Tick vaccine development

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An integrative approach to the development of a vaccine against the cattle tick, Rhipicephalus microplus

Industry Sector: Cattle and Small Stock

Research Focus Area: Animal Health and Welfare

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EXECUTIVE SUMMARY

Fast expansion of tick acaricide resistance in South Africa highlights the need for an improved tick control strategy. Without such a strategy, the spread of acaricide resistant ticks and the associated tick-borne diseases transmitted (e.g., lethal redwater, anaplasmosis, borreliosis) will be crippling for the agricultural industry and food security. To date, only one tick vaccine has been commercialized and was based on a tick gut membrane associated protein Bm86. This vaccine has since been removed from global markets (except for South America), due to variable efficacy in the field. In our laboratory, we were the first to identify 3 proteins that interact with Bm86 inside the tick gut. We therefore expressed these proteins and combined them into a BM86combi vaccine formulation for evaluation.

For the testing of improved vaccine formulations, a cattle trial with 16 Friesian calves was performed. These were subdivided into four groups: two control and two test groups. Whole body infestation was performed with approximately 4,000 *R. microplus* larvae per calve. Ticks were fed to engorgement, collected, weighed and incubated for ovipositioning. Cattle blood was collected for the obtainment of serum on day before first immunization, after the second and third immunization and before tick infestation to determine antibody titres against the injected proteins. Both vaccine formulations resulted in reduced tick numbers, but the Bm86 combi vaccine (Bm86 and 2 of its interacting partners) showed significance. This vaccine led to a tick reduction of 53%. This is very significant, as the Bm86 antigen alone offered no protection in South African cattle against the South African *R. microplus* strain. Enzyme-linked immunosorbent assay revealed a several fold increase in antibody titre over time for all antigens used. Further improvement of vaccine formulation, including eukaryotic expression systems to obtain glycosylated proteins (enhanced solubility and antigenicity) and modified adjuvant usage could further improve obtained results. These latter studies will be done in 2015 with funding provided by TIA.

To move the field of tick vaccine development into the next era, more insight into the protective responses from cattle against tick infestation is vital. Currently, available publications focus on skin and blood samples only, which are not a true representation of underlying immune responses. Therefore, the second aim of this study was the comparison of immune responses of three cattle breeds (tick resistant Bos indicus, tick susceptible Bos taurus and South African mixed bred cattle (Bonsmara)) determined by looking at lymph node tissue before and during tick infestation. This will aid in the identification of immunological markers that can lead to improved evaluation and formulation of future vaccines. Three biological repeats per breed were infested with ~7,000 *R. microplus* larvae. Lymphatic tissues were collected prior to infestation to serve as baseline to be compared to infested cattle (at 2 time points: larvae and adult infestation). RNA from lymph nodes was isolated and yielded very high purity material. Microarray technology was used for the identification of differentially expressed genes between the three cattle breeds allowing the identification of immune pathways influences by the different host immune responses. A total of 8 microarray experiments to determine the gene expression profile of the bovine lymph node tissue have

been completed with the remaining 19 microarray experiments currently being underway. For the latter, we will submit a new funding application in mid-2015 to the Red Meat Research Development Trust of South Africa.

Project Aims

1. To re-evaluate and improve the vaccine formulation and regime of two previously validated vaccine candidates (1 year project)
2. To compare the immune responses of three cattle breeds (tick resistant Bos indicus, tick susceptible Bos taurus and South African mixed bred cattle Bonsmara) determined by looking at lymph node tissue before and during tick infestation

Popular article

Status of tick vaccine development: obstacles, successes and future perspectives

Some 240 different species of ticks occur in Africa, with the greatest variety of species found in the eastern and southern parts of the continent. However, the species ranges in Sub-Saharan Africa are not necessarily constrained by host distribution (i.e., cattle) and expansion of suitable habitats is expected in the wake of global climate change. Currently, some 106 tick species occur in South Africa, of which only 23 (including 21 hard and 2 soft) are considered endemic to this country. This is of great concern to the livestock industry as 35 species are associated with domestic animals. Of these species 15 are competent vectors for transmitting pathogens associated with around 23 diseases or health syndromes. Moreover, several tick-borne zoonotic diseases transmitted between animal reservoirs (i.e., livestock or companion animals) and human hosts have been described to date (Table 1).

Cattle production and tick transmitted diseases

Cattle represent a major livestock production system, plagued by many tick-borne diseases (TBDs) as shown in Table 2. The southern cattle tick, *Rhipicephalus microplus*, is an invasive species in South Africa and is currently considered the principal pest of cattle in many regions of the world including: southern Asia, Madagascar, as well as the southern and eastern coasts of Africa. Moreover, it is regarded as the most economically devastating tick species worldwide due to its high adaptability to new distribution ranges (i.e., its introduction into the Ivory Coast in West Africa and recently Namibia), its competency as a vector for several economically important diseases (including redwater and gallsickness), as well as the occurrence of acquired resistance to all major chemical classes of acaricides. In South Africa, this species' range has expanded from the coastal regions of the Cape and Kwazulu Natal to include areas of the Eastern Cape, Free State, Mpumalanga, Limpopo, Gauteng and the North-West provinces. *Rhipicephalus microplus* has also been shown to displace the less pathogenic and endemic blue tick species, *R. decoloratus*. It is therefore expected that the impact of this species on the local cattle industry will only increase in the near future as climate change creates new favorable areas for infestation.

Chemical control

Current control measures are mostly centered on the use of chemical acaricides and in 2003 ectoparasiticides represented 22% of the total sales in the veterinary market in South Africa. However, the development of resistance to acaricides, especially investigated and reported for R. microplus, is a global problem. In South Africa, resistance of this species against acaricides was first reported in 1979 and has become increasingly problematic even after the introduction of novel acaricides. Resistance in field populations can be gained within two years for R. microplus due to this species' high reproductive potential, their short life cycle (as short as 7 weeks) that enables 4 to 6 generations to be produced per year and the intensive misuse of acaricides that sustains a high level of selection pressure on these populations. The further rapid spread of these resistant populations is then facilitated by the uncontrolled movement of cattle, especially in the rural areas of South Africa. Apart from resistance, several additional drawbacks are associated with acaricide use and include environmental pollution and the contamination of milk and meat products with chemical residues, all of which are a concern for human health. Hence, the introduction of new chemicals does not necessarily present a long-term solution on its own as new resistance occurs rapidly, in addition to new acaricides being generally more expensive than their predecessors. Therefore, monitoring and screening for markers associated with resistance will assist in the early detection of acquired resistance and will be an invaluable addition to the development of an integrated pest management program. Such a program has been initiated at the University of Pretoria to screen and study resistance in collaboration with Red Meat Research and Development South Africa (RMRD SA), Zoetis™ South Africa and ClinVet International (Pty) Ltd.

Economical impact of ticks and tick-borne diseases

The loss of cattle conditions directly due to tick infestation (i.e. anaemia and secondary infection) cattle losses resulting from tick-borne diseases, as well as damage to hides as a result of scarring around tick feeding sites, poses a severe problem to the revenue stream for both small-holder and commercial cattle farmers. According to the Department of Agriculture, Forests and Fisheries (DAFF), approximately 14 million head of cattle (incl. other livestock) are grazed on 69% of the available agricultural land in South Africa. As an important source of nutrition and revenue, the South African cattle industry was responsible for a gross income value of around R18.5 billion for 2013 (9.7% of the gross agricultural production for 2013). In contrast, losses to the agricultural industry recorded in 2011 due to the use of ectoparasiticides were estimated to be around R7,5 billion for sprays and dips alone. Currently, the annual capital losses due to tick burdens on beef production are estimated to range between R1.3 and R3.1 billion.

Immunological control via vaccination

Since the acquisition of resistance to chemical control has become a grave concern, the development of tick vaccines for use in cattle tick control has become a research priority at the University of Pretoria. Though current vaccines lack efficacy for stand-alone use, their inclusion into an integrated control strategy can give producers an edge in resistance and disease transmission management.

Selection of vaccine candidates

Immunological control of ticks via vaccination of host animals with tick-derived proteins (antigens) has been explored since the early 80's. Initial classical approaches for tick vaccine development included vaccination with crude crushed preparations of ticks, a method still in use today in other African countries such as Zimbabwe. However, it is difficult to standardize and the unintentional introduction of disease from infected ticks to susceptible livestock is a serious concern. Therefore, in 1986 cattle were vaccinated with purified protein fractions from R. microplus gut tissues and finally a tick vaccine based on a single antigen, Bm86, was commercialized and called TickGARDTM (Australia) and GAVACTM (South America). The efficacy of this vaccine in lowering tick burdens on cattle was sufficiently demonstrated in both controlled and field trials. Recently, a two year country-wide vaccination program involving 1.9 million head of cattle was initiated in the Republic of Venezuela using GAVACTM vaccines. Results showed an 87.8% reduction in acaricide use along with an associated cost saving of 81.5% on the use of traditional chemical control only. Unfortunately, trials performed in South Africa by our group using the Bm86-based vaccines did not confer protection against a local R. microplus strain. Therefore, research to identify the Bm86 antigen from South African tick populations and vaccine trials is currently underway.

Additional next generation vaccine candidates have been identified to date, however, only a single new antigen (called subolesin or 4D8) has been tested in cattle vaccination trials with good efficacy (Figure 1). Hence, identification and validation of new candidates for second generation vaccines are desperately needed. Our current approach to rational vaccine design uses available genetic data for the parasite in large-scale computer-based analyses (termed Immuno-informatics) to predict promising vaccine candidates. With these computer-based tools it is possible to predict the structure and function of many proteins that can be recognized, processed and elicit a particular immune response in the bovine host. However, computer-based approaches cannot replace laboratory and field experiments or the critical expertise of operators needed to evaluate a target intended for vaccine development. The biological function (i.e. feeding, development and reproduction) of a particular candidate may be critical for its success as a vaccine candidate. Moreover, whether a target occurs in a complex, working synergistically with other proteins and/or occurs within a particular metabolic pathway may be a pivotal consideration in multivalent (targeting several parasite functions) vaccine design. Also, the functional redundancy of the vaccine candidate must be considered. The R. microplus genome is very large and around 60% of the proteins that it encodes have no known function (Figure 2). One of the reasons for such a large store of genetic information is credited to DNA duplication creating several copies of the same gene. This allows the parasite to produce large families of related proteins that could “rescue” the function that the vaccine is targeting. This highlights the need for the development of a vaccine containing subunits (containing large regions) or peptides (containing very small lengths) of the target protein that is highly conserved within the family.

Vaccine candidate production

Since the 1980s researchers have been able to produce vaccine antigens for vaccination on a large-scale bacteria or yeast. Many tick vaccine candidates have been produced in this way for evaluation in cattle trials including some 13 candidates derived from R. microplus (Figure 1). However, the choice of protein production system can greatly enhance the vaccine efficacy of a target protein by producing it in its natural state and/or provide components that can increase the host immune responsiveness to the protein. Non-pathogenic microorganisms such as yeast and bacteria have been used extensively for synthetic production of proteins for biomedical purposes (i.e. the Hepatitis B vaccine for humans). Currently, our approach has identified 28 novel antigens targeting the cattle ticks’ metabolism and digestion as well as antigens associated with Bm86 that are being produced synthetically in yeast for testing in cattle vaccination trials (in collaboration with the CSIR and Faculty of Veterinary Sciences, Onderstepoort).

DNA vaccines

A relatively new alternative to protein-based animal vaccination is the use of DNA-based vaccines as to protect against tick infestation. However, this approach has only enjoyed limited attention to date. This approach was previously applied in mice challenged with the Lyme disease tick, I. scapularis. More recently the antigen, subolesin, has been used in an orally administered DNA vaccine that reduced larval infestation and reduced transmission of the pathogen, Borrelia burgdorferi. Additional undisclosed R. microplus antigens (ARS1 and ARS2), were reported to confer protection against infestation (~75% efficacy) using a DNA-based vaccine in cattle trials (Figure 1). Additional advances in this field in regards to increase vaccine stability, improved delivery and priming-boosting of host responses could eventually make tick DNA vaccines commercially feasible.

Antigen delivery vehicles

Nano- or microparticles are inert porous bead-like polymers that are used as unique drug delivery vehicles in the field of nanobiopharmaceutics. This approach has been used successfully to block transmission of a malaria parasite, Plasmodium berghei, from infected mosquitoes (Anopheles gambiae) in a mouse model vaccinated with mosquito derived antigen contained in a microparticle. These particles have a natural immune boosting ability, confer prolonged protection due to slow-release of antigen and have been shown to influence the type of immune response elicited. Therefore, a microparticle encapsulated antigen can be tailored to elicit a particular host response and increase vaccine efficacy. Alternatively, non-pathogenic viral particles have been developed for use in cattle vaccination against bovine adenovirus-3. In this case, viral coat proteins are used as a scaffold (or conjugate) onto which an antigen of choice can be attached so that it can prime the entire host immune system or it can be tailored via co-production of immunomodulatory proteins to elicit a particular host response. This will be tested with some of the candidate antigens discovered to date. However, what constitutes a protective response against tick infestation in cattle from an immunological perspective remains a grey area in our field. These so-called correlates of protection are needed to evaluate whether any given vaccine will elicit the necessary responses to confer protection in the immunized host. Factors such as physical (i.e. skin composition) and mechanical (i.e. grooming behavior) traits have been also implicated in resistance to tick infestation. However, whether differences in immune response alone are enough to confer protection in cattle is unknown. This is valuable information needed for formulation of a vaccine with the correct adjuvant, a substance mixed with all vaccine antigens to enhance the immune response. In this regard, the University of Pretoria has initiated a program to study the correlates of protection for several common cattle breeds including Holstein-Friesian (Bos taurus), Brahman (Bos indicus) and Bonsmara (mixed). Preliminary results indicate marked differences in host responses during tick infestation and further studies will identify which components are responsible for protection (i.e. inflammatory and/ or antibody-mediated responses), improving vaccine formulation for application in most cattle breeds.

Conclusion

Though the use of acaricides may remain the main method of current tick control, the development of an integrated strategy incorporating several different control measures that include an effective tick vaccine can alleviate the selection pressure for resistant ticks. Moreover, targeting several components and stages of the parasites' biology will cripple its ability to adapt and persist. A multidisciplinary research approach has established a platform for tick surveillance; candidate discovery and rational vaccine design that with the aid of independent and industry partners will add a new and much needed alternative to pest and disease management in South Africa.

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