

## Review Article

*J Vect Borne Dis* 44, June 2007, pp. 79–89

# Upcoming and future strategies of tick control: a review

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### Abstract

Ticks are distributed worldwide and significantly impact human and animal health. Due to severe problems associated with the continuous use of acaricides on animals, integrated tick management is recommended. Increasing public health concern over the tick-borne diseases demands the strategic control of ticks on animals that transmit diseases to human beings. Immunological control of tick vector of Kyasanur Forest Disease (KFD) on cattle and other wild reservoir hosts is one of the possible alternative strategy for reducing the transmission of KFD to man. Chemical-vaccine synergies have been demonstrated and a combination of chemical and vaccine for tick and tick-borne disease control has been identified as a sustainable option. Studies have suggested the possibility of vaccine strategies directed towards both tick control and transmission of pathogens. Besides tick vaccine, use of endosymbionts, which are essential for the survival of arthropod hosts, for the control of tick vectors will be one of the targeted areas of research in near future. India with huge natural resources of herbs and other medicinal plants, the possibilities of developing herbal acaricides is discussed. The future of research directed towards target identification is exciting because of new and emerging technologies for gene discovery and vaccine formulation.

**Key words** Endosymbionts – insecticide – tick control – tick-borne diseases – vaccine

### Introduction

On global basis, ticks transmit a number of pathogenic organisms like protozoans, rickettsiae, spirochaetes and viruses, than any other arthropod vector group, and are among the most important vectors of diseases affecting livestock, humans and companion animals<sup>1</sup>. Besides acting as vector, the direct effects of ticks have great economic importance since tick bite marks diminishes up to 20–30% of the value of skins and hides<sup>2</sup>. De Castro<sup>3</sup> estimated the global costs of ticks and tick-borne diseases (TTBDs) in cattle between US\$ 13.9 and US\$ 18.7 billion annually. In India alone the cost of TTBDs in animals has been estimated in the tune of US\$ 498.7 million (more than

2000 crores) per annum<sup>4</sup>. Over the past two decades, the incidence of tick-borne diseases (TBDs) increased and pose a major public health problem in Europe<sup>5</sup>. The recent report of increasing number of KFD in Karnataka state of India, despite routine vaccination indicates the insufficient efficacy of the vaccine protocol<sup>6</sup> and necessitates the need for strategic control of the tick vector. Although research efforts were directed towards the development of vaccines against the tick-borne pathogens, the results obtained till date are not satisfactory (Table 1). Therefore, at present, TTBDs control is mainly effected by widespread use of acaricides like organophosphates, carbamates, pyrethroids, BHC/cyclodines, amidines, macrocyclic lactones and benzoylphenylureas leading to various

**Table 1. Important tick-borne diseases of human and livestock in India**

Tick-borne diseases (TBD)	Pathogen/Parasite	Tick vector	Host	Vaccine status against TBD
Kyasanur Forest Disease (KFD)	KFD virus	<i>Haemaphysalis spinigera</i>	Man	Chick embryo tissue culture vaccine <sup>a</sup>
Crimean Congo Hemorrhagic Fever (CCHF) <sup>7</sup>	CCHF virus	<i>Hyalomma</i> spp?	Man	Nil
Indian Tick Typhus (ITT) <sup>8,9</sup>	<i>Rickettsia conorii</i>	<i>Rhipicephalus sanguineus</i> ?	Man	Nil
Bovine Tropical Theileriosis	<i>Theileria annulata</i>	<i>Hyalomma anatolicum anatolicum</i>	Cattle	Attenuated macroschizont vaccine <sup>b</sup>
Babesiosis	<i>Babesia bigemina</i>	<i>Boophilus microplus</i>	Cattle, Buffalo	Nil
	<i>B. motasi</i>	<i>Haemaphysalis</i> sp	Goat	Nil
	<i>B. canis</i>	<i>R. sanguineus</i>	Dog	Nil
	<i>B. ovis</i>	<i>Rhipicephalus</i> sp	Sheep	Nil
	<i>B. equi</i>	<i>Hyalomma</i> sp	Horse	Nil
Anaplasmosis	<i>Anaplasma marginale</i>	<i>B. microplus</i>	Cattle, Buffalo, Sheep	Nil
Ehrlichiosis	<i>Ehrlichia bovis</i> ,	<i>Hyalomma</i> sp	Cattle	Nil
	<i>E. canis</i>	<i>R. sanguineus</i>	Dog	Nil

<sup>a</sup>Not fully effective in field situation<sup>6</sup>; <sup>b</sup>Effective but not in large-scale use; ? Tick species not yet confirmed.

problems such as resistance, residues, environmental pollution and high cost. These factors reinforce the need for alternative approaches to control tick infestations.

The integrated pest/vector management (IPM) has been identified as the future sustainable option of tick control<sup>10,11</sup>. As an important component of IPM, cattle tick vaccine in the form of TickGARD<sup>TM</sup> (*Escherichia coli* expressed BM86 vaccine) and TickGARD plus<sup>TM</sup> (*E. coli* expressed BM86+BM91) and Gavac<sup>TM</sup> (*Pichia pastoris* expressed Bm86 vaccine), have been developed and commercialised<sup>12,13</sup>. The vaccine was based on a concealed glycoprotein (89 kDa) isolated from the mid gut of *Boophilus microplus*. Besides the concealed antigen approach, exposed antigens have been targeted to develop vac-

cine against tick species. For example, Mulenga *et al*<sup>14</sup> characterised a 29 kDa salivary gland-associated protein from *Haemaphysalis longicornis* and vaccination with recombinant protein produced in *E. coli* led to a significant reduction in adult female engorgement weight and 40% and 56% mortality of larvae and nymphs post-engorgement, respectively. Recently, a 15 kDa protein from *R. appendiculatus*, 64P, was identified as a putative cement protein involved in attachment and feeding of ticks. Vaccination of guinea pigs with recombinant versions of the 64P protein (64 TRPs) resulted in reduction of the nymphal and adult infestation rates by 48 and 70%, respectively<sup>15</sup>. An important impact of controlling tick infestations is the reduction of medically and economically important tick-borne pathogens. For example, in extensive field trials immunisation of cattle with Bm86

vaccines resulted in a reduction in the incidence of babesiosis as well as reduced tick infestations<sup>13,16</sup>. Similarly, transmission of tick-borne encephalitis (TBE) viruses have been prevented by immunisation of mice with recombinant tick cement protein, 64P<sup>15</sup>. Recombinant tick vaccines, therefore, could be targeted for control of TTBDs. The development of existing tick vaccines and their effect on the targeted vectors and pathogens has been reviewed<sup>17</sup>. The present review is focused on upcoming and future tick control strategies keeping in view of the technological development in the post-genomic era.

### Upcoming tick control strategies

#### (A) Tick vaccine

##### (i) Target identification

**Expression library immunisation and RNAi:** Expression library immunisation (ELI) in combination with sequence analysis of expressed sequence tags (ESTs), provides an efficient global approach for identification of vaccine antigens that is based on rapid screening of the expressed genes. This method allows for antigen identification without introducing prior criteria to direct the selection of candidate genes and thus may result in the discovery of novel and unexpected antigens<sup>18,19</sup>.

Although the application of ELI in combination with EST analysis represented an improved method for identification of tick protective antigens, experimental immunisation and challenge tick infestation of a large number of laboratory animals are laborious, expensive and difficult to standardise. While the laboratory animal model of tick infestations is restricted to selected tick species, many other tick species (especially *Boophilus* spp) would require larger animal hosts for tick feeding, such as sheep and cattle, and would thus contribute to the complexity of the screening experiments.

The genome and EST work has generated a huge pool

of coding sequences, but the functions of many of them are still unknown or poorly understood. RNA interference (RNAi) is becoming an increasingly powerful post-transcriptional gene silencing technique that is providing insight into gene function<sup>20,21</sup>. This reverse vaccinology approach reduces the use of animal challenge experimentation and allows rapid screening of protective tick antigens.

The RNAi process involves an ATP-dependent production of small  $\approx 21$ – $25$  nucleotide, short interfering RNA molecules (siRNAs) from double stranded RNA (dsRNA) by dicer ribonuclease and is believed to be responsible for targeting and destroying specific mRNAs facilitated by the formation of the RNA-induced silencing complex (RISC). The RISC RNA molecules complementary to the mRNAs seem to work as a guide, recruiting a ribonuclease that consequently cleaves only specific mRNAs<sup>22–24</sup>.

Aljamali *et al*<sup>25</sup> hypothesised that ticks have the machinery that processes the dsRNA into siRNAs, which target specific mRNAs as this homeostatic silencing pathway seems to be conserved in several other eukaryotes<sup>21</sup>. They reported the application of RNAi in the study of histamine binding protein (HBP) of female tick, *Amblyomma americanum*. The HBP dsRNA was synthesised from *A. americanum* HBP dsDNA by RNA polymerases (*in vitro* transcription). Unfed female *A. americanum* ticks were microinjected with  $10^{10}$  dsRNA molecules in injection buffer. Reduction in daily weight gain and extended feeding time were observed in the dsRNA injected ticks. Moreover, HBP transcript levels were low in experimental group ticks than the control group. Both the differences in feeding and the pronounced decrease in the HBP mRNA indicate the ability of dsRNA to spread from the injection site to the salivary glands.

**Bioinformatics:** In recent years due to the explosion in biological information, there has been a move from the classical linear approach of drug/vaccine target identification to non-linear and high throughput ap-

proach. It appears that the ability to generate vast quantities of data has surpassed the ability to use this data meaningfully. The genomics information can be effectively used for the identification and validation of vaccine targets. The genome project for *Ixodes scapularis* is underway and in near future the full genome sequence of other veterinary and medically important ticks will be available. The accumulation of these information into databases will help us to identify potential vaccine and acaricide targets and it can save much time and expenses on target identification by conventional methods.

The types of information need for the identification of potential vaccine targets include nucleotide and protein sequence, homologues, mapping information, domains, motifs, structure and function prediction, pathway information, disease associations, variants, protein expression data and species, taxonomic distributions among others. The protective homologues can be identified in a range of tick species and exploited for the purpose of recombinant vaccines. Comparative genomics help us to find protein families that are widely and taxonomically dispersed and those are unique to a particular tick species. This will help us to identify vaccine targets for a broad spectrum anti-tick vaccine. Due to the advances in the computational biology, finding an attractive target is no longer a problem but finding the targets that are most likely to succeed has become the challenge.

### (ii) Vaccine development

**DNA vaccination against ticks:** DNA vaccine development is still in its infancy for ticks of veterinary and medical importance. Since the BM86 antigen present in the commercial anti-*Boophilus* vaccine, located in the mid-gut of the tick, doesn't normally enter into contact with the bovine immune system, no booster to the primary vaccination is provided during subsequent natural infestation. Though the efficacy of the vaccination depends on the magnitude and persistence of the antibody, repeated boosting is essential

for the vaccine effectiveness. In DNA vaccination, the injected plasmid DNA molecules believed to enter nucleus actively and remain in side the nucleus as episomal DNA lifelong, thus generating the protective antigens continuously as long as the cell lives<sup>7</sup>. The continuous synthesis, processing and presentation of antigens to T-cells *in vivo* in DNA vaccinated animals could avoid the problem of repeated boosting to maintain high antibody titer. Moreover, as DNA vaccine consists only of plasmid DNA and no contaminating proteins, multiple or repeated vaccination would seem possible without generating immune response to vector DNA<sup>26</sup>.

De Rose *et al*<sup>27</sup> attempted the DNA vaccination of Merino crossbred sheep against *B. microplus* using Bm86 full length gene. Though the antibody titer of the immunised animals was low, a marginal decrease (not significant) in mean engorgement weight of female ticks was observed. Apart from that, the DNA vaccination had primed for a significant anamnestic response to protein antigen. In addition, covaccination with Bm86 and GM-CSF plasmids gave statistically significant reduction in the fertility of ticks. But in positive control, vaccination of sheep with TickGARD<sup>plus</sup> induced strong protection (25 times more effective than DNA vaccination) as measured by all parasitological parameters.

Since humoral immune response plays a major role in tick immunity, a DNA vaccine is to be designed to polarise the host immune response towards Th2 response. The levels of specific IgG1 which appear to be regulated by Th2 cells in cattle vaccinated with mid-gut antigens of *B. microplus* were found to correlate with protection. Placing appropriate secretory signal sequence downstream of the target gene would allow the target antigen to be secreted out to the extracellular compartment and elicit better humoral immune response. Moreover, coinfection with the other immunomodulatory genes like IL4 and IL10 would favour the selection of Th2 cells.

## Future strategies

### (A) Tick vaccine

#### (i) *Target identification*

**Endosymbiotic approach:** Endosymbionts of ticks are almost unexplored and appear to be a potential target for the control of ticks. This is because very few studies that involved the identification and characterisation of the endosymbiotic organisms of ticks<sup>28,29</sup>. They are commonly found in the arthropods that always depend on single source of diet that is deficient of some nutrients. As in the case of ticks they always depend on host blood as only food source that may not provide all the essential nutrients to the growth and development of ticks. So, ticks must have many primary endosymbionts that are not yet characterised. The endosymbionts are heritable and transmitted to the progenies through vertically or maternally. This association is obligate or beneficial to the arthropod host.

Since endosymbiotic organisms are essential for the survival of the arthropod host, elimination of the organism would be deleterious for their survival. Understanding the genetics and physiology of endosymbionts is essential in devising strategies which are based on interference with their endosymbionts for the control of these vectors.

Tick control in future can be attempted by exploiting endosymbionts, by chemotherapeutic, immunological and microbiological approaches. Before considering these approaches some basic studies on endosymbionts of different tick species like identification, microbiological and molecular characterisation and *in vitro* cultivation of the organisms should be initiated.

**Chemotherapeutic approach:** Recent field reports of ivermectin resistance amongst ticks<sup>30</sup> necessitates the search for another systemic antibiotic for the control

of ticks. Targeting the endosymbionts of tick species through antibiotics would be easy to apply against the blood feeding parasites like ticks, as antibiotics or antibacterial can be systemically administered to the animals so as to reach the endosymbionts inside the tick, especially in the gut region. As observed in most of the arthropod species, the endosymbionts of ticks would be essential for their growth and development and by disturbing the endosymbionts of ticks, the life of ticks could be jeopardised. The observations on the effect of antibiotics on the endosymbionts of tsetse fly and a filarial nematode *Litomosoides sigmodontis* give an optimistic look towards this approach. Sterility was observed in healthy tsetse flies fed with tetracycline (2500 µg/ml) due to damage to the mycetome bacterial endosymbionts<sup>31</sup>. Available antibacterial and antibiotics can be screened against the endosymbionts of ticks and subsequently can be tested systemically against challenge infestation. Of course, the non-availability of any *in vitro* cultivation methods for the endosymbionts of ticks can only defer but can't stop the testing of the above hypothesis in future.

**Immunological approach:** Animals can be immunised with the whole killed endosymbionts or purified antigens or recombinant antigens of the endosymbionts. The concealed antigens or the mid gut antigens of the blood feeding arthropods like ticks, glosina, mosquitoes are the potential vaccine targets being exploited by the researchers for the control of vectors<sup>7</sup>. Instead of targeting the host (vector) antigens, the endosymbionts could be targeted to disturb the symbiotic relationship between the vector and the symbiont. If the animals are immunised with antigens of endosymbiont, antibodies will be produced against it. Following ingestion of the blood from immunised animals, these antibodies together with other components of the immune system such as complement, will destroy the symbionts, leading either to death or to disruption of normal gut physiology of the tick and reduce growth and egg-laying ability. For the production of whole killed bacterial endosymbionts or the

native purified protective antigens of endosymbionts *in vitro* culture of the organisms is essential. But, the *in vitro* culture of the endosymbionts especially the primary endosymbionts is very difficult with the common laboratory media. Thus, a specific media for culturing these symbionts of ticks is to be developed to proceed further.

**Tick genome project:** Tick genomic study is expected to boost the development of novel methods for tick control and blocking parasite/pathogen transmission. The *Ixodes scapularis* is the first tick to be taken up for genome sequencing. This genome data will provide information about many aspects of tick biology and will enable the identification of unique tick genes and physiological process that could be exploited for TTBDs control. Identification of novel vaccine targets through genome analysis of *I. scapularis* is expected to widen the scope of tick research in India. The homologues genes of economically important Indian tick species, viz. *B. microplus*, *H. a. anatolicum*, *Haemaphysalis spinigera* can be screened for their candidature as vaccine target.

### (ii) Vaccine development

**Cocktail vaccines against tick and tick borne diseases:** Although reduction in disease transmission potential of ticks is achieved by vaccination against ticks<sup>13–15,32</sup>, a cocktail vaccine against both tick and pathogen/parasite is expected to give an even higher level of protection against TBDs. A possibility for the development of such a combination vaccine against *T. parva*–*R. appendiculatus* and *T. annulata*–*H. a. anatolicum* systems may exist. A novel subunit vaccine against *T. parva*, has been recently evaluated for its vaccine potential in cattle<sup>33,34</sup>. Additionally, the homologue of Bm86 has been discovered in *R. appendiculatus* (Ra86). The recombinant Tams 1 antigen of *T. annulata* (Parbhani strain) and the Bm86 homologue antigen of *H. a. anatolicum* (Izatnagar isolate), rHAA86, were produced in *E. coli* and *Pichia pastoris*, respectively in the Division of Parasitology,

Indian Veterinary Research Institute, Izatnagar, India<sup>35</sup>. A cocktail vaccine consisting of antigens Tams 1 and rHAA86 is expected to protect Indian cattle population against *H. a. anatolicum* and *T. annulata*.

**Broad spectrum anti-tick vaccine:** Multiple tick species infestation on animals is impedance to the use of tick vaccines with a narrow spectrum, having antigens of a single tick species. The recombinant BM86 included in commercial vaccine formulations Tick-GARD (Hoechst Animal Health, Australia) and Gavac (Heber Biotec S.A., Havana, Cuba) confers partial protection against phylogenetically related *Hyalomma* and *Rhipicephalus* tick genera. However, immunisation with BM86 failed to protect against the more phylogenetically distant *Amblyomma* spp<sup>36</sup>. Therefore, there remains a need to identify broad range vaccine candidates against tick infestations across phylogenetically distant species.

Tick genes encoding for protective antigens have been discovered that are expressed across tick species and in some circumstances across genera, thus making the use of these cross reactive antigens in broad range anti-tick vaccine formulations perhaps possible. These genes were cloned and associated polypeptides of *I. scapularis* were designated as 4D8 (also identified as subolesin), 4F8 and 4E6<sup>18,19,37,38</sup>. The sequence of 4D8 was shown to be conserved among different tick genera and the activity of subolesin protective antigen was demonstrated to extend to other tick species, including non-*Ixodes* tick species<sup>39</sup>. The 4D8 molecule is expected to present in important tick species of India.

Recombinant forms of the secreted *R. appendiculatus* tick cement antigens, 64 TRP's may act as a 'dual action' anti-tick vaccine in that they target both 'exposed' and 'concealed' antigens<sup>37,38</sup>. Ticks feeding on 64TRP's immunised guinea pigs induce a cutaneous inflammatory response and increased antibody titer, which is detrimental to engorged ticks. This dual action, acting at the feeding site and in the midgut,

confers a self-sustaining strategy for tick control boosted by natural infestations. Immunoblotting studies using tick tissue extracts with antisera to different 64 TRP constructs reveal antigenic cross-reactivity with *R. sanguineus*, *I. ricinus*, *A. variegatum* and *B. microplus*. In vaccine trials using guinea pigs, rabbits, and hamsters, the 64 TRP constructs provided cross-protection against *R. sanguineus* and *I. ricinus*, apparently by targeting antigens in the cement cone, midgut and salivary glands of adults and nymphs, causing mortality. These results may have important implications for the development of 64 TRPs-based tick vaccines for the control of multi-tick infestations and the transmission of tick-borne pathogens.

### **(B) Transgenic animals resistant to ticks**

Novel approaches to modify disease resistance or susceptibility in livestock are justified by economic and animal welfare concerns. Successful production of transgenic livestock has been reported for pigs, sheep, rabbits and cattle. Current research on the improvement of disease resistance by gene transfer focuses on three main strategies, such as: (a) somatic gene transfer—nucleic acid vaccines; (b) deletive germ-line gene transfer—gene knockout; and (c) additive germ-line gene transfer. These strategies aim at either the transient or stable expression of components known to influence non-specific or specific host defense mechanisms, or the disruption of genes known to cause susceptibility to disease. One significant advantage of transgenesis is in rapid genetic improvement of traits of interest as the selection for an animal for a particular trait involves many years.

Different cattle breeds that evolved independently may have accumulated different resistance genes. Identification of a range of resistance genes would considerably hasten the process of production totally resistant animals. These range of major anti-tick genes are also potential candidate genes for use in the development of transgenic cattle. The anti-tick gene can be introduced into the cattle through somatic gene

transfer (DNA vaccine) or additive germ-line transfer. It is impractical to select for or to introgress polygenic resistance into lowly resistance breeds. Transgenesis is the only option to improve the tick resistance of such breeds. Before experimentation on the production of transgenic cattle for tick resistance, the identification and complete characterisation of the tick resistant genes of the tropical cattle breeds is imperative.

### **(C) Newer generation acaricides**

In the current system of livestock production in developing countries, the tick control can not be imagined without the use of acaricide despite the increasing resistant tick population. Newer generation acaricides targeting previously not explored metabolic pathways or bio-molecules synthesis pathway should be generated and these acaricide should be kept in reserve to meet out any emergency situations expected to arrive by the multi-acaricide resistant tick population in future. Newer methodologies like combinatorial chemistry and computational biology along with high throughput screening against a target would yield newer generation acaricides. Monitoring and early detection of acaricide resistance is one of the essential components for successful integrated pest management (IPM). Such acaricide resistance screening tests should be available for all the acaricides which are being used in the field. Early detection of the resistance, subsequent withdrawal and replacement with another acaricide would sustain the susceptible tick population in the field.

### **(D) Herbal acaricides**

The ethno-veterinary and medical knowledge offers a range of herbs to be evaluated for their insecticidal and acaricidal properties. The ingredients of plants and herbs are known to possess insecticidal, growth inhibiting, anti-molting and repellent activities. A number of reports are available on the effect of different extracts of plant material on tick species<sup>39–45</sup>.

In our laboratory, the alcoholic extracts of sitaphal (*Annona squamosa*) and neem (*Azadirachta indica*) are being evaluated for their acaricidal property against different life stages of *H. a. anatolicum* and *B. microplus* and the initial results are highly encouraging. Final characterisation of the key active components of the tested extracts have the great scope for commercialisation and have potential to be a part of IPM for sustainable effect.

### Conclusion

The effect of TTBDs will continue to exact a huge economic loss on livestock industries worldwide. Control of these pests rests on continuous use of acaricides on and off the hosts. Long-term use of these chemicals is leading to the development of resistance, issues around the residues in livestock products and in environment and their undesirable effects.

Recent advances in vector biology open new possibilities in target identification and vaccine development. The efforts to characterise the genomes of *I. scapularis* and *B. microplus*<sup>46,47</sup> will impact on the discovery of new tick-protective antigens. The use of the information in conjugation with functional analysis using bioinformatics, RNAi, mutagenesis, immunomapping, transcriptomics, proteomics, ELI and other technologies will allow for a rapid, systematic approach to tick vaccine discovery. Vaccination trials can be designed to evaluate the effect of selected tick antigens in combination with other tick protective and pathogen-specific antigens, for improving the level of tick infestations and reducing transmission of tick-borne pathogens. The future of research on development of tick vaccines is exciting because of new and emerging technologies for gene discovery that facilitate the efficient and rapid identification of candidate vaccine antigens. These new tick vaccines will probably play a key role in future integrated tick control strategies in cattle and in companion animals. Tick genomics and proteomics are likely to evolve into projects addressing the sequencing, anno-

tation and functional analysis of entire tick genomes, providing invaluable information for the development of tick vaccines.

Reduction in the transmission of TBDs by vaccination against tick vectors is documented. The lack of effective vaccines against the TBDs of man and animals force us to look into strategic control of tick vectors in an integrated format for the effective control of TBDs. Globally two tick research groups<sup>14,15</sup> are trying to develop an effective vaccine against tick vectors of animals to reduce the transmission of TBE virus to man. In the same line, immunological control of *H. spinigera*, tick vector of KFD on cattle, and other wild reservoirs of KFD virus in the endemic area is expected to reduce the transmission of KFD to man. An oral vaccination strategy using baits could be an option to immunise the monkeys, the amplifier host of KFD virus. It is unfortunate that India that suffered the endemic of KFD had no programme or research group to explore the possibility of developing a vaccine against *H. spinigera*, tick vector of KFD. There is an urgent need to develop a multidisciplinary programme for the control of KFD and other tick transmitted human diseases utilising the principle of IPM.

For the future, we must conserve what we have and use it in combination with all the principles of IPM like strategic and focused treatments of animals, disease management, modification of the vectorial capacity and resistant breeds. Governments and different nodal agencies associated with livestock improvement particularly in developing countries should develop effective policies that ensure sustainable services and market development.

### Acknowledgement

The authors are grateful to the Department of Biotechnology, Government of India for providing financial support. Authors are also grateful to Prof. Jose de la Fuente of Oklahoma State University, USA and



Prof. Peter Willadsen of CSIRO Livestock Industries, Queensland, Australia for providing valuable literature. This work has been facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Programme of the European Union through Coordination Action Project No. 510561.

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*Received:* 23 October 2006

*Accepted in revised form:* 1 December 2006