Revista da Sociedade Brasileira de Medicina Tropical 46(3):265-269, May-Jun, 2013
http://dx.doi.org/10.1590/0037-8682-0014-2012

Review Article

Approaches towards tick and tick-borne diseases control

Ana Domingos[1], Sandra Antunes[2], Lara Borges[1] and Virgílio Estólio do Rosário[2]

[1]. Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Lisboa, Portugal. [2]. Instituto de Higiene e Medicina Tropical, Lisboa, Portugal.

ABSTRACT

Ticks are obligate haematophagous ectoparasites of wild and domestic animals as well as humans, considered to be second worldwide to mosquitoes as vectors of human diseases. Tick-borne diseases are responsible worldwide for great economic losses in terms of mortality and morbidity of livestock animals. This review concerns to the different tick and tick-parasites control methods having a major focus on vaccines. Control of tick infestations has been mainly based on the use of acaricides, a control measure with serious drawbacks, as responsible for the contamination of milk and meat products, as a selective factor for acaricide-resistant ticks and as an environmental contaminant. Research on alternatives to the use of acaricides is strongly represented by tick vaccines considered a more cost-effective and environmentally safe strategy. Vaccines based on the Bm86 tick antigen were used in the first commercially available cattle tick vaccines and showed good results in reducing tick numbers, affecting weight and reproductive performance of female ticks which resulted in reduction of cattle tick populations over time and consequently lower reduction of the pathogen agents they carry.

Keywords: Ticks. Tick-borne diseases. Control methods. Vaccines.

TICKS AND TICK-BORNE DISEASES

Ticks are arthropods with a complex life cycle, proven resilient and persistent in the environment. Three families are currently recognized: *Ixodidae* (hard ticks), *Argasidae* (soft ticks) and the recently identified *Nuttalliellidae*[1]. Hard ticks distinguish themselves by the presence of a *scutum* or hard shield that grows to accommodate large volumes of ingested blood, which, in adult ticks, reaches 200 to 600 times their unfed body weight. On the contrary, soft ticks accommodate smaller volumes of blood (5 to10 times their unfed body) and can resist to starvation, surviving for long periods of time without a blood meal[2]. The *Nuttalliellidae* family contains only one species, *Nuttalliela namaqua,* which exhibits intermediate characteristics in comparison with the other two[1]. Adult ticks, larvae or nymphs (pre-adult stages) can be infected horizontally by feeding on infected vertebrate hosts, or within the ticks, from the female to the eggs, maintaining the infection to the hatched larvae, a phenomenon denoted as transovarial transmission. There is also the transtadial transmission, which is the pathogen's diffusion from one tick life stage through a molt to the next instar[3].

These ecto-parasites have direct impact on the vertebrate hosts, leading to reduction of body weight, and in cattle, affect the milk and meat production, while ticks' bites reduce the quality of leathers. Among domestic animals, they are also responsible for anemia and severe dermatitis[4-6]. Apart from these direct effects, the most important feature of ticks is that they are vectors, as well as reservoirs, of multiple pathogens. Ticks and tick-transmitted parasites have co-evolved with various wild animal hosts, being part of the ecosystem's equilibrium[3]. Tick-borne diseases (TBDs), long known but often neglected, are progressively being recognized due to their economic impact in livestock, but also due to their impact in human health, to which they have become a threat.

Examples of TBDs transmitted to man are Lyme borreliosis by *Ixodes* sp. ticks, caused by at least three species of bacteria belonging to the genus *Borrelia* spp. and Rocky Mountain spotted fever, caused by *Rickettsia rickettsii* spread by *Dermacentor variabilis*. Changes in land use, reforestation, human demographics and behavior, are altering the interactions between human and infectious disease agents leading to the emergence of other infectious and zoonotic diseases[7]. Under these circumstances, arthropod vectors may enhance their potential to spread bacteria, viruses, protozoa and helminthes[8]. Nevertheless, the major medical and economic concerns with ticks and TBDs remain in the veterinarian field with a special emphasis on animal production. TBDs, as theileriosis, babesiosis, anaplasmosis and heartwater (also called cowdriosis), are considered the most important, concerning both health and management problems of cattle and small ruminants, especially in Latin America, Africa, Australia and Asia. Economically, the most important livestock ticks belong to the family *Ixodidae,* genera *Hyalomma spp., Rhipicephalus (Boophilus) spp.* and *Amblyomma spp.* Theileriosis and babesiosis are caused by the protozoan parasites *Theileria parva*, *T. annulata* and *Babesia bovis* or *B. bigemina*, correspondingly. While the heartwater and anaplasmosis are caused by the *Rickettsiales, Ehrlichia ruminantium* and *Anaplasma marginale*, respectively[2,8,9].

CONTROL METHODS

So far, the use of acaricides has been a major component of integrated tick control methods. Even before Smith & Kilborne (1893) proved the role of ticks as vectors of *Babesia* spp., animal

Address to: Dra. Ana Domingos. IHMT/UNL. Rua da Junqueira 100, 1349-008 Lisboa, Portugal.
Phone: 351 21 365-2600; Fax: 351 21 363-2105
e-mail: adomingos@ihmt.unl.pt
Received 01 October 2012
Accepted 11 December 2012

health authorities in the USA, Australia and Southern Africa were treating cattle with a variety of chemical agents, mainly mixtures of querosene, sulphur and lard, in an effort to control ticks. Acaricides are often inappropriately used, have residual effects in milk and meat subproducts, and are not environmentally friendly, being responsible for the increase of acaricide-resistant ticks[10,11]. Resistance is associated to mutations in genes related to drug susceptibility. The appearance of acaricides' resistance leads to the rise of individuals for which the lethal dose is higher than the one for the majority of determined specie. Nowadays, combinations of powerful acaricides are being used worldwide; products combining different active components are available in an attempt to include a diverse number of mechanisms of action, to reduce the emergence of insecticide resistance[12,13].

For companion animals, a formulation combining dinotefuran, permethrin and pyriproxyfen (Vectra 3D) was registered in the USA in 2007, and is indicated for the prevention and treatment of fleas, ticks, flies and mosquitoes, on dogs[14,15]. Others like imidacloprid/flumethrin collar [Seresto®, Bayer Animal Health, Investigational Veterinary Product (IVP)], a deltamethrin collar (Scalibor®, MSD, CP1), a fipronil/(s)-methoprene spot-on (Frontline Combo®, Merial, CP2), and an amitraz/fipronil/(s)-methoprene spot-on (Certifect®, Merial, CP4/CP5) against repeated infestations with *Rhipicephalus sanguineus* and *Ctenocephalides felis felis* are being tested for efficacy[15]. The example of pet animals is not valid in the scenario of animal production. George et al. offers an extended review on chemical control of ticks that can be consulted for further information[10].

Recent studies in Brazil and Mexico showed that the resistance to drugs such as cipermetrine and amitraz in *Rhipicephalus microplus,* and other ticks, is increasing[13,16-18]. The speed, with which resistance has appeared, along with the significantly more expensive pesticides, has restrained the companies to develop new drugs. The introduction of a new product in the market is time-consuming and has a huge economic burden; being the cost of discovering and developing a novel product estimated in US$100 million, with an average duration of 10 years[11]. This and the increasing concerns about resistance and side effects of insecticidal compounds, has led to the introduction of few new products over the years (e.g. spinosad)[12,19].

Recently, particular attention has been focused on the development of entomopathogenic fungi[20], such as *Metarhizium anisopliae* and *Beauveria bassiana,* as biocontrol agents against a range of several ticks under laboratory and field conditions, namely *Rhipicephalus annulatus*[21], *Ixodes scapularis*[22], *Rhipicephalus appendiculatus* and *Amblyomma variegatum*[23], *Argas persicus*[24] and *Ornithodoros lahorensis*[25]. Biocontrol agents usually favor both human and environmental safety, especially in comparison to the use of acaricides, but few biopesticide have been used in spite of their potential. The inability to successfully adopt biocontrol strategies includes factors like environmental stability (e.g., UV resistance, temperature tolerance), ability to initiate infection at low humidity, and potential unspecific damage to non-target invertebrates[24,25].

In this review the focus is on the new strategies becoming available for the control of ticks and associated pathogens in cattle.

VACCINES

Globally, most of the vaccines available to overcome TBDs are attenuated or live blood-derived. In theileriosis, the pathogens' life cycle involves three developmental stages: sporozoites, schizonts and piroplasms[26]. Cattle that recover from infection with *Theileria parva or Theileria annulata* are solidly protected against subsequent infections with homologous strains but can succumb to heterologous challenge. Immunization with simple mixtures of parasite strains results in an attenuated infection that produces an effective immune response[27,28]. The only commercialized *T. annulata* vaccine is based on attenuated shizonts produced in cell culture (Rak-shavac-T®, National Dairy Development Board, India). Cattle immunization with sporozoite surface antigen-1 or attenuated schizont-infected cells induces limited protection against homologous or heterologous sporozoite challenge, whereas a combination of recombinant and live vaccine results in survival of all vaccinates[29]. Attenuated vaccines have also been used to protect cattle against babesiosis and anaplasmosis, being these results evidence for the creation of improved immunity, by including sporozoite and schizont antigens in vaccines. Attenuated vaccines have successfully been used against babesiosis for example in Argentina, Israel and Australia. Moreover, Australian *Babesia bovis* and *Babesia bigemina* attenuated strains are being used to immunize cattle in other regions of the world namely, Africa South America and South-East Asia as described in Office of Environmental Information (OEI) report[30]. 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 and the possible reversion to virulence, requiring a cold chain system of maintenance. Therefore, an additional research is needed for the development of safer alternatives, more cost-effective and better defined live, or subunit, vaccines. Nonetheless, alternative approaches have been raised to control TBDs, which involve the development of anti-tick vaccines that can quell both vector and pathogens[8,31].

Recombinant vaccines became commercially available in the early 1990s, aiming to reduce the use of acaricides and their consequences. The feasibility of controlling tick infestations through immunization of hosts with selected tick antigens was achieved developing vaccines that reduced infestations on cattle. Vaccines against ticks allowing the inclusion of multiple antigens that could target a broad range of tick species and could also prevent transmission of pathogens[32].

Tick antigens are usually regarded as either exposed or concealed antigens. *Exposed* antigens are those that naturally come into contact with the host immune system during tick infestation. Hosts immunized with these antigens are boosted by continuous tick exposure. *Concealed* antigens are not exposed to the host immune system and therefore repeated immunizations are required to maintain high antibody titers. However, concealed antigens are more advantageous once ticks are unlikely to have evolved a mechanism to counteract the host immune response, contrarily to an exposed antigen[32,33].

A great handicap in the development of anti-tick vaccines, like other anti-parasite vaccines, is the identification of effective antigens. One of the major constraints when working with obligate intracellular parasites is a large excess of proteins of host or vector origin that interfere with pathogen protein detection. Among the important characteristics for a *concealed* antigen is the accessibility to antibody ingested during tick feeding and a vital physiological function of the tick[32]. The advances in characterization of tick genomes, along with the use of bioinformatics, ribonucleic acid interference (RNAi), mutagenesis, immunomapping, transcriptomics, proteomics, expression library immunization (ELI) and other technologies has allowed a rapid, systematic and comprehensive approach to tick vaccine discovery[32].

An effective antigen against ticks is the protein Bm86, specifically directed against the cattle tick *Rhipicephalus microplus*, it stands as the basis of two commercial vaccines, TickGARD Plus and Gavac Plus. The greatest effect was the reduction of larval infestations in subsequent generations, by reducing the number of engorging female ticks, their weight, and reproductive capacity[19,31,34,35].

Pipano et al. tested the efficacy of a Bm86 vaccine in protection against ticks and pathogens transmitted by those ectoparasites (*B. bovis* and *B. bigemina*)[36]. The results showed that immunized cattle, when challenged with *B. bovis*-infected ticks, continued to become infected, but in the case of *B. bigemina*, Bm86-immunized animals remained protected against infection[36]. Canales et al. have cloned ortholog genes (Ba86 and Bm86) from *R. annulatus* and *R. microplus*, respectively[37]. Cattle vaccination with Bm86 reduced the *R. annulatus* and *R. microplus* numbers, weight, oviposition and eggs' fertility. For *Rhipicephalus decoloratus*, Odongo et al., using a Bm86 based-vaccine, found a reduction on engorged adult female ticks, ticks weight and eggs weight[38]. Bastos, et al. studied the Bm86 silencing on the ability of *R. microplus* ticks to feed in *B. bovis* infected cattle, showing that this procedure decreased survival engorged ticks rate and eggs weight[39].

Rhipicephalus microplus Bm86/Bm95 antigens have proven their efficacy for the control of cattle tick infestations and transmission of tick-borne pathogens but only in some regions. In fact, the 900 tick species that have been documented are distributed by several geographic areas and animals are, therefore exposed to different tick stocks[40,41]. Several approaches have been made, like double vaccination with different active-principles and/or several doses along the time[42,43], and it became clear that for every region and tick species, should be formulated a different immunization procedure. Nonetheless, this approach is highly expensive and technically challenging[9,31].

Some examples of research studies aiming the identification of new vaccines are here described. The protein 64P from *R. appendiculatus* was found to be involved in ticks attachment and feeding, and was used to immunize guinea pigs, reducing nymph and adult infestation[44]. First studies on yolk pro-Cathepsin expressed in eggs of *R. microplus* suggested this aspartic proteinase as a promising antigen, however, when expressed as a recombinant protein in *Escherichia coli* and tested in a cattle trial, the efficacy was merely 25%[45]. Another example concerns to 5'-nucleotidase that when tested in sheep showed positive results, but no effect in a subsequent trial in cattle[36]. The gut-expressed iron storage protein, ferritin 2, is another antigen that has been evaluated in cattle trials. Silencing of ferritin 2 by RNA interference showed significant impacts on tick feeding, oviposition and larval hatch, indicating ferritin 2 as a candidate tick vaccine antigen[46].

Subolesin, first discovered in *I. scapularis*, is a highly conserved protein involved in modulation of tick feeding and reproduction, and had a protective effect against all tick developmental stages when used in recombinant protein immunization. Subolesin was silenced by de la Fuente et al. through RNAi in *D. variabilis*, leading to degeneration of several tick tissues, such as guts, salivary glands, reproductive tissues and embryos[32]. Therefore, production of sterile ticks was made possible through subolesin knockdown by RNAi. Consequently, the release of subolesin-silenced ticks, as a sterile acarine technique (SAT), for autocidal control of tick populations has been proposed[43]. By releasing enough numbers of sterile individuals that mate with wild ones, it is expected a decrease in the wild population overtime, due to the lowering of the reproductive potential[48]. The use of sterile insect technique has proved its utility in dealing with crop pests, but its potential in tick control has not yet been explored in a larger scale. Kocan et al. showed that subolesin knockdown in *I. scapularis*, *Dermacentor variabilis* and *Amblyomma americanum* also affected oviposition, eggs embryogenesis, larval hatching and fertility[47]. Vaccination with subolesin reduced *R. microplus* survival and reproduction rates and tick infection by *Anaplasma* spp. and *Babesia* spp.[41]. Their results demonstrated that *R. microplus* infestations where successfully controlled by combining vaccination and release of transgenic ticks, which suggests that the combination of methods increases the efficacy of cattle tick control, at least under some circumstances. Further studies are being developed concerning cattle vaccination in different regions of the world, to determine whether the promising results obtained in Mexico can be reproduced elsewhere[8,9,42].

Looking for new antigens, Antunes et al. have characterized *R. annulatus* genes differentially expressed in response to *B. bigemina* infection using suppression-subtractive hybridisation (SSH) and real-time reverse transcription polymerase chain reaction (RT-PCR). Genes confirmed as differentially expressed in infected ticks [tick receptor for OspA (TROSPA), calreticulin, ricinusin, serum amyloid A and Kunitz-type protease inhibitor (KTPI)] were functionally characterized using RNAi approach to analyze their role during pathogen infection in the tick vector[49].

Pal et al. had already described TROSPA in *I. scapularis*, studying the effect of anti-TROSPA antibodies and gene knockdown during a *B. burgdorferi* infection. Reduced *B. burgdorferi* adherence to the *I. scapularis* gut was observed *in vivo*, culminating in a deficient colonization of the vector, with subsequent levels reduction of pathogens transmission to the mammalian host[50]. Antunes et al. found a similar protein over-expressed in *B. bigemina*-infected *R. annulatus*, with high sequence identity to *Ixodes* spp. After TROSPA knockdown, significant decrease in infection was observed for both *R. annulatus* and *R. microplus*. These results suggested the possibility that *B. bigemina* uses a TROSPA ortholog receptor for infection of *Rhipicephalus* tick cells and encouraged research for the characterization of this molecule in *Babesia*-tick interactions and development of transmission blocking vaccines. Serum amyloid A is known for being involved in host response to tissue injury and inflammation. After gene knockdown in both *Rhipicephalus* spp. lower infections were observed without weight losses, suggesting that this protein may be part of tick response to the stress produced by *Babesia* sp. infection, but at the same time necessary for pathogen multiplication in *Rhipicephalus* spp. ticks[49]. Calreticulin, already described in tick's saliva by Jaworski et al. and Ferreira et al., seems to take a role during tick feeding, once gene knockdown resulted in a weight decrease of *R. annulatus*[51,52]. These results also suggest that this protein may be required for *B. bigemina* infection. Gene knockdown of ricinusin did not affect pathogen infection, thus suggesting that this molecule is not essential to control *B. bigemina* infection in *Rhipicephalus* spp. ticks[49].

The absence of full tick genomic data and the lack of a confirmed tick RNAi pathway can underestimate the off-target effects in current tick RNAi experiments[53]. Despite this, the use of long dsRNAs as gene knockdown treatments in ticks has been accepted as a routine method for validation/support of tick gene function[42,54,55]. Some of the *R. annulatus* genes discovered in this study such as serum amyloid A, calreticulin and TROSPA could contribute to the development of novel vaccines designed to reduce tick infestations and prevent or minimize pathogen infection in ticks and transmission to vertebrate hosts[49].

CONCLUSIONS

These revision concerns to a discussion on the methods used for tick and tick-borne parasites control and was mainly focused on the development of new recombinant vaccines.

Research in the post-genomic era is leading to the development of new control measures such as the recombinant vaccines. Despite these advances the establishment of non-living vaccines has been challenging. As a result, and despite several disadvantages, attenuated vaccines are still being used, adapted to conditions of each region.

After more than a decade, the two commercial tick recombinant vaccines are still being used in some countries such as Cuba, Australia and Mexico, though not worldwide due to commercial and technical constrains. These vaccines, however, when used in field trials, showed very positive results on tick and tick-borne diseases (TBD) reduction, improving cattle production and reducing dependency on acaricides. In parallel, they also showed to be a cost-effective and environmentally safe strategy, to tick control.

Tick infestation is rarely a one-species issue, and therefore, anti-tick vaccines should aim at a more global protection against the main species of economical and epidemiological interest. The great rise of acaricide resistance, still asks for the implementation of an effective vaccine. The discovery of potential antigens against tick and tick-pathogens proteins should result in improved vaccines, more advantageous in an overall approach to control TBDs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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