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Narrative Review

Parasitology

Comprehensive antigen identification and comparative analysis: significant approaches for controlling *Haemaphysalis longicornis* ticks

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ABSTRACT

Importance: Ticks transmit severe human and animal diseases, posing global health and economic risks. *Haemaphysalis longicornis* spreads infections like Rickettsia, Theileria, and Anaplasma, exacerbating concerns. Conventional tick control, including chemical acaricides, faces challenges like toxicity, non-target effects, and resistance. Innovative, sustainable strategies are essential. Advances in tick antigen research have identified molecular targets, paving the way for anti-tick vaccines as a promising, eco-friendly alternative to manage *H. longicornis* infestations and reduce tick-borne disease transmission. This review explores recent discoveries in tick antigens, the development of recombinant proteins, and their knockdown effects on *H. longicornis* infestations.

Observations: Several novel antigens target essential physiological processes for tick survival. Reproductive and developmental antigens, such as subolesin and subolesin+cystatin, regulate immunity and reproduction, reducing blood feeding, oviposition, egg mass, and hatching rates. Knockdown of recombinant P27/30 impairs embryogenesis, significantly reducing larval survival. Chitinase inhibition disrupts molting, impairing nymph development. Metabolic enzymes like enolase and GSK-3β regulate homeostasis and energy production; their inhibition reduces feeding efficiency and survivability. Additionally, ribosomal protein S27 and troponin I-like protein, essential for protein synthesis and muscle contraction, respectively, impact tick growth and mobility. These antigens may serve as valuable vaccine targets for controlling *H. longicornis*.

Conclusions and Relevance: Anti-tick vaccines offer a cost-effective, sustainable alternative to chemical controls. Advances in transcriptomics, genomics, and proteomics have identified promising antigens, with subolesin, chitinase, troponin I-like protein, GSK-3β, and enolase demonstrating strong potential. Enolase, affecting immunity, reproduction, and pathogen transmission, emerges as the most effective target for reducing *H. longicornis* infestations.

Keywords: *Haemaphysalis longicornis*; vaccine development; immunologic dose-response relationship; vaccine; tick control

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Conflict of Interest
The authors declare no conflicts of interest.

Data Availability Statement
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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INTRODUCTION

Haemaphysalis longicornis, a hard tick, is widely distributed in East Asia and Australia. It is a vector for fatal diseases in animals and people [1,2]. Ticks are the second most common vector of human disease after mosquitoes. The primary arthropod that indirectly spreads diseases to both domestic and wild animals. Tick-borne diseases are on the rise, including viruses (e.g., tick-borne encephalitis and Powassan), bacteria (e.g., Lyme disease, spotted fever rickettsiosis, human anaplasmosis), and protozoan parasites causing babesiosis. Many ticks are distributed, and they cause several tick-borne diseases, a significant problem worldwide, as illustrated in **Table 1** [3]. Moreover, *H. longicornis* is mainly found in Australia, New Zealand, New Caledonia, the Fiji Islands, Japan, the Korean Peninsula, and the

Table 1. List of tick species carried by pathogens in different regions

Country	Pathogen+ ticks species name	Reference
United States of America	*Ixodes scapularis* *Ixodes pacificus* *Amblyomma americanum* *Dermacentor variabilis* *Dermacentor aodersoni* *Otobius megnini* *Ornithodoros coriaceus* *Borrelia spp.* *Anaplasma phagocytophilum* *Ehrlichia spp.* *Babesia microti* *Francisella tulorensis* *Rickettsia spp.* *Powassan virus*	[12]
Brazil	*Amblyomma spp.* *Rhipicephalus microplus* *Rhipicephalus sanguineus complex* *Dermacentor nitens* *Ornithodoros spp.* *Rickettsia rickettsii* *R. parkeri strain Atlantic rainforest* *Borrelia spp.* *Babesia spp.*	[12]
Central America	*Amblyomma mixtum* *Amblyomma ovale* *Dermacentor nitens* *Rhipicephalus microplus* *Rhipicephalus sanguineus s.I.* *Alectorobius puertoricensis* *Alectorobius talaje* *Rickettsio spp.* *Ehrlichia spp.* *Anaplasma spp.* *Babesia odocoilei/B. vogeli* *Borrelia spp.* *Hepatozoon canis/Hepatozoon spp.*	[12]
Mexico	*Rhipicephalus microplus* *Rhipicephalus sanguineus* *Rickettsia rickettsia* *Borrelia bungdor* *Borrelia microti* *Anaplosma phagocytophilum* *Anaplasma platys* *Babesia vogeli* *Ehrlichia canis*	[12]

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Table 1. (Continued) List of tick species carried by pathogens in different regions

Country	Pathogen+ ticks species name	Reference
Australia	*Spotted fever group Rickettsia transmitted by ticks;* *Queensland tick typhus (QTT)* *Flinders Island spotted fever (FISF)* *Australian spotted fever (ASF)* *Q fever*	[12]
Korea	*Ixodes scapularis (Lyme disease)* *Dermacentor spp.* *Haemaphysalis longicornis (Severe fever with thrombocytopenia syndrome) (SFTS)* *Ixodes ricinus (Tick-borne viral encephalitis)*	[5]
Europe	*Ixodes ricinus* *Rhipicephalus spp.* *Dermacentor spp.* *Hyalomma marginatum*	[12]
Egypt	*Hyalomma spp.* *Rhipicephalus spp.* *Anaplasmosis* *Babesiosis* *Boreliosis* *Rhipicephalus spp.*	[13]
Nepal	*Ixodes spp.* *Haemaphysalis spp.* *Amblyomma spp.*	[12]

northeastern regions of both China and Russia, in addition to the diseases of veterinary and economic significance.

Dogs, camels, cattle, and chickens are heavily impacted by ticks, which not only suck their blood but also transmit serious pathogens. Controlling tick infestations and spreading tick-borne diseases continues to be a significant challenge for the cattle industry worldwide [4]. The effective execution of vector control within the Onchocerciasis Control Program in West Africa over the last 25 years is remarkable [5]. Furthermore, vector control can significantly contribute to preventing and managing African trypanosomiasis and lymphatic filariasis in various epidemiological environments [5-7]. These diseases significantly impact the economy, affecting around 80% of the world's cattle [8] and costing between USD 13.9 billion and $18.7 billion annually. Lyme disease costs per patient in the USA increased from USD 8172 in 2002 to USD 11,838 in 2019 [9]. Managing tick infestations is complicated; such diseases are especially severe in developing countries; for instance, Tanzania incurs USD 364 million in losses due to tick-borne diseases, with 1.3 million cattle deaths primarily from theileriosis (68%), anaplasmosis (13%) and babesiosis (13%) [10], alongside lumpy skin disease [11]. In the Republic of Korea, the presence of *Anaplasma* spp., *B. burgdorferi*, and *Ehrlichia canis* is widespread [12]. Hence, dogs may play a key role as sentinel animals of multiple zoonotic infectious agents in the country.

The rising resistance to drugs used to treat diseases carried by ticks and manage vector infestations is a major worry on the global level, and the exorbitant expenses associated with current control methods, such as chemical or essential oil strategies [13,14] only exacerbate the burden. Vaccination makes logical sense for tick infestation management because it is less expensive and safer for the environment than chemical treatments [15].

In the 1950s, chemicals were developed to control ticks [16]. Chemicals called acaricides are currently used extensively to control ticks [17]. There are several issues with this approach. The quest for alternate management strategies has been prompted by these outcomes and public worries about the safety of chemical residues in animal products and the adverse consequences of chemicals in the natural ecosystem [17]. Tick control by an immunological approach may offer significant benefits in controlling longevity and immunity to adverse environmental impacts [18]. Immunology has a substantial role in tick control. Extracts tick saliva [19] from salivary glands as an antigen source. It seems natural that salivary gland proteins would be a good source of antigens to prevent an infestation of ticks. In 1979, Allen and Humphreys introduced the concept of “concealed” antigens to elicit effective anti-tick response [20]. These antigens, not presented to the host immune system during tick feeding but are exposed through the blood meal, induce immunity against ticks. In 1989, Willadsen et al. [21] isolated and characterized an antigen from tick gut extracts, demonstrating its effectiveness in limiting *Rhipicephalus microplus* tick feeding and reproductive performance.

Furthermore, it has been shown in specific tick-host techniques that successfully employ tick crude extracts for vaccination against ticks' gut or internal organs [20]. A commercial vaccine has been developed based on the successful artificial immunization of cattle with hidden tick antigens [22]. The most remarkable outcomes were achieved with a purified protein derived from *Boophilus microplus*’s midgut [21]. Two commercial vaccinations have been developed to prevent tick infestations in cattle: Gavac in Cuba and TickGARD Plus in Australia. The recombinant antigen Bm86, found on the mid-gut digestive cell, is the basis for both vaccinations. The salivary glands and midgut of *B. microplus* contain the low-abundance glycoprotein Bm 91 (Riding et al. [21], 1994). Australian field testing has demonstrated the vaccine’s effectiveness [22] in Brazil and Cuba [23]. Mulenga et al. [18] have shown that development of an anti-tick vaccine against *H. longicornis* is possible. The conclusions drawn from these investigations offered significant data about the dependability and usefulness of biotechnology to find tick vaccine antigen candidates and generating enough of them.

This review begins by providing an overview of the challenges posed by *H. longicornis* ticks and the importance of antigen-based strategies in managing their populations. Molecular identification and characterizing new antigen candidates that are more conserved and capable of inducing cross-reactive immunity in host species is essential to advancing vaccine development. In addition to highlighting new antigens that show promise as vaccine candidates, particularly those effective against *Haemaphysalis* ticks, this paper provides an overview of both standard and RNA-based vaccines, researching their possible applications.

We analyze recent discoveries of new antigens by cloning and knockdown effects of specific genes of *H. longicornis* ticks and their comparative effects in the tick life cycle, evaluating their potential uses in conjunction with new antigens. These are exciting potential targets for future tick control strategies.

METHODS

In this review, we focused on research on *H. longicornis* antigens that are crucial to tick survival and disease transmission by conducting a comprehensive literature search across databases such as PubMed and Web of Science. In addition to evaluating studies on RNA interference, recombinant protein, and mRNA vaccine technologies, we also included research on conserved antigens involved in attachment, blood feeding, reproduction, and immunity. To evaluate their potential for wide-ranging tick control, a few studies were evaluated for vaccination efficacy and antigen conservation across tick species. The results were combined to offer strategic insights into potential targets and approaches for controlling *H. longicornis* ticks in a way that benefits the environment.

Discovery of antigens for controlling *H. longicornis*

Tick troponin I and chitinase were identified and localized from *H. longicornis*, and recombinant tick troponin I was produced in *Escherichia coli* described elsewhere [24,25]. After the induction of resistance to *H. longicornis* infestation in rabbits that had been immunized with recombinant *H. longicornis* P27/30 protein, rabbits immunized with recombinant P27/30 expressed in *Escherichia coli* showed significantly ($p < 0.05$), longer feeding duration for larval and adult ticks, lower engorgement rates for larval ticks (64.4%), and apparent reduction in egg weights, which together suggest that *H. longicornis* P27/30 protein is a candidate antigen for a tick vaccine for biological control of *H. longicornis* [26].

Another study used the same species of ticks in a mouse model. The recombinant P27/30 (rP27/30) expressed in *E. coli* was used to immunize mice, which were then challenge-infested with ticks at different developmental stages [27]. The *rP27/30* protein stimulated a specific protective anti-tick immune response in mice, evidenced by the significantly more extended pre-feeding periods in adult ticks and the considerably longer feeding periods in both larval and adult ticks. On the other hand, only larval ticks exhibited low attachment rates (31.1%). Immunization of mice with rP27/30 protein confers protection against hard tick *H. longicornis* infestation. These results suggest that the rP27/30 protein is a valuable vaccine candidate antigen for the biological control of ticks.

The appearance of *H. longicornis* chitinase in the epidermal cuticle implies it acts as a molting enzyme. These results indicate that *H. longicornis* chitinase is an essential enzyme for molting and controlling the turnover and porosity of the PM in ticks and, thus, is a major candidate as a bio-acaricide [25].

H. longicornis salivary cystatin (HlSC-1) and subolesin by RNA interference [28]. Multi-antigen vaccinations are recommended for tick control since they are more effective than single-antigen vaccines [25,29]. Thus, a promising field of research in creating anti-tick vaccinations is to find out how effective combination antigens are. Subolesin is an intracellular tick protein identical to insect and vertebrate akirins. It regulates the expression of another gene and affects the innate immune response, blood engorgement, digestion, reproduction, and development [17,30,31]. Another important salivary molecule, salivary cystatin (HlSC-1), plays a vital role during the early period of blood feeding [21]. Therefore, it could be an essential immuno-dominant molecule whose inhibitory function might hamper tick feeding and tick-borne disease transmission. In nematodes, cystatins impeded the release of proteases in APCs from the host's immune system [32]. The authors report that cystatin and synaptobrevin are essential for blood intake in *A. americanum*. Both subolesin and cystatin have significant roles in blood intake. When subolesin and cystatin are knocked down causes the reduction of tick engorgement, egg mass weight, and egg conversion ratio. Therefore, the elucidation of multiple antigens may be helpful for the future development of vaccines. In this study, we silenced salivary subolesin and/or cystatin using RNAi in order to determine their roles in feeding and reproduction. This information may help to identify multiple antigens as candidate vaccines.

Here, first identified and analyzed the transcriptional expression and functional status of ribosomal protein S27 (RPS-27) from *H. longicornis*, focusing on the role of RPS-27 in tick feeding. Recombinant RPS-27 (10-RPS-27) was successfully expressed in vitro using a pGEMEX-2 vector, resulting in a molecular mass of about 45 kDa, according to previous in vitro studies [33]. Using RNA interference (RNAi) to evaluate the functional importance of RPS-27, we found that both adult and nymph female ticks exhibited significantly decreased post-blood meal eating ability and engorgement weight ($p < 0.05$). Moreover, nymph molting rates decreased significantly ($p < 0.05$) after RPS-27 silencing. RPS-27 silencing in eggs led to abnormal morphology and impaired hatching. All of these results illustrate how important RPS-27 is to several stages of the tick life cycle, including feeding and mating suggesting that RPS-27 is a viable target for tick control in the future.

GSK-3β, a member of the serine/threonine kinase family, regulates the metabolism of glycogen, Wnt signaling, hormone levels, and the development of embryos in a variety of eukaryotes. When applying RNA interference (RNAi) to evaluate the functional role of GSK-3β, problems in egg development and hatching, as well as a significant ($p < 0.05$) decrease in eating and reproduction, were observed [34]. These results suggest that GSK-3β is a promising option for a multi-antigen vaccination targeted at controlling the tick population. GSK-3 is a vital enzyme that plays a key role in mammalian glycogen metabolism. GSK-3 occurs as two isoforms, GSK-3α and GSK-3β; both forms play multifunctional roles in glucose metabolism, apoptosis, embryonic development, and gene transcription [35,36].

The process of recombinant enolase purification from the salivary glands of the Jeju strain of *H. longicornis* ticks has been described in detail [37]. Enolase is crucial to the metabolic process known as glycolysis, which converts glucose into energy. It is also necessary for tick motility, adhesion, invasion, growth, and differentiation. In this study, recombinant enolase was used to immunize mice, generating polyclonal antibodies. Treated nymphs and larvae showed decreased attachment and engorgement rates compared to controls. Ninety percent of mice immunized with enolase derived from *E. coli* were able to resist tick infection [37]. Owing to its glycolytic nature and role in essential physiological functions, enolase is a desirable target for impeding tick survival and disease transmission. The development of polyclonal antibodies that recognize enolase resulted in a considerable decrease in the rates of attachment, feeding by ticks, and engorgement. According to our research, recombinant enolase is a promising vaccine candidate for treating *H. longicornis* infections in experimental mouse models [37]. Another recent research showed that Tick development, reproduction, and immunological functions are all hampered when subolesin and enolase (GenBank accession number ON871822) are silenced, interfering with vital functions. Since subolesin is essential for tick growth and immunological response, it lowers tick survival by impairing immune control and cell division. Enolase is involved in metabolism and energy generation; when it is inhibited, glycolysis is impacted, which lowers ATP synthesis and total energy levels, weakening the tick. When these genes are silenced together, tick viability and pathogen transmission are reduced [38].

Effects of different antigens on the life cycle of *H. longicornis* ticks

H. longicornis antigens are essential throughout the life cycle because they affect important biological functions (**Supplementary Fig. 1**). In order to ensure the tick's nourishment, several antigens influence blood-feeding efficiency by modifying blood coagulation or saliva secretion. For egg formation and embryonic development to be effective, reproductive antigens are necessary. Digestion-related antigens improve pathogen survival and nutrition absorption in the tick gut. Furthermore, immune-related antigens help ticks survive and transmit infections by enabling them to avoid host immunological responses. In this review, important antigens that are essential for tick development and helpful for various biological and physiological processes were discussed. Chitinase breaks down chitin, a key component of the tick exoskeleton and gut lining. By targeting chitinase, it's possible to disrupt tick molting, cuticle integrity, or digestive processes, which could reduce tick survival. Ecdysteroids induce chitinase to degrade the older chitin when molting [39,40], P27/30 as a troponin I-like protein [27] involved in tick feeding, reproduction, and general survival. Subolesin and cystatin [28] is a highly conserved protein that regulates gene expression related to tick feeding, reproduction, and pathogen transmission. The recombinant form has been studied in combination with other antigens (like cystatin) to enhance the immune response, suggesting that subolesin could be a core antigen in multi-antigen vaccines targeting tick control. RPS-27 [33] is involved in protein synthesis and has also been implicated in stress responses and survival mechanisms. Its role in tick biology makes it a potential antigen for tick control strategies. However, its direct contribution to tick feeding or reproduction is less clear than that of other antigens. GSK-3β [34], GSK-3β is a signaling protein involved in a wide range of cellular processes, including metabolism, immune responses, and cell proliferation. In ticks, it likely plays a role in survival and reproduction, making it an interesting target for disruption through immunization. Enolase is a glycolytic enzyme that also acts as a plasminogen receptor on the surface of pathogens, including ticks. It helps in host tissue invasion by degrading extracellular matrix components, making it a potential antigen for interfering with tick feeding and pathogen transmission [41]. Recombinant enolase is a laboratory-produced form of this enzyme and is studied as a candidate for vaccines, potentially reducing tick feeding efficiency and pathogen transmission. mRNA vaccines work by introducing messenger RNA (mRNA) into the host cell, where ribosomes read it and make the target protein. Frequently antigenic, these proteins are identified as foreign substances by the immune system. Antigen-presenting cells (APCs) stimulate the creation of protective or functional antibodies by delivering the proteins to immune receptors, so initiating an immune response. Antibodies and an early immunological response in the vaccinated host inhibit tick feeding. Although the great efficacy of mRNA vaccines, their usage has been limited in certain nations due to their instability in animal trials [42].

In ticks, knockdown of subolesin and enolase impairs impairs cell division, immunological response, and metabolism, which lowers energy, survival, and immune defense [38]. Collectively, these initial RNAi functional studies in ticks demonstrated the relevance of this methodology for enhancing the understanding of tick biology. Subsequent studies have included the knockdown of different tick genes to characterize multiple biological pathways involved in tick-host-pathogen interactions. By inhibiting this antigen by RNA interference, preparing recombinant vaccines that target these antigens may provide an effective method to manage tick populations and reduce the spread of disease.

RESULTS

Effects of different antigen parameters on tick attachment

Determining the effects of different antigen parameters on tick attachment is crucial to understanding the intricate dynamics of host-tick interactions. Antigens, key components of the host's immune system, can significantly influence the attachment process. For instance, hosts with a diverse range of antigens may experience varying degrees of tick attachment compared to those with a limited antigen profile. Different antigen attachment parameters given below recombinant P27/30 protein adult 100%, recombinant P27/30 protein larva

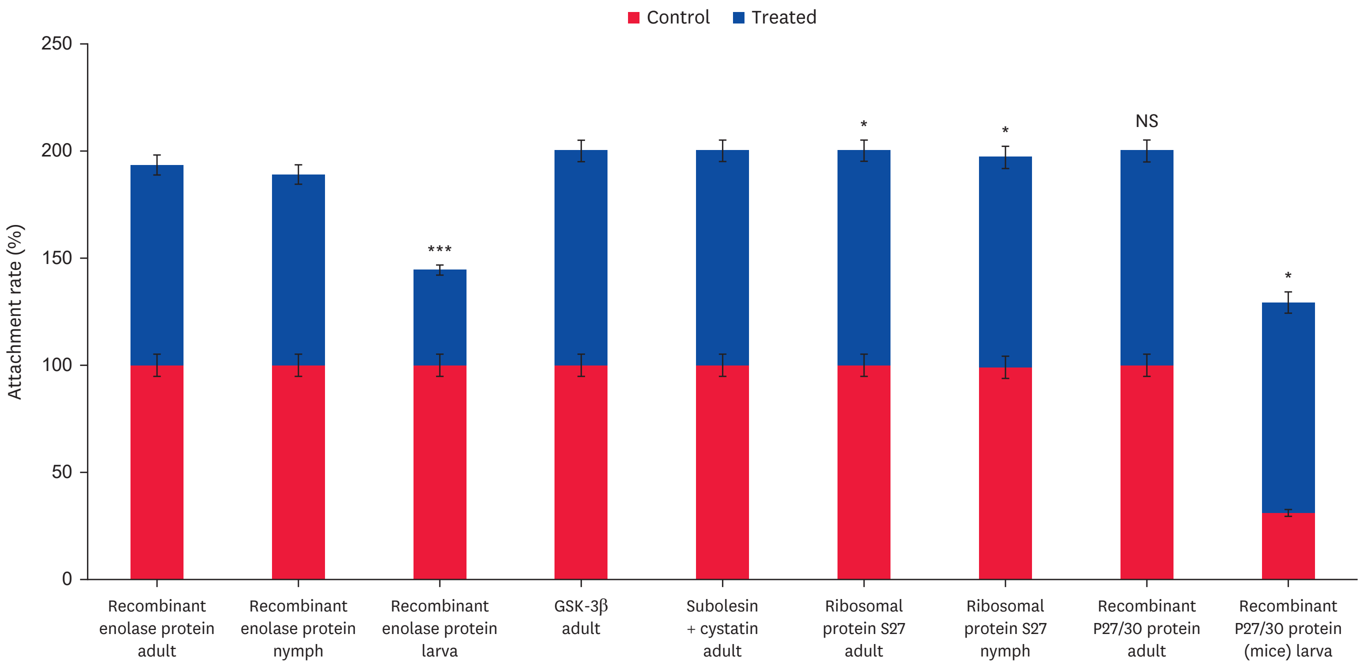

Fig. 1. Comparative attachment rates after treatment with different antigens for controlling adults, nymphs, and larvae of *Haemaphysalis longicornis* ticks.
NS, not significant.
$^{*}p < 0.05$; $^{***}p < 0.001$.

treated 93.4% compared to control 64.4% [26], subolesin and cystatin [28] adult 100%, RPS-27 adult 100%, RPS-27 nymph 99% treated and control 98% [33], recombinant enolase protein adult, 93.3% [37], recombinant enolase protein nymph, 88.9% recombinant enolase protein larva 44.4% [37], glycogen synthase kinase 3β (GSK-3β), adult 100% [34], comparative features are illustrated in **Fig. 1**. The specificity and variability of antigens present in hosts may impact the ability of ticks to attach and feed successfully. The statistically significant more extended feeding periods for adult ticks and the statistically significant longer feeding periods for larval and adult ticks, along with the low attachment rates in larval ticks (31%), indicate that mice immunized with a troponin I (P27/30) protein developed a significant level of resistance against *H. longicornis* infestations. Troponin I, expressed in E. coli, stimulated a specific protective anti-tick immune response in mice [24]. Investigating the effects of different antigen parameters provides valuable insights into the molecular mechanisms governing host defense response and the adaptability of ticks. Such research is essential for comprehending the nuances of tick-host relationships and may contribute to developing targeted strategies for managing tick-borne diseases.

Effects of antigen on feeding period

The exploration of diverse antigens during the feeding period of ticks has become a focal point in understanding the complex interactions between ticks and their hosts. Ticks introduce an array of antigens into the host during feeding, comprising salivary proteins with diverse functionalities already identified in adult *H. longicornis* ticks in our previous study [19]. These antigens often include immunomodulatory molecules, anticoagulants, and other bioactive compounds. The distinct compositions of these antigens can vary among tick species and may influence host immune responses differently. Some antigens play roles in mitigating host defenses and promoting successful blood feeding, while others may contribute to transmitting pathogens. Research efforts have sought to identify and characterize these different antigens to unravel their specific roles in tick-host interactions.

Improved knowledge in this area enhances our understanding of tick biology and holds promise for developing targeted strategies for tick control and preventing tick-borne diseases. As the field progresses, a comprehensive understanding of the diverse array of antigens that mediate the feeding period of ticks is crucial for advancing our capabilities in mitigating the impact of these arthropods on both animal and human health. Adult ticks that fed on rP27/30-immunized rabbits were observed to feed longer (12.5 ± 0.78 days) compared to the control (7.8 ± 0.83 days) ($p < 0.05$), as did tick larvae (4.9 ± 0.3 days vs. 4.4 ± 0.2 days) ($p < 0.05$, **Fig. 2**). However, no differences were observed in the duration of nymph feeding (5.6 ± 0.45 days) versus the control (5.3 ± 0.76 days) (**Fig. 2**) [26]. Both larval and adult ticks that fed on rP27/30 immunized mice showed a significant increase in feeding periods compared to the control group ($p < 0.05$). The feeding periods of larvae were 4.1 ± 0.17 days ($p < 0.05$), and adult ticks were 6.5 ± 0.5 days ($p < 0.05$). In comparison, the control showed 3.7 ± 0.07 days and 4.6 ± 0.3 days, respectively (**Fig. 2**). It can be seen that nymphal ticks feeding periods on rP27/30-immunized mice ranged 4.6 ± 0.3 days compared to 4.3 ± 0.8 days in the control with no significant difference observed (**Fig. 2**) [27]. Subolesin knockdown significantly ($p < 0.05$) increased feeding duration 8 days compared to controls 6 days. Cystatin knockdown caused a significant ($p < 0.05$) increase in feeding duration 10 days compared to controls 8 days (**Fig. 2**). To assess the synergistic role of feeding, a mixture of subolesin and cystatin dsRNA was injected into unfed females, which were then allowed to feed until spontaneous drop-down. The feeding duration was similar to that of the control group, seven days on average, with no significant difference observed (**Fig. 2**) [28]. RPS-27 dsRNA treated adult female ticks, there was substantial ($p < 0.05$) variation in feeding duration of 15 days compared with control groups 8 days; RPS-27 treatment extended the feeding period to 11 days for nymphs, compared to 6 days in the control group ($p < 0.05$) (**Fig. 2**) [33]. Finally, Recombinant Enolase protein-treated Both larval (1.3 ± 0.6 days) and adult (4.7 ± 0.6 days) ticks that fed on Recombinant Enolase-immunized mice significantly more extended feeding

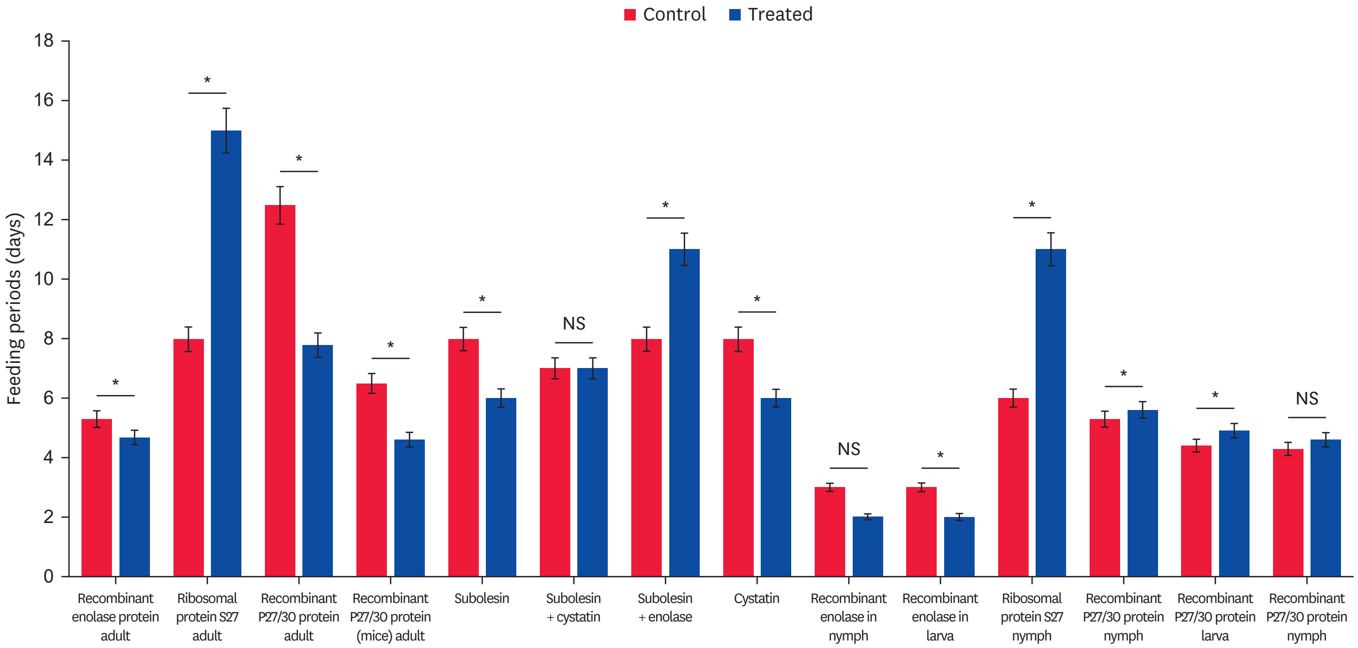

Fig. 2. Comparative feeding periods after treatment with different antigens for controlling adults, nymphs, and larvae of *Haemaphysalis longicornis* ticks.
NS, not significant.
$^{*}p < 0.05$.

periods ($p < 0.05$) compared to the controls (2.8 ± 0.6 days and 5.3 ± 0.6 days, respectively; **Fig. 2**). The nymphal tick feeding period in the Recombinant Enolase-immunized mice (2.8 ± 0.3 days) was lower than that in the controls (3.2 ± 0.8 days). Still, the difference was not significant [37].

Effects of different antigens on blood engorgement

Antigens play pivotal roles in influencing blood engorgement in ticks. Antigens, integral components of the host's immune system, can modulate the tick's ability to engorge blood successfully. Hosts with a diverse array of antigens may experience variations of blood loss.

Ticks that fed on rP27/30-immunized mice exhibited the engorged body weights of larval, nymphal, and adult ticks following feeding on rP27/30-immunized mice were 0.63 ± 0.05, 4.2 ± 0.1, and 258 ± 11.5 mg compared to the controls, 0.68 ± 0.05, 4.1 ± 0.1, and 262 ± 18.8 mg, respectively, and no significant reduction of engorged weights was observed in immunized mice [27]. Ticks that fed on rP27/30-immunized rabbits exhibited the engorged body weights of larval, nymphal, and adult ticks following feeding on rP27/30-immunized rabbits were 0.62 ± 0.05, 4.1 ± 0.2, and 257 ± 10.5 mg, respectively, compared to control values of 0.66 ± 0.05, 4.2 ± 0.2, and 263 ± 12.5 mg, none of which were significant ($p < 0.05$) (**Fig. 3**) [26].

The average engorgement weight of ticks injected with cystatin dsRNA was 81.38 mg, significantly lower than the control group's 174.13 mg. Similarly, subolesin dsRNA-injected ticks had an average engorgement weight of 78.5 mg, considerably lower than the control group ($p < 0.001$). Subolesin and cystatin ds RNA-injected ticks engorgement weight (average: 107.13 mg) was significantly ($p < 0.002$) reduced in comparison to the control group (average: 174.13 mg). The engorgement weight was reduced considerably ($p < 0.0001$, df4) by GSK-3β knockdown compared to the control group. The average tick engorgement weight in

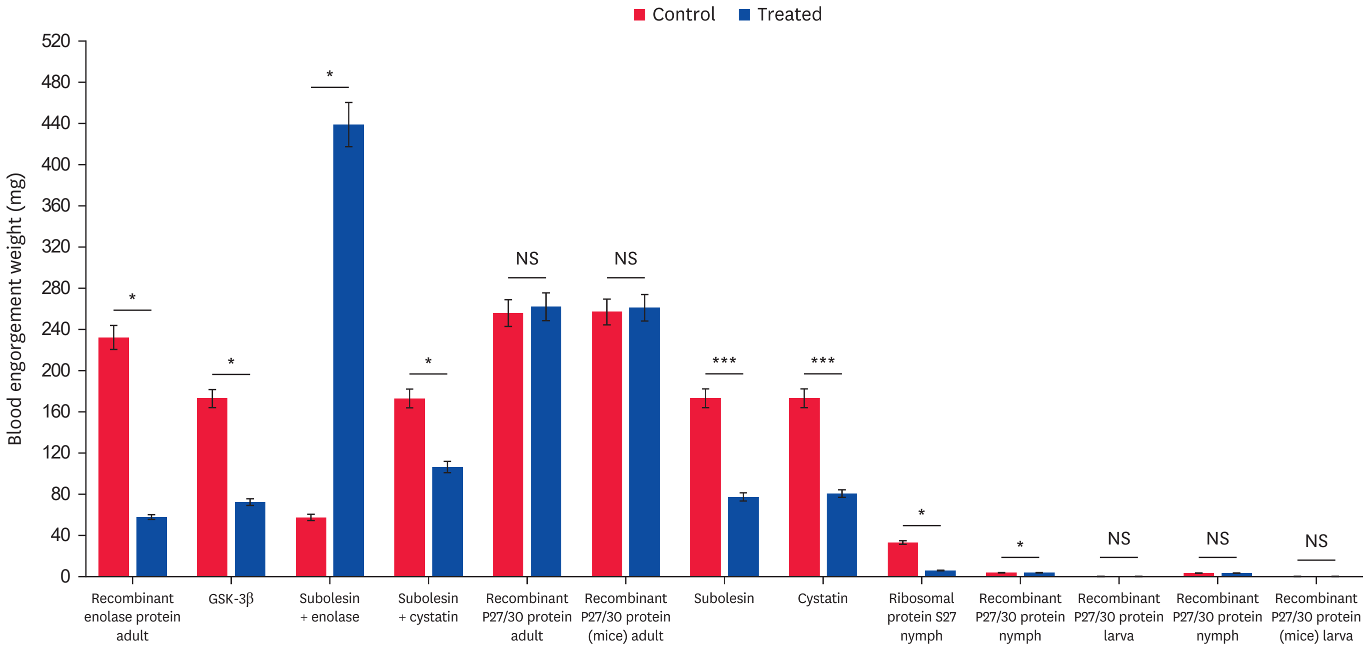

Fig. 3. Comparative blood engorgement weight after treatment with different antigens for controlling adults, nymphs, and larvae of *Haemaphysalis longicornis* ticks.
NS, not significant.
*$p < 0.05$; ***$p < 0.001$.

the GSK-3β dsRNA-injected group was 73.2 mg, compared with the control group's 282 mg (**Fig. 3**) [34].

Recombinant enolase protein treated ticks resulted in a significantly reduced average weight to 58.216 mg compared to 233 mg in the control group ($p < 0.05$) [37], RPS-27 dsRNA treatment on nymphal tick blood engorgement, there was significant ($p < 0.05$) variation in engorgement weight (6.33 ± 1.5 mg), compared with control groups (34 ± 2 mg).In the case of adult females, the average engorgement weight in the treatment group (6.3 mg/tick) was significantly ($p < 0.05$) lower than that of the control groups (165.66 mg/tick).Subolesin and enolase dsRNA treated ticks engorgement weight (average: 58.21 mg) was significantly reduced compared to the control group (average: 440 mg) ($p < 0.001$) (**Fig. 3**).

The interplay between specific antigens and the feeding behavior of ticks can significantly impact the success of blood feeding. Understanding the nuances of how different antigens affect blood engorgement is essential for unraveling the complex dynamics of host-tick interactions. This knowledge may contribute to the development of targeted approaches for managing tick-borne diseases, as it sheds light on the factors influencing tick-feeding success in the presence of various host antigens. Nymphal ticks that fed on chitinase-immunized mice had a lower molting rate (83.3%) compared to the control (100%). This result means that chitinase-immunized mice sera are implicated in the molting step [43].

Effects of different antigens on egg mass weight

A comprehensive review of the impacts of different antigens on egg mass weight in ticks reveals a critical dimension of the intricate host-tick relationship. Antigens, which are integral to the host’s immune response, exhibit varying effects on the reproductive success of ticks, particularly in the context of egg mass weight. Hosts with diverse antigen profiles may influence the weight and fecundity of tick egg masses differently than those with limited antigen diversity. A significant reduction in egg weights was observed for adult ticks fed on rP27/30-immunized rabbits (125 ± 20 mg vs. a control value of 140 ± 12 mg; $p < 0.05$) and mice (120 ± 22 mg) compared to the control (147 ± 12 mg) [27]. Subolesin and cystatin combined silencing resulted in a significant ($p < 0.001$) retardation of the average egg mass of 50 mg as compared to 79.87 mg for the control group (**Fig. 4A**) [28]. Adult female ticks treated with RPS-27 dsRNA were unable to lay eggs. The egg mass weight of varied significantly ($p < 0.05$) compared to control groups (150 mg/tick) [33], GSK-3β dsRNA caused significant reduction of egg production ($p < 0.0027$, df4) in treated groups 25 mg compared to control groups 140 mg [34]. However, ticks fed on Recombinant Enolase-immunized mice tended to have lower egg weights, 15 ± 0.39 mg, which varied significantly ($p < 0.05$) than those in the control groups 170 mg (**Fig. 4A**) [37]. This review sheds light on the complex interplay between specific antigens and the reproductive biology of ticks, offering insights into the factors contributing to variations in egg mass weight. This understanding is pivotal for elucidating the mechanisms governing tick reproduction and can provide valuable information for devising targeted strategies in tick control and management. Our review also underscores the need for further research to deepen our comprehension of how different antigens impact the reproductive fitness of ticks.

Effects of different antigens on hatching eggs

In the context of a comprehensive review, exploring the influence of different antigens on the hatching of tick eggs presents a multifaceted perspective on the host-tick relationship. Antigens, integral components of the host's immune system, play pivotal roles in shaping

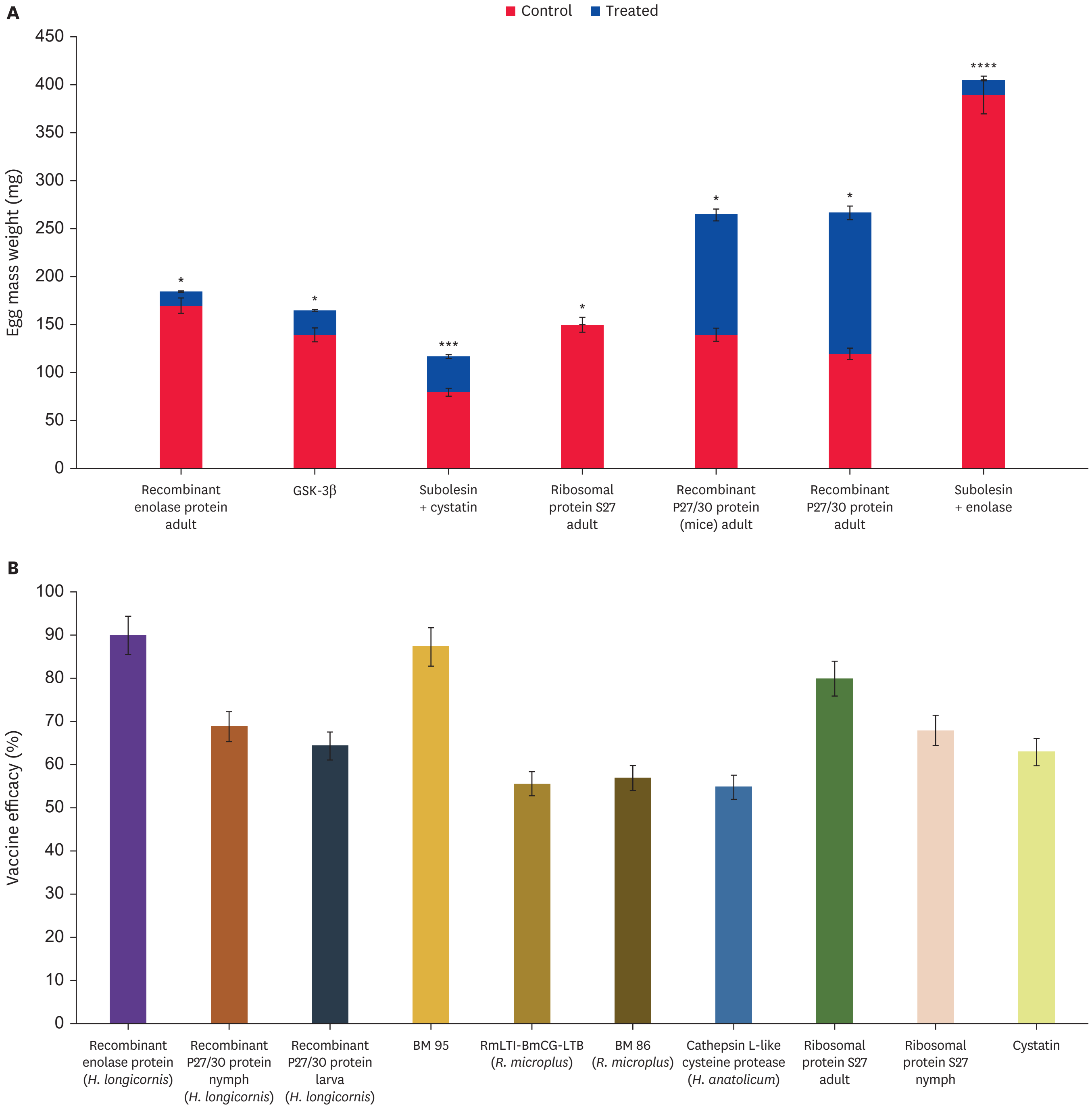

Fig. 4. Comparative effect of different antigens treated on *Haemaphysalis longicornis* ticks. (A) Egg mass weight. (B) Comparative efficacy rate of several vaccine controlling *Haemaphysalis longicornis* and other ticks.
$^{*}p < 0.05$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

the hatching success of tick eggs. The interaction between specific antigens and the embryonic development of ticks can have diverse effects on the hatching process. Hosts with varying antigen profiles may contribute to differential outcomes regarding hatching rates of *H. longicornis* ticks. Enolase protein 53% [37] compared to control 100%, GSK-3β 20% compared to control 90% [34]. Subolesin and/or cystatin no difference in hatchability was observed [28]. RPS-27 25% compared to control 100% [33]. Recombinant P27/30 protein no hatchability record observed [27]. This part of the review highlights the complex relationship between host immunity and tick reproductive success, providing valuable insights into the factors that affect egg hatching. Understanding this interplay enhances our grasp of tick biology and is crucial for developing precise strategies in tick control and management, which further underscores the importance of continued research in this field.

Comparative efficacy of different antigen

The effects of immunization were evaluated based on the reduction of the tick's engorged body weight, oviposition, and hatching. Calculations were made using the following formulas adapted from previous tick vaccination reports [44]. Researchers currently employ various approaches to assess the effectiveness of antigens against ticks [45-47]. For instance, commonly used formulas for evaluating vaccine impact on both one-host and three-host ticks focus on aspects such as female tick engorgement, oviposition, and egg hatchability [48]. An alternative formula has been proposed for gauging vaccine effectiveness against three-host ticks by examining the impact on the weight and quantity of ticks across different life stages [49]. Different efficacies were observed for single recombinant protein and multiple recombinant proteins in different animals that vary from those shown in this review (**Fig. 4B**). Several recombinant antigens have demonstrated promising but variable efficiency against tick infestations, indicating the necessity for continued research to improve the defense. Recent tick control research has investigated the effectiveness of several recombinant proteins and antigens; the results indicate a variety of protection levels that point to viable directions for vaccine development. Recombinant Enolase protein demonstrated high efficacy at 90%, indicating strong potential for reducing tick infestations [37]. Similarly, in BM95 infestations, recombinant antigens that target *Hyalomma anatolicum* had an effectiveness rate of 87.4% [50]. Moderate protection was observed with recombinant RmTI-CG-LTB protein at 55.6%, while BM86, designed for *R. microplus* control showed 57% efficacy [51,52]. Additionally, Cathepsin L-like cysteine protease, aimed at *H. anatolicum* control, had an efficacy of 54.8% (**Fig. 4B**) [53].

Among other antigens, Cystatin showed a 63% reduction in tick infestations, while RPS-27 showed significant effectiveness, achieving 80% in adult ticks and 68% in nymphs. Significant results were also demonstrated by the recombinant P27/30 protein, which had efficiency rates of 64.4% and 68.9% in nymphs across studies (**Fig. 4B**). These findings demonstrate how different antigens have differing levels of efficacy, which promotes future research to improve and even combine these alternatives for more effective tick prevention strategies.

DISCUSSION

Ticks are carriers of various of pathogens that cause illnesses in vertebrates, including bacteria, viruses, and protozoa [54]. By using RNAi to shed light on the interactions between pathogens and ticks, new management tactics that aim to slow the spread of these illnesses may be developed. To identify novel vaccine antigens from systems biology studies of tick-pathogen interactions, algorithms that pick the most efficient targets for reducing tick infestations and pathogen transmission [55] must be developed. RNAi has also been used to identify and characterize candidate protective antigens for the control of tick infestations and tick-borne pathogens [56]. This would make the application of autocidal control for the reduction of tick populations more feasible [57]. The mechanism of dsRNA-induced RNAi in ticks should be refined to contribute to a better understanding and utilization of this genetic approach in this species. Due to the significant economic impacts (**Supplementary Fig. 2**) of tick-borne diseases, it is necessary to develop vaccines for controlling ticks globally. In this review chitinase [39,40], toponin I like protein recombinant P27/30 protein [27], subolesin and cystatin [28], RPS-27 [33], GSK-3β [34], enolase [41], recombinant enolase [37], subolesin and enolase [38] the correlation between this antigen in tick control strategy is very remarkable. Enolase, GSK-3β, RPS-27, and chitinase which involves in metabolic and survival pathways, are just a few of the antigens that are important targets for lowering tick vitality and causing interference during their lifecycle. Ticks may efficiently feed because antigens like cystatin modulate the host immune system. They may be less able to avoid the host's immune system if these proteins are disrupted. Tick development, reproduction, and general survival are all influenced by proteins like subolesin and P27/30, which makes them potential targets for tick population reduction by preventing reproduction (**Supplementary Fig. 2**).

In many studies, the results were used to propose new candidate protective antigens for vaccine development or use as pharmaceutical targets. *Ixodes scapularis* ticks transmit numerous pathogens that cause human diseases. Recently the 19ISP mRNA vaccine was developed against *I. scapularis* [58] and tested by challenges with *I. scapularis* ticks. The results showed that the ticks struggled to attach and could not take normal blood meals, suggesting that 19ISP could be used alone or alongside traditional pathogen-based vaccines to prevent Lyme disease and possibly other tick-borne infections. Future extensions of this concept will be applied to mRNA vaccine strategies for tick-borne disease control. According to a previous study [59], standardizing procedures used in efficacy trials such as the animal model, challenge protocols, formulas for calculating efficacy, and the relationship between antibody titer and protection is also essential for the development of new anti-tick vaccines. It is difficult to compare findings from various research groups when there is a lack of standardization. Previous studies by Riding et al. [60] and Willadsen et al. [61] provided evidence that anti-tick immunity induced by a combination of vaccine antigens is more effective compared to an exposed single antigen vaccine. Our efforts are currently directed toward the characterization and in vitro expression of other concealed tick molecules for use in vaccine trials in combination with other important antigens. The findings from these studies provided important evidence for the reliability and practicality of using biotechnology to identify and produce sufficient amounts of candidate antigens for tick control vaccines. It is difficult yet possible to create a universal vaccine that protects against a variety of tick species by focusing on conserved antigens that are critical for tick survival, reproduction, and getting immunized Recombinant vaccinations that interfere with these processes provide an effective way to control tick populations and inhibit the spread of harmful diseases carried by ticks. Combining recombinant protein vaccines with new mRNA technology provides promise for more reliable and effective tick repellents, which would be a significant step in maintaining global health given the limitations of acaricides and the need for sustainable solutions.

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SUPPLEMENTARY MATERIALS

Supplementary Fig. 1

Important of controlling ticks.

Supplementary Fig. 2

Tick's anatomical features, with labeled antigens at their respective action sites.

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