[Frontiers In Bioscience, Elite, 10, 122-136, January 1, 2018]

Anti-tick vaccines in the omics era

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TABLE OF CONTENTS

1. ABSTRACT

Tick vaccines have been available for more than 20 years. They are useful and effective control agents when used properly. However, no new products have emerged since the Bm86-based Gavac vaccine was commercialized. Acaricide resistance is a problem with no abatement in sight and anti-tick vaccines are likely to be relied upon even more in the coming years. As human medicine and plant agriculture has embraced the various *Omics* technologies, the search for anti-tick vaccines would be well served to follow; so that new vaccine antigens and adjuvants might be developed to assist tick control programs. However, the simple outward appearance of ticks and their life cycle belies the complexity of their genomes which are computationally challenging to sequence and annotate. We review various *Omics* research efforts in light of research on anti-tick vaccines.

2. INTRODUCTION

Ticks belong to the phylum *Arthropoda*, subphylum *Chelicerata,* which is comprised of spiders, scorpions, mites and ticks, and the subclass Acari. The *chelicerates* include a diversified group of terrestrial and marine arthropods (1), and are the second largest group of arthropods after the insects. Ticks are important vectors of a significant number of pathogenic microorganisms, protozoa, *rickettsiae*, *spirochaetes* and viruses. Consequently, the tick and tick borne disease (TTBD) complex constitutes a global threat for livestock and human health (2). For example, *Dermacentor andersoni* affect human health because this tick is a vector for the disease known as Rocky Mountain Spotted Fever (RMSF) caused by the gram-negative *coccobacillus*, *Rickettsia rickettsia* an obligate intracellular pathogen (3). The beef and dairy cattle industries of the tropical and sub-tropical regions around the world are threaten by the cattle tick *Rhipicephalus microplus* which is vector of bovine tick fever (babesiosis and anaplasmosis) as well as other diseases such as equine piroplasmosis (*Theileria equi*) (2). Recent analysis estimated the global losses to the cattle industry caused by this TTBD complex are US$22–30b per annum (4).

The treatment of animals with chemical acaricides is the established method to control tick populations. This method has significant environmental drawbacks, including the potential for chemical residues in milk and meat and the selection of acaricide resistant ticks. Consequently, the focus of a number of scientific studies has been the development of different methods of tick control. Some of these include the use of naturally resistant cattle, biological control (biopesticides) and vaccines (5–9). The most important step forward in the area of vaccine development was the discovery in 1986 of the Bm86 glycoprotein localised on the surface of tick gut membrane cells. This protective antigen (10) induced a protective immune response in vaccinated hosts mediated by host antibodies that damage the tick gut subsequently affecting tick survival and egg viability (11–14). A second generation of vaccines based on Bm86 antigens has demonstrated effective control of *R. microplus* and *R. annulatus* infestations (Table 1) (6, 11, 12, 15–18). This antigen also induced partial protection

Table 1. Mosquitoes and Ticks genome sequencing projects

Species	Genome		ESTs	Protein Coding Genes	Diseases	Reference
	Gbp	% Sequenced				
I. scapularis	2.262	57	202,190	20,486	Lyme borreliosis	GenBank:gi|255764735|ref|NZ_ABJB000000000.1
I. ricinus	2.1	~24	1,974	6,415		GenBank: JXMZ00000000.2; http://www.ncbi.nlm.nih.gov/nuccore/1059430230
A. americanum	3.1		6,481	400		GenBank: GBBK00000000.1
A. cajennense	2.8	0.16	5,770	5,827		GenBank: GBBK00000000.1
D. andersoni	2.74		21,797	677		(43)
R. microplus	7.1	25.35	53,208	1.036		
R. appendiculatus		20.3 Mb	21,410	12,761		(44)
R. sanguineus				2,903		
Ornithodoros spp.[1]	1.09			4,013		
Anopheles gambiae	0.278	100		13,683	Malaria	http://www.ebi.ac.uk/ena/data/view/GCA_000005575.1
Culex quinquefasciatus	0.579	100		18,883		http://www.ncbi.nlm.nih.gov/bioproject/PRJNA29017
Aedes aegypti	1.38	100		17,387	Yellow Fever, Dengue, Chikungunya	http://www.ncbi.nlm.nih.gov/assembly/GCA_000004015.2

Ornithodoros parkeri (1600 ESTs), *Ornithodoros porcinus porcinus* (1482 ESTs), *Ornithodoros coriaceus* (930 ESTs)[1]

against *Boophilus decoloratus, R. appendiculatus, R. sanguineus s.l., Hyalomma anatolicum* and *H. dromedarii* (19). Bm86-based vaccines are capable of reducing the number, weight, and reproductive capacity of engorging female ticks, to the extent that subsequent tick generations show significantly reduced larval infestation. The Bm86-based vaccine has been successfully used in integrated control programs developed for cattle tick populations that include a component of limited acaricide applications for short-term control of unacceptable tick burdens (7). However, Bm86 vaccines have not shown efficacy against all tick stages and also have demonstrated unsatisfactory efficacy against some geographical *R. microplus* strains limiting widespread adoption (20). Consequently, researchers continue looking for new antigens in order to improve the current commercial tick vaccine.

3. TICK VACCINE ENTERS THE GENOMIC ERA

Until the late 1990s, vaccine development was based on the ‘‘isolation–inactivation–inoculation” principle. The “first generation” of vaccines consisted of live, attenuated or killed pathogens, while the “second generation” relied upon purified components of the targeted pathogen. Pasteur’s approach to vaccine development has been successfully applied to induce protection against several pathogenic bacteria and viruses. However, this approach is long and laborious even in successful projects (19). Additionally, vaccine research is often unsuccessful against pathogens not possessing a clear immuno-dominant vaccine candidate antigen, are unculturable, contain hyper-variable-antigens, or have complex life cycles (21). Hence, the recent advances in genomics and “omics” technologies in general have provided a “third generation” approach which might overcome these challenges. This new methodology is termed reverse vaccinology, and is rooted in functional genomics, bioinformatics, and systems biology.

During the second half of the 70s, Frederick Sanger developed a chain-termination-based DNA sequencing technique which led to the full sequencing of the genome for the virus X174 (22). However, at that time using available technology, the genome sequencing of more complex organisms as human, mouse, plant or bacteria was a very difficult and time consuming task. In 1990 the Celera Genomic Company launched the human genome initiative with an estimated cost of $3 billon. This company suggested the “shotgun sequencing” method which was used to successfully sequence the genome of the *Drosophila melanogaster* (fruit fly) (23). Also in 1990, Pal Nyrén developed pyrosequencing, a method based on sequencing-by-synthesis. Basically this protocol synthesizes the complementary DNA strand by incorporation of each nucleotide sequenced into the DNA strand (A, T, C or G) using a series of enzymatic reactions that result in a light signal read by an analyser (24). Later the 454 Life Sciences group further optimized this method utilizing the Polymerase Chain Reaction (PCR). The Genome Sequencer FLX from 454 Life Sciences was able to sequence 12.5. million bases per hour in a single instrument run, a capability that allowed sequencing of the human genome in

~ 10 days. In 1997, Shankar Balasubramanian and David Klenerman suggested (25) the use of clonal arrays and massively parallel sequencing of short reads using solid phase sequencing by reversible terminators. Basically, a fluorescently labelled reversible terminator is imaged as each dNTP is added, and then cleaved to allow incorporation of the next base. Since all 4 reversible terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias. The method minimises errors and missed calls that are often associated with strings of repeated nucleotides (homopolymers). This chemistry is the basis of the current Illumina SBS technology-based instruments that able to generate over 1 terabase (Tb) of data per instrument run.

The full sequencing of the *Haemophilus influenza* (26) genome in 1995 marked an important push into the genomic era with transcendental impact on the existing paradigm of vaccine development. To date, the genomes of 127 mammals, 76 fishes, 226 insects, 210 plants, 867 fungi, 210 *protists*, 8811 bacteria, 580 *archaea*, 5581 viruses and two ticks (*I. scapularis,* and *I. ricinus*) have been reported (http://www.ncbi.nlm.nih.gov/genome/browse/) (27–29) with many other genome-sequencing projects underway. The emergent genomic, proteomic, transcriptomic and comparative genomic datasets provide the foundation for studies identifying genes that encode putative protective antigens as novel targets for interventions. For example, reverse vaccinology, pioneered by Rappuoli and colleagues (21, 30), was used for the development of vaccines where the standard approaches failed. This method involves the *in silico* screening of the entire genome of a pathogen to identify genes with important immunogenic characteristics, followed by wet lab verification of immunogenicity and protection. Recently, groups in South Africa, the United States, and Australia have applied the reverse vaccinology method for the identification of important cattle tick vaccine candidates. The antigens selected by this methodology were tested in cattle with various levels of efficacy against cattle tick infestations (4, 31–33).

4. OMICS APPROACHES IN TICK VACCINE DEVELOPMENT

Tick genomes are among the largest genomes with sizes ranging from 1.0.4.-7.1. x10^9 bp (34, 35), and karyotype analyses have shown a sex determining system determined by XY or XO arrangement with different numbers of chromosomes. Genomics studies have been conducted in different ticks species, life stages, and tissues to enhance knowledge and understanding tick-host interaction, and as an important scientific tool assisting novel tick vaccine antigen identification. The sialotranscriptome of *A. variegatum* (36), *A. americanum* (37–39), *D. variabilis* (39), *I. scapularis* (40, 41), *I. ricinus* (42), *D. andersoni* Alarcon-Chaidez; (43) and *R. appendiculatus* (44) have been determined. *R. microplus* transcriptomes of larvae (45–49), engorged female gut (50), ovary (51), and synganglia (52) have been reported recently. The full-length coding sequences of numerous proteins and unique protein tick protein families have been described based in these analyses and their roles during host-parasite interaction described.

Proteins secreted in tick saliva are designed to counteract the innate and acquired host immune responses and facilitate the blood feeding process during the tick life cycle. Proteins such as, serpins, lipocalins, and proteases have been reported in all the tick transcriptomes that have been sequenced (53–57). These studies were mainly conducted utilizing pools of ticks or tissues collected at a specific stage of the tick life cycle. The methodologies used included Transcriptome analysis by Massively Parallel Signature Sequencing (MPSS), subtracted and cDNA libraries, and DNA microarray (Figure 1). For example, the role of the serpins family in ticks is to reduce the activity of specific proteases involved in important physiological pathways such as the complement cascade, blood coagulation, fibrinolysis, and extracellular matrix remodelling (58). Lipocalins comprise a multi-protein family with a low molecular weight and diverse functions (57, 59, 60). These proteins play an important role in modulation of the immune response, regulation of cell homeostasis and clearance of endogenous and exogenous compounds (61–67). Other functions associated with lipocalins include retinol and pheromone transport, olfaction, invertebrate colouration and prostaglandin synthesis (60). Sialome studies showed that saliva protein families are diverse and characterized by a high somatic variety based on gene polymorphism. Larger scale proteomic approaches have been used to identify proteins in *R. microplus* ovaries (68) and gut (69) of engorged females in response to infection with *Babesia bovis*. Out of these proteomic studies, several anti-tick vaccine antigens were identified and tested *in vitro* and *in vivo* (33, 70). As the relationship between transcript and protein abundance is not direct and cannot reliably be predicted, proteomic and transcriptomic provide complementary information and are not redundant.

The resources from the *I. scapularis* (57%) and *R. microplus* (~27%) genome sequencing projects will enable further gene discovery that can focus upon the tick – host interaction which might prove important for tick vaccine development (28, 71–73) (Table 1). Recently, de la Fuente and colleagues highlighted the scientific and practical implications of the recently released *I. scapularis* genome sequence (28). This resource provides a key reference for comparative genomics, tick-host and tick pathogen interaction, and facilitated knowledge about gene organization within this tick's genome (71). All tick genomes sequenced

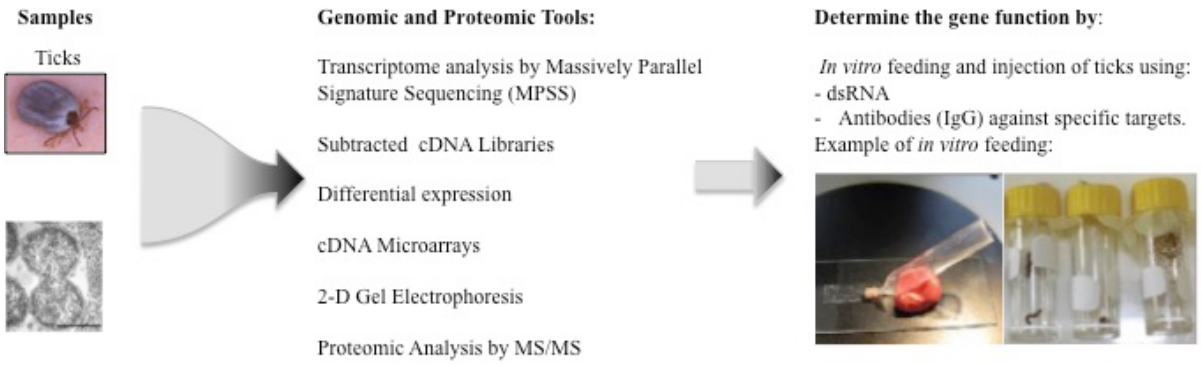

Figure 1. Functional genomics and tick vaccine development.

to date are characterized by a high content of tandem repeats and transposable elements. For example, the *I. scapularis* genome has ~ 70% of these genomic elements. Gulia-Nuss and co-workers suggested the possibility of horizontal transposable element transmission due to the high presence of the Non-LTR retrotransposons of the CR1, I and L2 clades which are common to mammals, bird and lizard (28). Additionally, the *I. scapularis* genome resource facilitated the sequencing of the *I. ricinus* genome (29). The *R. microplus* genome has been sequenced (F. Guerrero, unpublished) by a hybrid short read Illumina and long read Pac Bio approach (74, 75). The assembly of this large genome was computationally intensive and the results are currently being processed for submission to the scientific community through the peer-review process, DNA sequence databases, and CattleTickBase (72). As with *I. scapularis*, the *R. microplus* genome is complex and replete with repetitive elements (76). Genome sequencing projects have been initiated for *R. annulatus*, *A. variegatum*, and *Ornithodoros turicata* (F. Guerrero personal communication). Further sequencing efforts should be taken to complete the genomes of other tick species as an important step forward to develop novel control methods against these important vectors.

5. FUNCTIONAL GENOMICS AT THE TICK-VECTOR INTERFACE

Functional genomics refers to the use of the information in an organism's genome to study protein expression and function on a global scale (genome-wide or system-wide), using high-throughput or large-scale experimental methodologies together with bioinformatic, statistical and computational analysis of the results. Functional genomics permits the quantitative and qualitative study of the expression of one to thousands of genes under specific developmental stages of cell, tissues or an organism of interest (77). RNA interference (RNAi) is the most common technique used to study tick gene function, and it has been used in *Amblyomma, Ixodes, Haemaphysalis, Dermacentor,* and *Rhipicephalus* species (78, 79). A typical RNAi experiment involves *in vivo* injection or *in vitro* tick feeding with a solution containing a gene-specific dsRNA followed by determination of the efficiency of gene silencing and its effect on tick survival, engorgement and oviposition (Figure 1). Another method to study functional genomics involves *in vitro* tick feeding using blood or serum containing antibodies against specific molecular targets (80–83).

These experiments have provided valuable information about the function of genes involved in regulating tick feeding and reproduction (79). The RNAi pathway was identified in *R. microplus* by using sequence similarities to orthologous proteins present in *D. melanogaster*, and the effects of their silencing by RNAi using tick cell cultures and adult female ticks (84). Recently, it was shown by RNAi that Lysine-ketoglutarate reductase/saccharopine dehydrogenase (LKR/SDH) enzyme plays an important role in egg production, reproduction and development of the tick (85). RNAi experiments conducted with the vitellogenin receptor of the *ixodid* tick, *Haemaphysalis longicornis* Neumann showed this gene is essential for vitellogenin uptake and egg development, and transovarial transmission of *Babesia* parasites in this tick (86). Also, the effect of Protein Kinase B (AKT) / Glycogen Synthase Kinase (GSK) during tick embryogenesis was determined by introduction of dsRNA by electroporation of tick eggs (87). A significant reduction in tick feeding was observed after silencing the *A. americanum* (Aam) CD147 receptor by RNAi (88). Finally, four proteins, threonyl-tRNA synthetase (2C9), 60S ribosomal proteins L13a (2D10) and L13e (2B7), and interphase cytoplasm foci protein 45 (2G7), were identified using RNAi as potential anti-tick vaccine antigens against *A. americanum*, although only 2D10 and 2G7 affected both nymph and adult stages (89). Recently, nonspecific down- and up-expression of seemingly unrelated genes was reported while performing RNAi studies with Ubiquitin-63E dsRNA (sized 594 bp) and this was found to result from off-target effects of the dsRNA. This report

showed the necessity of optimization and dsRNA selection during the experimental design phases (84). The availability of the *I. scapularis* and *R. microplus* genome sequence will facilitate RNAi functional genome studies, thus improving our knowledge of tick physiology, development and gene regulation.

Functional genomics has been applied to study the tick and tick borne disease (TTBD) complex and for tick vaccine development. Tick-borne pathogens interact with their respective tick vectors through complex molecular mechanisms. Hence, different studies have been conducted to determine these mechanisms using functional genomics in ticks and tick cell lines. For example molecular studies have been conducted to study the tick-*Anaplasma* spp. interface. Diverse cellular pathways are perturbed during *Anaplasma marginale* and *A. phagocytophilum* infection of ticks. RNAi was used to study the effect of *Anaplasma* infection on the expression of the Subolesin gene (90). Similarly, MSP1, MSP2, MSP5 and other unknown surface proteins present in *Anaplasma* have been studied during their complex interaction with ticks (90, 91). The temporal transcriptional response of *A. marginale*-infected *R. microplus* showed tissue-specific differences in the number of transcripts expressed between the midgut and salivary gland. The relative low effect observed in the number of high or down regulated genes confirmed that *A. marginales* is well adapted to his vector (92). Similar studies conducted in *B. bovis*-infected *R. microplus* showing the tick's transcriptome response to infection by the apicomplexan parasite (45, 50, 51). Bastos in 2009, and Hussein 2015 (33, 70) utilized gene silencing in *R. microplus* to study gene expression induced upon infection or in association with *B. bovis* infection.

6. PERSPECTIVE

In 1912, Robert Nabours reported that F2 generation from crosses of *Bos indicus* and *Bos taurus* were resistant to tick infestations. These animals showed an inherited genetic trait for tick resistance that could be genetically segregated (93). In 1939, William Trager (94) reported that acquired immunity is responsible for tick resistance. This efforts to develop immunologically-based controls method was followed by many publications related with the host-parasite interactions and control methods (95). Two major types of antigens have emerged regarding the development of tick vaccines: 1) Secreted saliva proteins and 2) Concealed antigens (73, 96, 97). Secreted antigens have the advantage that they are re-exposed to the immune system throughout the tick's feeding process, inducing an important immunological memory. However, they are often members of protein families composed of numerous proteins with very low similarity and redundant functions (57, 98), consequently they can induce only partial protection or may fail as other family members act to effectively restore the function of the targeted molecule. Bm86 is a glycoprotein localised on the surface of the *R. microplus* gut cells and represents all the concealed antigens reported as an effective vaccine antigen in pen and field trials (5, 6, 19). The principal limitation of the concealed antigen is that they cannot boost by natural routes the host immune response; hence, booster shots are necessary to stimulate the host memory cells. There can also be sequence variants of the antigen that differ among tick populations from different geographic regions. In addition, there can be physiological issues (99) responsible for the variable efficacy of the vaccines based on Bm86 antigen. Other concealed antigens that have been evaluated for efficacy against the cattle tick are the ribosomal protein P0, showing 96 % efficacy in pen trial (100), Subolesin, showing efficacy from 0–86 % (4), and 68 and 75 % with aquaporin (33, 101). The antibody-antigen recognition process is poorly understood in the tick gut, hemolymph, and the intracellular space where tick digestion occurs.

Correlation between antibodies titre and protection from tick infestation was observed in animals vaccinated with Bm86. Therefore herds of cattle with similar mean antibody response following vaccination will also have the same average vaccine-induced protection against ticks (11, 16). However, tick infestation level, bovine breed, and physiological stage such as pregnancy, are factors affect the antibody-protection correlation. The antibody titers necessary to induce protection can be enhanced by optimized vaccine, adjuvant, dose, and treatment schedule in order to induce a more effective quantitative and qualitative antibody response. These specific responses can vary from the naturally occurring geographic antigen sequence variant. Additionally, the recombinant antigens utilised in tick vaccines lack 3D structural information that could be used to predict conformational epitopes in addition to sequential B epitopes that are currently predicted by bioinformatic algorithms. The challenges to obtain an effective vaccine against ticks remain immense and the unveiling of the *I. scapularis* and *R. microplus* genomes are essential steps forward to achieve this goal. To this point, the advent of the "*Omic*" era has only produced a small number of anti-tick vaccine antigens, most with only partial efficacy (Table 2). Genomics, functional genomics, and structural biology studies must be merged with the common goal of developing novel tick control technologies. The organs on the different life stages of the tick, particularly salivary gland and gut, should be comprehensively screened for novel antigens. Also, it will be very useful to conduct "*Omics*" studies at the individual cell level or specific tick organs, rather than maintaining focus upon pooled samples of whole ticks. Further

Table 2. Tick antigens developed and approaches tested for vaccine development

Tick antigens	Tick species	Protein identity	Approach	% Efficacy or reduction	Reference
BM86 and orthologs	*R. microplus*	Midgut membrane-bound prot.	PRC[1], 2nd generation	0–100	(19, 102–104)
	R. annulatus				
	R. decoloratus				
	H. dromedarii				
RAS-3, RAS-4, RIM36	*R. appendiculatus*	Serpins	PRC, 2nd generation		(105)
RaFER2/RmFER2	*R. microplus*	Ferritin, iron transporter	PRC, 2nd generation	64 & 72	(106)
	R. annulatus				
	I. ricinus				
64TRP	*I. ricinus*	Putative tick cement protein	PRC, 2nd generation	62 & 47 (Mortality)	(107, 108)
	R. appendiculatus				
	R. sanguineus				
GP80/VIT87	R. microplus	Vitellin/vitellogenin	Purified components	68	(14)
BlmLTI/BmTI/BmTI-A	*R. microplus*	Trypsin inhibitors	PRC, 2nd generation	18, 32 & 72	(109)
GLP	*H. dromedarii*	Glycoproteins	Purified components	63 (reduction in egg hatch)	(110)
Ef1a	*R. microplus*	Elongation factor	PRC, 2nd generation	31	(111, 112)
Subolesin	*R. annulatus*	Regulator factor	Immunisation screening by injecting naked cDNA library	0–83	113, 114)
	R. microplus				
	I. scapularis				
pP0	*R. sanguineus*	Acidic ribosomal protein P0	Synthetic peptide, 2nd generation	96	(100)
	R. microplus				
UBE	*R. microplus*	Ubiquitin	PRC, 2nd generation	13 & 55	(112)
	R. annulatus				
GST-HI	*R. microplus*	Glutathione-S transferase	PRC, 2nd generation	57	(115)
4F8	*R. microplus*	5'-Nucleotidase	PRC, 2nd generation	0	(116)
BM91	*R. microplus*	Angiotensin converting enzyme	PRC, 2nd generation	6 (reproduction)	(117)
BMA7	*R. microplus*	Mucin	Purified components 2nd generation	21 (reduction of egg weights)	(118)
Aquaporin	*R. microplus*	Aquaporin	Reverse Vaccinology, 3th generation	68 & 75	(101)
179 different Antigens	*R. microplus*	N/A	Reverse Vaccinology, 3th generation	N/A	(31)
24 different Antigens	*R. microplus*	N/A	Reverse Vaccinology, 3th generation	87	(32)

[1]Purified Recombinant Components

innovation in ideas and funding strategies will be necessary to turn discoveries into novel vaccines for tick control (73).

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Key Words: Ticks, Anti-Tick Vaccines, Genomics, Review

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