64TRP) produced protective immune response in guinea pigs. Nymphal and adult infestation rates were reduced up to 48 and 70%, respectively. Vaccine based on 64TRP was reported to have dual action and induced humoral and delayed type of hypersensitivity (Trimnell *et al*. 2005). This candidate antigen resulted in cross protection against *R. sanguineus* and *I. ricinus* (Trimnell *et al.* 2002), and protected against the transmission of tick borne encephalitis thus it is also considered as transmission blocking vaccine (Nuttall *et al*. 2006)

Metalloprotease: Metis 1-metalloprotease from salivary gland *of I. ricinus* when expressed in recombinant form was tested on rabbits. It resulted in reduction in engorgement weight of female ticks and their ability to lay eggs (Decrem *et al.* 2008a, Decram *et al.* 2008b).

Vaccination with recombinant Hl MP 1 (metalloprotease from *Haemaphysalis longicornis*) resulted in increased mortality of nymphs and adults (Imamura *et al.* 2009). BrRmMP 4 (metalloprotease against *R. microplus*) reduced the number of engorged females and overall efficiency was found to be 60% (Ali *et al.* 2015).

Cystatins: These are the group of cystine protease inhibitors, inhibiting variety of cystein peptidases that are involved in modulation of host immunity and inflammation (Schwarz *et al.* 2012). Feeding of *I. scapularis* on guinea pigs vaccinated with Sialostatin-2 resulted in reduction of nymphs as well as ability of nymphs to imbibe blood (Kotsyfkis *et al.* 2008). Mice vaccinated with recombinant OmC2 (cystine from *Ornithodoros moubata)* resulted in reduction in number of *Ornithodoros moubata* nymphal stage. It is present in both salivary and gut tissue, therefore speculated to have dual action (Salat *et al.* 2010).

Salp 15: It is a salivary protein with immunosuppressive properties. It combines with outer surface proteins C (OSP c) of *Borrelia burgdorferi* facilitating the survival of spirochetes and pathogen transmission. It conceals OSP c from host immune response (Ramamoorthi *et al.* 2005). When mice were immunized with recombinant Salp15, antibodies directed against Salp15 separated Salp15 from OSP c leaving it exposed to host immune response thus blocking the transmission of spirochetes (Dai *et al.* 2009). Salp15 homologue found in *I. ricinus*, binds with *B. garini* and *B. afzelii* OspC to facilitate transmission (Hovius *et al.* 2008).

Salp 25D: It is expressed in *I. scapularis* gut and salivary glands (dual action) and is homologous to peroxiredoxin antioxidant. It protects *Borrelia* from oxygen metabolites produced by neutrophills and facilitates *Borellia* acquisition by ticks (Das *et al.* 2001, Barr and Gedamu 2003). Immunization with recombinant Salp15D reduces *Borellia* acquisition by *I. scapularis.* Therefore, it can be used as vaccine to interrupt life cycle of spirochetes but does not influence the transmission of pathogen from tick to mammalian host (Narasimhan *et al.* 2007).

Tick histamine release factor (tHRF): It was identified from *I. scapularis* and is secreted in tick saliva. It has a role in tick engorgement and transmission of *B. burgdorferi* (Dai *et al*. 2010). Silencing tHRF by RNAi resulted in impaired feeding of ticks and decreased the levels of *B.burgdorferi* infection in mice. Transmission of spirochetes was also reduced.

Tick salivary lectin pathway inhibitor (TSLPI): These are salivary protein expressed in *I. scapularis* which are responsible for protection of *B. burgdorferi* by preventing it from direct killing by host complement system. Silencing TSLPI mRNA reduces *Borrelia* load in nymphs and impairs transmission to mice but immunization with rTSLPI does not completely block bacterial transmission from tick to host (Schuijt *et al*. 2011).

Gut antigens

Vaccination of cattle with pure native Bm 86 antigen (present in micro-villi of *R. microplus)* with FCA and FIA resulted in reduction in number of engorged ticks (70 and 61% in two trials), reduction in weight of surviving ticks (29 and 37%) and reduction in egg laying capacity with overall reduction of greater than 90%.

The major problem to overcome was to produce the adequate quantity of antigen cheaply for a vaccine to become cost effective, which became possible by adopting recombinant DNA technology (Tellam *et al*. 1992).

Tick guard and Gavac are the two commercially available vaccines in Australia and Cuba respectively, which have been produced by recombinant DNA technology based on Bm 86 antigen (89 kDa). The concealed Bm86 antigen has been expressed both in *Escherichia coli* and *Pichia pastoris.* Protective mechanism of these is based on the development of antigen specific antibodies in immunized host that interact and subsequently effect the biological function of target antigen within the tick thus impairing tick feeding on the immunized host. Bm 86 vaccination trials resulted in reduction in incidence of babesiosis as well as reduced tick infestations in vaccinated cattle (Willadsen. *et al*.1995, De la Fuente *et al*. 1998). Three additional membrane bound antigens, viz. Bm91 (86 kDa), BmA7 (63 kDa) and Bm95 have been identified from the gut and salivary glands of *B. microplus*. Bm 95 is homologue of Bm 86, also expressed in microvilli of midgut of ticks (Willadsen 2001). Vaccines based on these antigens have also produced effective control of *R. microplus* and *R. annulatus*. These vaccines also induced the partial immunity against *B. decoloratus, R. appendiculatus, H. anatolicum* (De vos *et al*. 2001, Leal and Ferreira 2021). Tick guard plus which comprises of *E. col*i expressed Bm 86 and Bm 91, was produced in Australia. Vaccination with tick guard plus resulted in reduced number of engorged ticks, reduced production and hatchability of eggs (Jonsson et al. 2000, Tramboo *et al*. 2021). A Bm 86 ortholog of *H. anatolicum anatolicum* Haa86 has been identified and experimentally tried at IVRI which reduced the transmission of *Thelaria annulata* and protected cattle against homologous tick challenge (Azhabianambi *et al.* 2009).

Serine protease inhibitors: A number of host defence reactions such as blood coagulation and complement activation are mediated by cascades of serine proteases. Recombinant serine protease inhibitors (serpins) from several tick species have been examined as candidate vaccine targets (Maritz Olivier *et al.* 2007). A protease inhibitor from *H. longicornis* (HlS2) resulted in 40% mortality of nymphs and adults fed on immunized rabbits (Sugino *et al.* 2003). Four serpins from *R. appendiculatus* RAS1-4 were tested either as mixture of recombinant RAS-1and RAS-2 (reduced the no. of engorged nymphs by 60% and mortality of males and females by 43% and 28% respectively) or cocktail composed of RAS-3, RAS-4 and immunodominant RIM3 (affected the mortality of *Thelaria parva* infected female (Imamure *et al*. 2006). Recombinant serpin from *I. scapularis* IRIS resulted in significant protection against nymphs and adults fed on vaccinated

rabbits (increased mortality by 30% and decreased weight gain) but had no effect on mice (Prevot *et al*. 2007).

Aquaporins: Ticks require an effective water transport mechanism so as to concentrate the blood components for effective digestion. These aquaporins originally called as water channels allow the regulation of water transport across highly hydrophobic lipid bilayer of cell membrane, therefore aquaporins present another target for vaccine development. IrAQP1- aquaporins from *I. ricinus,* resulted in 50% reduction in weight of semi engorged females that were fed for 5 days. (Campbell *et al*. 2010). RmAQP1-aquaporin from *R. microplus* showed 75% efficacy in cattles, therefore, a promising vaccine candidate (Guerrero *et al*. 2014).

Acid peptidases: Blood digestion occurring in digestive cells of tick gut epithelium is performed by acid peptidases like aspartic peptidase of cathepsin D type, cysteine endo and ecto peptidases of papain type (cathepsin B, C and L) and asparginyl endopeptidase of leguminin type. Therefore these digestive enzymes offer multiple potential antigens for tick control (Sojka *et al.* 2013). Immunization of rabbits with cocktail of recombinant cathepsin D, B, L, C and leguminin from *I. ricinus* resulted in high antibody titre, but only slight mortality of *I. ricinus* females (Franta *et al*. 2011).

Ferritins: These are the iron storing protein and have a critical role in homeostasis of iron during feeding thus forming another target for vaccination (Kopacek *et al*. 2003). Ferritin 2 (rmFER2) knockdown by RNAi and vaccination of rabbits with recombinant protein resulted in reduction in feeding, oviposition and fertility of *I. ricinus, R. microplus* and *R. annulatus* (Hajdusek *et al.* 2010)

Other miscellaneous antigens

Vitellogenesis and fertility enzymes: Impairment of vitellogenesis especially embryogenesis and fertility by vaccines would be promising for the control of ticks especially *R. microplus*. Three enzymes involved in vitellogenesis in *R. microplus* have been identified as 2 aspartic peptidase, *Boophilus* yolk cathepsin (BYC) and cathepsin L like vitellogenin degrading cystine endopeptidase (VTDCE) (Logullo *et al.* 1998, Sorgine *et al.* 2000). Immunization of bovines with BYC and VTDCE and GST resulted in reduction of semi-engorged females by 50% and increased body weight in vaccinated calves but individually these antigens had low efficacy (Parizi *et al.* 2011 and Seixas *et al*. 2008).

Glutathion-s-transferases: They have important role in metabolic detoxification, reactive oxygen species, heme and other toxic components (Zhan *et al.* 2005). Potential of *H. longicornis* GST was examined in cattle against *R. microplus* infestation. It resulted in 50% reduction in number of ticks (Parizi *et al.* 2011).

Transmission blocking vaccines

TROSPA: TROSPA (Tick receptor for outer surface protein A) is the receptor for *B. burgdorfer*i OspA (Outer surface protein) found in tick gut and is essential for colonization of *B. burgdorferi*. Blocking of TROSPA with TROSPA anti sera or via RNA interference reduce the adherence of spirochete to the gut of *I. scapularis* thus decreasing bacterial colonization and pathogen transmission. OspA has been used as vaccine candidate against pathogen transmission (Pal *et al*. 2004). Vaccination of mice with OspA inhibits the transmission of pathogen to tick (Tsao *et al.* 2001). Combination of OspA and TROSPA enhances the vaccine efficacy.

64TRP and Salp 15: It is the cement antigen (salivary protein) from *R. appendiculatus.* Since it blocks the transmission of tick borne encephalitis, it is also considered as the candidate antigen for transmission blocking vaccine. Similarly, Salp15 which is the immunosuppressive protein identified from *I. scapularis* and vaccination with the candidate antigen separates Salp15 from binding with OSP of *Borellia*. Hence, it is considered as vaccine candidate for transmission blocking vaccine.

Vaccines directed against multiple species

Tick subolesin: Subolesin (4D8), ortholog of insect and vertebrate akirin is a highly conserved protein involved in modulation of tick feeding and reproduction and was discovered in *Ixodes scapularis.* Only one subolesin gene has been discovered in invertebrates which is evolutionary and functionally related to AKR2 (vertebrate akirin). It has a role in tick immunity. Knockdown of SUB by RNAi affects the genes involved in cellular pathway, reduces innate immunity of ticks resulting in higher levels of infection. Indirectly, it affects tick tissue structure, function and expression of genes required for pathogen infection (de la Fuente *et al*. 2006 a and b, Zivkovic *et al.* 2010). Preliminary experiments using the recombinant *I. scapularis* subolesin (SUB) have shown a good response against *Dermacentor variabilis* and *A. americanum* (Almazan *et al*. 2003). Vaccines containing SUB epitopes have been shown to protect animals against ticks, mosquito and sand fly infestation thus suggesting the possibility of developing universal vaccine against arthropods (Pulcini *et al.* 2013).

DNA Vaccines

To control the tick infestations, various researchers focused on developing DNA vaccines (Rodriguez-Vivas *et al*. 2007, Wikel 2018). DNA fragments encoding specific sequences or whole gene is introduced into body via bacterial plasmid. The injected plasmid DNA enters the nucleus and remains inside the nucleus as episomal DNA generating protective antigens continuously as long as cell is alive (Myhr 2017, Ghaffarifar 2018). Humoral immune response plays an important role in tick immuniny, therefore, the DNA vaccines should be designed in such a way that it restricts the immune response of host towards Th2 response (Abbass *et al*. 2023). DNA vaccine development is still in its infancy for ticks of veterinary and medical importance (Ghosh *et al*. 2007).

DNA vaccination of Merino cross breed sheep against *Boophilus microplus* using Bm 86 full length gene, resulted in marginal decrease in mean engorgement weight of female ticks. Co vaccination with Bm 86 and GM-CSF plasmids caused significant reduction in fertility of ticks however, Tick guard plus was found to be 25 times more effective than DNA vaccination (De Rose *et al*. 1999).

Para myosin (Pmy) a myofibrillar protein in invertebrates is an immunomodulatory protein, which plays an important role in immune reactions. Eukaryotic expression plasmid DNA incorporating gene coding for Pmy was constructed and used to vaccinate the rabbits. Pmy DNA vaccine elicited specific immune response and partially protected against *H. longicornis* and ultimately resulted in significant reduction in engorgement weight, oviposition and size of adult female tick (Zhang *et al*. 2017).

Anti-tick microbiota vaccine

Tick microbiota can impact the tick physiology and vector competence. Larvae with perturbed micro biota results in alteration of gut peritrophic membrane and consequently binding of spirochetes to epithelium (Wu-Chutang *et al*. 2021). Inhibition of peritrophic membrane chitin binding protein altered PM integrity in *I. scapularis* and hence altered *B*. *burgdorferi* colonization and transmission to mice (Yang *et al*. 2021).

In addition to microbiome, the adult females of various ticks are dominated with endosymbionts like *Coxiella* (*D.silvarum*), *Rickettsia* (*I. affinus*), *Francisella* like endosymbionts (*Hyalomma lusitanicim*). They are suggested to be nutritional endosymbionts, provides cofactors for vit-B, amino acids and de novo synthesis of folate (Duron *et al*. 2018). Endosymbionts of arthropod vectors can be manipulated in different ways to control vector borne diseases. It can be attempted by utilizing *Wolbachia* by chemotherapeutic, immunological and *Wolbachia* cytoplasmic incompatibility based approach. Immunization of animals with whole killed endosymbionts or purified or recombinant antigen will render them immune to tick vector. The concealed antigens or mid-gut antigens of ticks are the potent targets being exploited. Following the injection of blood meal antibodies in the immunized host will be passed in the gut of ticks destroying the endosymbionts in turn leading to death or disruption of tick gut physiology (Gupta *et al*. 2012).

Conclusion

As the world is heading towards post-insecticidal and acaricide era there is an urgent need for the development of new and novel vaccines against ectoparasites in general and ticks in particular. Evaluation of new vaccine formulations (candidate antigen) against different tick species in multiple hosts with special emphasis on development of broad spectrum tick vaccine. Animal trials should be constructed to evaluate different adjuvants and delivery systems including DNA vaccination, identification of biological functions of antigens and regulation of gene expression, characterization of host immune response generated by vaccine antigens, and incorporation of antigens in tick vaccine that block the transmission of tick borne pathogens. Integrated tick control strategy in which tick vaccination would be major component may be most productive, cost-effective and environmentally sound approach to control ticks in future.

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