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The Progress and Process of the Development of Anti-tick Vaccine Against Cattle Ticks (*Boophilus microplus*) in Thailand

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ABSTRACT

Tropical cattle ticks (*Boophilus microplus*) are the most important ectoparasites that have the impact on livestock development in Thailand due to their responsible for severe economic losses through direct damages of blood feeding and as important pathogen vectors. Acaricides have been used extensively to control ticks with partial success. However, this approach suffers drawbacks such as selection for pesticide resistance, environmental contamination and residue problems. Recent advances have introduced possibility for immunological control of the parasites through use of an anti-tick vaccine. Anti-tick vaccines by contrast have the potential to be non-contamination, sustainable and low-cost technology, potentially applicable to a wide variety of hosts. There is considerable evidence that the major protective mechanism in the vaccine is the production of antibody to 'concealed antigen'. In Thailand, there has been the pilot study for the trend of semi-crude anti-tick vaccine trial with outstanding successful outcome. Therefore, this consequence was leading to the identification, characterization, cloning, and expression of candidate antigen proteins such as Bm86, Bm91, and Bm95 derived from *B. microplus*' midgut origin. Subsequently, the complete sequence of the gene and translated sequence encoded these proteins were obtained. The efficiency trial in natural hosts to measure effect of the vaccine is still underway. The outcome might be potential for the commercial anti-tick vaccine in Thailand in the near future.

Keywords: *Boophilus microplus*, anti-tick vaccine, gut antigens, Thailand

INTRODUCTION

Problems and challenges of successful ectoparasite control are epitomized by situation with *Boophilus microplus* in Thailand. Tick control is a continuing global priority because of losses in livestock production (Snelson, 1975; Steelman, 1976; McCosker, 1979). The traditional control methods include the use of chemicals, with partially successful results, but acaricides also has some adverse effects such as the high incidence of resistance within tick populations, harmful effects on vertebrate hosts, human beings and the environment due to residual problems, and the high cost of treatment (Wikel, 1988; Willadsen and Kemp, 1988; Nolan *et al.*, 1989). The control of

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ticks exacts cost itself can create its own problems. The tick also transmits a number of diseases, most notably bovine babesiosis and anaplasmosis. The cost associated with production losses and control of the parasite in Thailand has been questionable. However, based on the number of cattle in Thailand are around 7 millions; so the cost might be exceeding 700 million baht per year.

The development of tick control strategies is complicated due to the interaction of a range of factors. Briefly, these are the disparate nature of cattle production enterprises in tick infested areas such as the interaction between cattle breeds, tick susceptibility and market demands for meat as well as the factors affecting traditional chemical control of tick including the emergence of parasites resistant to the chemicals and the demand for residue-free food.

In Thailand, tick-infesteded cattle are raised on pasture under tropical environmental conditions. The style of husbandry varies from poor to good management with increasing the chance of contact between animals and ticks. There are no single approach for tick control that might have the universal applicability.

Traditionally, tick control has been used of chemicals such as arsenic compounds in the nineteenth century and proceeding through DDT (chlorinated hydrocarbons), organophosphates, carbamates, amidine and synthetic pyrethroids, then developed as chemical resistance (Nolan and Schnitzerling, 1986; Nolan *et al.*, 1989; Schnitzerling *et al.,* 1989). The occurrence of widespread acaricide resistance, and multiple chemicals resistances within a single isolate of ticks, poses great problems for the effective chemical control of the parasites.

Increasingly, there is consumer concern about the occurrence in food products of chemical residues including agents used in tick control. The issues raised are perplexing due to awareness of polluted environment, contaminated to food products of animal sources, and be fundamental cost of animal production. Under such circumstances, the desirability of alternative, environmentally friendly technology, such as immunological control, is clear. This has lead to the development, in Thailand, of the first anti-tick vaccine in Thailand.

An important component of tick control is the use of naturally acquired, immunologically, mediated resistance by cattle to ticks. This is acquired after extended exposure to the tick and it is due largely to cutaneous allergic reactions (Willadsen, 1980). This resistance may be high for *Bos indicus* cattle but with rare exceptions it is low for *Bos taurus* or cattle with a large proportion of *Bos taurus* in their parentages (Utech *et al*., 1978). Increasingly however, milk market forces favor dairy cows with a high proportion of *Bos taurus*, that is, cattle which are tick susceptible.

Development of anti-tick vaccines

The development of anti-tick vaccines against *B. microplus* in Thailand has taken 3 years (2001 to 2004) with some progression and promising. There were four steps of development. **Step 1** was the demonstration of feasibility in the laboratory under controlled conditions. To achieve this, it would be necessary to show that it might be possible to vaccinate against ticks, to identify the responsible antigens, to produce the antigens as a recombinant protein and to show that this recombinant protein was protective. **Step 2** would be demonstration that the vaccine could actually offer significant protection against tick infestation under realistic field conditions. **Step 3** would be registration that fulfilled of manifold conditions of efficacy, safety and general applicability that had to be satisfied before commercial sale be allowed. **Step 4** was the final stage that would be the totally commercial one by integrating a new product into commercial cattle production.

Step 1: Demonstration of feasibility

Development of the vaccine against *B.*

microplus began with a very conventional experiment in which homogenized salivary gland and midgut from semi-engorged adult female tick were used to vaccinate dairy cows (Jittapalapong *et al*., 2004a). Not only the numbers of engorged ticks after a subsequent larval tick challenge were considerably reduced, but also the survivors showed evidence of internal damages, after ingestion of the host blood (Pichitarsilp *et al.*, 2004). The idea of damaging haematophagous parasites by producing antibody to its own internal components has been reported (Tellam *et al.*, 1992) and the experiment itself was similar to one with *Dermacentor andersoni* by Allen and Humphreys (1979).

The selection of potential candidate antigen for evaluation as vaccine candidates depended on how we understood what was both really essential to a tick and accessible to host immune system. Fortunately, rapid development of molecular technologies was having an impact on the identification of antigen and potential antigens. Isolation of the single, protective antigen from extremely complex crude extracts used in the initial experiment was carried out by a series of recombinant protein system. Antigens such as Bm86, Bm91, and Bm95 were glycoproteins with an extracellular location on the digest cells of the tick gut (Gough and Kemp, 1993). The protein had a high disulphide content, 10% of all amino acid residues being disulphide-bonded cysteines (Rand *et al.,* 1989; Willadsen *et al.,* 1989; Cobon and Willadsen, 1990; Tellam *et al.,* 1992). The purification, sequencing and structure of these antigens of Thai *B. microplus* have been reported (Jittapalapong *et al.,* 2005).

A variety of expression system has been used for production of a recombinant antigen. The expression of *E. coli* with *Baculo* virus vectors and downstream processing of the bacterially expressed protein have been established. However, this system seems to be not efficient enough for the large scale production. Therefore, this would bring yeast expression system by using yeast *Pichia pastoris* (Rodriguez *et al*., 1994).

Step 2: Efficacy in field trials

Efficacy of vaccination could be measured in a modern system in which cattle with susceptible exposure to ticks were infested with *B. microplus* larvae and the engorged adult female ticks which mature approximately 21 days later collected, counted, weighed and their ability to lay eggs estimated. There was also some effect on the viability of larvae after hatching.

Typical results with recombinant protein of vaccine in trials showed a 20-30% reduction in the numbers of ticks engorging, a 30% reduction in the weight of engorging ticks and a 60-80% reduction in the weight of eggs laid per gram of engorged female ticks (Willadsen *et al.,* 1995). If the impact of the vaccine was measured through its effect on the reproductive capacity of a single generation of ticks, from larvae through to the eggs laid by the surviving, mature adult females, then the parameters listed above give an overall vaccine effect of 90% (Tellam *et al.,* 1992).

In the field trial, several unexpected events might be occurred such as the tick population in the field, which was very dependent on a variety of factors, principally climatic condition, which caused difficulty in prediction of the tick population.

Step 3: Product registration

Among features that set the anti-tick vaccine aside from more conventional vaccine or from current chemical methods of tick control, none was more important than the nature of the protective effect. Because the major effect of vaccinations was a reduction of the eggs laid per engorging female, the benefits derived by an individual vaccinated animal were relatively low. Benefit arised principally from reduction in the total number of tick larvae available for infestation.

The process of the product registration

demanded that the claimed action of the vaccine should be demonstrable under a wide variety of conditions with cattle of different sexes and breeds in a range of geographical locations and weather patterns, using a range of tick isolates from different areas and displaying varying patterns of acaricide resistance.

Examples of the factors considered in the registration process:

(1) **General efficacy of vaccination**

Factors such as the timing of vaccinations, maintenance, cattle breed, and the duration of immunity.

(2) **The stability of the vaccine**

Vaccine preparation and potency

(3) **Safety of the vaccine**

The vaccine was consisted of a sterile protein of high purity formulated in an oil adjuvant. Specifically, it should be established that vaccination of young animals or pregnant heifer or pregnant cows do not affect pregnancy rate, calving rates. The vaccine did not have an effect on growth rate or the value of the carcass.

Step 4: The product in use

Just as the progression from an experimental vaccine being tested under controlled laboratory conditions to a vaccine being under field conditions represents a major transition, so the progression from field experiments to a commercial product represent another transition of equal difficulty and complexity. Several factors bear on this.

1. In field trials conducted under close supervision, the vaccine could be used in the optimal way such as antibody titer synchronized with the development of the tick population, cattle maintained in good condition and movement of cattle in or out of the restricted experimental areas. These conditions were frequently not met in a commercial situation, where a vaccination for tick must fit with other husbandry requirements.

2. The vaccine was a potential competitor with established and accepted technology, namely the chemical control of ticks. The effects of chemical control were familiar, namely, a high level of short term mortality, followed by a waning effect. The vaccine operated in almost the reverse way, with little immediate mortality but offering long term control. In practice, it has been vital to demonstrate that cattle producer could implement the new strategies for tick control and satisfactorily combine conventional treatments with vaccine to obtain commercial benefits.

Efficacy improvement of the vaccine

Despite the success of the current, single antigen vaccine, improvement in efficacy to the stage where it would be a reliable, stand-alone means of tick control in all situations would clearly be desirable. It was tantalizing that experiments with partially purified, native antigens have indicated that considerably better tick control is possible (Jittapalapong *et al.*, 2004). Whereas Bm86 on its own, as either a native or recombinant antigen, produced a 90% reduction in the total reproductive capacity of tick relative to those maturing on control cattle, 99% reduction has been achieved with partially purified antigens. It seem likely that vaccine's efficacy could, in principle, be substantially improved. The identification of antigen other than Bm86 such as Bm95 and Bm91 which could be used to increase the efficacy of the existing vaccine.

The Control of other tick species

Cross-reactivity between antigens of different tick species has been frequently reported for the past decade. Cross-protection however is observed much less frequently even when the species are quite closely related and the efficacy of cross-protection is often low. In same cases, it seems intrinsically unlikely that the antigen cross-reactivity was such that cross-protection will occur. Some of this cross-reactivity might be due to

carbohydrate epitopes (Wheeler *et al.*, 1991) which may not be protective. This has been observed with other parasites (Xu and Powell, 1991) and occurred with the Bm86 antigen as well (Willadsen and McKenna, 1991).

The effect of vaccination on the transmission of tick-borne pathogens

In principle, tick vaccines could affect the transmission of disease in two possibilities.

(1) By affecting tick numbers, they could directly influence disease incidence. The effects might be positive or possibly, if vaccination against tick prevented the achievement of endemic stability to tick-borne diseases, deleterious.

(2) Since it was increasingly clear that disease transmission could involve complex interactions between host, tick and disease organism, it was possible that by disturbing the tick, the vaccine also more subtly but more directly affects the disease.

Field data from Cuba and Thailand has shown a very significant reduction in the incidence of babesiosis and anaplasmosis following sustained use of the Bm86 and crude extract of salivary gland proteins (Jittapalapong *et al.*, 2004b). In a retrospective study, prolonged vaccine usage led to a decline in the incidence of tick-borne disease in some but not all areas (de la Fuente *et al.*, 1998) but eventually to a reduction of 98%, a surprising but intriguing result. More strikingly, there was evidence, though on a smaller scale, that the use of vaccine in cattle infested with *B. annulatus* prevents the transmission of *Bebesia bigemina* and reduces the frequency or severity of disease due to *B. bovis* (Pipano *et al.*, 2003). Tentatively this might be attributed to the fact that with this tick species, in contrast to *B. microplus*, the engorgement of both larvae and nymphs, the stage that transmit these diseases, was severely affected by the vaccine.

The cost of vaccine comparable to pesticides

From a commercial perspective there were a number of other potential advantages of vaccines which are less frequently discussed.

(1) A recombinant vaccine can potentially be produced at low cost using a multi-purpose manufacturing facility so that dedicated facilities are not required. The cost of purpose-built manufacturing facilities and chemical synthesis are a critical barrier to the commercial development of many potential pesticides.

(2) The registration cost of a new vaccine should be substantially less than that of a new pesticide. A significant part of the cost of the development and registration of a new pesticide is in the demonstration of safety towards target and non-target species, in particular, humans. It is highly likely that for a vaccine based on a defined protein antigen, the regulatory hurdles will be very significantly less.

Table 1 Relationship between vaccine efficacy and Bm86 sequence relative to Thai Bm86.

B. microplus isolates	Sequence difference (%)	Efficacy (%)	References
Y-strain (Australia)	0	89	Tellam *et al.*, 1992
Tuxpan (Mexico)	5.7	51	Garcia-Garcia *et al.*, 1999
Mora (Mexico)	8.6	58	Garcia-Garcia *et al.*, 1999
Mexico	3.3	89	Cobon, 1997
Field-strain (Argentina)	-----	55	Lamberti *et al.*, 1995
A-strain (Argentina)	1.6	10	Garcia-Garcia *et al.*, 2000
Camcord (Cuba)	0.2	84	Montesino *et al.*, 1996
Columbia	-----	81	Patarroyo *et al.*, 2002
Thai strain	7.2-8.0	?	Jittapalapong *et al.*, 2005

(3) There are theoretical reasons for expecting that the development of resistance to a vaccine is less likely than for a pesticide. There are numerous evidences where a single point mutation in the target molecule is sufficient to render a pesticide ineffective, while there is the expectation that such point mutations are likely to be of little relevance to most vaccines. With some limitations, this is the case for the current vaccines against *B. microplus*. This is because in most cases a vaccine is likely to target multiple epitopes on a single protein antigen. Finally, while the introduction of a tick vaccine for a farming community used to parasite control through pesticides might be initially difficult, in the longer term vaccines should be less subject to some of the serious patterns of misuse seen with pesticides. Therefore, there might be not frequently needed for the product registration for a new vaccine compare to pesticides.

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