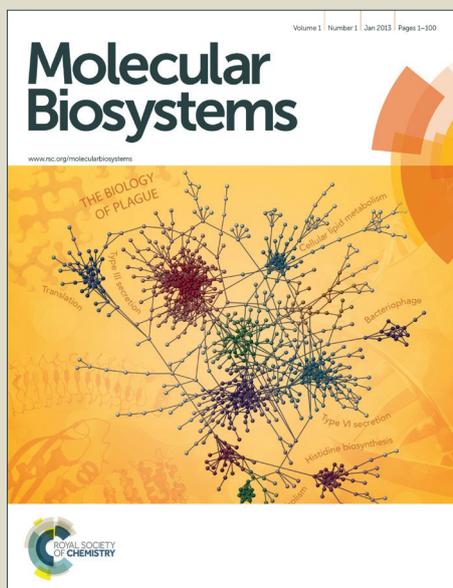


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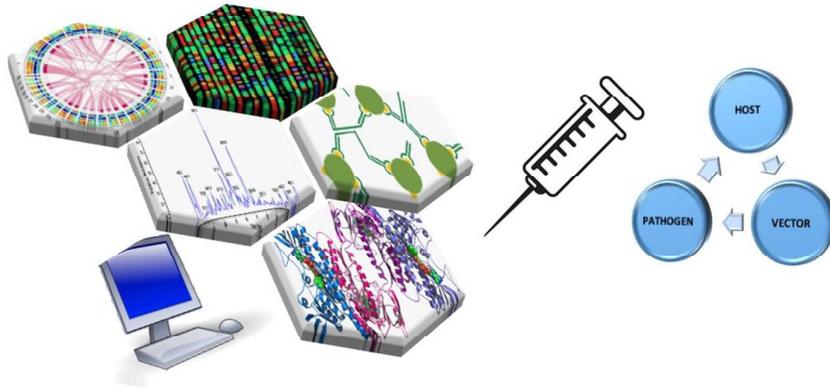


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Availability of omics datasets coupled to high-throughput and bioinformatics analyses enabled rational and faster identification of new generation vaccine candidates.

## New approaches and omics tools for mining of vaccine candidates against vector-borne diseases

Josipa Kuleš<sup>a,\*</sup>, Anita Horvatić<sup>a</sup>, Nicolas Guillemin<sup>a</sup>, Asier Galan<sup>a</sup>, Vladimir Mrljak<sup>a</sup>,  
Mangesh Bhide<sup>a,b,c</sup>

<sup>a</sup> ERA Chair VetMedZg project, Internal Diseases Clinic, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

<sup>b</sup> Laboratory of Biomedical Microbiology and Immunology, Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Kosice, Slovakia

<sup>c</sup> Institute of Neuroimmunology, Slovakia Academy of Sciences, Bratislava, Slovakia

\* Correspondence: Josipa Kuleš, ERA Chair VetMedZg project, Internal Diseases Clinic, Faculty of Veterinary Medicine, University of Zagreb, Heinzlova 55, 10 000 Zagreb, Croatia. Phone: +385 1 2390 329, email: [jkules@vef.hr](mailto:jkules@vef.hr)

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**Abstract**

Vector-borne diseases (VBD) present major threat to the human and animal health, as well as they put substantial burden on livestock production. As a way of sustainable VBD control, focus is set on vaccine development. Advances in genomics and other “omics” over the past two decades have given rise to a “third generation” of vaccines based on technologies such as reverse vaccinology, functional genomics, immunomics, structural vaccinology and systems biology approach. Application of omics approaches is shortening the time required to develop the vaccine and increasing the probability of discovery of potential vaccine candidate. Herein, we review the development of new generation vaccines for VBD, discuss technological advancement and overall challenges in vaccine development pipeline. Special emphasis is given on the development of anti-tick vaccines that can quell both, vector and pathogens.

## Introduction

Vector-borne diseases (VBD) are defined as infectious diseases of human and animals caused by pathogenic agents such as viruses, bacteria, protozoa and helminths transmitted by hematophagous arthropod vectors (mosquitoes, ticks and sand flies). Major contributors to the spread of VBD include habitat changes, ecological and climatic changes, development of insecticide and drug resistance, globalization and the significant increase in international trade, tourism and travel.<sup>1</sup> VBD impose an important global burden on public health, including widespread human diseases that were formerly zoonotic, such as malaria and dengue, as well as zoonotic diseases, such as Lyme disease, leishmaniasis, West Nile fever and ehrlichiosis.

Chemical tick control, treatment of animals, chemoprophylaxis and vaccination are among the measures available to limit losses incurred by VBD. Chemical control is limited owing to selection of acaricide-resistant ticks. Intensive acaricide use also interferes with enzootic stability, rendering animals susceptible to the diseases. Chemoprophylaxis can be effective, but only for a short period. Vaccines offer green solutions to control disease as they are sustainable, and reduce reliance on pharmacological drugs and pesticides. The use of vaccines has multiple benefits such as improving animal health and welfare by controlling animal infections and infestations; improving public health by controlling zoonoses in animals; solving problems associated with resistance to acaricides, antibiotics and anthelmintics; keeping animals and the environment free of chemical residues and maintaining biodiversity.<sup>2</sup> Therefore, sustainable control has been based on vaccination.<sup>3</sup> All of these attributes should lead to improved sustainability of animal production and economic benefit.

The dominant technology of vaccine development until the late 1990s have been relying on „isolate – inactivate – inject“ principle. With this conventional approach, protection against a wide range of bacterial and viral pathogens (but not parasitic pathogens) has been achieved,

and whole-organism vaccines still represent a large proportion of the vaccines licensed today. Vaccines produced following Pasteur's principles allowed the control and, in some cases, the eradication of many important infectious diseases. These first and second generation of vaccines consisted of live, attenuated or killed pathogens (first generation), or purified pathogen component (second generation). A major advantage of whole-organism vaccines (first generation) is that they do not require prior knowledge of immune responses associated with protection, or of the pathogen genome or proteome.<sup>4</sup> Importantly, whole-organism vaccines provide an extensive repertoire of pathogen-derived B cell and T cell epitopes for recognition by the immune system of the vaccine in the context of multiple genetic backgrounds found in heterogeneous human populations, although only a fraction of these epitopes may be important targets of protective immunity. Despite these advantages, whole-organism killed, inactivated or live attenuated vaccines are typically complex and their poorly characterized products have often raised safety concerns including reversion to virulence for live attenuated pathogens, induction of autoimmunity, or unacceptable reactogenicity associated with the induced inflammatory response.<sup>5,6</sup> Development of the second generation vaccines was allowed by advances in cell culture (*in vitro* growth of viruses), polysaccharide chemistry, recombinant DNA technology and immunology.<sup>6</sup> Antigen identification typically started with the cultivation of the target pathogen, and the component proteins are then assayed in a cascade of *in vitro* and *in vivo* assays, leading to the identification of a subset of proteins associated with protective immunity. However, not all pathogens can be cultivated outside the host, many proteins are expressed only transiently during the course of infection, and not all proteins are abundant enough to be detected by *in vitro* assays. Furthermore, identification of vaccine antigens by this approach may take decades.<sup>7</sup>

Despite several successes, the Pasteur's approach to vaccine development took a long time to generate vaccines against those pathogens for which the solution was feasible, but failed to

produce vaccines for those viruses, bacteria and parasites that do not have obvious immunodominant protective antigens or are yet uncultivated, pathogens with antigenic hypervariability or those with complex life-cycle.<sup>8</sup>

Advances in genomics and other „omics“ over the past two decades have given rise to a “third generation” of vaccines based on technologies such as reverse vaccinology, functional omics and systems biology approach. To overcome challenges of conventional approach in vaccine development, vaccine design has become more tailored, focusing on the antigen moieties targeted by protective immune responses,<sup>4</sup> with a broad perspective of the pathogen and its interaction with the host immune system.<sup>9</sup> Thus, the modern era of vaccinology is increasingly reliant on novel omics approaches that incorporate high-throughput cutting edge technologies such as genomics, proteomics and transcriptomics, with improved understanding and technological innovations in basic immunology, host-pathogen biology, immunomics, advanced bioinformatics and computational modeling. Herein, we review the development of new generation vaccines for VBD. We also highlight the role of advanced omics tools in rational design of vaccines against VBD.

### **New generation of vaccine development technologies for new generation of vaccines**

The availability of the first bacterial genome sequence in 1995 marked the beginning of a genomic era that has allowed scientists to change the paradigm of vaccine development. To date, genomes of 70 animals, 36 plants, 56 fungi and 32 protists as well as approximately 2490 bacteria, 170 archaea and 3339 viruses have been sequenced (<http://www.ncbi.nlm.nih.gov/genome/browse/>). Furthermore, several thousand genome sequencing projects are currently underway. The ever growing body of genomic, proteomic, transcriptomic and comparative genomic datasets provides the foundation for studies

identifying genes that encode putative protective antigens as novel targets for interventions. New strategies for vaccine development are based on the key elements of new paradigm in vaccine development, that are the omics technologies, sophisticated bioinformatics and computational modeling tools (Table 1).

Reverse vaccinology, one of the novel approach pioneered by Rappuoli and colleagues, was used for the development of vaccines against pathogen for which the applications of Pasteur's principles have failed.<sup>10</sup> It comprises high-throughput *in silico* screening of the entire genome of a pathogen to identify genes that encode proteins with characteristics associated with vaccine-induced immunity, and the systematic evaluation of those proteins for immunogenicity.<sup>11</sup> Thus vaccine development in reverse vaccinology approach is starting from the genomic information rather than growing the causative microorganism. The development of a serogroup B *Neisseria meningitidis* (MenB) vaccine against meningococcal meningitis transmitted by airborne droplets, represents the first example of the successful application of reverse vaccinology.<sup>12</sup> In just 18 months more surface exposed antigens have been identified than in 40 years of conventional vaccinology and a vaccine based on three of these novel antigens and outer membrane vesicles is now licensed in 30 countries.<sup>13</sup>

Another innovative approach for vaccine development, a “pan-genome approach” represents further advancement in the reverse vaccinology, since it overcomes the problems such as gene variability.<sup>8</sup> Pan-genomic reverse vaccinology aims to identify conserved vaccine candidates in antigenically diverse species. Comparative analysis of the newly sequenced genomes revealed that a pathogen species can be described by its “pan-genome”.<sup>14</sup> The pan-genome can be defined as the global gene repertoire referring to a species, and it includes a “core genome” containing genes present in all strains and a “dispensable genome” composed of genes absent from one or more strain and genes that are unique to each strain. Dispensable genes are responsible for species diversity and might encode additional biochemical pathways

and functions that can confer selective advantages, such as antibiotic resistance or colonization of the host. The pan-genome concept was first successfully applied to group B *Streptococcus* (GBS) vaccine discovery. Using various computational algorithms, pan-genome approach was used to predict potential vaccine candidate for both highly virulent species of *Leishmania*, *L. major* and *L. infantum*. Total proteome of both species were compared to identify common proteins, and from 8,122 common proteins, only 19 epitopes were analyzed for their binding efficiency to both major histocompatibility complex (MHC) class I and class II alleles. Verification of these candidates is to be justified through the confirmation made by wet lab analysis.<sup>15</sup>

The third promising approach, a subtractive reverse vaccinology, was developed on the principle that is based on subtractive comparative genome analysis to compare genome of a pathogenic isolate to a nonpathogenic or commensal isolate. This approach could provide identification of antigens critical in pathogenesis and genes conserved between pathogenic and nonpathogenic isolates could be discarded, reducing the number of candidates and consequently, reducing the time for development of a vaccine.<sup>11</sup> A very recently adopted methodology is based on subtraction of sequence between host and parasite proteome. Availability of whole genome sequence for human and pathogenic microbes has been utilized to find vaccine and therapeutic targets against *Borrelia burgdorferi*<sup>16</sup> and *Leishmania donovani*<sup>17</sup>. Thus sorted key pathogenic proteins essential for parasite survival but non-homologous to host, are subsequently checked for their cellular localization within the pathogen, exo-membrane topology and role in metabolic pathways to be used for drug or prophylactic targets. Usually, conserved secreted and membrane proteins from pathogens are selected to design novel vaccines.<sup>18</sup> Weak immunogenicity of secreted proteins due to their inability to induce maturation of dendritic cells, can be overcome in part by adjuvant formulations.<sup>19</sup> A preliminary work based on computational subtractive genomics has

identified protein kinase, adenylate kinase and MAP kinase homolog of *L. donovani* for regulating normal cell cycle and differentiation of the parasite.<sup>17</sup> Further prediction of functional sites and exploration of *Leishmania* proteome are required to get the full benefit of this approach for a cross-protective vaccine against visceral leishmaniasis. Moreover, it is advisable to consider all the pathogenic strains undertaking *in silico* mutagenesis approach and pan-genomics data base, for further improvement of this basic strategy.<sup>18</sup>

Advances in structural proteomics had led the substantial progress in the structural vaccinology. Detailed three-dimensional (3D) structure, domain organization, and dynamics of surface proteins of pathogens offer molecular targets that can guide the design of effective vaccines and better immunogens by stabilizing native conformations or combining, exposing, and/or improving the immunogenicity of epitopes.<sup>20</sup> Important goals of this relatively new vaccine development technology are to stabilize a conformation of an antigen capable of eliciting protective responses or to selectively present the conserved determinants of complex and variable antigens in order to focus immune response to conserved epitopes.<sup>21</sup> Detailed analysis of immune responses revealed by structural vaccinology, for example by pyrosequencing of expressed antibody genes or by B cell sorting to isolate monoclonals for crystallographic and other analysis, should provide a wealth of data to guide further improvements in immunogen design.

As yet there are no commercial vaccines available based on structure-based design and most of the structure-based vaccine candidates are in the preclinical stages of development. Computational design and bioinformatics analysis together with *in vitro* screening and evolution methods will be integrated with biophysical, structural and immunological evaluation in innovative vaccine design approach to develop vaccines with highly optimized and specifically tailored immune responses.<sup>20</sup>

Structural vaccinology was employed to develop a vaccine for Lyme disease, based on the rationale that protective epitopes should be sufficient to induce immune responses.<sup>22-24</sup> The Lyme disease vaccine was based on the outer surface protein A (OspA), an antigenic protein of *Borrelia*. The study was based on the observation that anti-OspA monoclonal antibodies that block *Borrelia* transmission bind to conformational epitopes in the C-terminal domain of OspA, suggesting the possibility of using the C-terminal domain alone as a recombinant protein-based vaccine.<sup>22</sup>

A novel concept, systems biology (which includes systems immunology and systems vaccinology) was developed during the last decade to study the immune response in an integrated perspective rather than studying isolated components, in order to identify immune correlates of protection or signatures of immunogenicity.<sup>25</sup> This approach possesses some of essential elements, such as (i) monitoring all components of the system in response to perturbations, (ii) integration of data from multiple types and (iii) creation of mathematical models to predict the structure and behaviour of the informational system.<sup>26</sup> Such studies are expected to provide a comprehensive understanding of the host-pathogen interaction and its regulation, to identify novel immune mediators and pathways, and correlates vaccine efficacy, facilitating vaccine evaluation in the clinic.<sup>6</sup> Thus, a systems biology approach also holds great promise as a methodology to identify, model and predict the overall dynamics of the host-pathogen interactome. It is believed that such approach will be essential in future for the rational design of both animal and human vaccines.<sup>27</sup> The challenges in using systems biology in application to vector-borne diseases will be related to the integration of “omics” data (various omics approaches are described below) from host-vector-pathogen interactions. It is important to note that omics data must be from different developmental phases of pathogens in both host and vector. Another challenge in the development of vaccines against most of the

VBD is related to the wide spectrum of animal hosts in which vaccines must perform effectively (e. g. *E. ruminantium* can affect cattle, sheep, goats, and water buffalo).<sup>28</sup>

### Omics approaches in vaccine development

Functional genomics, transcriptomics, proteomics and immunomics are key omics approaches in vaccine development. Functional genomics and transcriptomics contribute to vaccine development in the selection of appropriate vaccine candidates not by examining directly the genetic content but by the transcription and gene expression profiles. Development of bioinformatics tools and availability of pathogen genomes enabled accessible web databases for different VBD, e.g. for babesiosis (<http://piroplasmadb.org/piro/>), malaria (<http://plasmodb.org/>, <http://www.genedb.org/>) and Lyme disease (<http://borreliabase.org/>).

For vaccine development it is of key importance to know what genes are expressed during host infection. In fact, proteins that are expressed during disease represent the most likely protective vaccine components. Gene expression can be analyzed in either pathogen or host, thus allowing investigation of both sides of the host–pathogen interaction. The transcriptome of a pathogen (the complete set of RNA transcripts expressed under a specified condition) can be used to identify genes that are differentially expressed upon infection, during different stages of multi-stage pathogens, or in response to particular factors and nutrients available in various host environments. The most widely used methods to identify specific subsets of genes involved in pathogenesis that may represent potential targets of protective immunity are DNA or RNA microarrays and, most recently, RNA sequencing (RNAseq). To date, the most widely used method for transcriptome analysis is DNA microarray, a high-throughput technique that has also been successfully applied in VBD studies.<sup>29, 30</sup> The application of transcriptome analysis to vaccine development is expected to greatly advance with improving

technologies for differentially extracting pathogen RNA from tissues during *in vivo* experiments, as shown for bacterial pathogens such as *B. burgdorferi*.<sup>31</sup> The increasing availability of microarrays and the new wave of microarray-independent gene-expression analyses based on advances in ultra-high-throughput pyrosequencing technology, which have enabled the rapid sequencing of cDNA and quantification of sequence reads, should enable further transcriptome-based advances in vaccine development.<sup>32</sup> Expression microarray and RNA-sequencing approaches were also employed to compare transcriptome profiles of two *Babesia bovis* strain pairs, with each pair consisting of a virulent parental and its attenuated derivative strain.<sup>33</sup>

Proteomics-based approaches have rapidly developed and are now widely considered to be effective technologies that are complementary to classical genomic-based approaches for discovering surface-associated, immunogenic proteins that could be potential vaccine candidates.<sup>32</sup> Advances in protein separation technologies, combined with mass spectrometry and genome sequencing, have made the elucidation of total protein components of a given cellular population a feasible task and facilitated the application of proteomics to antigen discovery. Proteomic discovery methods could be more advantageous than transcriptomic methods because the expression of a gene does not necessarily correlate with protein translation and post-translational modifications might play a role for antigenicity.<sup>34</sup>

*In silico* analysis of proteome data has led to the definition of several new “omes” relevant to antigen discovery, such as the surfaceome, exportome, secretome, and interactome.<sup>7</sup> For most bacterial pathogens, proteins able to elicit a protective immune response are either secreted proteins or surface-exposed proteins, and these represent the most promising vaccine candidates. *In silico* analysis of the complete sequence of several bacterial genomes predicts that surface-associated proteins constitute 30%–40% of all bacterial proteins. Using this information, a novel proteomic-based approach was developed to specifically isolate bacterial

surface proteins.<sup>35</sup> The method uses proteolytic enzymes to “shave” the bacterial surface under conditions that preserve cell viability, and the peptides released are analyzed by mass spectrometry (MS). The peptide sequences are then matched with predicted gene sequences from published genomes, permitting a fast and selective identification of all proteins partially or entirely exposed on the bacterial surface. The major limitation of this approach is that bacteria are grown under rich conditions *in vitro* and harvested at a single growth phase, which does not reflect *in vivo* conditions. Furthermore, this approach would not be suitable to identify protease resistant protein complexes such as pili, which were demonstrated to be promising vaccine candidates for some cases.<sup>7</sup> Similar proteomics approach using biotinylation was used for the identification of the proteins on the surface of malaria-infected erythrocytes.<sup>36</sup>

Immunomics remains most favorable among omics approaches for vaccine development. It studies the subset of pathogen-derived proteins or their epitopes that are recognized by the host immune system. Immunomics-based antigen identification strategies integrate and validate *in silico* and *in vitro* approaches by evaluating whether previously selected or newly identified proteins are targets of clinically relevant immune responses (e.g. production of specific cytokines, activation of cell types, protection).<sup>37</sup> Thus, immunomics specifically addresses the interface between the host immune system and the pathogen proteome.

Generating vaccine-mediated protection is a complex challenge. Currently available vaccines have largely been developed empirically, with little or no understanding on how they activate the immune system. Their early protective efficacy is primarily conferred by the induction of antigen-specific antibodies, produced by B lymphocytes. Contemporary approaches to vaccine design recognise adaptive immunity as a highly sophisticated biological response involving antibodies and T cell receptors as recognition systems that have evolved in response to the high mutation rate of pathogens and intracellular replication.<sup>19</sup> Antigen recognition by

the cellular elements of the adaptive immune system initiates a focused, highly specific immune response, finally resulting in elimination of the pathogen and termination of the disease. The identification of appropriate antigenic structures involves various other considerations, based on the desired type of immune response. For example, if a neutralising antibody response is needed to protect from infection, usually an antigenic structure from the bacterial/viral cell surface is selected.<sup>4</sup> T lymphocytes represent distinct mode of antigen recognition when compared to antibodies, that allows the immune system to recognize and fight intracellular pathogens. Unlike extracellular pathogens, for which protective immunity is caused mostly by the elicitation of functional antibodies, immune protection against host cell-invading pathogens usually is mediated by the concerted action of both humoral and cell-mediated immunity.<sup>38</sup> Therefore, the development of efficacious vaccines against obligate and facultative intracellular pathogens depends largely upon the ability to identify antigen formulations that induce effective B- and T-cell responses. The first demonstration of the role of T lymphocyte subsets in immunity to spotted fever group rickettsiae showed that immune T lymphocyte subsets was capable of conferring protection against a lethal dose of *R. conorii* and that clearance of spotted fever group rickettsiae from endothelial cells requires immune CD8+ T lymphocytes.<sup>39</sup>

Although both genomic and proteomic strategies have been applied successfully to discover B-cell-stimulating vaccines, the identification of antigens eliciting effector T cells generally is considered less amenable to high-throughput approaches. Presently, the methodical challenges of working with T-cells need to be addressed because most of the vaccines that remain to be produced require a strong T-cell component to afford significant protection.<sup>38</sup> Empirical methods for identification of antigens recognized by T-lymphocytes rely on T-cells from animals or individuals that are immune to the pathogen. Those memory T-cells had been selected during the physiological immune response to persist and recognize a limited number

of antigens (i.e., immunodominant antigens). Thus, methods that use memory T-cells for antigen identification are more likely to miss potentially protective subdominant antigens. One strategy for T-cell antigen identification that is not biased towards immunodominant antigens is genomic immunization or expression library immunization (ELI). This method allows the priming of naïve T-cells by the expressed cloned microbial genes regardless of whether they are subdominant or dominant during a natural infection as long as the appropriate T-cell receptors are present.<sup>40</sup>

The study of immune biomarkers or antigens is not new and classical methods such as agglutination, enzyme-linked immunosorbent assay or Western blotting have been used for many years to study the immune response to vaccination or disease. However, in many of these traditional techniques, protein or peptide identification has often been the bottleneck. Recent advances in genomics and proteomics, has led to many of the rapid advances in proteomics approaches. A rapidly growing collection of approaches have the common goal of identifying and measuring antigenic peptides or proteins. This includes gel based, array based, mass spectrometry based or *in silico* approaches.<sup>41</sup>

The set of proteins identified by this approach, which are able to elicit a humoral immune response during the course of infection, represents the “immunoproteome” or “antigenome” of that pathogen. A number of methods have been developed to enable high-throughput display of the proteome of a pathogen to the host immune system, including SERPA (SERological Proteome Analysis), high-throughput proteome wide screening of antibody targets (proteome arrays), glycopeptide arrays, antigen discovery using expression arrays (SEREX), immunocapture mass spectrometry (MAPPING), epitope mapping.

A common immunomics-based approach uses sera or plasma as selection agent to screen protein libraries derived from genomic sequence data. Originally, small (linear epitopes) or medium-sized (potential conformational epitopes) protein fragments were displayed on the

surface of *E. coli* via outer membrane proteins. More recently, a variety of microarrays displaying whole cells, phages or purified macro-molecules has been used for serological antigen screening.<sup>7</sup>

SERPA (SERological Proteome Analysis) combines top down proteomics based approaches with serological analysis for the identification of *in vivo* immunogens suitable as vaccine candidates. 2D-PAGE resolves the majority of proteins in a sample to a single protein spot, giving the potential to readily identify the antigenic proteins within the resolved proteome. Gels are then transferred to membranes and probed with sera from animal models or humans and developed as per any traditional Western blotting experiment.<sup>41</sup> Example of applied SERPA approach is study focused on visceral leishmaniasis that led to detection of different immunogenic antigens.<sup>42</sup> Immunoproteomics using two-dimensional gel electrophoresis (2-DE), matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and Western blot analyses from clinical isolates was used for identification and evaluation of responses to parasite T-cell epitopes as putative vaccine targets. Results of this study reflected specificity pattern of the immune responses against complex pathogens.

As an example of high-throughput immunomics being a powerful platform to discover potential targets of host immunity in vector-borne diseases, can also serve a study by Chen et al.<sup>43</sup> In this study, *in silico* data mining by comparative genomics combined with high-throughput profiling antibody using high density protein microarray screening was used to study responses against blood-stage *P. vivax* infection. A total of 151 highly immunoreactive antigens were identified, and there were 40 proteins that exhibit potential for antibody-surveillance applications.

### **Applications and examples of vaccine development against VBD**

The major economic concerns with VBD remain in the veterinary field with a special emphasis on animal production. Veterinary vaccines comprise only approximately 23% of the global market for animal health products; but this sector is growing consistently due to new technological advances in vaccine development, the continuous development of drug resistance by pathogens, and the emergence of new diseases.<sup>44</sup> Four groups of VBD are of importance to the livestock production: theileriosis, babesiosis, anaplasmosis and heartwater (also called cowdriosis), posing major health and management problems of cattle and small ruminants in affected areas.<sup>45, 46</sup> Globally, most of the veterinary vaccines available to overcome these diseases are attenuated or live blood-derived. Though these vaccines can be effective, little is known about their full mechanism of action. They comprise important drawbacks, as a short shelf life, the potential transmission of other pathogens, the risk of reversion to virulence, requirement of a cold chain system of maintenance and difficulties in standardizing the vaccine dose.<sup>45</sup> Therefore, an additional research is needed for the development of safer alternatives, more cost-effective and better defined vaccines.

Reverse vaccinology approach was used to identify potential vaccine candidates for protection against *Ehrlichia ruminantium*, an obligate intracellular bacterial pathogen which causes heartwater, a serious tick-borne disease of ruminants throughout sub-Saharan Africa.<sup>47</sup> Applied approach involved four steps: (i) *in silico* selection of open reading frames (ORFs) from the annotated genome; (ii) cloning of selected genes, and the expression and purification of the corresponding recombinant proteins; (iii) identification of recombinant proteins that induce IFN- $\gamma$  production in ELISPOT and/or qPCR assays; and (iv) cytokine profiling of IFN- $\gamma$ -inducing recombinant proteins using qPCR. By *in vitro* cellular immune response 11 antigens were identified.

Another successful application of reverse vaccinology was in East Coast fever, highly fatal lymphoproliferative disease of cattle caused by *Theileria parva*, with the aim to identify a

vaccine candidate antigen of *T. parva* that is the target of schizont-specific cytotoxic T lymphocytes (CTL) from immune cattle.<sup>48</sup> The availability of the complete genome sequence data and bioinformatics tools was used to identify genes encoding secreted or membrane anchored proteins that may be processed and presented by the MHC class I molecules of infected cells to CTL. From the 986 predicted ORFs on chromosome 1, bioinformatics was used to select 55 genes encoding proteins that could potentially access the host cell cytosol. From the selected genes, 36 were successfully cloned into a eukaryotic expression vector and screened for recognition by schizont-specific CD8<sup>+</sup> CTL derived from 13 cattle immunised against *T. parva* by a live infection and treatment immunization regime. One antigen, termed Tp2, was identified by CTL from 4 different animals. Subsequent analysis has defined multiple CTL epitopes on this molecule. A more thorough characterization of the genotypic and antigenic diversity of the *Theileria parva* is on-going, and currently the Muguga cocktail vaccine, which provides broad-spectrum immunity to East Coast fever is now a registered product in eastern Africa.<sup>49</sup>

Despite rapid advances in the diagnosis of bacterial infections and the availability of effective antibiotics, Lyme disease, the most common vector borne disease, continues to represent a substantial public and global health problem. Availability of *Borrelia burgdorferi* ZS7 proteome, the causative organism of Lyme disease, has enabled *in silico* analysis of protein sequences for the identification of drug and vaccine targets. A subtractive genomics approach was used to identify proteins exclusively present in the pathogen by deducing the homologous proteins.<sup>16</sup> From 272 essential proteins that were identified, 42 proteins were unique to the microorganism. The study further identified 15 membrane localized drug targets, which can be characterized for the development of novel vaccine epitopes and novel antibiotic therapy against *Borrelia burgdorferi*.

For viral diseases, the development of effective universal vaccines has been slowed by the elevated mutation rate and the high antigenic diversity associated with the pathogens. Recent studies using next generation sequencing technologies highlight viral dynamics and genomic diversity even within the host.<sup>50</sup> Biological studies of antigenic diversity require great experimental effort, even for a single viral protein. Rapid accumulation of sequence data from both classical and functional genomic approaches makes the experimental studying of antigenic diversity difficult and time-consuming. Therefore, a bioinformatics approach is necessary to support large-scale antigenic analysis of viral diversity, which can complement laboratory experiments. In the case of dengue virus, a systematic bioinformatics approach has been applied to define the antigenic diversity of viral strains by analyzing more than 9000 dengue protein sequences, and identifying peptide combinations sufficient to capture antigenic diversity of T-cell epitopes.<sup>51</sup>

The major challenge in developing dengue vaccine is to induce a broad durable immune response against all four serotypes of dengue virus simultaneously. Ramanathan et al.<sup>52</sup> have developed a universal vaccine construct, DNA SynCon, which was found to induce a humoral response in mice. The construct consists of a consensus region from domain III of the E protein from all four serotypes, which was cloned into a mammalian expression vector. The safety and immunogenicity of this candidate needs to be further evaluated.

Many protozoan parasites have complex life cycles within their host species and they have evolved different strategies to evade or work with the immune response of the host to enable them to survive, multiply and differentiate to new forms. The net outcome of the wide array of different immune responses may have different consequences for the host parasite relationship.<sup>2</sup> One well studied example of protozoan vector-borne disease is leishmaniasis. There is freely accessible LeishCyc database that houses a comprehensive bank of gene

products, metabolites and biochemical pathways from transcript, protein and metabolome profiling studies of *L. major* in an integrated format.<sup>53</sup>

The first large-scale evaluation of T cell epitope prediction tools to identify new *Leishmania major* antigens and evaluate the performance of this immunoinformatics approach appears to be a very powerful strategy for rational antigen identification, particularly for a pathogen with a large genome such as *Leishmania*.<sup>54</sup> In this study, 8272 sequences from the whole *L. major* genome were analyzed to generate consensus predictions of potential T cell epitopes and the immunogenicity of 26 of the best candidates were evaluated in mice. Fourteen epitopes resulted immunogenic, indicating an elevated rate of successful predictions, thus establishing the usefulness of this strategy for vaccine development.

### **Tick vaccines**

Alternative approaches have been developed to control vector-borne diseases, which involve the development of anti-tick vaccines that can quell both, vector and pathogens. In particular, ticks are second to mosquitoes as vectors of disease and can transmit a wide variety of infectious agents. In addition, ticks are the most important vectors of pathogens that cause disease in cattle.<sup>55</sup> The *Ixodes ricinus* species alone transmits viruses, bacteria, and protozoa that cause in humans tick-borne encephalitis, Lyme disease, and babesiosis, respectively. *Rhipicephalus microplus* (cattle tick) is the most significant in terms of impact on livestock in particular the beef and dairy cattle industries of tropical and sub-tropical regions of the world.<sup>56</sup> Globally, 80% of the world's cattle populations are at risk of VBD with the huge economic losses caused by ticks and tick-borne diseases.<sup>57</sup>

The development and application of tick vaccines includes proper knowledge of the host and tick species involved, characterization of tick-host-pathogen interactomes and identification of protective antigens. The ultimate goal of arthropod vector vaccines is the control of tick infestations and vector-borne diseases. The effect of tick vaccines on VBD could be obtained

by (i) reducing ticks populations and thus the exposure of susceptible hosts to vector-borne pathogens, (ii) reducing the arthropod vector capacity for pathogen transmission, and, preferably, (iii) a combination of these factors.<sup>58</sup> The limiting step in the development of tick vaccines is the identification of protective antigens. Tick antigens for vaccine candidates have been classified as either exposed or concealed antigens.<sup>59</sup> Exposed antigens are those that naturally come into contact with the host immune system during tick infestation, such as antigens secreted in tick saliva during attachment and feeding in a host. Concealed antigens are those not normally presented to the host, and associated with some vital function for the tick, e.g. BM86 antigen from the tick gut wall that induce an antibody-mediated host immune response detrimental to tick survival. Recently, a third group of antigens has been distinguished that combines the properties of both exposed and concealed antigens, offering the prospect of a broad-spectrum vaccine and also showing transmission-blocking and protective activity against a tick-borne pathogen.<sup>60</sup> The feasibility of controlling tick infestations through immunization of hosts with selected tick antigens was previously demonstrated using recombinant antigens such as BM86 and homologs, subolesin, serpins, ferritins, salivary proteins and others (Table 2).

The advances in characterization of tick genomes has allowed a rapid, systematic and comprehensive approach to tick vaccine discovery. Genomics databases have been generated for different tick species such as the *Ixodes scapularis* genome project and the *R. microplus* database “CattleTickBase”.<sup>61, 62</sup> These studies have led to the identification of multiple genes that are critically important for tick survival during the host-parasite interaction process.<sup>61</sup> Candidate tick protective antigens have been identified using advanced technologies allowing rapid, systematic and global antigen screening and providing a comprehensive approach for the selection of candidate vaccine antigens (Table 3). High throughput screening platforms used for the identification of tick protective antigens include protein fractionation and testing

in vaccinated hosts, expression library immunization (ELI), suppression subtractive hybridization (SSH), microarray hybridization, in vitro tick feeding systems and RNA interference (RNAi).<sup>63</sup> High-throughput technologies used in reverse vaccinology approach to identify candidate tick protective antigens include a microarray hybridization, *in silico* prediction of protein localization and protective antigenicity corroborated by immunoassays.<sup>29</sup> Expression library immunization is a high-throughput technology to discover vaccine candidate genes by using the immune system to screen the entire genome of a pathogen for vaccine candidate.<sup>64</sup> It is based on the immunization of groups of mice with subsets of an expression library of a pathogen, followed by the identification of the subsets providing protection against an infectious challenge. The main advantage is that it makes no assumptions on which type of proteins may be a good vaccine candidate, nevertheless ELI is still very time consuming and labor intensive. A combination of cDNA ELI and sequence analysis resulted in the identification of individual protective antigens against *I. scapularis* infestations.<sup>65</sup> In order to identify protective antigens, a cDNA expression library was constructed from a continuous *Ixodes scapularis* cell line (IDE8). cDNA clones were subjected to several rounds of screening in which mice were immunized with individual pools and then challenge-exposed by allowing *I. scapularis* larvae to feed on the immunized and control mice. Immunity against tick infestation was determined by the reduction in the ability of the larvae to feed to repletion and molt to the nymphal stage. Individual clones in pools that induced immunity to larval infestations were partially sequenced and grouped according to their putative protein function by comparison with sequence databases. The screening identified several individual antigens that induced a protective immune response against *I. scapularis* infestations. This was the first study to demonstrate that cDNA -ELI combined with analysis of expressed sequence tags (EST) is a powerful and efficient tool for identification of candidate antigens for use in vaccines against ticks.

RNA interference (RNAi) is becoming the most widely used gene-silencing technique in ticks and other organisms where alternative approaches for genetic manipulation are not available or are unreliable.<sup>66</sup> Study by de la Fuente et al.<sup>67</sup> applied the posttranscriptional gene silencing by RNAi to pools of *I. scapularis* cDNAs used previously in ELI experiments, for identification of tick protective antigens. Through RNAi, it is possible to screen a large number of genes for potential vaccine candidates in a relatively short time and with minimal use of laboratory animals. Selected antigens could then be characterized and evaluated as recombinant proteins in controlled vaccine trials. RNAi could serve as a more rapid and cost-effective tool for vaccine antigen discovery in ticks than expression library immunization, however, one drawback of this approach is the necessity of beginning the screening process with large pools of dsRNAs in order to increase the number of clones analyzed while reducing the number of treatments.

Tick vaccines became commercially available in the early 1990's for the control of cattle tick infestations. TickGARD (in Australia) and Gavac (in Latin American countries) are both derived from *R. microplus* midgut membrane-bound recombinant protein BM86. However, these vaccines were not effective against all tick stages and have demonstrated unsatisfactory efficacy against some geographical *R. microplus* strains limiting widespread adoption.<sup>68</sup> Additionally, several boosts of TickGARD® were required per year to achieve optimal protection and TickGARD® has not been commercially available since 2010.

New generation tick vaccines will likely combine tick antigens with different protective mechanisms alone or in combination with pathogen-derived antigens to ultimately result in the reduction of tick infestations while affecting pathogen infection and transmission to control VBD.<sup>69</sup> Recent results using vaccination with the combination of tick subolesin with *A. marginale* major surface protein 1a (MSP1a) as a membrane-exposed chimeric antigen showed an effect on reducing tick infestations and pathogen infection under field conditions.<sup>70</sup>

Other directions to improve tick vaccine efficacy include the use of novel formulations based on more effective adjuvant and antigen presentation and the possibility of developing vaccines with tick knock-down effects.<sup>69</sup>

Apart from ticks, there are increasing number of studies that focus on other vectors of VBD, as mosquitos and sand flies.<sup>71-73</sup> Research on tick vaccine development is, however, more advanced than that reported for other major ectoparasites. Recent studies using subolesin/akirin have shown how vaccination with these orthologue proteins in ticks and insects protects against multiple ectoparasites and the infection with vector-borne pathogens.<sup>74</sup> Vaccination with subolesin/akirin has shown an effect on the reduction of infestations by soft and hard ticks (*I. scapularis*, *I. ricinus*, *R. microplus*, *R. annulatus*, *R. sanguineus*, *Amblyomma americanum*, *Dermacentor variabilis*, *Ornithodoros erraticus*, *O. moubata*), mosquitoes (*Aedes albopictus*), poultry redmites (*Dermanyssus gallinae*), sand flies (*Phlebotomus perniciosus*) and sea lice (*Caligus rogercresseyi*). Subolesin and akirin are functionally important for arthropod innate immunity to pathogens and, at least in ticks, for other molecular pathways, including those required for tissue development and function, and for pathogen infection and multiplication.<sup>55</sup>

Mosquitoes belonging to the genus *Aedes* transmit many viruses and parasites, and *Aedes aegypti*, the main vector of the yellow fever and dengue viruses, is the best-characterized species of subfamily Culicinae.<sup>75</sup> A study using 2D DIGE followed by MALDI-MS/MS was carried out to investigate protein responses in the *A. aegypti* midgut to Chikungunya and dengue virus.<sup>76</sup> A transcriptomic study of the *A. gambiae* salivary gland response to blood feeding, identified candidate genes that are involved in hematophagy.<sup>77</sup> Also, a catalog of mosquito *A. gambiae* salivary transcripts and proteins is freely available for studying the role of salivary proteins in malaria research,<sup>78</sup> as well as description of the *A. aegypti* salivary gland proteome.<sup>79</sup>

Using massive cDNA sequencing, proteomics and customized computational biology approaches, the most abundant secreted proteins from the salivary glands of the sand fly *Lutzomyia longipalpis* (an important vector of *Leishmania chagasi*) have been isolated and identified.<sup>80</sup>

The number of potential vaccine antigen candidates has continued to increase rapidly over recent years but there have not been many reports of their actual assessment in vaccination trials. Also, despite all of these efforts, currently there are no effective or widely accepted vaccines for the prevention of tick infestations in cattle. Very few antigens appear to be highly effective on their own suggesting the need for a multi-antigen or chimeric vaccine that incorporates critical tick and pathogen antigenic epitopes.<sup>58</sup>

## Conclusions

Two essential aspects of vaccine development are antigen identification and definition of immunological correlates of protection to guide the selection of vehicles, vectors, schedules and adjuvants. Mining of vaccine candidates against vector-borne diseases is, nevertheless the first step in vaccine design. There are many other aspects that require consideration, such as choice of recombinant production system, dose, adjuvants/immunomodulators, boosters, antigen delivery systems, etc. In the case of those pathogens where no economic animal model is available, a major bottleneck is vaccine trial. The complication and high cost of vaccination trials hinder VBD vaccine research have so far seriously limited the systematic examination of antigen candidates, and only a handful of promising vaccine candidates have been tested in vaccination-challenge experiments.

Recent development of reverse vaccinology approaches and the knowledge from functional and structural genomics provide novel strategies for a more rapid identification of antigens

leading to a third generation of vaccines. In fact, antigen identification represents the most important bottleneck in vaccine development against any pathogen, as this was usually achieved through rather empirical, time-consuming and labor-intensive *in vivo* and *in vitro* experiments. Availability of novel omics technologies coupled to high-throughput protein expression and purification, appropriate immunogenicity assays and bioinformatics tools enabled more rational and faster identification of antigens among large numbers of pathogen and host proteins. The combination of all strategies can offer the highest chance of identifying the best genes and proteins of potential interest for vaccine development in a very brief period of time. By joining of acquired knowledge through wealth of omics datasets with an integrative systems biology approach, researchers should be able to present efficient next generation vaccines.

### Competing interests

The authors declare that they have no competing interests.

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**Tables****Table 1.** Examples of different new generation approaches in the development of vaccines against vector-borne diseases.

<b>Pathogen</b>	<b>Disease</b>	<b>Approach</b>	<b>Status of vaccine development</b>	<b>References</b>
<i>Plasmodium</i> spp.	Malaria	Immunomics (with genomics, proteomic and transcriptomic data)	Vaccine candidates	<sup>81-83</sup>
<i>Leishmania major</i> <i>Leishmania infantum</i>	Leishmaniasis	Reverse vaccinology – pan-genome approach	19 potential common epitopes identified	<sup>15</sup>
<i>Leishmania major</i>	Leishmaniasis	Reverse vaccinology: genome to T-cell epitope prediction	14 immunogenic epitopes identified	<sup>54</sup>
<i>L. donovani</i>	Visceral leishmaniasis	Proteome-serological approach	Immunogenic antigens	<sup>42</sup>

<i>Leishmania donovani</i>	Visceral leishmaniasis	Proteomic approach (2-DE based peptide mass-mapping)	33 T-cell stimulating antigens from soluble proteins of <i>L. donovani</i> clinical isolate	<sup>84, 85</sup>
<i>Theileria parva</i>	East Coast fever (cattle)	Reverse vaccinology Immunoscreening	Identified cytotoxic T lymphocyte (CTL) target antigens	<sup>48, 86</sup>
<i>Babesia bovis</i>	babesiosis	Bioinformatic prediction of the exportome	Three novel proteins identified	<sup>87</sup>
<i>Babesia bovis</i>	babesiosis	Reverse vaccinology - <i>in silico</i> identification of GPI-anchored proteins	Predicted repertoire of GPI-anchored proteins of <i>B. bovis</i>	<sup>88</sup>
<i>Anaplasma marginale</i>	Anaplasmosis	Reverse vaccinology, genome-wide screening	21 OMPs capable of inducing bovine IgG2 opsonizing	<sup>89</sup>

			antibodies	
<i>Anaplasma marginale</i>	Anaplasmosis	Predicted gene function and reverse genetics coupled with antibody response to candidates in protectively immunized animals	Identification of outer membrane antigens AM854 and AM936 (negative results in challenge trials)	<sup>90</sup>
<i>Ehrlichia ruminantium</i>	Heartwater	Reverse vaccinology	11 proteins eliciting an <i>in vitro</i> cellular immune response	<sup>47, 91</sup>
Dengue virus	Dengue fever	Immunomics (large-scale analysis of antigenic diversity of T-cell epitopes in Dengue virus)	Identified short regions of sequences (~<100 aa) within viral antigens that are specific targets of immune	<sup>51</sup>

			responses	
Dengue virus	Dengue fever	Tetravalent DNA vaccine	A synthetic consensus (SynCon) DNA vaccine that elicits immunity against all four dengue serotypes	<sup>52</sup>
<i>Borrelia burgdorferi</i>	Lyme disease	Genome-wide protein array Structural vaccinology	Immunogenic antigens identified	<sup>92</sup>
<i>B. burgdorferi</i>	Lyme disease	Structural vaccinology	Multivalent OspA-based vaccine	<sup>23</sup>
<i>Borrelia burgdorferi</i>	Lyme disease	Subtractive genomics approach	15 membrane localized vaccine targets	<sup>16</sup>
<i>Borrelia burgdorferi</i>	Lyme disease	Reverse vaccinology, <i>in silico</i> identification of	Development of peptide based vaccine candidates	<sup>93</sup>

		protective epitopes		
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**Table 2.** Overview of tick antigens tested for tick vaccines development

Tick antigen	Vector/tick species	Protein identity	Pathogen	References
BM86  BM orthologs and homologs	<i>R. microplus</i> <i>R. appendiculatus</i> , <i>R. decoloratus</i> , <i>R. microplus</i> , <i>Hyalomma</i> <i>anatolicum</i> , <i>H. dromedarii</i>	Midgut membrane- bound protein	<i>Babesia</i> spp. <i>Anaplasma</i> spp.	94 68 95
RAS-3, RAS-4, RIM36 cocktail	<i>Rhipicephalus</i> <i>appendiculatus</i>	Serpins (serine protease inhibitors)	<i>Theileria parva</i>	96
RaFER2/Rm FER2	<i>R. microplus</i> , <i>R.</i> <i>annulatus</i> , <i>I.</i> <i>ricinus</i>	Ferritin, iron transporter	<i>Babesia</i> spp. <i>Anaplasma</i> spp.	97
64TRP	<i>I. ricinus</i> , <i>R.</i> <i>appendiculatus</i> , <i>R. sanguineus s.l.</i>	A putative tick cement protein	TBEV	98 99
GP80/VIT87	<i>R. microplus</i>	Vitellin/Vitellogenin		100

BlmLTI/Bm TI/BmTI-A	<i>R. microplus</i>	Trypsin inhibitors		101
GLP	<i>Hyalomma dromedarii</i>	Glycoproteins		102
Ef1a	<i>R. microplus</i>	Elongation factor		70, 103
Subolesin	<i>R. annulatus, R. microplus, I. scapularis</i>	Regulator factor	<i>A. phagocytophyllum, A. marginale, B. bigemina, B. burgdorferi</i>	104 74
pP0	<i>R. sanguineus s.l., R. microplus</i>	Acidic ribosomal protein P0		105
UBE	<i>R. microplus, R. annulatus</i>	Ubiquitin		103
GST-HI	<i>R. microplus</i>	Glutathione S transferase		106
4F8	<i>R. microplus</i>	5'-nucleotidase		107
BM91	<i>R. microplus</i>	Angiotensin converting enzyme		108
BMA7	<i>R. microplus</i>	mucin		109
Aquaporin	<i>R. microplus, R. annulatus</i>	Aquaporin		110

TROSPA	<i>I. scapularis</i>	a tick receptor for <i>B. burgdorferi</i> OspA	<i>B. burgdorferi</i>	<sup>111</sup>
TSLPI	<i>I. scapularis</i>	salivary protein TSLPI (Tick Salivary Lectin Pathway Inhibitor)	<i>B. burgdorferi</i>	<sup>112</sup>
tHRF	<i>I. scapularis</i>	tick histamine release factor	<i>B. burgdorferi</i>	<sup>113</sup>
Salp15	<i>I. scapularis</i>	secreted salivary protein	<i>B. burgdorferi</i>	<sup>114</sup>
Salp25D	<i>I. scapularis</i>	secreted salivary protein	<i>B. burgdorferi</i>	<sup>115</sup>

**Table 3.** Examples of omics approaches for tick vaccine development

Tick	Pathogens	Approach	Status of vaccine development	References
<i>Rhipicephalus microplus</i>	<i>Anaplasma</i> spp., <i>Babesia</i> spp., <i>Theileria</i> spp., <i>Ehrlichia</i> spp.	Functional genomics and <i>in silico</i> reverse vaccinology approach (microarray hybridization platform)	Candidate tick protective antigens identified	<sup>29</sup>
<i>Rhipicephalus microplus</i>	<i>Anaplasma</i> spp., <i>Babesia</i> spp., <i>Theileria</i> spp., <i>Ehrlichia</i> spp.	Transcriptomics (suppressive subtractive hybridization)	Stage-specific differentially expressed sequences identified	<sup>116</sup>
<i>Ixodes pacificus</i>	<i>Borrelia burgdorferi</i>	Transcriptomics	Transcriptome of the salivary glands	<sup>117</sup>
<i>Ixodes scapularis</i>	<i>Borrelia burgdorferi</i> ,	cDNA expression library, next	24h tick saliva immuno-	<sup>118</sup>

	<i>Anaplasma phagocytophilum</i> , <i>B. miyamotoi</i> , <i>Babesia microti</i> , Powassan virus	generation sequencing, <i>de novo</i> assembly, and bioinformatics analyses	transcriptome	
<i>Ixodes scapularis</i>	<i>B. burgdorferi sensu stricto</i> , <i>A. phagocytophila</i>	Expression library immunization (ELI) combined with analysis of expressed sequence tags (EST)	Two of these cDNAs conferred protection when used individually to immunize mice	<sup>65</sup>
<i>Ixodes scapularis</i>	<i>Borrelia burgdorferi</i> , <i>Anaplasma phagocytophilum</i> , <i>Babesia microti</i> , tick-borne encephalitis virus	Transcriptomics - RNA interference (RNAi)	Candidate tick protective antigens	<sup>67</sup>
<i>Rhipicephalus microplus</i>	<i>Anaplasma</i> spp., <i>Babesia</i> spp., <i>Theileria</i> spp., <i>Ehrlichia</i> spp.	Functional genomic study	Comparison of the influence of tick resistant and	<sup>30</sup>

			susceptible bovine breeds on the transcriptomes	
<i>Rhipicephalus microplus</i>	<i>Anaplasma</i> spp., <i>Babesia</i> spp., <i>Theileria</i> spp., <i>Ehrlichia</i> spp.	Transcriptomics	Identified 3 aquaporin-like full length ORFs from <i>R. microplus</i> transcriptome	<sup>110</sup>
<i>R. microplus</i>	<i>Anaplasma</i> spp., <i>Babesia</i> spp., <i>Theileria</i> spp., <i>Ehrlichia</i> spp.	Immunization with the three recombinant proteins together	A multi- antigenic vaccine composed by BYC, VTDCE and GST-HI recombinant proteins against <i>R. microplus</i> infestation in cattle	<sup>106</sup>
<i>Haemaphysalis flava</i>	Tick-borne encephalitis	High-throughput sequencing	Midgut transcript	<sup>119</sup>

	virus, <i>B. burgdorferi</i>	technology	expression during different blood feeding stages	
<i>Dermacentor silvarum</i>	<i>Babesia equi</i> , <i>Babesia caballi</i> , <i>Rickettsia</i> spp.	SERPA	3 antigenic proteins identified	<sup>120</sup>
<i>R. microplus</i> and <i>Rhipicephalus annulatus</i>	<i>Anaplasma</i> spp., <i>Babesia</i> spp., <i>Theileria</i> spp., <i>Ehrlichia</i> spp.	RNA interference (RNAi)	Vaccination trials on glutathione-S transferase, ubiquitin (UBQ), selenoprotein W, elongation factor-1 alpha, and subolesin (SUB)	<sup>121</sup>
<i>Ixodes scapularis</i>	<i>Borrelia burgdorferi</i>	Suppressive subtractive hybridization	Ten unique salivary gland- associated cDNAs that are up-regulated during feeding	<sup>122</sup>

<i>Amblyomma americanum</i>	<i>Rickettsia</i> spp.	RNA interference (RNAi)	Four cDNA clones were selected for vaccine studies in cattle	<sup>123</sup>
<i>Amblyomma. americanum, A. cajennense, A. variegatum</i>	<i>Rickettsia</i> spp., <i>Babesia</i> spp., <i>Theileria</i> spp., <i>Ehrlichia</i> spp. Crimean-Congo Hemorrhagic Fever virus <i>B. burgdorferi</i>	Proteomics study (phyloproteomics)	The first report on the proteome of the most important <i>Amblyomma</i> tick species	<sup>124</sup>
<i>Lutzomyia longipalpis</i> (sand fly)	<i>Leishmania chagasi</i>	cDNA sequencing, proteomics and customized computational biology approaches	Secreted proteins as vaccine candidates	<sup>80</sup>
<i>Anopheles gambiae</i> (mosquito)	<i>Plasmodium</i> spp.	Proteomics study	Characterised midgut's peritrophic matrixproteome	<sup>125</sup>

			of the main malaria vector	
<i>Aedes aegypti</i> (mosquito)	<i>Chikungunya virus (CHIKV)</i> <i>dengue virus (DENV-2)</i>	Proteomics study (2D DIGE followed by MALDI-MS/MS)	The first analysis of the protein response of <i>Aedes aegypti</i> 's midgut infected with viruses belonging to different families	<sup>76</sup>
<i>Anopheles gambiae</i> (mosquito)	<i>Plasmodium</i> spp.	A global transcriptome analysis of salivary gland (microarray analysis, RNAi)	Identified candidate genes involved in hematophagy	<sup>77</sup>

## Biography

Josipa Kuleš, PhD, MSc. Med. Biochem.



Josipa Kuleš holds an MSc in Biochemistry and a PhD in Veterinary Medicine. From 2008 to 2014 she was employed as assistant – scientific researcher at the Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine of the University of Zagreb, and from 2014 as a postdoctoral researcher. In July 2015 Dr Kuleš joined the ERA Chair team at the Faculty of Veterinary Medicine of Zagreb. Her major research interests are vector borne diseases and host-pathogen interactions. Dr Kuleš was awarded as the best young scientist in 2014 by the Croatian society of medical biochemistry and laboratory medicine.

Anita Horvatić, PhD



Anita Horvatić gained her PhD in Chemistry (2014) from the Faculty of Science, University of Zagreb. From 2005 to 2009 she was employed in Pliva R&D in Laboratory for Liquid Chromatography and Mass Spectrometry. From 2009 to 2014 she was working as a research assistant at the Ruđer Bošković Institute, and from 2015 as a postdoctoral researcher. Her scientific work has been focused on biomarker discovery, pathogen diagnostics and protein stability using high-throughput proteomic methods and mass spectrometry. In 2015 Dr Horvatić joined the ERA Chair team at the Faculty of Veterinary Medicine, Zagreb. In 2013 she obtained the L'Oréal-UNESCO Award for Women in Science.

Nicolas Guillemin, PhD



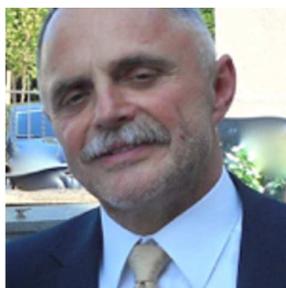
Nicolas Guillemin obtained a PhD in molecular physiology and genetics at the National Institute for Agricultural Researches (INRA, France) in 2010. He set up a new Dot-Blot technique for fast protein quantification and identified protein markers in muscle. He completed a post-doctoral fellowship at the University Laval, Canada, in 2013. He set up a new methodology for SNP markers detection, using genomics and transcriptomics, and identified new SNPs related with fertility. He has experience in interactome analysis and prediction models. Dr Guillemin integrated the ERA Chair team at the Faculty of Veterinary Medicine of Zagreb in July 2015.

Asier Galan, PhD



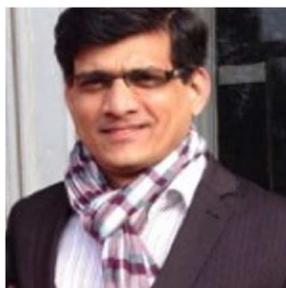
Asier Galan gained his PhD in Biochemistry and Molecular Biology from University of the Basque country, Leioa (Spain). In 2003 to 2004 he was a postdoctoral fellow at the Technische Universität München, Munich (Germany). From 2005 to 2011 he worked as Head of Proteomics Department at OWL Metabolomics SL, a biotechnological company in Derio (Spain), developing diagnostic procedures for liver metabolic disorders. Dr. Galan has followed up his career as senior postdoctoral fellow in diverse areas of proteomics at Palacky University Olomouc (Czech Republic) and at Ruđer Bošković Institute Zagreb (Croatia). He joined the ERA Chair team at the Faculty of Veterinary Medicine of Zagreb in July 2015.

Prof. Vladimir Mrljak, DVM, PhD



Prof. Vladimir Mrljak gained his PhD in Veterinary Medicine by the Faculty of Veterinary Medicine, University of Zagreb. He is currently project coordinator of FP7-ERA Chairs project and the main researcher on the research project of the Croatian science foundation. Prof. Mrljak is also member of the COST project NGP-net (COST Action BM 1405: Non globular proteins in molecular physiopathology). He has taken active part at numerous veterinary and human medicine meetings, symposia and congresses. His main areas of scientific and professional interest are pathogenesis of vector-borne diseases, and clinical laboratory diagnostics.

Assoc. Prof. Mangesh Bhide, DVM, PhD



During his doctoral and post-doctoral research (Marie Curie fellowship, Laboratory of Special Pathogens, NCM, Carlos III, Madrid, Spain) he gained expertise in the gel based proteomics, protein-protein interaction analysis and other omics tools. His main research interest is to decipher the underlying molecular principles involved in the translocation of the neuroinvasive pathogens across the blood-brain barrier and find mechanisms of complement evasion. His research activities are focused to convert acquired knowledge in the development of therapeutics (chimeric vaccines and nanobodies) to hinder the neuroinvasion. The pathogens of interest are *Borrelia*, *Neisseria*, *Trypanosoma* and *Streptococci*.