ISSN 2449-8955 European Journal of Biological Research *Review Article*

DOI: http://dx.doi.org/10.5281/zenodo.6386931

Tick saliva antigen-based vaccines, disease protection and prophylaxis

Nidhi Yadav, Ravi Kant Upadhyay *

Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur 273009, U.P. India

* Corresponding author: E-mail: rkupadhya@yahoo.com

Received: 04 December 2021; **Revised submission:** 01 March 2022; **Accepted:** 23 March 2022

https://jbrodka.com/index.php/ejbr

ABSTRACT: This review emphasizes the immune responses to tick infestation and the administration of vaccine to save the life of man and his livestock. There are so many vaccines in operation in various parts of the world. These vaccines have been developed by using tick saliva toxins or recombinant antigens synthesized. This article explains the use of modern molecular tools such as genomics and proteomics in identification and search of new potent antigens which could prepare sizable defense against tick-borne pathogens. The present article also highlights explorations on salivary gland secreted molecules, genes and their expression for preparation of the highly efficacious targeted anti-tick vaccine. There is a need to search feeding inhibitors of ticks so that pathogen transmission can be blocked and easy disruption of enzootic cycle become possible. In addition, protein antigens from tick midgut must be searched to have a new multi-target vaccine to counter-attack tick infestation in various animal and human hosts.

Keywords: Tick; Vaccine; Tick-borne.

1. INTRODUCTION

Ticks are ubiquitous blood-sucking (hematophagous) arthropod external parasites. These suck blood and feed on various classes of terrestrial animal hosts mainly livestock and wild mammals. These are major vectors of various human pathogens and animals worldwide. Tick parasitism is maintained by its various life stages and completes among different hosts and mainly domestic, wild and zoo and dairy farm animals. The main factors of tick parasitism are blood-sucking and saliva secretion, host immune status, age, breed and local ecology. All these factors play important role in the tick-borne pathogen harbor, release and morbidity in man and other animals [1]. For feeding ticks grasps the skin, cut the area of attachment and insert their feeding tube and cut into the surface [2]. Ticks transmit various disease pathogens and cause morbidity and pathogenesis in humans and animals around the world. Tick infestation is noted almost in America, Europe, Africa, Australia and Asia, *R. microplus, Rhipicephalus (Boophilus) microplus*, *Amblyomma* and *Ixodes* ticks are the medical tick species which transmit so many tick-borne pathogens in humans. Ticks use hypostome to puncture the skin, during feeding ticks discharge saliva in the blood of host, through which easily transmit disease pathogens. Moreover, anticoagulatory molecules found in tick saliva interfere with the host defense mechanisms. These pathogens evoke immune responses after an interaction to cells and tissues of immune function. Thus, ticks salivary secretions transmit a variety of disease pathogens bacteria, viruses, and

protozoans in man, livestock and wild animals [3]. Ticks are the major cause of morbidity and mortality; they generate significant economic losses to dairy owners [4]. Hard ticks feed very slowly but establish long-term blood-feeding and much extended parasitic morbidity and show specific host responses [5].

There is one unique property of ticks that they are resistant to saliva pathogens and have evolved molecules of immune defense. These provide tolerance to pathogens; and assisted in the development of immune protection. The main factor which establishes the ticks on hosts is salivary secretion and the multistage life cycle of ticks. Saliva upon secretion acts like a carrier for diverse group pathogens and easily transfers them into the bloodstream of human and animal hosts. Due to their adaptability to adjust always to new conditions makes ticks more perfect transmitters of pathogens of several human diseases and other animals. Ticks transmit pathogens during the normal course of feeding, from an infected host to an uninfected host. After blood-feeding on infected host, pathogens easily transfer into the gut, from where they reach to salivary glands via hemolymph. Again during feeding, ticks transfer these pathogens to another subsequent host [2]. Many tick species secrete a cement-like substance that keeps them firmly attached during the meal. It is a complex protein that assists ticks in blood-feeding the transfer of disease pathogens. More specifically both tick salivary gland secretions and host-derived compounds modulate long-term tick feeding [6]. In addition, some pathogens live inside the ovarian tissue of the tick and transmit pathogens directly to their progeny. In ticks, saliva plays important role in feeding, the establishment of hemostasis and defying host immune responses. It also assists in the formation of a favorable niche for the survival and inoculation of various disease pathogens.

Tick bite causes severe inflammation on biting sites, swelling and pain. After 2 days it converts into red patches on the skin and marks as allergic signs on the skin. This allergic dermatitis develops due to contact of saliva components during its release on the skin, and due to expression of IL-6, CXCL-8 and CCL-2 genes and synthesis of pro-inflammatory chemokines. More often, low expression of these genes affects the production of salivary gland proteins that also decrease host immunity, inflammation and coagulation [7]. This shows a delayed-type response in susceptible hosts. To combat allergy granulocytes and T lymphocytes are recruited [7]. However, vaccines prepared gut proteins or antigens obstruct both feeding and transfer of pathogen at the tick feeding site [8].

Though, for the protection of livestock so many countermeasures have been developed. But repeated tick infestations result in the development of natural immunity in livestock. It protects from tick bites and blocks the transfer of disease pathogens. But it does not seem satisfactory in controlling wider tick attacks. However, for control of ticks various acaricides are used but these were not proved sufficient in successful control of ticks. Its repetitive use and exposure cause problems to host skin as these absorb through the skin and reach in the blood. It has become a major cause of drug resistance in ticks; contamination of dairy products and fouling of surroundings. It is true that dairy products obtained from treated dairy animals contain acaricide residues which persist for a longer duration. These also impose adverse effects on the environment and human beings. Therefore, to avoid undesirable physiological effects of acaricides and environmental contamination vaccines are proved much better and appropriate solution.

However, before the development of an appropriate vaccine all related factors which essentially target tick saliva feeding must be known. These major factors are host-parasite interaction, its participatory molecules, pathways, gaps and interconnections, tick immunity mainly cellular and molecular components of humoral and cellular immunity must be known. In addition, adverse effects of tick antimicrobial compounds on signaling pathways, metabolism and physiology of the host body must be known [9]. In addition, various

antigens from tick saliva or gut proteins released by ticks during feeding must be identified. These could be used as appropriate molecules for generating potential vaccines to stop blood-sucking by ticks. Feeding inhibition by administration of vaccine will also help in obstruction pathogen transmission from the tick to hosts. There is a need to purify and obtain new novel and highly effective vaccine antigens to control tick-borne pathogen attacks [10]. However, for the preparation of effective and appropriate vaccine tick tissue extracts or saliva toxins could be used as antigens.

2. TICK-BORNE DISEASES AND ECONOMIC LOSS

Ticks are ectoparasites and major disease transmission vectors among all arthropods. Tick menace is the biggest challenge for livestock and dairy owners in tropical and subtropical regions of the world. Though for control of tick menace so many traditional control methods have been applied to kill this dreadful parasite, but it has gained resistance to acaricides and made heavy to the cattle industry [11]. There are so many species of soft and hard ticks which parasitize different hosts and doing annual losses of billions of dollars. Tick menace is severely affecting dairy farming units as the annual loss has been enhanced up to ~ $22-30 billion annually around the globe. Around 80% of the global loss is only due to *Rhipicephalus microplus* transmitted infection [12]. *Rhipicephalus microplus*, is a monoxenous tick that causes massive damage to livestock in India and the world. There was obtained variability in tick infestation Cattle breeds mainly taurine breeds display higher loads of the tick parasite while indicine breeds are somewhat lesser [13].

3. HOW TO COUNTER-ATTACK TICK INFESTATION

For tick control, various strategies and methods have been applied so far. All these are based on controlling the spread of tick-borne pathogens which after transmission affect human and animal health. Tick-borne diseases spread with the close interactions of human contact with livestock and wild animals. Therefore, interaction and exposure of wild animals to humans must be reduced [14]. Hence, there is a need to understand the complex cycle of ticks, its associating hosts and various pathogens transmitted in the human population. There is a need of disruption of tick life cycle among various mammalian hosts by using alternative approaches. It could become possible by applying cultural, acaricidal rotation, use of synergists, mixed pesticide formulations, use of biopesticides, safe animal care methods, and selection for host resistance, nutritional and environmental management. For providing protection cover regular vaccination of farm animals is highly important with dip bathing by use of plant origin acaricides, plant extracts and essential oils. However, the systematic use of different control methods will help to eliminate the acaricide-resistant tick population [15].

Tick saliva contains pathogens which after release into the host bloodstream show immune interactions and the host body starts preparing both innate and acquired immune defenses. In response to control agents ticks have developed resistance to them, ticks also subvert immune defense prepared by various hosts [16]. Ticks also have evolved adaptations to chalk out immune functions of crossbreed livestock and challenged its genetic constitution [17]. This leads to a lower efficacy of recombinant vaccines. Hence, there is a need to find certain uniqueness in modules and gaps to conquer and finish the tick menace. There is a need to focus on the search of new noble antigens, biologicals, signaling molecules, complement molecules, cytokines, death programmers, feeding inhibitors, antimicrobials, antibodies and vaccines [2]. Lastly, new molecules could generate strong innate immune responses and break the adaptational cover made by various tick species in form of immune tolerance and efficiency to resistance to their own pathogens [2].

Vaccines are more effective against ticks as they easily disrupt feeding in ticks, thereby blocking pathogen transmission among hosts and much able to break enzootic cycles [3]. It should reduce exposure of infected ticks to humans, domestic and wild animals [3]. Recombinant vaccines provide long-term cover as they generate enough immune protection against diverse tick-borne pathogens. These are cost-effective and do not put any harm to the surrounding environment. These are the best alternative to replace chemical acaricides. Hence, there is an immense need to search for new protective antigens for vaccine development. For this purpose, host-pathogen interaction, transmission methods, mechanisms of acquisition, and persistence of parasitism must be known. More often, all concerned attack points and elements of the tick life cycle must be identified [18]. It will not only help in the success of vaccine programs but also prove a sharp-edged weapon to kill acaricide-resistant tick populations [19]. There is a need to search a series of noble and unique feeding inhibitory devices, molecules, genes, biochemicals to repel ticks from taking a blood meal and target the adaptational shield of ticks [12]. However, combined methods and combined efforts are essentially needed to finish the biggest challenge given by ticks to medical and veterinary scientists and public health institutions to control tick-transmitted pathogens [17].

There is a need to develop commercial and low-cost vaccines, but till date only two vaccines have been prepared which are in use and available in the international market. A single vaccine has been developed by using tick midgut protein Bm86 as an antigen. It protects from tick bites and the transfer of disease pathogens. It shows limited efficacy to only some tick species and is currently used for field applications [20]. A midgut glycoprotein Bm86 was used as an antigen to prepare TickGARD and Gavac vaccines. It was found highly effective against *R. microplus* tick found in different geographical areas [21].

4. HOST ANTIBODIES

Sera from tick-susceptible Holsteins contain antibodies, which can neutralize tick salivary proteins involved in parasitism. Alternatively, antibodies can prohibit tick feeding and thereby control tick infestations [22]. Antibodies synthesized against tick-protective antigens can successfully control tick-borne pathogens [9] and blood-feeding by nymphs [23]. However, administration of a live vaccine having Enterobacteriaceae bacterium *Escherichia coli* strain BL21 produces anti-*E. coli* and anti-α-Gal IgM and IgG. These antibodies efficiently kill *I. ricinus* nymphs by inhibition of feeding in them [24]. Aquaporin antigen was used to make a vaccine that targets infestations of *R. microplus*. Being an active ingredient in cattle vaccines it has increased the effectiveness of vaccines and reduced the numbers of adult female ticks from blood-feeding [25].

5. HEMOSTASIS

For establishing parasitism in various hosts, ticks synthesize and secrete many anti-haemostatic molecules such as vasodilators and a group of peptides which inhibit blood coagulation. These act as platelet aggregation inhibitors and increase fibrin breakdown in the host [26]. These anticoagulants disrupt elements of both the intrinsic and extrinsic pathways. These stay at the injury spot or cut mark, and assist in providing uncoagulated uninterrupted supply of blood to the ticks from the blood pool. Thus, host hemostasis is challenged due to the release of antagonistic molecules or vasodilators at ticks biting sites on host skin. Most of these vasodilators are lipid derivatives, mainly prostacyclin and prostaglandins [27]. Ticks also secrete histamine release factor (tHRF) protein molecules, which stimulate histamine, interleukin 4 and IL-3 production from IgE-sensitized basophils and mast cells inside the host body. Ticks also synthesize four categories of serine protease inhibitors i.e. Kunitz domain inhibitors, Kazal domain inhibitors, trypsin

inhibitor-like cysteine-rich domain (TIL) inhibitors, and serpins. Serine proteases or serpins secreted in tick saliva stop the action of a hemostatic system [28]. These also inhibit the activity of trypsin and thrombin [26] and obstruct platelet aggregation and blood clotting [29,30]. Tick salivary serpins do indirect inhibition of mainly factor X and thrombin (IIa). Few tick species also synthesize Salp14 and tissue factor pathway inhibitors. These inhibit the action of factor X and factor VIIa [26,31]. In addition, there are numerous salivary proteins i.e TIX-5 which act as an anticoagulant and halt the action of factor V and Xa. Few of them are reported in *I. scapularis* saliva, which accelerates fibrinolysis, degrades fibrin clots and acts as metalloproteases [32].

Due to multiple tick bites and mixing of salivary molecular repertoire mainly anticoagulants hosts due to invasion and tick multiple bites severely destroy and damage blood vessels and capillary endothelium. In response to tick bites, several agonists such as ADP, thrombin and collagen are secreted from the host body. These bind to specific platelet membrane receptors and activate platelets. Tick saliva proteases inhibit platelet activation and aggregation at different stages. More often these substances block the binding of fibrinogen to activated platelets [26]. Ticks *I. scapularis* transmit *B. burgdorferi*, secrete proteins that directly or indirectly support the multiplication of *B. burgdorferi*. After blood-feeding both tick pathogens secreted molecules and tick components interact with each other that support pathogen persistence and transmission [33]. Lipocalin (ISCW005600) is an inhibitor of lectin pathway inhibitor is secreted by *I. scapularis* [34]. Thus, ticks maintain continuous blood-feeding throughout life. Similarly, in response to repetitive tick bites host body also develop a multi-layered defense system against foreign pathogens and ectoparasites, coagulants try to check blood loss and maintain homeostasis [34].

6. SEARCH OF ANTIGENS AND VACCINE PRODUCTION

6.1. Identification of elements related to tick pathogens' life-cycle

Various recent molecular and genetic methods have been used to find novel antigens (Figure 1) for the generation of vaccines for tick control. For this purpose, salivary gland transcriptome analysis is performed to find functional genes which are responsible for the synthesis of toxins [35]. By using genomics, and proteomic methods to novel antigens can be identified for the preparation of a vaccine against ticks. The main focus is required on saliva secreted antigens associated with tick gut. Tick salivary secretions are quite important for their reproduction and survival. Once the action of saliva proteins will be inhibited pathogen transmission is also inhibited. By using a similar concept anti-tick vaccines were made by using inhibitory molecules as antigens. The administration of these vaccines in livestock prevents tick feeding and efficiently prevents infections [36] finally controlling blood-feeding nymphs and adults [6,8].

Tick saliva is a natural source of toxins which could be used as an antigen. It interplays between ticks and host's immune system is affected by various antigens, these evoke sizable immunity in host's body and mostly used for the production of vaccine. For the generation of natural protection tick salivary extracts from ticks are administered as immunogen. It was found successful when salivary gland extracts of *Rh*. (*B*.) *annulatus* tick were injected in cattle. It leads to the synthesis and secretion of protective antibodies against saliva antigens. It is a safer non-chemical method of tick control [37]. Thus, immunization with anti-tick vaccines inhibits feeding efficiency in ticks that also stop the spread of various disease pathogens. Therefore, the production of an effective and appropriate vaccine production, identification, purification and characterization is highly important to have a noble antigen. Few cell-surface proteins are expressed which are also known as exposed antigens are released in tick saliva during blood feeding. The second type of antigens

is hidden antigens which are very hard to collect and concealed type. The third type of antigens is very hard to identify and possess properties of both exposed and concealed type vaccine. This third category of antigens is widely used for the production of vaccines with broad-spectrum anti-pathogen efficacy. These were found effective against both immature and adult stages of ticks [36]. In comparison to acaricides, vaccines are the environmentally safer method to control ticks. This is true that appropriate anti-tick vaccines are required to combat tick-borne diseases [3].

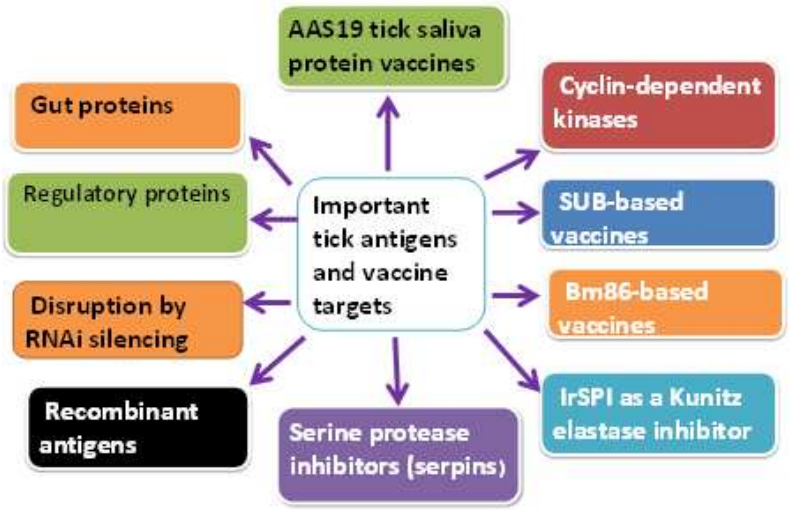

Figure 1. Important tick vaccine antigen targets for control of pathogenicity and tick infestation.

Genes which are responsible for the synthesis of toxins could be used to make the highly efficacious vaccine. These are based on protective antigens which provide protection cover against tick bites and tick-borne diseases. By using genetic and DNA sequencing methods new genes can be identified and their expression libraries can be used for searching new antigens for immunization. Therefore, by using expression sequence tags of various genes and their annotations, and comparison with available antigens can accelerate the antigen screening. It will also assist in the opening of the new comprehensive way for having an appropriate antigen/s for generation of highly effective against tick-borne pathogens [38]. Differential expression analysis is also being done to identify differentially expressed genes (DEGs). Many of these genes found in hosts are related to pathogen susceptibility or resistance to tick bites and feeding. Besides this, certain genes might control stress response during blood feeding [35].

The dilemma of tick-host and pathogen interaction can be resolved by identifying and encountering tick-origin infectious agents and antigens [21]. These unique molecules could control tick feeding and pathogen transmission by so many species of ticks not only *Rhipicephalus* (*Boophilus*) *microplus* [35]. In addition, there is a need to find molecules which inhibit gene expression of saliva proteins which are responsible for hemostasis, inflammation and inhibit coagulation and platelet aggregation anticoagulation and suppress host immunity [7].

For searching novel, tick-protective antigens for the formulation of anti-tick vaccines various molecular and immunological methods are used [39]. For identification of functional gene transcripts of salivary proteins and for detection of parasite antigen encoding genes high-throughput microfluidic RT-PCR is used [40]. Immunogenetic and molecular biology tools are also used for epidemiological surveys and improve TBD prevention and control of ticks [40].

Further, exploration of molecules can also help in the analysis of cattle cross-breeding tolerance and tick infestation [42] and may prove highly beneficial for control programs to combat tick-borne diseases [10]. These novel candidates can serve as tick protective antigens for making potential vaccine prevention and control of many tick species [34]. Further, for control of ticks inhibitors of microRNAs (miRNAs) should be searched, because these are important regulators of gene expression. Once action microRNAs is blocked, the synthesis of saliva proteins is inhibited that will result in blood-feeding inhibition in ticks. Further by using, genetic methods manipulation of vector microbiome is also possible that can assist in tick control at a large scale. Genetically produced tick salivary glycoproteins are highly specific and viable vaccine targets to inhibit tick feeding and are found as anti-tick vaccine candidates [43].

For better control, tick feeding and its parasitism must be explored. All different tick species transmit pathogens through blood-feeding; it is also responsible for inter-host transmission or migration of pathogens from the gut lumen to the hemocoel. Through blood-feeding ticks release large numbers of pathogens salivary secretions which mix in host blood. Thus both saliva and its derived proteins transfer pathogens in the number of vertebrate hosts. These modulate responses at the site of attachment and biting sites and frame conditions, to make blood-feeding essays, uninterrupted. Besides, pathogens (spirochaetes *Lyme borreliosis*) tick saliva also contains diverse molecular components i.e. Iris, Salp15 which modulate T cells while complement proteins i.e. ISAC, Salp20, TSLPI activate B cells. Similarly, a close interaction has been observed between outer surface proteins of unfed ticks spirochaetes and OspA receptors found in gut region of ticks [45,46]. Spirochetes synthesize OspC a lipoprotein during the tick blood-feeding; this protein is down-regulated after transfer of *B. burgdorferi* to the mammalian hosts. While after feeding is upregulated [47]. OspC is very essential for *B. burgdorferi* infestation and transfer of this pathogen from gut to tick salivary glands for initiation of infection [48,47]. More specifically, OspC binds to Salp15 and saves spirochaetes from antibody-mediated killing. g. It is also responsible for their transmission into the host [49]. Tick antigens useful for vaccine production are presented in Table 1.

Table 1. Various tick antigens used for vaccine production with their efficacy and protection.

Vaccine candidate	Function	Method applied	Tick stage during discovery	Antibody/ screening used during discovery	References
Salivary proteins					
Sialostatin L2	Specifically inhibits cathepsin L activity in cytotoxic T lymphocytes	*I. scapularis* salivary gland cDNA expression library and sialome	*I. scapularis* salivary glands from fed nymphs and (un)fed adults	Inhibition of tick feeding by anti-sialantibodies	[64]
TSLP	Tick Salivary Lectin Path inhibitor	Gel filtration, salivary protein facilitating *B. burgdorferi*	Inhibiting the host lectin complement pathway	Inhibition of feeding in *I. scapularis*	[65]
BPTI-Kunitz thrombin inhibitors	ixolaris, pethalaris, Salp14 and BmTIs/RsTIs	Gel filtration, tick anti-haemostatic antigens	Block binding of fibrinogen to activated platelets	Inhibition of feeding in *I. scapularis* due to blood clotting	[26]
TIX-5	An anticoagulant salivary protein	Gel filtration, TIX-5 in salivary gland extract of *I. scapularis* adults	Inhibition of tick feeding	Render tick immunity	[75]
Tick cement cone protein 64P	Structural protein	Gel filtration escape host rejection during tick infestation	Escape host rejection during tick infestation	Render tick immunity	[77]
Ferritins	FER1 and FER2	Gel filtration iron during blood feeding and	Reduce tick infestations and the transmission of tick-borne pathogens	Anti-ferritin vaccine reduce tick feeding in *R. microplus*, *R. annulatus* and *H.*	[20]

Vaccine candidate	Function	Method applied	Tick stage during discovery	Antibody/ screening used during discovery	References
				longicornis	
HlFER and HlFER2	Iron-binding protein ferritin (HlFER), an intracellular 1 and a secretory HlFER2	Gel filtration, iron during blood-feeding	Inhibition of blood-feeding in ticks	HlFER2 anti-tick vaccine effective against multiple tick species	[20]
AAS19	Immunogenic tick saliva protein	Gel filtration, serine protease inhibitor serpin, AAS	Anti-haemostatic functions	Similar to trypsin-like proteases	[80,117, 123]
Concealed antigens	A heterogeneous group of proteins		Host immune system		[10,78]
		Gut proteins			
TROSPA	Gut-protein, binding partner of the *Borrelia* protein OspA	Screening of *I. scapularis* cDNA library	*I. scapularis* protein involved in pathogen colonization is TROSPA	*Borrelia* protein OspA, impaired *B. burgdorferi* acquisition	[9,46]
ISDLP and ixofin3D	Two new *Ixodes* gut proteins	Gel filtration	Reduced *Borrelia* loads in the skin 7 days after tick challenge	Antibody/vaccine obstruct tick feeding	[86,87]
		Regulatory proteins			
Subolesin	Signal transduction, impaired tick feeding	cDNA expression library immunization and analysis of expressed sequenced tags	*I. scapularis* cell line (IDE8) derived from tick embryos	Tick-immunised mouse sera, Subolesin based vaccine obstruct tick feeding	[90,91]
OspA a Cyclin-dependent kinases CDKs	Participate in cell cycle control	cDNA expression library of Outer surface proteins	Recombinant CDK10 followed by tick challenge	Anti-cdk based vaccine obstruct tick feeding	[95]
			Recombinant antigens		
B. annulatus Bm86 ortholog, Ba86	Midgut membrane glycoprotein	rDNA technology	Expressed in yeast *Pichia pastoris*	Bm86-based vaccine obstruct tick feeding	[12]
IrSPI and IrLip1	Recombinant protein, Kunitz elastase inhibitor	cDNA expression library	Enhance tick engorgement and molting and decrease tick	immunomodulator implicated in *I. ricinus* feeding	[5,19,126]
Salp14	Factor Xa inhibitor *I. scapularis*	Salivary gland cDNA expression library	*I. scapularis* salivary gland	Tick immune rabbit sera	[74]
Salp15	Impair *B. burgdorferi* transmission and inhibits activation of murine CD4 positive T cells and human dendritic cells	Salivary gland cDNA expression library	Expression library engorged *Ixodes ricinus*	Tick-immune rabbit serum	[49,59,84]
Salp20	*I scapularis* complement inhibitor	Salivary gland cDNA expression library	Engorged *I. scapularis* nymphs	Tick-immune rabbit serum	[45,46]
Salp25D	Anti-oxidant protein	Salivary gland cDNA expression library	Engorged *I. scapularis* nymphs, impaired *B. burgdorferi* transmission	Tick-immune rabbit serum	[33]
64P/TRP	Tick cement cone, reduce tick mortality, and TBEV transmission, *Ixodes ricinus* tick feeding	Immunoblot analysis adult *Rhipicephalus appendiculatus* ticks	Salivary glands extracts	Antibody, tick-immune guinea pig serum	[77]
FER2	Binding and transport of iron, impaired tick feeding	Isolated and cloned from cDNA libraries	Unfed adult *I. ricinus* ticks and larvae of *Ornithodoros moubata*	cDNAs encoding ferritin	[84]
tHRF	Tick histamine release factor (tHRF) in *I. scapularis* saliva	2-dimensional fluorescence difference gel electrophoresis (DIGE)	*B. burgdorferi*-infected and uninfected *I. scapularis*	Salivary glands extracts none	[29]
Isac-1	*I. scapularis* complement inhibitor	Random screening of a salivary gland cDNA library by sequencing	Partially engorged female *I. scapularis* ticks feeding for 3-4 days on a rabbit	None, impaired *B. burgdorferi* transmission	[66]

Vaccine candidate	Function	Method applied	Tick stage during discovery	Antibody/ screening used during discovery	References
IRAC-I	*I. ricinus* complement inhibitor	Isolation from transcriptome of *I. ricinus* salivary glands	*I. ricinus* salivary gland of engorged adult female ticks	Antibody response, marginally impaired tick feeding	[29]
IRAC-II	*I. ricinus* complement inhibitor	Transcriptome of *I. ricinus* salivary glands	*I. ricinus* salivary gland of engorged adult female ticks	None	[29]
TSLPI	Tick salivary lectin pathway inhibitor	Immunoscreening of *I. scapularis* salivary gland yeast surface display library	*I. scapularis* salivary gland	Tick immune rabbit sera, impairs neutrophil phagocytosis and chemotaxis	[75]
Iris	*I. ricinus* suppress T cell proliferation, induces a Th2 immune response, inhibits production of IL-6 and TNFα	Analysis subtractive library from salivary glands	Unfed and 5 day fed female *I. ricinus* ticks	None, impaired tick feeding	[58]
Tix-5	Anticoagulant	Immunoscreening *I. scapularis* salivary gland yeast surface display library	Adult *I. scapularis* salivary glands	Tick immune rabbit sera	[62]
CDK10	Cyclin-dependent kinases participate in cell cycle control	Bioinformatic search for CDK homologues	*R. microplus*	None, impaired *Ixodes* feeding	[95]
Ir86-1 and Ir86-2	Bm86 homologue, gut protein *I. scapularis*	Bioinformatic search for Bm86 homologues	*I. ricinus*	None, no effect on weight/attachment	[108]
ISDLP	Gut-protein, binds to *Borrelia* and involved in *Borrelia* migration	Probing of *I. scapularis* midgut yeast surface display library	*I. scapularis* midgut	*Borrelia* proteins	[65]
Ixofin3D	Gut-protein, binds to *Borrelia* and involved in *Borrelia* migration	Probing of *I. scapularis* midgut yeast surface display library	*I. scapularis* midgut	*Borrelia* proteins vaccination, no effect on tick feeding	[87]

6.2. Salivary gland proteins

Tick saliva is important for pathogen transmission, as it takes place through saliva secretions during tick feeding. Tick salivary gland proteins are the main operators of blood-feeding and establishment of parasitic life on the host's body surface [50]. Repetitive tick feeding and secretion of various salivary proteins also partially raised immunity in the cattle herds. It plays a protective role against tick inflammation and inhibits feeding. These saliva proteins have pharmacological and immunological significance. These are the most appropriate candidate molecules for making a potential anti-tick vaccine which can work against various tick species and their associated pathogens [51]. More often, multiple tick saliva toxins and multiple antigens could be used to make a cocktail of tick antigens or multivalent vaccine [52]. Vertebrate host proteins have been detected post blood-feeding in tick midguts. Host blood is the main source of nutrients for ticks. For easy intake of blood meals ticks synthesize anticoagulants and other inhibitory proteins from their salivary glands during tick feeding and release pathogens which are responsible for pathogenesis. Thus both tick saliva toxins and pathogen secreted molecules interact to host immune molecules, interfere in signaling pathways and affect synthesis and secretion of protective metabolites and invade the host body. These molecules which are responsible for host-pathogen-interaction must be identified to explore new targets for the development of potent vaccines eradication of ticks and tick-borne diseases [53]. Few of them have been identified in *Rhipicephalus bursa* salivary glands genes which show differential expression in response to blood-feeding. These genes synthesize saliva secreted toxins which also raise adaptive immunity that is possibly induced upon TBEV infection and vaccination [54]. Few other protective antigens such as lachesin, vitellogenin-3, and

cement proteins have been identified which can be used as suitable antigens development of novel tick vaccines [55]. However, functional characterization of these tick saliva toxins is an important issue regarding vaccine and antibody generation [56]. These proteins could be used to develop anti-paralysis vaccines. Thus, both vector and/or pathogen secreted molecules could be used as protective antigens which might be cross-reactive to different tick species and most appropriate for the development of novel control strategies [55-57].

Till date, so many salivary proteins have been isolated and characterized tick saliva which have an essential role in tick-host and pathogen interaction. Certainly, these will prove the best candidates for the potential vaccine. Iris is a protein secreted from ticks from female *I. ricinus* salivary glands both by nymphs and adults. It causes impairment of tick feeding in vaccinated experimental rabbits [58]. Similarly, another protein Iric-1 a homolog of Salp15 is secreted by *I. ricinus*, that is essential for the transmission of *B. burgdorferi*s. *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii* and save these pathogens from antibody-mediated killing *in vitro* [62].

Ticks transfer so many pharmacologically active chemical components during blood feeding inside hosts. Normally hosts are repeatedly bitten by ticks. These releases a few immunomodulatory proteins like Sialostatin L2 which also generate adaptive immune responses after repeated tick bites. This protein acts as an antigen and is used in the active immunization of animals [64].

A tick salivary protein tHRF is secreted to do multiple functions. It induces the release of histamine from basophils and mast cells of mammalian hosts. It participates both in IgE-dependent and IgE-independent mechanisms. It leads to an increase in the recruitment of pro-inflammatory cells and increase vascular permeability and produces an itching response. This protein increases vascular permeability and blood flow just after the tick bite site [29]. Immunization with the recombinant protein blocks *Borrelia* transmission and tick feeding. This tHRF blocking could become the best strategy to develop vaccines which might inhibit the feeding of blood and transfer of tick-borne pathogens [29]. Similarly, a protein TSLPI, from *I. scapularis* inhibits the host lectin complement pathway and produces antibody response to infectious toxin antigens from various ticks species when mixed in host blood [65].

There are thousands of compounds isolated from tick saliva and most of them have been identified by transcriptomic gene sequencing methods [66-69]. Besides this, there are dozens of genes which are expressed in tick salivary glands [69]. The expression of these genes is responsible for homeostasis and pathogen transfer. A few important proteins are Salp15, lipocalins, metalloproteases whose function is still unknown [70,71]. Normally these proteins were detected post 24 hours of feeding; these assist in blood feeding and pathogen transmission [68]. Ticks and tick-borne pathogens need a suitable environment for pathogen establishment [72].

6.3. Tick anti-haemostatics

Tick saliva is a reservoir of pathogen-induced inhibitory proteins which interfere in host immune response and signaling. More specifically, tick *Ixodes ricinus*, secrete tick salivary glands. Iripin-3 protein shows anti-haemostatic activity i*n vitro* and immunologically active [50]. Iripin-3 impairs proliferation of $CD4^+$ T lymphocytes, cut down T helper type 1 immune response, and induction of regulatory T cell differentiation. It does proteolytic inhibition of serine proteases kallikrein and matriptase. Iripin-3 also inhibits the blood coagulation pathway and cut down the production of pro-inflammatory cytokine interleukin-6 by lipopolysaccharide-stimulated bone marrow-derived macrophages [52]. It modulates the adaptive immune response and decreases the survival of mouse splenocytes [50]. These generate make a favorable environment

for pathogen transmission. Other important proteins are salivary cystatins, these target two host cysteine proteases, cathepsin S and cathepsin C . Cathepsin S is required for antigen- and invariant chain-processing, while cathepsin C plays a critical role in processing and activation of the granule serine proteases [73]. Cystatin OmC2 found in the saliva of ticks inhibits the activity of several lysosomal cysteines [73].

Tick saliva also contains BPTI-Kunitz thrombin inhibitors, ixolaris, pethalaris, Salp14 and BmTIs/RsTIs, all these act as anti-hemostatic antigens [26]. Both BmTIs and Salp14 are injected in the host body to trigger immune response and obstruction of tick feeding through skin surface [26, 74]. Similarly, a recombinant anticoagulant salivary protein TIX-5 is also administered for immunization of experimental rabbits. Its homologs are also injected which show very high tick feeding inhibition and prepare a sizable immune defense in hosts [75].

For stabilization of parasitic life, survival and continuity on host body ticks essentially need blood feeding. Blood feeding is highly required for normal development and reproduction in ticks. In blood-feeding ticks, saliva and its derived molecules work as anti-hemostatic and immunomodulatory candidates. Among these proteins are lipocalins, metalloproteases, protease inhibitors including the Kunitz/BPTI-family, proteins with phospholipase A2 activity, acidic and basic tail proteins, and vitellogenins. These proteins also prepare tick immune protection [76].

6.4. Structural components

Tick saliva contains a large number of various non-proteinaceous substances and proteins are differentially produced and secreted during tick feeding on the blood of hosts. Ticks have also a cement-like substance that holds them during feeding. This is a complex protein polymerization substance secreted by ticks. A protein 64P assists in host attachment formation of cone-like structure [77]. It shows similarity mammalian skin proteins, and play role in host rejection during tick infestation if not secreted [78]. Truncated versions of the protein (64TRPs) showed significant adult and nymphal mortality in guinea pigs. It generates potent adaptive immune response [79].

Tick saliva and salivary glands contain exosomes which could play important role in therapeutics [80]. These can be isolated from the saliva of blood unfed ixodid ticks. Exosomes inhibit wound healing *via* downregulation of C-X-C motif chemokine ligand 12 (CXCL12) in vivo and upregulation of interleukin-8 (IL-8) at the tick–human skin interface [80]. Further, inhibition of IL-8 or CXCL12 delays exosome-mediated cell migration, wound healing, and repair process. In contrast, exogenous treatment of CXCL12 protein completely restored this delay and enhanced the repair process [80].

Ticks release various Kunitz inhibitors, serpins, and cystatins in saliva during feeding. Among Kunitz inhibitors serve as anti-hemostatic agents and obstruct blood coagulation and platelet aggregation. Serpins and cystatins work as anti-hemostatic effectors proteins. All these are potent modulators of the host immune system and manage tick-host-pathogen interaction.

Besides this, enzyme inhibitors mainly serine protease inhibitors (SPIs), have their involvement in various tick biological processes. SPIs are also secreted during tick feeding, and inhibit blood clotting but assist in blood digestion and nutrient extraction. SPIs secreted from tick hemocytes make innate immune defenses in ticks but severely affect host defense mechanisms.

6.5. Concealed antigens

'Concealed' antigens are those which do not stimulate an immune response during a natural parasite infection, typically because of their physical location. More specifically, these are heterogeneous proteins

which are excreted in intracellular space; these are not limited to a particular tick species. Among them are intrinsic membrane glycoproteins Bm86, which function as antigen synthesized inside tick gut. Livestock synthesizes antibodies against Bm86 but it remains unidentified from the host's immune system during a natural infestation. It may be due to carbohydrate determinants on many tick glycoproteins are cross-reactive immunologically and show non-specific reaction with carbohydrate determinants on tick glycoprotein [10] [78]. But its recombinant forms evoke immunogenicity when administered vertebrate host [78]. Antibodies generated against these antigens were effective and obstruct the transmission of pathogens by feeding inhibition. These could be generated in the required amount to raise sufficient levels of antibodies.

Bm86 is a gut protein secreted by tick *B. annulatus*, but its multiple orthologs are produced. This is an appropriate target of an anti-tick vaccine. Similarly, an Is86 antigen contains EGF-1, EGF-2, and EGF-3 domains. It is upregulated during *B. burgdorferi* infection but does not assist for tick engorgement during feeding. It only cutdown spirochete loads in the host skin. It may be useful in making anti-tick measures and fighting against tick-borne illnesses. Contrary to this, a cocktail of various tick antigens may be more appropriate to generate a multivalent vaccine to inhibit feeding in nymphs of *R. appendiculatus* and ably break the natural cycle of tick pathogen transmission [52]. Besides this, genes which encode tick gut proteins show differential expression to susceptible and resistant hosts. Possible inhibition of synthesis of these proteins mainly sialoproteins could help to achieve the target of complete feeding inhibition in ticks [81]. In addition, the level of immune functions may differ from host to host; hence, hosts with strong immune defense are resistant and they affect gene expression of proteins in tick salivary glands. Alum-adjuvanted antigens generate strong immunogenicity, by having more T-cell epitopes and preparing much better antibody responses against ticks [82, 83]. Alum-adjuvanted vaccines based on recombinant proteins have shown much better efficacy against tick feeding.

6.6. Ferritins

Ticks also generate iron-binding proteins i.e. ferritins during blood feeding [20,84]. FER1 is an intracellular form (FER1) while FER2 acts as an iron transporter in the tick hemolymph and is expressed in all life stages of ticks. By employing gene silencing and interference of RNA blood feeding and reproduction in ticks can be controlled [84]. Moreover, a vaccine generated by using recombinant FER2 protein mainly *I. ricinus* (IrFER2) found 98% efficacious against ticks bites [84]. It successfully inhibits feeding in *R. microplus*, *R. annulatus* and *H. longicornis* [20,84]. Ferritins are secreted almost in all tick species; hence a cross-reactive vaccine also becomes possible. Similar proteins HlFER and HlFER2 have been identified and characterized in hard tick *Haemaphysalis longicornis.* These are also highly effective antigen targets to make the anti-tick vaccine effective against multiple tick species [20]. Similarly, RNAi silencing of the fer1, fer2, and irp1 genes impose an adverse impact on the hatching rate and decrease post-blood meal weight in tick females [85]. Salp15 is a protein that is secreted by *Ixodes persulcatus* and *Ixodes pacificus* salivary glands. It is responsible for the transmission of Lyme disease spirochetes in hosts [85].

6.7. Gut proteins

Tick survival depends on gut synthesized proteins play an important role. These proteins assist pathogens multiplication, transfer to various hosts and generation of immune responses. TROSPA is a gut protein delivered in intercellular spaces and the luminal surface of the gut [48]. Its expression is seen in almost every life stage of tick. It is an important candidate for the generation of antibodies which significantly inhibited feeding in ticks [9]. Similarly, dystroglycan-like membrane-bound proteins ISDLP and ixofin3D are

synthesized by *Ixodes* gut [86,87]. ISDLP protein is upregulated upon both feeding and infection. Silencing by RNA interference (RNAi) of ISLDP reduced *Borrelia* in the salivary glands of the tick low down tick infestation. Vaccination against ixofin3D did not inhibit tick feeding and cutdown *Borrelia* loads significantly 1 week after tick feeding [87].

Tick *Hyalomma* (*H.*) *dromedarii* gut synthesizes their glycoproteins (range 40-97 kDa) [88]. Vaccines generated against these proteins successfully cut down the reproductive index and egg hatchability. GLPs are good immunogens and can be useful in the vaccination of cattle against tick infestation. An RNA-mediated interference does silencing of *pixr* in mice, PIXR impairs the ability of *B. burgdorferi* to colonize the tick gut [89].

Few other vaccine types were also generated by using a synthetic peptide. These were specially designed to target the neuropeptides which innervate *Ixodes Ricinus* salivary glands and hindgut. Administration of this vaccine makes a sizable immune defense against nymphs or larvae and of *Anaplasma phagocytophilum.* These myoinhibitory synthetic peptides (MIP) have been used to make multiple antigenic peptide constructs (MAPs) and used for immunization. These generate robust IgG antibody response and gave sizable protection against tick borne pathogens [19].

6.8. Regulatory proteins

Subolesins are gene regulatory proteins isolated from ticks; these are responsible for pathogen transmission to the hosts during blood-feeding. These proteins remain involved in signal transduction pathways. Subolesins are used to generate vaccines which work against diseases vectors, mosquitos, and sandflies other than ticks [90]. Effect of Subolesins was tested in cDNA and/or recombinant protein immunization experiments; it has provided protection against all tick developmental stages [91]. SUB-based vaccines are mainly used to provide cross-vector protection and could be used for the control of important tick species by inhibiting the transmission of pathogens [92,93]. In another method ticks essentially need blood hemoglobin for embryonic development, if the hemoglobin-depleted serum is, ticks embryogenesis could be stopped. More specifically, the acquisition of exogenous heme is essential for tick reproduction [94]. If heme and iron metabolism is interrupted it will help to manage anti-tick interventions [94].

6.9. Cyclin-dependent kinases

Cyclin-dependent kinases (CDKs) control both cell division and modulate transcription after receiving extracellular and intracellular signals in eukaryotes [95]. Cyclin-dependent kinases enzymes phosphorylate specific target proteins. This attached phosphate group during phosphorylation functions like a switch, making the target protein more or less active. But cyclins are proteins, associated to a Cdk, activate the Cdks. These are thought to be appropriate antigens and are used for the generation of the anti-tick vaccine. Upon immunization, these vaccines display inhibition in tick feeding and reproduction. Besides, protein antigen vaccines, Pmy DNA (Paramyosin DNA) vaccine generated a sizable immune response and show protection against *Haemaphysalis longicornis* blood feeding. It induces effective cellular and humoral immune responses in rabbits and protect against *H. longicornis* infection [96]. DNA vaccines show lesser side effects than the vaccines generated against attenuated pathogens [97]. Similar immune protection was obtained after immunization with *Toxoplasma gondii* 14–3-3/pSAG1 protein. It has generated high levels of IgG antibody responses in experimental animals [98]. Besides, the phylogeny of *Haemaphysalis doenitzi* was decided based on mitochondrial 16S rDNA [99]. A similar vaccine generated by using *Plasmodium berghei* circumsporozoite protein provided protection against malarial sporozoite infection as it cut down mosquito

bites [100]. Pmy vector series is a batch of expression plasmids that are used for recombinant production of single proteins and protein complexes in bacterial cells. These plasmids could be used to recombine the most appropriate molecular candidate for the production of an anti-tick vaccine [101]. In addition, pcDNA3.1 (+)-Pmy plasmid was also constructed that also display immune protection against ticks infections [102].

7. TARGETING BOTH TICKS AND TICK-BORNE PATHOGENS

Host blood proteins are essential for body metabolism and reproduction of ticks; therefore, blood feeding is highly important for ticks. However, obstruction of tick feeding and killing of pathogens released into the blood of hosts must be prohibited by adopting any method. Tick saliva proteins induce innate and acquired immune responses in host animals. As ticks rely on blood, these are established ecto-parasites, and no human protective vaccines is available against them. There are protein vaccines prepared from outer surface protein, OspA from *B. burgdorferi* . This was found active against both *Ixodes* species and its pathogen. This *Borrelia* antigen is approved by FDA in 1998 and a vaccine against it is available [103]. Though, these autoantigens are not of broad immunological use [104]. TROSPA and Salp15 interact are parasite proteins which were also found suitable for tick-pathogen combined vaccination [75,105]. Many antigens have been isolated from *Borrelia*, and the use of this multiantigen vaccine has much greater efficacy protection against tick-borne diseases [71].

Production of anti-Salp15 antibodies has shown much broader protective efficacy than OspA and OspC antibodies [29]. Hence, both tick and pathogen antigens could be used for making a more perfect vaccine that may be found active against many species of ticks [75]. More specifically, the structurally stable antigen will provide high efficacy against ticks [10]. In addition, anti-vector vaccines provide better protection cover against lethal vector-borne pathogens [106]. However, an anti-tick vaccine derived from a tick cement protein (64TRP) of *Rhipicephalus appendiculatus.* It shows greater protection in experimental mice against tick-borne encephalitis virus (TBEV) transmitted by infected *Ixodes ricinus* ticks [106]. The 64TRP vaccine also interrupts pathogen transmission but shows a local cutaneous inflammatory immune response at the tick-feeding site [106].

8. BM86-BASED VACCINES

Tick population shows polymorphism in Bm86 antigen genes, it is the main reason that existing Bm86 based vaccines are not so much efficacious. Hence, there is a need to make hybrid recombinant Bm86 antigens for having more effective vaccines against *R. microplus* and *R. annulatus*. BM86 is a midgut protein that is used for production of vaccine to immune cattle for tick control [107]. RNA interference of these gut proteins target feeding in ticks, and such vaccines provide wiser control against *R. microplus* infestations [108]. Bm86 gene is the most stable and found expressed in all life phases of many tick species. More specifically, Bm86 is expressed in lifelong in *R. appendiculatus* and *R. microplus*. This expression differs in one-host life cycle and in three-host tick *R. appendiculatus* [109]. Vaccination with Ba86 recombinant *B. microplus* gut antigen significantly cut down spread of tick borne pathogens. Ba86 blocks oviposition and egg fertility in two major species of ticks *B. annulatus* and *B. microplus,* respectively [109]. There is a need of induction of vaccine-mediated enhancement tick control by checking blood feeding from host skin [19]. Two more antigens subolesin and TROSPA are used to prepare vaccines which could inhibit feeding in ticks. These are specially used to control cattle tick *Anaplasma marginale* and its transmitted pathogen *Babesia bigemina* [90].

A recombinant Bm86 was prepared by using Gavac and Mozambique strains (99.6%) [91]. Gavac shows 55-100% efficacy against *B. microplus* infestations in grazing cattle 4-9 months after the first vaccination [11]. Bm86-based vaccine provides also shows high efficacy against some tick species having lower Bm86 sequence homology [109]. It shows 83.0% homology with Ba86 from *B. annulatus* [91]. This vaccine is used in Cuba, Colombia, Brazil and Mexico for veterinary uses. A similar vaccine prepared by using recombinant Ba86 antigen from Israel strain is administered to livestock from *B. annulatus* infection. Besides this, combined antigens are also used to increase the protection cover of tick vaccines [12]. For better efficacy of a vaccine, interactions among ticks and their resident pathogens will help to combat tick-borne illnesses [110]. EGF antigens show a partial reduction in spirochete loads in the skin [110].

Tick aquaporins (AQPs) are intrinsic membrane proteins which assist in the transport of water, glycerol and small solutes such as urea across the cell membrane. By using transcriptomic studies a cDNA that codes for aquaporin was characterized in cattle tick, *Rhipicephalus microplus* [25]. Both genetic and DNA sequencing methods were used to identify new genes of tick antigen proteins for immunization. From cDNA expression libraries annotations, and comparison with available antigens, novel antigen candidates can be identified. This most appropriate antigen/s can be used for the generation of highly effective against tick-borne pathogens [38]. More specifically, microRNAs (miRNAs) regulate gene expression in ticks. After infection rickettsial *Anaplasma phagocytophilum* infection, starts down-regulation of tick microRNA-133 (miR-133). It induces organic anion transporting polypeptide (isoatp4056) gene expression in *Ixodes scapularis.* Inhibition of microRNAs activity may stop bacterial survival in the ticks and its transfer to the vertebrate host [41].

8.1. Bm95 recombinant tick antigen-based vaccines

Bm95 recombinant antigen is used for immunization of calves, this antibodies generate against this antigen showed protective efficacy against *Rhipicephalus* (*Boophilus*) *microplus* ticks. These also cut down oviposition and affected egg hatchability in ticks parasitize over immunized calves [111]. The overall protective efficacy noted in Bm95 recombinant cattle tick antigen was 81.27% [111]. BM95 is a glycoprotein that shows more than 90% homology to Bm86 antigen protein. For achieving better efficacy the recombinant chimeric protein comprising tick BM95 immunogenic peptide is fused to the *A. marginale* MSP1a N-terminal found on the *Escherichia coli* membrane. This shows significant elevation in protective efficacy against *R. microplus* infestations in rabbits. Chimeric vaccines protection against multiple cattle tick infestations [112].

8.2. SUB-based vaccines

Subolesins are antigenic proteins which manage blood-feeding in ticks. These were first identified in *Ixodes scapularis* as gene regulatory proteins. These are responsible for the transfer of pathogens from ticks to the hosts during blood feeding. SUB-based vaccines after injection generate high levels of immune responses in cattle ticks [90] and stop feeding in ticks and inhibit the transfer of infectious agents in dairy farm animals [113]. These are used for the preparation of potential vaccines to control *Rhipicephalus appendiculatus*, *R. decoloratus* and *Amblyomma ariegatum* ticks species. These are major vectors of farmyard animals which impose multiple morbidities in ticks and affect milk production in them [114]. Similarly, the SUB-based vaccine control tick infestations and inhibits pathogen infection/transmission in cattle and sheep [113]. SUB vaccine antigens physically interact with histone. These are real actors of interaction between tick-host and pathogen and regulate all these important activities. In all the ways, subolesin suppress tick blood-feeding and reproduction using RNAi treatment. Moreover, importin-α interaction with subolesin reduces tick weight in

sub RNAi-treated ticks as it obstructs feeding [115]. *H. longicornis* Subolesin (HlSu) was identified and expressed as a recombinant protein using *E. coli* [115].

8.3. Disruption by RNAi silencing

Disruption of mRNA activity by RNAi silencing is an important molecular method for control ticks by inhibition of feeding on blood meals. Moreover, RNase III- causes degradation of dsRNA into small interfering RNAs (siRNAs), which form an RNA-induced silencing complex. Disruption of RNAi silencing is a unique way to control ticks. AAS19 is an immunogenic tick saliva protein that assists in blood meal feeding in *Amblyomma americanum*. This is an inhibitor of a serine protease (serpin, AAS) and trypsin-like proteases. It acts as a blood clotting cascade that shows anti-hemostatic functions. More specifically, if its mRNA is disrupted by RNAi silencing, blood-feeding could inhibit in experimental animals. In experiments rAAS19 was found highly immunogenic and as immunized rabbits show lesser infestation egg-laying. Tick engorged females which feed on AAS19 immunized rabbits were fully stopped from egg-laying. It also results in the termination of infestation. This is protein shows homology in its functional domain, which is 100% conserved across so many tick species [7]. No doubt rAAS19 can be used generation of cocktail tick vaccine [116]. Unfortunately, RNAi silencing was not found successful for Is86 genes. It could not influence tick engorgement or *B. burgdorferi sensu stricte* persistence from blood-feeding [110].

AP-1 is a transcriptional activator protein that is responsible for the transmission of pathogens by infected Ixodes scapularis ticks during feeding. This acts as a molecular switch and regulates expression of iafgp gene. RNAi-mediated silencing of ap-1 expression affects the synthesis of saliva proteins that inhibits feeding in *A. phagocytophilum* nymphal ticks [117].

Similarly, knockdown of the expression of kat mRNA alone or in combination with isoatp4056 mRNA significantly affected *A. phagocytophilum* survival and isoatp4056 expression in tick cells [118]. *A. phagocytophilum* specifically up-regulates *I. scapularis* organic anion transporting polypeptide, isoatp4056 and kynurenine aminotransferase (kat). This kat gene is involved in the production of tryptophan metabolite xanthurenic acid (XA), for its survival in ticks [118]. Non-coding RNAs or microRNAs regulate gene expression. These miRNAs are transcribed from DNA sequences into primary miRNAs and processed into precursor miRNAs, and finally mature miRNAs. If the function of these miRNAs is obstructed, survival of various bacterial pathogens in ticks in vertebrate hosts can be finished. Inhibition of miRNA activity by its homologs miR-133 mimic lower down isoatp4056 expression and bacterial burden in ticks *Anaplasma phagocytophilum* [119].

8.4. IrSPI as a Kunitz elastase inhibitor

Ixodes ricinus secretes serine protease inhibitor (IrSPI) which showed much similar activity to Kunitz elastase inhibitor. Its inhibition increases molting and mortality. It also acts as an immunomodulator and obstructs feeding in ticks [120]. After passing into host blood through the salivary gland secretions during blood-feeding find their way into the host body. It represses the proliferation of CD4+ T lymphocytes and proinflammatory cytokine secretion from both splenocytes and macrophages. It also acts work as an immunomodulator in ticks [120]. In addition, ticks midgut synthesizes thousands of proteins and secretes them during a blood meal. These modulate various host defense mechanisms [120]. Unfortunately, IrSPI and IrLip1 antigen-based vaccines failed to check pathogenicity against *I. ricinus* nymph transmitted pathogens.

Ticks salivary glands infected with *A. phagocytophilum* show differentiated regulation of apoptosis pathways. These were identified in *I. scapularis* nymphs and adult female midguts. The bacterial infection

was found enhanced after *A. phagocytophilum* infection. Bacterial infection inhibited the intrinsic apoptosis pathway in tick salivary glands. This was due to RNA interference-mediated porin knockdown that significantly increases tick colonization by *A. phagocytophilum*. It resulted in the inhibition of Cytochrome c release as the anti-apoptotic mechanism to facilitate bacterial infection [121]. These tick-host interactions can be used to make anti-tick vaccines targeting this immunomodulator implicated in *I. ricinus* feeding [122].

8.6. Serine protease inhibitors (serpins)

Serine protease inhibitors or (serpins) are responsible for tick's invasion of the host's serine protease-mediated defense pathways. Serpins in general inhibit multiple enzymes and control serine proteases activity, these are responsible for inflammation and blood clotting [122]. Serpins also block the action of digestive enzymes. Moreover, these also inhibit tick salivary molecules which are responsible for pathogen transmission and do tick-mediated skin immunomodulation and affect immune defense in many unique ways. These could be used for designing and production of new potential anti-tick vaccines [123]. Similarly, genes related to tick parasitism are differentially expressed and have a wide concern with the level and stage of host immunity. These could be used for the development of new sustainable molecular and immunological methods for tick control [13].

8.7. Inhibition of extracellular vesicles mediated pathogen transmission

Unfed ixodid tick saliva contains exosomes are small membrane-bound extracellular signaling vesicles which are secreted in tick saliva and salivary glands of partially fed or unfed ixodid ticks [124]. These vesicles assist in pathogen transmission from arthropods to humans and other animals diseases [80]. These are rich in CD63 ortholog protein and heat shock protein 70 (HSP70) [80]. If the formation of extracellular vesicles is stopped, lethal pathogens can be finished by using targeted therapeutic molecules. It has been experimentally proved as lethal pathogen *Francisella tularensis* transmitted by *Dermacentor andersoni* was stopped by using this method [125]. It was also found effective against Blacklegged tick, *Ixodes scapularis* [126]. These also block transmission of pathogens of some other tick-borne pathogen diseases like Lyme disease, anaplasmosis, babesiosis, *Borrelia miyamotoi* disease, Powassan virus disease, and ehrlichiosis associated with *Ehrlichia muris eauclarensis* [127].

9. CONCLUSION

Blood feeding is an important and highly essential step for the establishment of the tick life cycle. Blood meals are the sole source of metabolically required components which are also essential for the reproduction and survival of ticks. Once tick feeding is inhibited parasitic life of ticks will finish, it will not only control ticks but also tick-borne pathogenesis. Certainly knowledge about molecular, physiological and genetic pathways and functioning of antigen and other biomolecules will make real and needful assessments about achieving optimal protection after inducing or administration of antigens. However, for searching new novel antigens for single and multi-target vaccines genomic and proteomic tools will be more helpful. Identification of new protective antigens and their functional transcripts can revolutionize the vaccination and prophylaxis for the management of ticks. Further, there is an immense need to find out gaps among host and tick immunity, pathogen interaction to host and resistance and immune tolerance. There will require a complete discourse on ticks saliva toxins and gut secreted anti-hemostatic molecules. Inhibition of gut protein synthesis and their secretion is a highly important step that can solve the problem of tick menace. In this work, both proteomic and transcriptomic studies can assist in finding new alternatives related to vaccine

development. These will also provide direction and production of saliva-focused vaccines and vaccine strategies. For this purpose, besides the study of parasitology of ticks, a study of genetics, cell biology, immunology, and pharmacology will be more helpful to understand ticks and host acquired resistance. There is a need for more clarifications on digestion physiology and tick salivary secretions to end tick-borne diseases. More often, a combination of immunological, ecological, and veterinary methods and microbiology tools could lead resolve the complexity of tick–host and pathogen interaction and achieve more successful vaccine strategies.

Authors' Contributions: RKU and NY were responsible for conception, literature review, writing and revising the manuscript. Both authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgments: Authors are thankful to H.O.D., Department of Zoology for research facilities.

REFERENCES

1. Hrnková J, Schneiderová I, Golovchenko M, Grubhoffer L, Rudenko N, Černý J. Role of Zoo-Housed Animals in the Ecology of Ticks and Tick-Borne Pathogens-A Review. Pathogens. 2021; 10(2): 210.
2. Fogaça AC, Sousa G, Pavanelo DB, Esteves E, Martins LA, Urbanová V, et al. Tick Immune System: What Is Known, the Interconnections, the Gaps, and the Challenges. Front Immunol. 2021; 12: 628054.
3. Perveen N, Muzaffar SB, Al-Deeb MA. Ticks and Tick-Borne Diseases of Livestock in the Middle East and North Africa: A Review. Insects. 20211; 2(1): 83.
4. van Oosterwijk JG, Wikel SK. Resistance to Ticks and the Path to Anti-Tick and Transmission Blocking Vaccines. Vaccines. 2021; 9(7): 725.
5. Blisnick AA, Foulon T, Bonnet SI. Serine Protease Inhibitors in Ticks: An Overview of Their Role in Tick Biology and Tick-Borne Pathogen Transmission. Front Cell Infect Microbiol. 2017; 7: 199.
6. Villar M, Pacheco I, Merino O, Contreras M, Mateos-Hernández L. Tick and Host Derived Compounds Detected in the Cement Complex Substance. Biomolecules. 2020; 10(4): 555.
7. Franzin AM, Maruyama SR, Garcia GR, Pereira R. Immune and biochemical responses in skin differ between bovine hosts genetically susceptible and resistant to the cattle tick *Rhipicephalus microplus.* Parasit Vectors. 2017; 10: 51.
8. Kim TK, Tirloni L, Bencosme-Cuevas E, Kim TH, Diedrich JK, Yates JR. *Borrelia burgdorferi* infection modifies protein content in saliva of *Ixodes scapularis* nymphs. BMC Genomics. 2021; 22(1): 152.
9. Merino O, Alberdi P, de la Lastra JMP, de la Fuente J. Tick vaccines and the control of tick-borne pathogens. Front Cell Infect Microbiol. 2013; 3: 30.
10. Willadsen P. Anti-tick vaccines. Parasitology. 2004; 129 Suppl: S367-87.
11. De la Fuente J, Rodriguez M, Montero C, Redondo M, Garcia-Garcia JC. Vaccination against ticks (*Boophilus* spp.): the experience with the Bm86-based vaccine gavac. Gen Anal Biomol Eng. 1999; 15: 143-148.
12. Ndawula C Jr, Tabor AE. Cocktail Anti-Tick Vaccines: The Unforeseen Constraints and Approaches toward Enhanced Efficacies. Vaccines. 2020; 8(3): 457.
13. Garcia GR, Chaves Ribeiro JM, Maruyama SR, Gardinassi LG, Nelson K, Ferreira BR, et al. A transcriptome and proteome of the tick *Rhipicephalus microplus* shaped by the genetic composition of its hosts and developmental stage. Sci Rep. 2020; 10(1): 12857.
14. van Oosterwijk JG, Wikel SK. Resistance to Ticks and the Path to Anti-Tick and Transmission Blocking

Vaccines. Vaccines. 2021; 9(7): 725.

15. Rodriguez-Vivas RI, Jonsson NN, Bhushan C. Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitol Res. 2018; 117(1): 3-29.
16. Brossard M, Wikel SK. Tick immunobiology. Parasitology. 2004; 129: S161-S176.
17. Tabor AE, Ali A, Rehman G, Garcia GR, Zangirolamo AF, Malardo T, Jonsson NN. Cattle Tick *Rhipicephalus microplus*-Host Interface: A Review of Resistant and Susceptible Host Responses. Front Cell Infect Microbiol. 2017; 7: 506.
18. Rego ROM, Trentelman JJA, Anguita J, Nijhof AM, Sprong H, Klempa B. Counterattacking the tick bite: towards a rational design of anti-tick vaccines targeting pathogen transmission. Parasit Vectors. 2019; 12(1): 229.
19. Almazán C, Fourniol L, Rouxel C, Alberdi P, Gandoin C, Lagrée AC, et al. Experimental *Ixodes ricinus*-Sheep Cycle of *Anaplasma phagocytophilum* NV2Os Propagated in Tick Cell Cultures . Front Vet Sci. 2020; 7: 40.
20. Galay RL, Umemiya-Shirafuji R, Bacolod ET, Maeda H, Kusakisako K, Koyama J, et al. Two kinds of ferritin protect ixodid ticks from iron overload and consequent oxidative stress. PLoS One. 2014; 9(3): e90661.
21. Rodríguez-Mallon A. Developing Anti-tick Vaccines. Methods Mol Biol. 2016; 1404: 243-259.
22. Franzin AM, Maruyama SR, Garcia GR, Oliveira RP, Ribeiro JM, Bishop R. Immune and biochemical responses in skin differ between bovine hosts genetically susceptible and resistant to the cattle tick *Rhipicephalus microplus*. Parasit Vectors. 2017; 10(1): 51.
23. Kim TK, Tirloni L, Pinto AFM, Diedrich JK, Moresco JJ, Yates JR, et al. Time-resolved proteomic profile of *Amblyomma americanum* tick saliva during feeding. PLoS Negl Trop Dis. 2020; 14(2): e0007758
24. Mateos-Hernández L, Obregón D, Maye J, Borneres J, Versille N. Anti-Tick Microbiota Vaccine Impacts *Ixodes ricinus* Performance during Feeding. Vaccines. 2020; 8(4): 702.
25. Guerrero FD, Andreotti R, Bendele KG, Cunha RC, Miller RJ, Yeater K, Pérez de León AA. *Rhipicephalus* (*Boophilus*) *microplus* aquaporin as an effective vaccine antigen to protect against cattle tick infestations. Parasit Vectors. 2014; 7: 475.
26. Maritz-Olivier C, Stutzer C, Jongejan F, Neitz AW, Gaspar AR. Tick anti-hemostatics: targets for future vaccines and therapeutics. Trends Parasitol. 2007; 23(9): 397-407.
27. Ribeiro JM, Evans PM, MacSwain JL, Sauer J. *Amblyomma americanum*: characterization of salivary prostaglandins E2 and F2 alpha by RP-HPLC/bioassay and gas chromatography-mass spectrometry. Exp Parasitol. 1992; 74: 112-116.
28. Kotal J, Langhansova H, Lieskovska J, Andersen JF, Francischetti IM, Chavakis T, et al. Modulation of host immunity by tick saliva. J Proteom. 2015; 128: 58-68.
29. Dai J, Narasimhan S, Zhang L, Liu L, Wang P, Fikrig E. Tick histamine release factor is critical for *Ixodes scapularis* engorgement and transmission of the Lyme disease agent. PLoS Pathog. 2010; 6: e1001205.
30. Chmelar J, Calvo E, Pedra JH, Francischetti IM, Kotsyfakis M. Tick salivary secretion as a source of antihemostatics. J Proteom. 2012; 75: 3842-3854.
31. Narasimhan S, Koski RA, Beaulieu B, Anderson JF, Ramamoorthi N, Kantor F, et al. A novel family of anticoagulants from the saliva of *Ixodes scapularis*. Insect Mol Biol. 2002; 11: 641-650.
32. Francischetti IM, Mather TN, Ribeiro JM. Cloning of a salivary gland metalloprotease and characterization of gelatinase and fibrin(ogen)lytic activities in the saliva of the Lyme disease tick vector *Ixodes scapularis*. Biochim Biophys Res Commun. 2003; 305: 869-875.
33. De S, Kitsou C, Sonenshine DE, Pedra JHF, Fikrig E, Kassis JA, Pal U. Epigenetic Regulation of Tick Biology and Vectorial Capacity. Trends Genet. 2021; 37(1): 8-11.

34. Contreras M, Alberdi P, De Mera IGF, Krull C. Vaccinomics Approach to the Identification of Candidate Protective Antigens for the Control of Tick Vector Infestations and *Anaplasma phagocytophilum* Infection. Front Cell Infect Microbiol. 2017; 7: 360.
35. Giachetto PF, Cunha RC, Nhani A Jr, Garcia MV, Ferro JA, Andreotti R. Gene Expression in the Salivary Gland of *Rhipicephalus (Boophilus) microplus* Fed on Tick-Susceptible and Tick-Resistant Hosts. Front Cell Infect Microbiol. 2020; 9: 477.
36. Hajnická V, Vančová-Štibrániová I, Slovák M, Kocáková P, Nuttall PA. Ixodid tick salivary gland products target host wound healing growth factors. Int J Parasitol. 2011; 41(2): 213-223.
37. Nikpay A, Nabian S. Immunization of Cattle with Tick Salivary Gland Extracts. J Arthropod Borne Dis. 2016; 10(3): 281-90.
38. de la Fuente J, Blouin EF, Kocan KM. Infection exclusion of the rickettsial pathogen *Anaplasma marginale* in the tick vector *Dermacentor variabilis*. Clin Diagn Lab Immunol. 2003; 10(1): 182-184.
39. Bhowmick B, Han Q. Understanding Tick Biology and Its Implications in Anti-tick and Transmission Blocking Vaccines Against Tick-Borne Pathogens. Front Vet Sci. 2020; 9: 319.
40. Gondard M, Cabezas-Cruz A, Charles RA, Vayssier-Taussat M, Albina E, Moutailler S. Ticks and Tick-Borne Pathogens of the Caribbean: Current Understanding and Future Directions for More Comprehensive Surveillance. Front Cell Infect Microbiol. 2017; 7: 490.
41. Gillingham EL, Hansford KM, Meadows S, Henney J, Hernández-Triana LM, Muscat I. Ticks on the Channel Islands and implications for public health. Ticks Tick Borne Dis. 2020; 11(3): 101405.
42. Ali A, Khan MA, Zahid H, Yaseen PM, Khan MQ, Nawab J. Seasonal Dynamics, Record of Ticks Infesting Humans, Wild and Domestic Animals and Molecular Phylogeny of *Rhipicephalus microplus* in Khyber Pakhtunkhwa Pakistan. Front Physiol. 2019; 10: 793.
43. Kurokawa C, Lynn GE, Pedra JHF, Pal U, Narasimhan S, Fikrig E. Interactions between *Borrelia burgdorferi* and ticks. Nat Rev Microbiol. 2020; 18(10): 587-600.
44. Kazimirova M, Stibraniova I. Tick salivary compounds: their role in modulation of host defences and pathogen transmission. Front Cell Infect Microbiol. 2013; 3: 43.
45. Neelakanta G, Li X, Pal U, Liu X, Beck DS, DePonte K, et al. Outer surface protein b is critical for *Borrelia burgdorferi* adherence and survival within ixodes ticks. PLoS Pathog. 2007; 3: e33.
46. Pal U, Li X, Wang T, Montgomery RR, Ramamoorthi N, Desilva AM, et al. TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. Cell; 2004; 119(4): 457-468.
47. Pal U, Yang X, Chen M, Bockenstedt LK, Anderson JF, Flavell RA, et al. Ospc facilitates *Borrelia burgdorferi* invasion of *Ixodes scapularis* salivary glands. J Clin Invest. 2004; 113: 220-230.
48. Grimm D, Eggers CH, Caimano MJ, Tilly K, Stewart PE, Elias AF, et al. Experimental assessment of the roles of linear plasmids lp25 and lp28-1 of *Borrelia burgdorferi* throughout the infectious cycle. Infect Immun. 2004; 72(10): 5938-5946.
49. Ramamoorthi N, Narasimhan S, Pal U, Bao F, Yang XF, Fish D, et al. The Lyme disease agent exploits a tick protein to infect the mammalian host. Nature. 2005; 436(7050): 573-577.
50. Chlastáková A, Kotál J, Beránková Z, Kaščáková B, Martins LA, Langhansová H, Prudnikova T. Iripin-3, a New Salivary Protein Isolated From *Ixodes ricinus* Ticks, Displays Immunomodulatory and Anti-Hemostatic Properties. *In Vitro*. Front Immunol. 2021; 12: 626200.
51. Lynn GE, Diktas H, DePonte K, Fikrig E. Naturally Acquired Resistance to *Ixodes scapularis* Elicits Partial Immunity against Other Tick Vectors in a Laboratory Host. Am J Trop Med Hyg. 2021; 104(1): 175-183.

52. Olds CL, Mwaura S, Odongo DO, Scoles GA, Bishop R, Daubenberger C. Induction of humoral immune response to multiple recombinant *Rhipicephalus appendiculatus* antigens and their effect on tick feeding success and pathogen transmission. Parasit Vectors. 2016; 9(1): 484.
53. Lock O, Perez E, Villar M, Flores D, Rojas R. Bioactive Compounds from Plants Used in Peruvian Traditional Medicine. Nat Prod Commun. 2016; 11(3): 315-337.
54. Kubinski M, Beicht J, Gerlach T, Volz A, Sutter G, Rimmelzwaan GF. Tick-Borne Encephalitis Virus: A Quest for Better Vaccines against a Virus on the Rise. Vaccines. 2020; 8(3): 451.
55. Sanches GS, Couto J, Silva-Pedrosa R, Ferrolho J, Santos AS, Santos-Silva MM, et al. Molecular heterogeneity of *Rhipicephalus sanguineussensulato* and screening for Ehrlichia canis in mainland Portugal. Ticks Tick Borne Dis. 2018; 9(6): 1383-1390.
56. Esteves E, Maruyama SR, Kawahara R, Fujita A, Martins LA, Righi AA, et al. Analysis of the Salivary Gland Transcriptome of Unfed and Partially Fed *Amblyomma sculptum* Ticks and Descriptive Proteome of the Saliva. Front Cell Infect Microbiol. 2017; 7: 476.
57. de la Fuente J, Kocan KM. Strategies for development of vaccines for control of ixodid tick species. Parasite Immunol. 2006; 28(7): 275-283.
58. Prevot PP, Adam B, Boudjeltia KZ, Brossard M, Lins L, Cauchie P. Anti-hemostatic effects of a serpin from the saliva of the tick *Ixodes ricinus*. J Biol Chem. 2006; 281: 26361-26369.
59. Dai J, Wang P, Adusumilli S, Booth CJ, Narasimhan S, Anguita J, Fikrig E. Antibodies against a tick protein, Salp15, protect mice from the Lyme disease agent. Cell Host Microbe. 2009; 6(5): 482-492.
60. Hojgaard A, Biketov SF, Shtannikov AV, Zeidner NS, Piesman J. Molecular identification of Salp15, a key salivary gland protein in the transmission of Lyme disease spirochetes, from *Ixodes persulcatus* and *Ixodes pacificus* (Acari: Ixodidae). J Med Entomol. 2009; 46: 1458-1463.
61. Hovius JW, Van Dam AP, Fikrig E. Tick-host-pathogen interactions in Lyme borreliosis. Trends Parasitol. 2007; 23: 434-438.
62. Hovius JW, Schuijt TJ, De Groot KA, Roelofs JJ, Oei GA, Marquart JA, et al. Preferential protection of *Borrelia burgdorferi* sensu stricto by a Salp15 homologue in *Ixodes ricinus* saliva. J Infect Dis. 2008; 198: 1189-1197.
63. Schwalie PC, Schultz J. Positive selection in tick saliva proteins of the Salp15 family. J Mol Evol. 2009; 68: 186-191.
64. Kotsyfakis M, Anderson JM, Andersen JF, Calvo E, Francischetti IM, Mather TN, et al. Cutting edge: immunity against a 'silent' salivary antigen of the Lyme vector *Ixodes scapularis* impairs its ability to feed. J Immunol. 2008; 181: 5209-5212.
65. Wagemakers A, Coumou J, Schuijt TJ, Oei A, Nijhof AM, Van't Veer C, Van der Poll T. An *Ixodes ricinus* tick salivary lectin pathway inhibitor protects *Borrelia burgdorferi* sensu lato from human complement. Vector-Borne Zoonotic Dis. 2016; 16: 223-228.
66. Chmelar J, Anderson J, Mu J, Jochim R, Valenzuela J, Kopecky J. Insight into the sialome of the castor bean tick, *Ixodes ricinus*. BMC Genomics. 2008; 9: 233.
67. Cramaro WJ, Revets D, Hunewald OE, Sinner R, Reye AL, Muller CP. Integration of *Ixodes Ricinus* genome sequencing with transcriptome and proteome annotation of the naive midgut. BMC Genomics. 2015; 16: 1-15.
68. Lewis LA, Radulovic ZM, Kim TK, Porter LM, Mulenga A. Identification of 24h *Ixodes scapularis* immunogenic tick saliva proteins. Ticks Tick-Borne Dis. 2015; 6: 424-434.
69. Schwarz A, Tenzer S, Hackenberg M, Erhart J, Gerhold-Ay A, Mazur J, Kuharev JJ. A systems level analysis reveals transcriptomic and proteomic complexity in *Ixodes ricinus* midgut and salivary glands during early attachment and

feeding. Mol Cell Proteom. 2014; 13: 2725-2735.

70. Chmelar J, Kotal J, Kopecky J, Pedra JH, Kotsyfakis M. All for one and one for all on the tick-host battlefield. Trends Parasitol. 2016; 32: 368-377.
71. Gulia-Nuss M, Nuss AB, Meyer JM, Sonenshine DE, Roe RM, Waterhouse RM, et al. Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. Nature Commun. 2016; 7: 10507.
72. Wikel S. Ticks and tick-borne pathogens at the cutaneous interface: host defenses, tick countermeasures, and a suitable environment for pathogen establishment. Front Microbiol. 2013; 4: 337.
73. Zavašnik-Bergant T, Vidmar R, Sekirnik AL, Kopáček P, Turk B. Salivary Tick Cystatin OmC2 Targets Lysosomal Cathepsins S and C in Human Dendritic Cells. Front Cell Infect Microbiol. 2017; 7: 288.
74. Narasimhan S, Montgomery RR, DePonte K, Tschudi C, Marcantonio N, Anderson JF, et al. Disruption of *Ixodes scapularis* anticoagulation by using RNA interference. Proc Natl Acad Sci USA. 2004; 101: 1141-1146.
75. Schuijt TJ, Hovius JW, Van der Poll T, Van Dam AP, Fikrig E. Lyme borreliosis vaccination: the facts, the challenge, the future. Trends Parasitol. 2011; 27: 40-47.
76. Oleaga A, Soriano B, Llorens C, Pérez-Sánchez R. Sialotranscriptomics of the argasid tick Ornithodoros moubata along the trophogonic cycle. PLoS Negl Trop Dis. 2021; 15(2): e0009105.
77. Trimnell AR, Hails RS, Nuttall PA. Dual action ectoparasite vaccine targeting 'exposed' and 'concealed' antigens. Vaccine. 2002; 20: 3560-3568.
78. Nuttall PA, Trimnell AR, Kazimirova M, Labuda M. Exposed and concealed antigens as vaccine targets for controlling ticks and tick-borne diseases. Parasit Immunol. 2006; 28: 155-163.
79. Trimnell AR, Davies GM, Lissina O, Hails RS, Nuttall PA. A cross-reactive tick cement antigen is a candidate broad-spectrum tick vaccine. Vaccine. 2005; 23: 4329-4341.
80. Zhou W, Tahir F, Wang JC, Woodson M, Sherman MB, Karim S, et al. Discovery of Exosomes From Tick Saliva and Salivary Glands Reveals Therapeutic Roles for CXCL12 and IL-8 in Wound Healing at the Tick-Human Skin Interface. Front Cell Dev Biol. 2020; 8: 554.
81. Garcia GR, Maruyama SR, Nelson KT, Ribeiro JM, Gardinassi LG, Maia AA. Immune recognition of salivary proteins from the cattle tick *Rhipicephalus microplus* differs according to the genotype of the bovine host. Parasites Vectors. 2017; 10(1): 144.
82. Kopácek P, Galay RL O, Buresová V, Daffre S. Tick innate immunity. Adv Exp Med Biol. 2010; 708: 137-162.
83. Parizi LF, Githaka NW, Logullo C, Konnai S, Masuda A, Ohashi K, da Silva VI. The quest for a universal vaccine against ticks: cross-immunity insights. Vet J. 2012; 194(2): 158-165.
84. Dai O, Sojka D, Kopacek P, Buresova V, Franta Z, Sauman I, et al. Knockdown of proteins involved in iron metabolism limits tick reproduction and development. Proc Natl Acad Sci USA. 2009; 106(4): 1033-1038.
85. Hojgaard A, Biketov SF, Shtannikov AV, Zeidner NS, Piesman J. Molecular identification of Salp15, a key salivary gland protein in the transmission of Lyme disease spirochetes, from *Ixodes persulcatus* and *Ixodes pacificus* (Acari: Ixodidae). J Med Entomol. 2009; 46: 1458-1463.
86. Coumou H, BelEH. Improving the diagnosis of eosinophilic asthma. Expert Rev Respir Med. 2016; 10(10): 1093-1103.
87. Krause PJ, Narasimhan S, Wormser GP, Barbour AG, Platonov AE, Brancato J, Lepore T. Tick Borne Diseases Group. *Borrelia miyamotoi sensu lato* seroreactivity and seroprevalence in the northeastern United States. Emerg Infect Dis. 2014; 20(7): 1183-1190.
88. van den Hoek, HL, Bos WJ, de Boer A, van de Garde EM. Statins and prevention of infections: systematic review and meta-analysis of data from large randomised placebo controlled trials. BMJ Clin Res. 2011; 343.

89. Narasimhan V, Wilson K, Britto M, Warrier S, Lynch AC, Michael M, et al. Outcomes Following Cytoreduction and HIPEC for Pseudomyxoma Peritonei: 10-Year Experience. J Gastrointest Surg. 2020; 24(4): 899-906.
90. Canales M, Naranjo V, Almazán C, Molina R, Tsuruta SA, Szabó MP, et al. Conservation and immunogenicity of the mosquito ortholog of the tick-protective antigen, subolesin. Parasitol Res. 2009; 105(1): 97-111.
91. De la Fuente J, Almazan C, Blouin EF, Naranjo V, Kocan KM. Reduction of tick infections with *Anaplasma marginale* and *A. phagocytophilum* by targeting the tick protective antigen subolesin. Parasitol Res. 2006; 100: 85-91.
92. Bensaci M, Bhattacharya D, Clark R, Hu LT. Oral vaccination with vaccinia virus expressing the tick antigen subolesin inhibits tick feeding and transmission of *Borrelia burgdorferi*. Vaccine. 2012; 30(42): 6040-6046.
93. Havlíková S, Ličková M, Klempa B. Non-viraemic transmission of tick-borne viruses. Acta Virol. 2013; 57(2): 123-129.
94. Perner J, Sobotka R, Sima R, Konvickova J, Sojka D, Oliveira PL, et al. Acquisition of exogenous haem is essential for tick reproduction. Elife. 2016; 5: e12318.
95. Gomes H, Moraes J, Githaka N, Martins R, Isezaki M. Vaccination with cyclin-dependent kinase tick antigen confers protection against Ixodes infestation. Vet Parasitol. 2015; 211(3-4): 266-273.
96. Lu H, McClung CR, Zhang C. Tick Tock: Circadian Regulation of Plant Innate Immunity. Annu Rev Phytopathol. 2017; 55: 287-311.
97. Rosendal GK, Myhr AI. GMO assessment in Norway: societal utility and sustainable development. EMBO Rep. 2009; 10(9): 939-940.
98. VanBlargan LA, Himansu S, Foreman BM. An mRNA Vaccine Protects Mice against Multiple Tick-Transmitted Flavivirus Infections. Cell Reports. 2018; 25(12): 3382-3392.e3.
99. Chen X, Yu Z, Guo L, Li L, Meng H, Wang D, Liu R, Liu J. Life cycle of Haemaphysalis doenitzi (Acari: Ixodidae) under laboratory conditions and its phylogeny based on mitochondrial 16S rDNA. Exp Appl Acarol. 2012; 56(2): 143-150.
100. Weiss BL, Reuben Kaufman W. The relationship between 'critical weight' and 20-hydroxyecdysone in the female ixodid tick, *Amblyomma hebraeum*. J Insect Physiol. 2001; 47(11): 1261-1267.
101. Zhan L, Cao W, Chu C, Jiang B, Zhang F, Fang L, Habbema JD. Tick-borne Agents in Rodents, China. Emerg Infect Dis. 2009; 15(12): 1904-1908.
102. Hu Y, Zhang J, Yang S, Wang H, Zeng H, Zhang T, Liu J. Screening and molecular cloning of a protective antigen from the midgut of *Haemaphysalis longicornis*. Korean J Parasitol. 2013; 51(3): 327-334.
103. Steere AC, Sikand VK, Meurice F, Parenti DL, Fikrig E, Schoen RT, Nowakowski J. Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein with adjuvant. Lyme disease vaccine study group. New England J Med. 1998; 339: 209-215.
104. Abbott A. Lyme disease: uphill struggle. Nature. 2006; 439.
105. Hovius JW, Ramamoorthi N, Van't Veer C, De Groot KA, Nijhof AM, Jongejan F, et al. Identification of Salp15 homologues in *Ixodes ricinus* ticks. Vector-Borne Zoonot Dis. 2007; 7: 296-303.
106. Almazan C, Tipacamu GA, Rodriguez S, Mosqueda J, Perez de Leon A. Immunological control of ticks and tick-borne diseases that impact cattle health and production. Front Biosci. 2018; 23: 1535-1551.
107. Merino O, Antunes S, Mosqueda J, Moreno-Cid JA, Pérez de la Lastra JM. Vaccination with proteins involved in tick-pathogen interactions reduces vector infestations and pathogen infection. Vaccine. 2013; 31(49): 5889-5896.
108. Nijhof AM, Balk JA, Postigo M, Rhebergen AM, Taoufik A, Jongejan F. Bm86 homologues and novel ataq proteins with multiple epidermal growth factor (egf)-like domainsfrom hard and soft ticks. Int J Parasitol. 2010; 40: 1587-

1597.

109. Yang X, Koči J, Smith AA, Zhuang X, Sharma K, Dutta S, Rana VS. A novel tick protein supports integrity of gut peritrophic matrix impacting existence of gut microbiome and Lyme disease pathogens. Cell Microbiol. 2021; 23(2): e13275.
110. Kumar A, Garg R, Yadav CL, Vatsya S, Kumar RR, Sugumar P, et al. Immune responses against recombinant tick antigen, Bm95, for the control of *Rhipicephalus (Boophilus) microplus* ticks in cattle. Vet Parasitol. 2009; 165(1-2): 119-124.
111. Kasaija PD, Contreras M, Kabi F, Mugerwa S, de la Fuente J. Vaccination with Recombinant Subolesin Antigens Provides Cross-Tick Species Protection in *Bosindicus* and Crossbred Cattle in Uganda. Vaccines. 2020; 8(2): 319.
112. Canales M, Labruna MB, Soares JF, Prudencio CR, de la Fuente J. Protective efficacy of bacterial membranes containing surface-exposed BM95 antigenic peptides for the control of cattle tick infestations. Vaccine. 2009 7(52): 7244-7248.
113. Khanal S, Taank V, Anderson JF, Sultana H, Neelakanta G. Arthropod transcriptional activator protein-1 (AP-1) aids tick-rickettsial pathogen survival in the cold. Sci Rep. 2018; 8(1): 11409.
114. Pacheco I, Prado E, Artigas-Jerónimo S, Lima-Barbero JF, de la Fuente G, Antunes S, et al. Comparative analysis of *Rhipicephalus* tick salivary gland and cement elementome. Heliyon. 2021; 7(4): e06721.
115. Lee SH, Li J, Moumouni PFA. Subolesin vaccination inhibits blood feeding and reproduction of *Haemaphysalis longicornis* in rabbits. Parasites Vectors. 2020; 13: 478.
116. Kim TK, Radulovic Z, Mulenga A. Target validation of highly conserved *Amblyommaamericanum* tick saliva serine protease inhibitor 19. Ticks Tick Borne Dis. 2016; 7(3): 405-414.
117. Torina A, Moreno-Cid JA, Blanda V, Fernández de Mera IG, de la Lastra JM. Control of tick infestations and pathogen prevalence in cattle and sheep farms vaccinated with the recombinant Subolesin-Major Surface Protein 1a chimeric antigen. Parasit Vectors. 2014; 7: 10.
118. Taank V, Dutta S, Dasgupta A, Steeves TK, Fish D, Anderson JF, et al. Human rickettsial pathogen modulates arthropod organic anion transporting polypeptide and tryptophan pathway for its survival in ticks. Sci Rep. 2017; 7(1): 13256.
119. Taank V, Ramasamy E, Sultana H, Neelakanta G. An efficient microinjection method to generate human anaplasmosis agent *Anaplasma phagocytophilum*-infected ticks. Sci Rep. 2020; 10(1): 15994.
120. Regmi P, Khanal S, Neelakanta G, Sultana H. Tick-Borne Flavivirus Inhibits Sphingomyelinase (*Is*SMase), a Venomous Spider Ortholog to Increase Sphingomyelin Lipid Levels for Its Survival in *Ixodes scapularis* Ticks. Front Cell Infect Microbiol. 2020; 10:244.
121. Mans BJ. Chemical Equilibrium at the Tick-Host Feeding Interface: A Critical Examination of Biological Relevance in Hematophagous Behavior. Front Physiol. 2019; 10: 530.
122. Ayllón N, Zweygarth E, Passos LM, Broniszewska M, Villar M, Kocan KM, et al. Molecular and immunological characterization of three strains of *Anaplasma marginale* grown in cultured tick cells. Ticks Tick Borne Dis. 2015; 6(4): 522-529.
123. Vandegrift KJ, Kapoor A. The Ecology of New Constituents of the Tick Virome and Their Relevance to Public Health. Viruses. 2019; 11(6): 529.
124. Šimo L, Kazimirova M, Richardson J, Bonnet SI. The Essential Role of Tick Salivary Glands and Saliva in Tick Feeding and Pathogen Transmission. Front Cell Infect Microbiol. 2017; 7: 281.
125. Park JM, Oliva Chávez AS, Shaw DK. Ticks: More Than Just a Pathogen Delivery Service. Front Cell Infect Microbiol. 2021; 11: 739419.

126. Rizzoli A, Silaghi C, Obiegala A, Rudolf I, Hubálek Z, Földvári G, et al. *Ixodes ricinus* and Its Transmitted Pathogens in Urban and Peri-Urban Areas in Europe: New Hazards and Relevance for Public Health. Front Public Health. 2014; 2: 251.
127. Eisen RJ, Eisen L. The Blacklegged Tick, Ixodes scapularis: An Increasing Public Health Concern. Trends Parasitol. 2018; 34(4): 295-309.