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# Genome-Based Vaccinology Applied to Bovine Babesiosis

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## Abstract

Genomics approaches in veterinary research have been a very useful tool to identify candidates with potential to be used in prevention of animal diseases. In *Babesia*, genome information analysis has elucidated a wide variety of protein families and some members are described in this chapter. Here, we present some of the most recent studies about *B. bovis* and *B. bigemina* genomes where some proteins have been identified with potential to prevent infections by these parasites.

**Keywords:** bovine babesiosis, bioinformatics, vaccines, genomics

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## 1. Introduction

Bovine babesiosis is a tick-transmitted disease caused by apicomplexan parasites of the genus *Babesia*. This disease is caused by *Babesia bovis* and *B. bigemina* in the Americas including Mexico, where it is distributed in tropical and subtropical regions, occupying 51.5% of the national territory [1, 2]. This disease was reported for the first time by Viktor Babes in Rumania in 1888. However, it was until 1893 when Smith and Kilborne demonstrated that the disease is transmitted to cattle by infected ticks [3, 4]. In Mexico, the first report of bovine babesiosis occurred in 1905; however, it is believed that it was first introduced to the American continent by the Spaniards during the conquest. To date, measures used to control bovine babesiosis include vector control, an early diagnosis, treatment of sick animals and vaccination. The negative, severe impact that cattle fever tick and babesiosis have in the cattle industry in Mexico and the world has not diminished due mainly to a lack of commercially available, safe and effective vaccines. Vaccines based on approaches using genomics and bioinformatics are a

promissory solution to this problem [5]. It has been shown that experimental vaccines based on recombinant antigens have been developed successfully in apicomplexan parasites like *Plasmodium*, *Toxoplasma* and *Theileria* [6–8]. With the completion of the *B. bovis* genome [9] and the partial sequencing of the *Babesia bigemina* genome (<http://www.sanger.ac.uk/>), it is now possible to study these pathogens to the genomic level, taking advantage of the bioinformatics tools developed for this purpose. This approach is now generating valuable information on the essential characteristics of the genome structure and allows comparative analyses with genomes of other apicomplexan pathogens of importance in human and animal health, as well as the identification of genes with a potential use in diagnostics, vaccines or therapeutics. More specific analyses are also possible with the generation of expressed sequence tags (EST) obtained for *B. bovis*, which allow the analysis of those genes specifically expressed in the different stages of the parasite's life cycle [10] and, finally, implementation of methods for genome-wide analysis like microarrays which will be in short available for their use [11]. Additionally, research on this important disease is complemented with all the information generated so far about those genes codifying antigens with a potential as candidates in vaccines, diagnostics or therapeutics, which have been discovered in the last 30 years. Equally important is the knowledge about the life cycle of the parasite, the interaction with the vector tick and the genes involved in this interaction, which are poorly studied so far. In the following sections, we describe the most relevant aspects of the *B. bovis* and *B. bigemina* genomes and genes characterized to date.

## 2. Babesiosis

### 2.1. *Babesia bovis* genome

Although *Babesia bovis*, *B. bigemina* and *B. divergens* are causative agents of bovine babesiosis, *B. bovis* is regarded as the most important and has a bigger impact in the livestock industry due to its virulence and high mortality rate. For this reason, the *B. bovis* genome was the first to be sequenced. This was done by Washington State University in collaboration with the Agricultural Research Service and The Institute for Genomic Research (TIGR) in the USA. The genome sequence was obtained from the T2Bo strain, a virulent strain isolated from a clinical case in Texas, USA.

The genome of *Babesia bovis* has a length of 8.2 Mbp, contains 3671 genes and consists of four chromosomes, three of them are acrocentric: chromosome 1, has a length of 1.25 Mbp, is the smallest and contains a gap, which is estimated to be 150 Kpb long. Chromosome 2 is fully sequenced and contains 1.73 Mbp in length. Chromosome 3 is also fully sequenced and is 2.59 Mbp in length. Finally, chromosome 4, which is the only submetacentric one, it is partially sequenced because it contains an assembly gap that has not been solved and is 2.62 Mbp in length. The structural features of the *B. bovis* genome are similar to those of *Theileria parva* but have major differences with *Plasmodium falciparum* (**Table 1**), despite the fact that *B. bovis* and *P. falciparum* share similar clinical and pathological features [9].

*B. bovis* contains two extrachromosomal genomes: a lineal mitochondrial genome of 6 Kbp and an apicoplast genome that is circular and consists of 33 kpb with 32 genes and 25 sequences of tRNA.

| Features                                             | Species              |                 |                 |
|------------------------------------------------------|----------------------|-----------------|-----------------|
|                                                      | <i>P. falciparum</i> | <i>T. parva</i> | <i>B. bovis</i> |
| Size (Mbp)                                           | 22.8                 | 8.3             | 8.2             |
| Number of chromosomes                                | 14                   | 4               | 4               |
| Total G+C composition (%)                            | 19.4                 | 34.1            | 41.8            |
| Size of apicoplast genome (kbp)                      | 35                   | 39.5            | 33              |
| Size of mitochondrial genome (kbp)                   | ~6 linear            | ~6 linear       | ~6 linear       |
| Number of nuclear protein coding genes               | 5,268                | 4,035           | 3,671           |
| Average protein coding gene length (bp) <sup>a</sup> | 2,283                | 1,407           | 1,514           |
| Percent genes with introns                           | 53.9                 | 73.6            | 61.5            |
| Mean length of intergenic region (bp)                | 1,694                | 405             | 589             |
| G+C composition of intergenic region                 | 13.8                 | 26.2            | 37              |
| G+C composition of exons (%)                         | 23.7                 | 37.6            | 44              |
| G+C composition of introns (%)                       | 13.6                 | 25.4            | 35.9            |
| Percent coding                                       | 52.6                 | 68.4            | 70.2            |
| Gene density <sup>b</sup>                            | 4,338                | 2,057           | 2,228           |

<sup>a</sup>Not including introns.

<sup>b</sup>Genome size/number of protein coding genes.

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**Table 1.** Genome characteristics of *B. bovis*, *T. parva* and *P. falciparum* [9].

The apicoplast, which is an organelle conserved in the phylum apicomplexa, is a nonphotosynthetic plastid essential for survival [12]. The apicoplast genome was first sequenced in 1974 from *Plasmodium lophurae* and was thought that it could be mitochondrial DNA. In 1994 it was finally related to plastids DNA when a gene coding for a protein of 470 amino acids in length was identified and it contained a 50% identity with a protein only described in the plastome of red algae [13]. It is believed that the plastid is derived from a secondary endosymbiosis from red algae like in dinoflagellates [14]. Furthermore, *Chromera velia* has a plastid originated from red algae that is closely related with the apicoplast [15].

### 3. *Babesia* multigenic families

#### 3.1. Variant erythrocyte surface antigen-1

Even though the apicoplast is an apicomplexa organelle, they share a complex of organelles that is characteristic of the apicomplexa: the apical complex. This complex is composed of spherical body, rhoptries and micronemes; in this organelle, different proteins involved in the life cycle are generated, and some of these are secreted to the media or directed to the membrane [16–18]. In the erythrocyte stage, some *Babesia* parasites, including *B. bovis* and *bigemina*, can invade the host erythrocytes in a directly way without a pre-erythrocyte stage. Antigenically, the surface of infected erythrocytes is different between *Babesia* strains and the

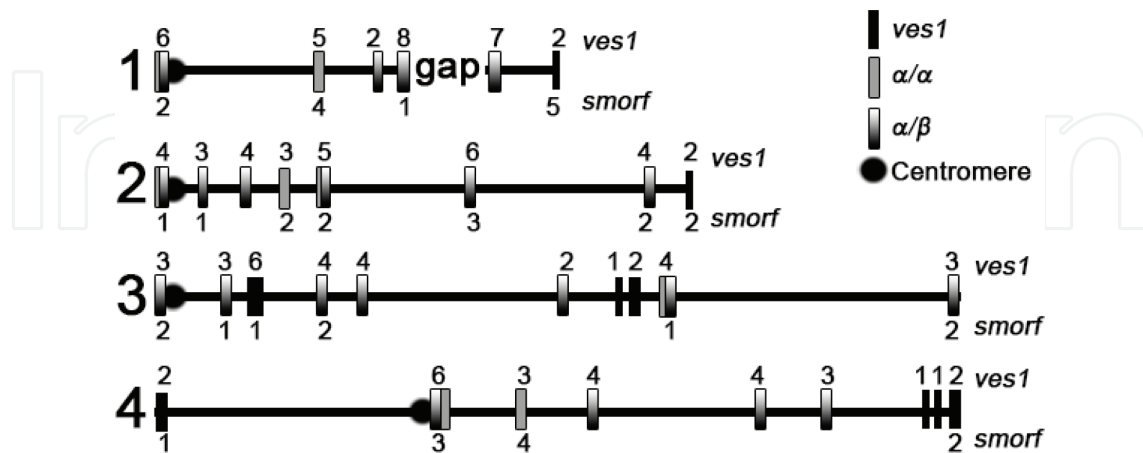
parasitized erythrocytes present in an infected bovine could fluctuate widely over time, in a process called antigenic variation [19–21]. This process on the infected erythrocytes is carried out by the antigenically variant protein named the variant erythrocyte surface antigen-1 (VESA1) that is constituted by two subunits (a and b) and encoded in a multigene family; the genes *ves-1* involved in this family are related too in cytoadherence and are distributed in the four chromosomes of *Babesia* [19, 22, 23]. Although more of 350 genes *ves-1α* and more of 80 genes *ves-1β* were previously reported, in *B. bovis* genome only 119 were evidenced [9].

3.2. SmORFs

The family of genes *ves-1* is associated across all four chromosomes with another multigene family of proteins that are smaller in size than the *ves-1* genes (Figure 1), due to small open reading frames (SmORFs). This family is the second largest in the *B. bovis* genome and comprises 44 genes without significant sequence identity to any protein or gene sequence available in databases. Of 44 genes, 42 are codified in a single exon, but from these 44 proteins that are extracellular just one does not have a signal peptide [9]. Sequence analysis in the T2Bo and Mo7 strains demonstrated that the repertoire varies between strains and has multiple semi-conserved and variable blocks; this family comprises two major branches called SmORFs A and B, and these branches are defined by a large hypervariable insertion in 20 genes [24]. Although the function of these proteins is unknown, it is believed that it could play a functional role in VESA1 biology or contribute to the antigenic variation and immune evasion as a consequence [9, 24].

3.3. VMSA

In American strains, the variable merozoite surface antigen (VMSA) family contains the proteins MSA-1, MSA-2a<sub>1</sub>, MSA-2a<sub>2</sub>, MSA-2b and MSA-2c, while in Australian strains only tree genes were found: *msa-1*, *msa-2c* and *msa-2a/b* [25]. The genes that conform this family reside



**Figure 1.** Representation of *B. bovis* chromosomes and the localization of the centromeres, *ves1*, and *smorf* genes. The chromosomes are depicted by black lines, with the chromosome number shown on the left. *ves1* loci are depicted with boxes: Black boxes represent unclassified *ves1* genes; Grey boxes represent at least one *ves1α/ves1α* pair within the cluster; Shaded boxes represent at least one *ves1α/ves1β* pair within the cluster. The number of *ves1* and *smorf* genes is shown above or below each locus, respectively. Finally the centromeres are represented as black circles. Modified from Brayton KA, et al., 2007 [9].



on chromosome 1, and the four copies of *msa2* gene are arranged tandemly in a head-to-tail fashion as long as *msa1* gene is located 5 kbp upstream from the *msa2* genes [9]. These five proteins have a conserved GPI domain and are involved in the first attachment to the erythrocyte. However, the exposed epitopes are not conserved between these proteins of this family and between different strains around the world [18, 26, 27]. Even though these proteins are variable, some studies have been shown that *msa-2c* gene is the most conserved of this family. Monoclonal antibodies against this protein can recognize strains from different geographic regions, and polyclonal antibodies have an effect on the invasion process, suggesting its utility as recombinant vaccine antigen or in diagnostic tests [18, 26, 28–33]. These results have not been observed in the other MSA proteins; the MSA-1 protein is immunogenic and avoids the invasion process in vitro, but the immunogenic response is not protective [30]. It could be due to the fact that *msa-1* gene has an important allelic variation in strains from the nearby geographical regions. This variation suggests that the antibodies generated could not have a cross-reaction between different strains [34, 35].

### 3.4. SBP

The spherical body protein (SBP) constitutes another family in *B. bovis*, and these proteins that are located in the spherical body of the apical complex are known as SBP1, SBP2, SBP3 and SBP4. In the invasion process, SBP2 is released from the spherical bodies to the cytoplasmic membrane of the erythrocyte [36]. Twelve truncated copies and just one complete copy of *sbp2* gene were identified, showing a conserved 3' region in these copies [9, 37]. The complete copy and one truncated are located in the chromosome 4, the other truncated copies are located in the chromosome 3, and some of these truncated copies are expressed in erythrocytic stages of *B. bovis* [10].

### 3.5. Bbo-6cys

A novel family of genes that codify proteins with similarities to 6cys family of *Plasmodium* were identified in *B. bovis* genome. This family contains six genes (6cys-A, B, C, D, E and F), and these genes are located in tandem in the chromosome 2 except for 6cys-F that is located in a distal region. To identify this family was employed the sequence of the *P. falciparum* PFS230 protein as a query that has a higher homology with Bbo-6cys-E gene. Antibodies against this protein have an inhibitory effect on the invasion process, suggesting its importance in control methods against *B. bovis* infection [38].

### 3.6. Bovipain

Inhibitors of cysteine proteases have been shown to hamper intraerythrocytic replication of *B. bovis*, and four papain-like cysteine proteases are found in *B. bovis* genome. The bovipain-2, which is the orthologous gene of *P. falciparum* falcipain-2 that is involved in hemoglobin digestion [39], is located in chromosome 4 by an ORF of 1.3 kb without introns, the characterization of this protein shown a molecular weight of 42 kDa, and a transmembrane region and is highly conserved between *B. bovis* strains of North and South America [40]. The bovipain-2 could be employed as a vaccine or as a target of drugs in the babesiosis control.

## 4. Vaccine antigens

### 4.1. RON proteins and AMA-1

In *Toxoplasma*, the characterization of the invasion process allowed the identification of a complex of proteins generated in the rhoptry neck, called rhoptry neck proteins (RONs), this family of proteins consists of RON2, RON4, RON5 and RON8 that are related to AMA-1 in the formation of the moving junction (MJ) in the invasion process [41]. The RON complex is inserted into the host cell; meanwhile, AMA-1 is released to the parasite membrane; this process is described in *P. falciparum*, where the specific interaction between the host membrane and the parasite membrane is mediated by AMA-1 and RON2; the disruption of this interaction avoids the invasion process [42, 43]. RON2 was identified in *B. divergens* and *B. microti*, has a full-length sequence of 4053 bp that codifies to a protein of 170 kDa and has apical localization; antibodies against this protein are inhibitors of parasite invasion like in other apicomplexan parasites; The *B. divergens* RON2 protein has a closely related sequence in *B. bovis* identified by BLASTp [42, 44, 45]. The first protein described to participate in the invasion process as part of the MJ in apicomplexan parasites was AMA-1. This protein is stored in the micronemes and secreted to the apical end during the invasion process. In *Babesia bovis*, AMA-1 contains 606 amino acids and it is codified by a 1818 bp-long gene, without introns. AMA-1 is a type I transmembrane protein with a N-terminal ectodomain, which is divided into three subdomains containing 14 cysteines [18, 46, 47].

### 4.2. RAP-1

One of the most studied proteins of the rhoptries identified in *B. bovis* is the rhoptry-associated protein 1 (RAP-1). The gene is constituted by only one exon and has a length of 1698 bp with two copies separated by a noncoding sequence of 1 kbp in *B. bovis* [48]. However in *B. bigemina*, rap-1 is represented by three genes: *rap-1a*, *rap-1b*, and *rap-1c*, arranged in tandem, as explained latter [49]. The members of this family have a signal peptide, four cysteine residues and a 14 aa motive and, moreover, contain immunogenic epitopes B and T that elicit a Th1 humoral response in the host that avoids the attachment of the parasite to the erythrocyte; its structure is conserved between different isolates and is expressed in the sporozoite [37, 50–55].

### 4.3. MIC

In *B. bovis* was described a gene that codifies to a protein like to the *T. gondii* protein 1 of the micronemes (MIC1). This gene is located in the chromosome 3 and is highly conserved between strains of *B. bovis*. Its function is involved in the cito-adherence process through the binding to sialic acid, and antibodies against the recombinant protein and synthetic peptides designed on antigenic regions of *B. bovis* MIC-1 avoid the invasion process on the in vitro culture of the parasites [56].

### 4.4. HSP-20

The heat shock protein 20 (HSP-20) is a protein of 20.2 kDa associated with other small proteins related to heat shock in mammals and plants [57]. The *hsp-20* gene consists of 686 bp with an intron of 153 bp that makes a polypeptide of 177 aa [58]. Antibodies against this protein

recognize both *B. bovis* and *B. bigemina*, suggesting that HSP-20 contains conserved epitopes in these species [59, 60].

## 5. *Babesia bigemina*

During the first decade of twenty-first century, the sequencing and description of *B. bovis* genome [61] have helped to find genes that play important roles during its life cycle, currently. The Sanger Institute is leading the sequencing project of *B. bigemina* genome, which is estimated in 10 Mb size distributed in four chromosomes [62]. The advances in genomics of both *Babesia* species that affects cattle in America are allowing researcher to find, analyze, compare and predict proteins involved directly in pathogenesis and its life cycle. For more than five decades, researchers have been trying to develop a vaccine against piroplasmosis; before 1980, several studies were carried out to immunize cattle in an effective way. The first attempts were directed in animals that were infected on purpose and healed from babesiosis as a strategy to avoid undesirable infections [63]. Some studies were focused on trying to find a way to reduce the virulence of high infective *B. bigemina* strains through inoculum passages in several animals [64], and some even tried to immunize calves in utero [65]. As we know now, the development of a of an effective and low-cost vaccine is more complex than initially thought. Nowadays some countries produce vaccines against bovine babesiosis. The Queensland Government in Australia offers a vaccine made of parasitized bovine blood [66]; this live attenuated parasite vaccine must be stored at  $-196^{\circ}\text{C}$  and during its production is necessary a batch of splenectomized calves that must remain in quarantine three times before the first procedures of manufacturing [67]. It is evident that piroplasmosis vaccination involves long periods of production, surgery, and maintenance of animal infected blood donors and thorough procedures to achieve high standards of bioethical considerations.

Currently, research is focused on developing vaccines that avoid complex production procedures and the use of live animals; new technologies have arrived bringing opportunities to develop a vaccine using high throughput production. For this, certain obstacles must be solved before an effective vaccine is produced.

In vaccination, developing gene polymorphisms and antigenic variation is one of the first problems that researchers must cope with, and the selection of most suitable antigen candidates is a crucial step. With gene databases, the analysis of sequence variations has been made easier to find differences in distant geographical strains. In this sense, several studies have been carried out to find whether some proteins are conserved and how auspicious its election as vaccine candidate would be.

Antigenic variation is used by microorganisms as an evasion mechanism of the immune response, and in *Babesia*, the vesa family is the most studied group of genes used to “escape” from the host immune system. As described above for *B. bovis*, the vesa family is composed of two multicopy genes, *ves1 $\alpha$*  and *ves1 $\beta$* , which are distributed within the four chromosomes, and it is estimated that there are 72 and 43 copies of them, respectively. Both genes are located in opposite transcription directions and are governed by a bidirectional promoter followed by small sequences that seem to be incomplete fragments of the same recombined gene. The



mechanism proposed for the multiple versions of the protein product of *ves1 $\alpha$*  and *ves1 $\beta$*  has to do with the fact that along the genome are *ves* pseudogenes that act like reiterative donors of divergent sequences during several rounds of DNA replications, while the genes that are being transcribed are located in a locus of active transcription, which means that during this multigenic conversion segment event new versions of VESA proteins of *B. bovis* are being generated [68, 69].

Even though in *B. bigemina* antigenic variation as *vesa* family in *B. bovis* is not described, there is information about important genetic differences between strains from diverse geographical locations. On the next lines, we are going to review in general terms some of the genes that are promissory vaccine candidates in *B. bigemina*.

### 5.1. AMA-1

The apical merozoite antigen (AMA-1) is a protein that has been related to the tight junction complex formation; during this step in the red blood cell invasion, the protein interacts directly with the Rhoptry proteins to anchor both membranes; this process is well studied in the apicomplexan parasite *T. gondii* [70]. In *Babesia* species, AMA-1 has been described as a low-diversity protein; in *B. divergens*, *ama-1* was sequenced from nine isolates from France, in which only two punctual mutations were observed compared with the reference strain [71], in *B. bovis*, the analysis of Sri Lankan strains showed that *ama-1* is a conserved member with about 95% of identity; the *msa* genes of these strains were mapped and showed variability. As mentioned previously, *msa* family is highly polymorphic. This last statement proves that the isolates analyzed are different because of their genotype, and among them, there is a highly conserved *ama-1* gene [72]. The *B. bigemina* *ama-1* seems to be conserved as well, and Italian strains have a conserved sequence among them and considerable differences in comparison with Australian reference strains; however, when these are compared with Mexican and Argentinian reference strains, the sequence matches in a 99% of identity [73]. The conservation level makes AMA-1 an excellent target for vaccine development.

### 5.2. RAP-1

The Rhoptry Associated Proteins are part of a multigenic family composed in *B. bigemina* of five genes arranged in tandem designated as *rap-1a*; between them, there are two other genes designated as *rap-1b* which is present in the same number of copies as *rap-1a* and at the end of the locus a single copy gene called *rap-1c*. All *rap-1* family members are co-transcribed in merozoites, and some members seem to be conserved in geographical strains [49]. It has been demonstrated in *B. bovis* that specific antibodies are capable of reducing sporozoite invasion to red blood cells in vitro [53]; in *B. bigemina*, antibodies against RAP-1 reduced parasitemia in comparison with an ovalbumin control in calves inoculated with iRBC [74].

### 5.3. GP45

The product of the *gp45* gene is a glycosylphosphatidylinositol-anchored protein of 45 KDa, which has been related to the adhesion step during the invasion process to red blood cells; it is postulated that play the same role of *msa* family in *B. bovis* [26]. Some studies proved that immunization of calves with the purified GP45 reduced significantly the parasitemia when

challenged with a Mexican *B. bigemina* isolate [74]. At this point, GP45 seemed to be an ideal vaccine candidate, however, several years later, other studies demonstrated that the gene is not present among all *B. bigemina* strains. Southern Blot analysis revealed that Puerto Rico and St. Croix isolates do not possess *gp45* sequence and also that in Texcoco strain the upstream sequence has polymorphisms and consequently there are nonfunctional promoters. As a result of this, there is a lack of transcription [75]. This last information put in doubt if GP45 would be a good immunological target, not because of its neutralization efficiency but because of that this would not be a good candidate if the purpose of the vaccine is to have a broad-spectrum protection that includes several strains from distant geographical locations.

#### 5.4. Profilin

Profilin is a protein that participates in cytoskeleton ensemble [76]; in *Toxoplasma gondii*, due to its characteristic gliding motility where the cytoskeleton takes part of, profilin has been involved as an important protein to invade host cells and its antigenicity has been proved for its recognition by Toll receptors [77]. There is new evidence that profilin is present in *B. bigemina*, *B. bovis* and *B. microti*, and more interesting is that sera from infected cattle with *B. bovis* and *B. bigemina* are capable to cross-react with recombinant profilin from both species and even with *B. microti*; the recombinant cattle babesial profilin is capable of conferring immunity in mice against *B. microti* [78]. Even though there is no information about protective activity in cattle of profilin immunization against *B. bigemina* and *B. bovis*, profilin seems to be another promissory target to work on to achieve an effective vaccine.

The genes mentioned are examples of genes with low variability that can be used as a target to prevent babesiosis by *B. bigemina*; unfortunately, for vaccines developers the variation of sequences and gene products does not follow a high conservation rule. In this sense, experimental strategies have been built up to find a more suitable way to neutralize *Babesia* infection. Taking advantage of the information available on databases and the sequence analysis is possible to track the most appropriate targets. Alignment tools allow researchers to display the protein sequences from several distant geographical strain similarities among their proteins and find the most suitable candidates.

### 6. New strategies against apicomplexan parasites

There are several studies on *Babesia* proteins, which have an important role on the parasite's life cycle and their immunogenicity. However, even though the protein role on the parasite development has been described in detail, currently there is not a single protein proposed as vaccine candidate against *B. bovis* or *B. bigemina* that generates an immunological protective response as effective as the one produced by the live, attenuated vaccines; the reason of it might be with the fact that one single antigen used as immunogen is not enough to display a strong immunological response. In an attempt to achieve protection against microorganisms, new strategies have been raising and one of them is the design of multiepitopic vaccines; in these novel strategies, *Plasmodium* genus and *Toxoplasma gondii* are some of the microorganisms within the apicomplexan parasites where these methodologies are being applied.

Researchers have been developing vaccines using more than one antigen. Such is the case of *P. falciparum* Chimeric Protein 2.9 (PfCP-2.9) composed of two blood-stage antigens, the carboxyl-terminal region of the protein known as Apical Membrane Merozoite Surface Protein 1 (MSP1-19) and domain III of the Apical Membrane Antigen 1 (AMA-1 III). The PfCP-2.9 resulted in being highly immunogenic in rabbits and in primates and is capable of producing an antibody titer 18-fold higher than both antigens administered in a mixture. The neutralization assays demonstrated that the fusion protein reduces substantially the parasite growth [79].

One concern of last decades is that scientists are predicting that some vector-borne diseases will increase as a consequence of expansion of vector habitats due to global warming [80]. In addition to the fact that vectors are acquiring resistance to pesticides that have been using as a mean of eradication and control, today there are reports that malaria vector exhibits multiresistance to diverse chemical families used for its control leaving any suitable choice to reduce mosquito population [81, 83]. As a novel alternative to cope with this situation, multistage vaccines have been designed in an attempt to interrupt the life cycle in both vertebrate and invertebrate hosts. Using antigens from blood stages as the glutamate-rich protein (GLURP) that had been recognized as a natural antigen in acquiring immunity against malaria [82] and by the usage of sexual stage antigens *Pfp48/45* that are involved in gamete fusion during sexual reproduction within the mosquito vector [83], the central objective of this alternative multiepitope vaccine is to confer immunity in the people that is at risk to acquire the infection and to reduce in long term the infection in the vector avoiding transmission [84]. The usage of more than two antigens is also an opportunity to confer immunity against parasites and to ensure the success to block the infection. In *T. gondii*, an alternative vaccine prototype was designed as a chimeric protein with six predicted epitopes from surface proteins all of them bound in a single polypeptide sequence. This synthetic protein proved to be a good immunogen, and to stimulate CD8<sup>+</sup> T cells from seropositive patients in comparison with a mixture of the same antigens and tachyzoite lysates, also the survival percentage in murine models infected with parasites increased substantially in the immunized individuals [85]. The list of new vaccine candidates against *Plasmodium* and *T. gondii* still grows. The information that is generated in these two well-studied models serves as a starting point to extrapolate the strategies and propose new ones in the research of vaccines against apicomplexan parasites. This last section is a very narrow landscape of a long list of options that have been generated against other parasites that for sure are helping scientists to find the effective vaccines against cattle babesiosis.

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