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## Progress in development of vaccine against babesiosis

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### Abstract

In worldwide, babesiosis is one of the most important tick-borne disease caused by *Babesia* spp. occur in livestock which causes economic losses to the farming community by reducing the milk and meat production. Hence, mostly the field veterinarians administer some anti-babesial drugs resulting in presence of chemical residues in meat and milk which have detrimental effect in public health concern. Sometimes applications of both anti-babesial drugs as well as acaricidal drugs with indiscriminate dose rate develop of resistance against the causative organisms and vectors. Therefore, particularly in countries where large numbers of animals are at risk, important research is directed towards improved vaccination strategies. However, solid immunity develops after infection and this feature has been exploited with the use of live attenuated organisms as immunogens. Although existing live vaccines give protection, they have considerable disadvantages. Some killed vaccines have also been used on a limited basis and consist of antigens extracted from cultured material or blood of infected calves, and given with adjuvant. The degree and duration of immunity against heterologous challenge is not well documented. Irradiation has been used as a vaccine too with some successful results. The developments of novel non-live and/or live vaccines using parasite antigens involved in host cell invasion and in pathogen-tick interactions, as well as the protective immunity against infection are discussed in the present communication.

**Keywords:** Babesiosis, vaccine, livestock

### 1. Introduction

*Babesia*, the intraerythrocytic tick-borne protozoan parasite which may be piriform, round, or rod-shaped parasites that lack conoids and flagellae in all stages, without oocysts and with sexual stages associated with the formation of a large axopodium like strahlen causes red water fever mostly in cattle, buffaloes, sheep, goat etc. throughout the world [1]. Because of the importance of the disease most of the animals are treated with anti-babesial drugs. So, residues in meat and milk have raised public health concerns which have led to the withdrawal of babesicidal drugs in many countries [2]. Moreover, there is increasing toxicity and persistence of chemical residues in the environment [3]. On the other hand due to repeated uses of acaricide has lead to the development of resistance in ticks and therefore, the costs associated with its use has limited acaricides as a control measure [4]. Vaccines, on the other hand, are safe, leave no chemical residues (and therefore no with-holding periods for animals) are environmentally friendly and acceptable to consumers [5].

#### 1.1 Strategies for Vaccine Development

In areas where there is a continuous inoculation of cattle with *Babesia* spp. by infected ticks, calves are likely to be in contact with the parasite during the first 10-12 months of life, when typically they do not show any clinical manifestations. *Babesia* parasites are able to establish persistent infections in these animals that thus develop into parasite carriers with strong acquired immunity and resistance to disease. In regions of enzootic instability, or when cattle are relocated from tick-free to tick-infested regions, prophylactic immunization has proved an effective method to prevent the occurrence of babesiosis outbreaks [6].

### 2. Currently available vaccines

The development of vaccines against bovine babesiosis was prompted by early observations indicating that cows that recovered from natural *Babesia* spp.

infections developed long-lasting immunity; and inoculation of their blood into susceptible cattle resulted in a less virulent form of the disease. Thus, the first vaccine formulations consisted of blood from donor bovines that had recovered from infection [7].

This carrier donor method also known as premunition has several major limitations:

- Unreliable potency
- Unpredictable reactions
- The risk of contamination

### 2.1 Infection & Treatment (ITM)

Immunization against *B. bigemina* infection was practised by the inoculation of infected blood & the subsequent treatment of animals with a babesiacidal drug to prevent severe/fatal diseases [8]. The basis of this immunization is that the antimicrobial reduces protein synthesis in parasite cells & mitochondrion which gives ample time for host to generate immune response against parasite and therefore animals develop solid immunity. ITM has to deal with certain challenges such as it protects only against homologous strains so animals remain susceptible to heterologous strains. Furthermore immunized animals may become carriers thus resulting in further spreading of infection. There is possibility of introduction of foreign strains. The cost involved on infrastructure is huge. Inoculation rate (quantum of sporozoites required) is determined by titration in ticks feeding on live cattle. Also there is the need of cold chain facility right from labs to field till immunization.

### 2.2 Attenuated by Blood passage

The mechanisms underlying attenuation is that parasites lose their capacity to express certain virulence-mediating genes and a subpopulation of parasites with a mild pathogenicity phenotype, present in the initial pathogenic field isolate is selected [9]. A breakthrough in the development of bovine babesiosis vaccines was achieved by Australian researchers, who observed that rapid successive blood passages of *B. bovis* between splenectomized calves resulted in progressive virulence decrease, with diminished post-vaccination changes in body temperature and haematocrit [10]. Later, attenuation of *B. bigemina* was also achieved, but in this case the procedure involved slow successive passages among spleen-intact calves [11]. Attenuation also leads to the waning of nervous symptoms in the case of *B. bovis* and is sometimes, but not always, associated with a loss of tick transmissibility [12]. Since the spleen is important in the trapping and destruction of infected erythrocytes, the use of splenectomized bovines yields adequately high parasitaemias in the case of *B. bovis* [6]. Current vaccines against *B. bovis* and *B. bigemina* are based on these attenuation procedures. The mechanisms underlying attenuation are still unknown. However, the attenuation scenario is likely more complex than just a selection procedure since, on one hand, an attenuated strain can be composed of virulent and avirulent subpopulations and, on the other, an avirulent clone can reverse its phenotype to a virulent one upon passage through a spleen-intact bovine [13].

**Table 1:** Commercially available live vaccines against bovine babesiosis

Country	Vaccine Name/ Institution	Composition	Storage	Reference
Argentina	Vacuna Contra La Babesiosis	Bbo/ Bbi, Acent Bbo/Bbi, Acent	R UF	Echaide <i>et al.</i> (1993a,b) [16, 17] Mangold <i>et al.</i> (1996) [18]
Australia	Combavac 3 in 1 concentrate Trivalent tick fever vaccine	Bbo/ Bbi, Acent Bbo/ Bbi, Acent	UF R	Block & de Vos (2001) [19] Standfast <i>et al.</i> (2003) [14]
Brazil	Embravac Hemopar Eritrovac n2®/Hemopar Eritrovac®/Hemopar	Bbo/ Bbi, Acent Bbo, Bbi, Acent Bbo, Bbi, Acent	UF UF R	Kessler <i>et al.</i> (1987) [20] <a href="http://www.hemopar.com.br/index.php/produtos.html">http://www.hemopar.com.br/index.php/produtos.html</a> <a href="http://www.hemopar.com.br/index.php/produtos.html">http://www.hemopar.com.br/index.php/produtos.html</a>
Colombia	Anabasan®	Bbo, Bbi, Acent	UF	Benavides <i>et al.</i> (2000) [21]
Israel	Kimron Veterinary Institute	Bbo, Bbi, Acent	UF	Pipano <i>et al.</i> (2002) [22]
Malawi	Central Veterinary laboratory, lilongwe	Bbo, Bbi, Acent	UF	Tjornehoj <i>et al.</i> (1997) [23]
Mexico	Vacuna Contra La Babesiosis bovina/cenid-pavet- inifap	Bbo, Bbi,	UF	Cantó Alarcón <i>et al.</i> (2003) [24]
South Africa	Frozen African Redwater Vaccine for cattle (babesia bigemina)/onderstepoort veterinary institute (ovi) frozen asiatic redwater vaccine for cattle ( <i>Babesia bovis</i> ) /ovis	Bbo, Bbi,	UF	<a href="http://www.obpvaccines.co.za/Cms_Data/Contents/OBPDB/Folders/Product/~contents/PR22ENFFGZ9V7NZA/RedwaterAsian.pdf">http://www.obpvaccines.co.za/Cms_Data/Contents/OBPDB/Folders/Product/~contents/PR22ENFFGZ9V7NZA/RedwaterAsian.pdf</a>
Uruguay	Hemovac c / Cibeles	Bbo, Bbi, Acent	UF	Solari <i>et al.</i> (1992) [25]

Bbo= *Babesia bovis*, Bbi= *Babesia bigemina* and Acent= *Anaplasma centrale*. UF= Ultra frozen and RF= refrigerated

Live vaccines against *B. bovis* and *B. bigemina* can be prepared typically as a bivalent formula that contains around  $10^7$  erythrocytes infected with each of these parasites, although a reduced dose of  $2.5 \times 10^6$  infected erythrocytes has also been reported as effective for protection against *B. bigemina* in Australia [14]. Often, a trivalent formula is commercialized that also contains  $10^7$  erythrocytes infected with the rickettsia *Anaplasma centrale* providing cross-protection against *Anaplasma marginale*, another intra-erythrocytic tick-borne pathogen causing a related disease with wide distribution in tropical and temperate regions [15].

Live *Babesia* vaccines are recommended to be applied to 4- to 10-month-old calves that generally show good tolerance, though a transient clinical response to vaccination can

sometimes take place [26]. Adult animals, on the other hand, can develop acute babesiosis upon vaccination, for which daily monitoring for up to 21 days is suggested and a babesiacide treatment is often needed [7]. Protective immunity develops 3–4 weeks after vaccination and normally lasts at least 4 years [15]. It was observed in South Africa that, after chemo sterilization of infections, sterile immunity to *B. bigemina* lasted for only 16 months, without further boosting of immunity from tick-acquired infections, while immunity to *B. bovis* lasted for over 3 years [27]. Thus, complete tick control after vaccination is discouraged, so that natural infections through tick bites can aid in the acquisition of a long-term protected status [6].

### 2.2.1 Disadvantages of Live Vaccines

- Maintenance of infection in the field
- The return to virulence
- The possibility of co-infection with contaminating organisms particularly viruses
- Temperature liability, storage and Transportation
- Limited shelf life lasting between 4 & 7 days at 4 °C

### 2.3 Irradiated Babesial Vaccines

Immunogenic properties of various irradiated parasites were studied with the first report specific to *Babesia* species being that of Phillips. Little presented evidence that basic lesion caused by heat or excitation of ionizing radiation caused free OH formation that could result in a range of effects from temporary impairment of cellular function to cell death. On experimentation with *B. rodhaini* infection of rats and mice, Phillips concluded that an exposure of 400 Gy (Gray) radiation dose or more rendered the parasite non-infective<sup>[27]</sup>. Irvin *et al.*<sup>[28]</sup> observed that there was a linear reduction in hypoxanthine uptake and lengthening of prepatent period proportional to increasing doses of radiation above 5 Gy & that there was a rapid fall in infectivity with doses greater than 400 Gy. Wright *et al.*<sup>[29]</sup> observed that irradiated parasites produced negligible amounts of protease in comparison to those fully virulent & concluded that its presence was an indicator of virulence of parasite. Experimentation by Mahoney *et al.* 1973<sup>[30]</sup> to calibrate the dose of irradiation 350 GY of gamma radiation was a suitable irradiation level to render *B. bovis* avirulent. Irradiation reduced the number of viable parasites infected and it had no effect on the virulence of the *B. divergens* strain used and that it was unlikely that a safe & practical *B. divergens* vaccine could be produced by irradiation of parasite<sup>[31]</sup>. There has been one report of the use of irradiated *B. ovis* to try to stimulate protective immunity in sheep & in that it was concluded irradiation dose of 300 GY was optimum for the isolate used<sup>[32]</sup>.

### 2.4 Culture derived *Babesia* exoantigens as immunogens

It took nearly a century from the discovery of the etiology and the vector of *Babesia* species to develop continuous microaerophilous stationary phase (MASP) method for cultivation of *Babesia bovis*. Animals vaccinated with culture derived soluble immunogens of *B. bovis*, *B. bigemmina* and *B. canis* were clinically protected against tick & needle challenge. Consequently a vaccine against babesiosis based on organism free antigens (exoantigens) has been developed. Exoantigens describes a group of proteinaceous substances released into plasma of infected animals/ into the supernatant medium of *in-vitro* culture of *Babesia* organisms. *In vitro* cultivation of bovine *Babesia* spp. allows vaccine preparations, cheap maintenance of field strains for antigen characterization, drug testing, seroneutralization assays, production of transgenic variants, morphological studies and invasion assays<sup>[33]</sup>.

#### 2.4.1 Production of *Babesia canis* vaccine

Blood is taken from the jugular vein of an infected dog. Heparin is added to prevent clotting. The blood is washed three times in culture medium at 4°C. The resulting pellet is diluted in medium (HEPES media) supplemented with normal dog serum to obtain a 5% (v/v) red blood cell suspension. The cells are put into culture at low oxygen concentration 1-5% (v/v), 5% (v/v) CO<sub>2</sub>, and 90-94% (v/v) N<sub>2</sub>, at 38°C in a humidified atmosphere. Every 12 h, cultures are decanted and

centrifuged. The supernatant is collected. Fresh medium and uninfected red blood cells are added to the cultures and incubation resumed. Adjuvant (saponin) is added to the pooled supernatant. The product is aliquoted and freeze-dried.

#### 2.4.2 Pirodog/Nobivac Piro

- It is a soluble parasite antigen (SPA) of supernatants of *in vitro* culture (*B. canis* & *B. rossi*)
- It gives 80% protection & immunity lasts for about 6 months
- It is given at 6 months of age & booster vaccination is required 3 to 6 weeks after initial vaccination & thereafter revaccination after every 6 months by i/m route
- The vaccine may produce local reaction at the site of injection
- Not recommended for pregnant bitches
- However, efficacy of vaccine is still questionable

#### 2.4.3 Advantages

- Culture – derived *Babesia* exoantigen containing immunogens devoid of erythrocytic components have been proposed as a practical & realistic approach for control of Babesiosis (Ristic *et al.* 1981)
- These being essentially free from erythrocytic stromal antigens don't induce formation of iso-antibodies
- Abundant supply of parasite antigens from supernatants fluids of *Babesia-infected* cell cultures
- In search of vaccines that are safe, efficacious & cost effective, immunogens comprising soluble exoantigens are prime candidates to satisfy these important criteria

#### 2.4.4 Drawbacks of in-vitro cultivation

According to OIE, (2010)<sup>[15]</sup>, the following drawbacks are found in in-vitro cultivation

- Labour intensive,
- Requires permanent supply of erythrocytes and serum from a suitable donor animal,
- Adequate laboratory equipment and trained personnel.
- Standardized conditions need to be followed to maintain the attenuated state Immunogenicity of vaccinal parasites including monitoring the number of *in-vitro* passages
- Carrying out periodic inoculation of naïve cattle
- Thorough testing of vaccine donors

### 2.5 Identifying protective antigens

#### 2.5.1 Empirical approach

Merozoite proteins or culture supernatants (exoantigens) have been fractionated in a variety of ways and individual fractions tested for induction of protective immunity in animal models (Schettters *et al.*1995)<sup>[34]</sup>. Four secreted *B. bovis* merozoite antigens were identified that were neither serologically immunodominant nor particularly abundant:

- 77–80-kDa protein (Bv80) also called Bb-1/spherical body protein 1 (SBP1)<sup>[35]</sup>
- 38-kDa cysteine-rich protein designated 12D3
- 60kDa Rhopty protein designated T21B4/also called Bv60 and Rhopty-associated protein1(RAP-1)<sup>[36]</sup>
- A high molecular weight antigen designated 11C5

The RAP-1 molecules are expressed in merozoites, sporozoites and other asexual stages, are able to bind erythrocytes and contain neutralization sensitive B-cell epitopes, and have been a leading candidate for vaccine development<sup>[37]</sup>.

### 2.5.2 Antibody-proteomic approach

Based on identifying merozoite surface proteins and apical complex proteins recognized by bovine immune serum or monoclonal/polyclonal antibodies raised in mice or rabbits are taken into consideration in antibody- proteomic approach. The rationale for this approach was to target proteins that may be important for erythrocyte invasion by eliciting neutralizing antibodies. Among the *B. bovis* proteins identified as antigenic were:

- 60-kDa RAP-1
- 42-kDa = merozoite surface antigen-1 (MSA-1)
- 44-kDa MSA-2.
- 77 KDa SBP-1
- 225 KDa SBP-2
- 135KDa SBP-3

MSA-1 was an attractive vaccine candidate because:

- Encoded by a single copy gene
- Is merozoite surface-exposed
- Highly antigenic in the native state &
- Antibodies against native or recombinant MSA-1 neutralized merozoite infectivity *in vitro*, suggesting its importance in merozoite invasion [38].

However, when put to the test, MSA-1 was not an effective immunogen, as it failed to elicit protective immunity in cattle against homologous strain challenge [39]. The MSA-2 proteins are more complex, encoded by a family of four tandem genes in the American isolates (*msa-2a1*, *msa-2a2*, *msa-2b*, and *msa-2c*) of which all but *msa-2a2* are expressed as 30–44-kDa proteins and elicit antibody responses upon infection. Bovine antisera specific for MSA-2a1/MSA-2a2, MSA-2b, and MSA-2c significantly blocked attachment and invasion of merozoites to erythrocytes and antibody raised in cattle against recombinant MSA-2c neutralized merozoite infectivity *in vitro* by approximately 50% [40].

In *B. bigemina*, the notable antigens identified by antibodies directed against the merozoite surface are RAP-1, GP45 and GP55 proteins [41]. RAP-1 is encoded by a polymorphic gene family consisting of transcripts coding for RAP-1a, RAP-1b and RAP-1c [42]. Native *B. bigemina* RAP-1a protein conferred partial protection, defined by reduction in parasitaemia following challenge with the homologous *B. bigemina* strain [41, 42]. Gp45 is a merozoite surface antigen encoded by a single copy gene and is expressed at the protein level in the Mexico strain of *B. bigemina* [43]. Immunization of cattle with native gp45 from the Mexico strain induced partial protection against homologous strain challenge suggesting that this protein may be useful as a component of a vaccine.

### 2.5.3 T cell proteomic approach

In immunized animals protected from challenge or in persistently infected animals that continually control parasitaemia, antigen-specific CD4+ T cells are believed to be required for the adaptive immune response by producing IFN- $\gamma$ . This approach identified several known antigens:

- Heat shock protein (Hsp) 70 and Hsp 90 (94), a 20-kDa Hsp belonging to the  $\alpha$ -crystalline protein family which acts to stabilize folding of other proteins [44].
- Fatty acyl coenzyme A synthetase (ACS1) that is involved in activation of fatty acyl coA for use in lipid biosynthesis [45].
- A Phosphoribosomal protein, P0, that in other organisms is involved in a complex that interacts with ribosomal RNA and is critical for cell viability [46].

### 2.5.4 Genomic Approach

Availability of genomic sequences of *B. bovis* has permitted identification of vaccine candidate antigens by genetic identity with homologous proteins in other protozoa. This approach also identified several antigens:

- 82-kDa AMA-1
- 75-kDa TRAP

AMA-1 = micronemal protein expressed on the surface of merozoites, where it becomes processed to smaller soluble fragments during invasion of erythrocytes. Antibodies against AMA-1 block merozoite invasion in mouse models [47] whereas, TRAP proteins are believed to function in host cell binding, *Babesia bovis* merozoites were shown to express TRAP, which localized to the apical end, and was also secreted. The raised antisera against TRAP peptides significantly inhibited erythrocyte invasion [48]. A novel family of genes that codify proteins were identified in *B. bovis* genome (Bbo-6cys). 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. Antibodies against this protein have an inhibitory effect on the invasion process, suggesting its importance in control methods against *B. bovis* infection.

Profilin is a protein that participates in cytoskeleton ensemble [49]. 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* [50].

### 3. Conclusion

Development of effective vaccines against apicomplexan pathogens, including *Babesia* is cumbersome and exceedingly difficult. Many of the live vaccines have not gained widespread acceptance because of a requirement for maintenance the cold chain between production and use in the field. *In vitro* methods have been used to grow organisms for preparation of live vaccine, however, the duration of protection against heterologous challenge have varied. The use of different cry preservatives and/or combinations to improve the viability of frozen products is being investigated. Genome sequencing projects have led to the current post-genomics era which is boosting a considerable amount of knowledge on the parasites, their antigens and their interactions with the host that can be exploited for the development of safer and industry-friendlier subunit vaccines. Progress in vaccine development will also require improved understanding of the mechanisms of immunity in natural hosts of these parasites, so that antigens can be delivered in a way that mimics the innate protective response that can lead to development of protective adaptive immunity.

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