

# Control of ticks of ruminants, with special emphasis on livestock farming systems in India: present and future possibilities for integrated control—a review

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**Abstract** India is predominantly an agricultural country with about 70% of her population dependent on income from agriculture. Although India accounts for a significant share of world's livestock resources, livestock production is greatly affected by ticks and tick-borne diseases (TTBDs). Therefore, India represents a particularly interesting scenario for the study of TTBDs. Herein, we review the problems and opportunities for the integrated control of ticks of ruminants with special emphasis on livestock farming systems in India. Developments discussed in the review in the area of tick vaccines and other tick control measures should have an impact on the future of Indian livestock production.

**Keywords** Tick · Tick-borne diseases · Tick control · Acaricide · Vaccine · India

## Introduction

On a global basis, ticks are second to mosquitoes as vectors of infectious pathogens to humans, and they form the most important group of arthropods to transfer disease pathogens from one animal to another. Ticks and tick-borne diseases (TTBDs) affect 80% of the world cattle population and are widely distributed throughout the world, particularly in tropical and subtropical countries (de Castro 1997), which

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cause significant production losses (Jongejan and Uilenberg 2004). De Castro (1997) estimated the global costs of TTBDs in cattle between US\$ 13.9 and US\$ 18.7 billion annually. The use of acaricides as the principal mean for tick control results in the selection of chemical-resistant ticks along with contamination of the environment and animal products. These consequences, combined with public concerns over different environmental issues, have led to the search for alternative methods of control that are consistent with the principles of sustainable agriculture (Donald 1994). The integrated pest management (IPM), in which application of two or more technologies are integrated in an environmentally compatible and cost-effective manner to control target pest population, has been identified as the future option. Several authors have suggested that the most cost-effective approach of tick control is to combine vaccination with natural resistance, particularly in extensive pastoral systems (Willadsen et al. 1995).

Livestock is an integral part of the agricultural production system in India and plays an important role in national economy as well as in socio-economic development of millions of rural households. Livestock is an important source of animal protein for farm families and also used for draught power in agriculture and transport, and their dung is used to increase soil fertility. Although India is ranking first in the total milk production, the livestock sector is suffering from a number of disease problems caused by bacteria, viruses, fungi and parasites. Among the parasitological problems, the damage caused by TTBDs is considered very high and the control of TTBDs has been given priority (Bansal 2005). The present review is focused on effect of TTBDs on sustainable livestock farming and future control programmes in India.

### **Livestock farming scenario of India**

India is predominantly an agricultural country with about 70% of her population dependent on income from agriculture. Farmers are keeping animals for milk, meat, wool and hide production and also for various farm operations. India accounts for a significant share of world's livestock resources with nearly 57% of world's buffaloes, 16.5% of cattle, 16.3% of goats, and 5.7% of sheep (FAO 2004a). The livestock sector accounted for 25.5% of agricultural GDP and about 5.6% of total GDP in 2001–2002. The share of livestock in the gross value of agricultural outputs has reached 35.5% in 2001–2002 (CSO 2003). The agricultural sectors of India contain a variety of farming systems, including relatively large-scale commercial systems. In terms of small-scale producers (who constitute the maximum contribution of livestock to GDP), three main systems can be identified: smallholder dairy, crop–livestock and livestock-dependent.

*Smallholder dairy cattle systems* these are mixed farming systems occurring in areas with productive soil and adequate rainfall; they are concentrated in peri-urban or densely populated rural areas where land availability is limited. Cross-bred or exotic cattle and indigenous animals are important components of these systems. Ticks and TBDS are considered as one of the most important bottleneck in economical livestock rearing.

*Crop–livestock systems* in these systems, indigenous livestock are closely integrated with other farm enterprises with less emphasis on milk production. The indigenous

animals are well adapted to local conditions and significantly resistant to clinical form of TBDs but tick infestation is a common problem.

*Livestock-dependent systems* in these systems, indigenous livestock are playing a major role in the owner's livelihood. In financial terms, these animals constitute the principal asset of a family and have been valued as a 'bank'.

### Tick species and breeds of livestock in India

Table 1 depicts the name of the important breeds of cattle, buffaloes, sheep and goats, the tick species reported from these animals, and the established vectors of different diseases commonly infecting the species of animals. The species of ticks infesting humans has also been marked. It is observed that although cattle and buffaloes are reared together, the number of tick species reported from cattle is significantly higher than the tick species reported from buffaloes.

### Distribution of predominant tick species of India infesting cattle, buffalo, sheep and goat

As per the last compilation report on tick species of India, approximately 106 tick species belonging to two families of Ixodidae and Argasidae are reported to infest domestic, wild and game animals (Geevarghese et al. 1997). Among the reported species of ticks, *Amblyomma testudinarium*, *Dermacentor auratus*, *Haemaphysalis bispinosa*, *H. spinigera*, *H. intermedia*, *Hyalomma anatolicum anatolicum*, *H. marginatum isaaci*, *H. hussaini*, *H. detritum*, *H. kumari*, *Boophilus microplus*, *Ixodes acutitarsus*, *I. ovatus*, *Nosomma monstrosus*, *Rhipicephalus haemaphysaloides* and *R. turanicus* have been considered the most widely distributed tick species infesting cattle, buffalo, sheep and goats (Fig. 1). *H. a. anatolicum*, *H. m. isaaci*, *B. microplus* and *R. haemaphysaloides* ticks are reported from almost all the states of India. Reports of *I. acutitarsus* and *I. ovatus* are mostly available from east and northeastern zones of India. Similarly, *D. auratus* infestation is not reported from the western zone and also from southern states except Karnataka. The tick species, *Nosomma monstrosus* is not reported from north eastern states, central zones, Rajasthan and southern states except Andhra Pradesh and Kerala. The tick, *A. testudinarium* is mainly reported from north eastern zones, states of West Bengal, Punjab, Madhya Pradesh, Orissa, Karnataka and Tamil Nadu. Of the three predominant *Haemaphysalis* species, *H. bispinosa* is prevalent throughout India except Uttar Pradesh, while *H. spinigera* is restricted in southern states, central zones, Orissa in eastern zone and Meghalaya in north eastern zone. The *H. spinigera* is the only tick species reported from the Andaman and Nicobar Islands. *H. intermedia* is reported in all the southern states except Kerala, central zones, eastern zones except West Bengal, northern zone except Uttar Pradesh and Haryana, however, reports are not available from western zones. Among the seven north eastern states, *H. intermedia* is reported from Assam only.

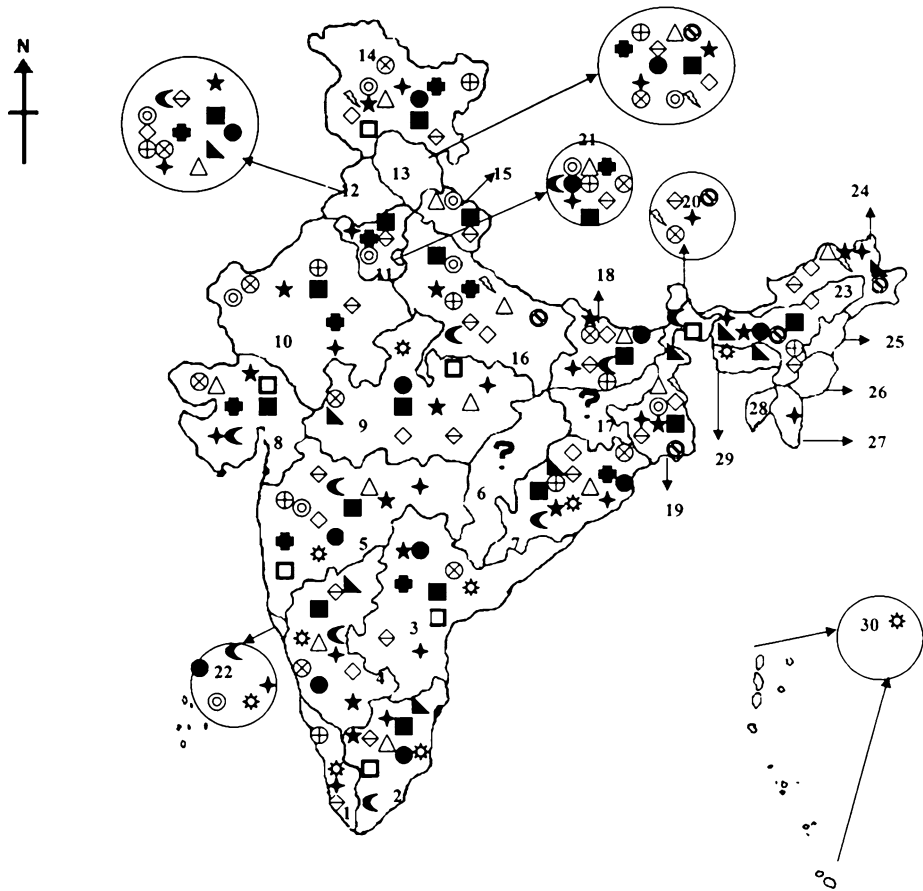
In most of the southern states except Kerala all the predominant tick species are reported from cattle, buffaloes, sheep and goats. In Kerala, the following tick species are reported viz., *H. kumari*, *H. spinigera*, *H. bispinosa*, *H. turturis*, *R. haemaphysaloides* and *B. decoloratus* (Prakasan and Ramani 2003).

**Table 1** Name of breeds of cattle, buffaloes, sheep and goats, the tick fauna recorded from these livestock species and the parasitic diseases transmitted by the established vectors

Breed	Reported tick species	Tick species and pathogen transmission
<b>Cattle</b>	Kankrej, Tharparkar, Hariana, Ongole/Nellore, Red Sindhi, Sahiwal, Gir	<i>H. a. anatolicum</i> — <i>T. annulata</i> , <i>B. microplus</i> — <i>B. bigemina</i> , <i>A. marginale</i> , <i>B. microplus</i> — <i>Ehrlichia bovis</i> , <i>H. m. isaaci</i> — <i>T. annulata</i> , <i>Haemaphysalis</i> sp.— <i>E. bovis</i> , <i>Hyalomma</i> sp.— <i>E. bovis</i>
<b>Buffalo</b>	Murrah, Jaffarabadi, Surti, Mehsana, Nili, Ravi, Godavari, Sambalpur	<i>Amblyomma integrum</i> , <i>A. testudinarium</i> , <i>Dermacentor auratus</i> *, <i>Haemaphysalis cornupunctata</i> , <i>H. aponommoides</i> *, <i>H. garhwalensis</i> , <i>H. birmanniae</i> , <i>H. himalaya</i> , <i>H. nepalensis</i> , <i>H. obesa</i> , <i>H. anomala</i> , <i>H. hispinosa</i> *, <i>H. davisi</i> , <i>H. ramachandrai</i> , <i>H. shimogadeer</i> , <i>H. spinigera</i> *, <i>H. intermedia</i> , <i>H. montgomeryi</i> *, <i>H. paraturturis</i> , <i>H. turturis</i> , <i>Hyalomma anatolicum anatolicum</i> , <i>H. hussaini</i> , <i>H. detritum</i> *, <i>H. kumari</i> , <i>H. marginatum isaaci</i> , <i>Ixodes acutitarsus</i> *, <i>I. kashmiricus</i> , <i>I. ovatus</i> *, <i>Nosomma monstrosus</i> *, <i>Rhipicephalus haemaphysaloides</i> , <i>Boophilus microplus</i>
<b>Goat</b>	Marwari, Kutchi, Black Bengal, Jamunapari, Beetal, Osmanabadi	<i>A. integrum</i> , <i>D. auratus</i> , <i>H. cornupunctata</i> , <i>H. aborensis</i> , <i>H. himalaya</i> , <i>H. anomala</i> , <i>H. hispinosa</i> , <i>H. ramachandrai</i> , <i>H. spinigera</i> , <i>H. a. anatolicum</i> , <i>H. m. isaaci</i> , <i>H. hussaini</i> , <i>H. kumari</i> , <i>B. microplus</i> , <i>N. monstrosus</i>
<b>Sheep</b>	Nilgiri, Kashmir merino Nali, Marwari, Chokla, Bellary, Chottanagpur, Malpura, Nellore, Mandya, Kanguri, Mechari, Ramnad, Madras red	<i>D. atrosignatus</i> , <i>D. raskemensis</i> , <i>H. cornupunctata</i> , <i>H. garhwalensis</i> , <i>H. himalaya</i> , <i>H. nepalensis</i> , <i>H. cholodkovskiyi</i> , <i>H. kashmirensis</i> , <i>H. sulcata</i> , <i>H. anomala</i> , <i>H. hispinosa</i> , <i>H. davisi</i> , <i>H. ramachandrai</i> , <i>H. intermedia</i> , <i>H. minuta</i> , <i>H. montgomeryi</i> , <i>H. turturis</i> , <i>H. a. anatolicum</i> , <i>H. m. isaaci</i> , <i>H. hussaini</i> , <i>H. detritum</i> , <i>H. kumari</i> , <i>B. microplus</i> , <i>I. acutitarsus</i> , <i>I. kashmiricus</i> , <i>I. ovatus</i> , <i>R. turanicus</i>
		<i>H. a. anatolicum</i> — <i>T. buffeli</i> ?, <i>B. microplus</i> — <i>B. bigemina</i> , <i>A. marginale</i>
		<i>H. a. anatolicum</i> — <i>T. lestoicardi</i> ( <i>hirci</i> ), <i>Haemaphysalis</i> sp.— <i>B. motasi</i> , <i>Rhipicephalus</i> sp.— <i>B. ovis</i>
		<i>H. a. anatolicum</i> — <i>T. lestoicardi</i> ( <i>hirci</i> ), <i>B. microplus</i> — <i>A. marginale</i> , <i>Rhipicephalus</i> sp.— <i>B. ovis</i>
		<i>A. integrum</i> , <i>D. raskemensis</i> , <i>H. cornupunctata</i> , <i>H. garhwalensis</i> , <i>H. himalaya</i> , <i>H. nepalensis</i> , <i>H. cholodkovskiyi</i> , <i>H. kashmirensis</i> , <i>H. sulcata</i> , <i>H. sundrai</i> , <i>H. hispinosa</i> , <i>H. davisi</i> , <i>H. intermedia</i> , <i>H. minuta</i> , <i>H. montgomeryi</i> , <i>H. spinigera</i> , <i>H. a. anatolicum</i> , <i>H. m. isaaci</i> , <i>H. hussaini</i> , <i>H. detritum</i> , <i>H. kumari</i> , <i>B. microplus</i> , <i>I. acutitarsus</i> , <i>I. kashmiricus</i> , <i>I. ovatus</i> , <i>R. haemaphysaloides</i> , <i>R. turanicus</i>

\* Tick species infests man and transmits human diseases

? To be confirmed



**Fig. 1** The map of India depicting the distribution of tick species was generated based on the published reports/literature. Since there has been no systematic study conducted throughout India on the prevalence of TTBDs, the absence of reports on a tick/pathogen spp. from a state does not indicate the non-existence of the spp. in that state. The tick distribution pattern of the states Madhya Pradesh and Bihar depicted in the map is based on the information published before the bifurcation of the states. Distribution of predominant Ixodid tick species of ruminants in India: *Hyalomma anatolicum anatolicum*, ■; *H. marginatum isaaci*, △; *H. dromedarii*, ●; *H. detritum* ⊙; *H. hussaini*, ⊗; *H. kumari*, ⊕; *Boophilus microplus*, ★; *Rhipicephalus haemaphysaloides*, ⊗; *R. turanicus*, □; *Haemaphysalis bispinosa*, ✦; *H. spinigera*, ⊛; *H. intermedia*, ●; *Ixodes acutitarsus*, ⊙; *I. ovatus*, ⊗; *Dermacentor auratus*, ◇; *Amblyomma testudinarium*, ▽; *Nosomma monstrosus*, ◐; ?, no reports are available from these newly formed states (Chattisgarh and Jharkhand). 1, Kerala; 2, Tamil Nadu; 3, Andhra Pradesh; 4, Karnataka; 5, Maharashtra; 6, Chattisgarh; 7, Orissa; 8, Gujarat; 9, Madhya Pradesh; 10, Rajasthan; 11, Haryana; 12, Punjab; 13, Himachal Pradesh; 14, Jammu & Kashmir; 15, Uttranchal; 16, Uttar Pradesh; 17, Jharkhand; 18, Bihar; 19, West Bengal; 20, Sikkim; 21, Delhi; 22, Goa; 23, Assam; 24, Arunachal Pradesh; 25, Nagaland; 26, Manipur; 27, Mizoram; 28, Tripura; 29, Meghalaya; 30, Andaman & Nicobar Islands

Since no systematic study was conducted on tick infestations in the north eastern states, reports are not available from Tripura, Manipur and Nagaland states. The severity of the infestations is more during the rainy season when the weather is hot and humid but some species of ticks are found throughout the year. Both indigenous and cross-bred animals are infested with ticks but some of the indigenous breeds of

cattle (Ongole/Nellore, Sahiwal) and Buffaloes are observed to have significant degree of resistance to tick infestations. Some of the non-descript, low milk producing cattle breeds located in the hilly regions (Kumaon, western Himalaya) of the country have been observed to have a very high level of resistance to tick infestations (Ghosh, personal observation). Among the bovines, buffaloes have been considered as the most important milk producing animals in India due to their capability to withstand the environmental conditions and resistance to many diseases including TTBDs.

### **Incidence of tick borne diseases in India**

The species of tick-borne haemoparasites infecting Indian farm animals are listed as *Theileria annulata*, *T. buffeli*, *T. lestoardi (hirci)*, *Babesia bigemina*, *B. motasi*, *Anaplasma marginale*, *Ehrlichia bovis* and *E. phagocytophila*. The incidence of *T. annulata* and *B. bigemina* is reported from the whole of India while the incidence of *A. marginale*, *E. bovis* and *E. phagocytophila* is confined to restricted zones. Since most of the reports available are related to the incidence of *T. annulata* and *B. bigemina*, the incidence of other species of *Theileria* and *Babesia* is not clearly known. In spite of cattle and buffaloes co-exist in nature, the prevalence of TBDs in buffaloes is comparatively less than in cattle. Although the *B. bovis* infection had been reported but the existence of the parasite in Indian animals needs further confirmation.

### **Impact of TTBDs on livestock economy of India**

The lack of accurate data on the epidemiology of TTBDs in India makes it difficult to determine their impact. However, since each TBDs is vectored by particular tick species, the potential distribution of each disease can be estimated from the distribution of its vectors. This potential distribution, however, is not always an accurate reflection of present disease incidence. For example, in some cases, the vector is present but the disease is not reported. Alternatively, other unrecognized tick vectors may be present. Nevertheless, tick distribution is the principal indicator used to estimate the distribution of TTBDs.

Tick surveys were performed in the past but updated or additional tick studies are being undertaken. Data on TTBDs incidence, which may vary widely with management system and climate, are especially difficult to obtain. Furthermore, estimates of costs by different authors are not always comparable because of the problem of distributing tick control costs between the different diseases being controlled. For example, Devendra (1995) estimated that a potential loss due to TTBDs is over US\$ 800 million per annum while a recent estimate of 498.7 million US\$ per annum has been calculated as the cost of TTBDs in cattle in India (Minjauw and McLeod 2003).

Farmers in high risk areas consider tick worry to be an important constraint and for this reason use both hand removal and acaricides for tick control. The most common combined effect of TTBDs in Indian dairy system is a reduction in milk yield. Loss of 14% of the lactation, as suggested by McLeod and Kristjanson (1999), would result in a significant reduction in income and would be particularly serious for livestock dependent system.

Besides reduction in milk production, the direct effect of tick infestation has tremendous effect on the availability of good quality hides for the leather industry which is suffering from huge shortfall of 3,000 millions pieces of hides and skin per year (Leatherware 2002). It has been estimated that India is producing only 9.8%, 63.3%, 9.2%, and 6.0% of world cattle, buffalo, goat and sheep hides, respectively, although the country possesses the highest livestock population (Leatherware 2002). Among the different factors implicating the non-availability of good quality raw material for leather industry, tick bite marks have been identified as one of the factors causing 20–30% depreciation in normal value in the market (Biswas 2003). Besides the ticks with a long hypostome such as *Hyalomma* spp., the brevirostrate species like *Boophilus* ticks also cause significant damage to the skin when present in large numbers as reported by Jongejan and Uilenberg (2004). Nevertheless, leather export from India has increased significantly and reached 1814.18 million US\$ in the year 2002–2003, after only 822.22 million US\$ in the year 1992–1993. The gap between demand and supply of good quality hides and skins can be reduced if the direct effect of tick infestation can be minimized which in turn will help in achieving the target of 5% share of global leather and leather products amounting US\$ 5 billion.

Ticks with a long hypostome may induce abscesses because of secondary bacterial infection which in turn attract myiasis producing flies, another arthropod related problem facing the animal owners of India.

### Tick control methods in India

The most widely used method for the control of ticks is the direct application of acaricides to host animals (Table 2). In most of the cases, the application of acaricides is repeated after 21–30 days. A recent survey on tick resistance conducted through questionnaire reported a large scale tick resistance in India (FAO 2004b). Insecticides consumption in Indian agriculture has been increased more than 100% from 22,013 tones to 61,357 tones during 1971–1995 (<http://www.indiastat.com>).

**Table 2** Commonly used acaricides and their efficacy in India

Acaricide type	Compounds	Method of application	Efficacy (%)	References
Pyrethroids	Cypermethrin	Wash	100	Khan (1996)
	Cypermethrin	Wash	96–100	Khan and Srivastava (1992) Banerjee and Sangwan (1990)
	Deltamethrin	Spray, Dip	96–100	Pathan et al. (2003) Gupta et al. (1998)
	Flumethrin	Pour-on	100	Ravindra Kumar et al. (2001)
	Permethrin	Wash	90–100	Khan 1996 Singh and Chabra (1991)
Organophosphates	Fenvalerate	Wash	90–100	Khan (1996)
	Hexafen	Swab	90	Khan (1999)
	Coumaphos	Swab	95–100	Khan and Srivastava (1993) Talukdar et al. (1998)
Carbamates	Diazinon	Wash	97–100	Shahardar et al. (2002)
	Carbaryl	Spray	100	Basu and Haldar (1997)
Macrocytic lactones	Ivermectin	Injection	100	Maske et al. (1992)

However, acaricides are expensive and detrimental to the environment and human beings as residues are present in milk and meat and so their use should be minimized and integrated with alternative approaches. Depending on the abundance and importance of the various tick species, strategies such as seasonal treatments at the peak of tick activity may be sufficient to avoid economic losses due to ticks and TBDs. Tick infestation of animal sheds can be avoided by plastering all surfaces with smooth cement to block cracks and crevices, but this is only true for the owners who have a separate shed for keeping their livestock. Although the control of ticks relies heavily on the use of chemicals, the selection of resistant tick strains to the available compounds is a serious threat to the sustainability of this approach.

Designing an economical, integrated tick control strategy for a particular production system in a specific area is one of the most difficult challenges faced by the veterinary scientists of India since extension activities are not sufficiently strengthened to provide farmers with the information necessary to enable them to evaluate sustainable strategies suitable for the control of TTBDs.

### **Current research and future opportunities**

The important development related to the sustainable control of ticks in India and other opportunities are discussed below.

#### Progress on development of vaccines against ticks in India

The subject can be divided into two broad categories: work done on laboratory animals and on natural hosts using crude and partially purified antigens and the experimentation using purified antigens and their testing as vaccine candidates.

#### *Use of crude and partially purified antigens*

The work on immunization of animals against targeted tick species is initiated in India in the early nineties to develop immunoprophylactic measure that would be more effective against local tick strains (Manohar and Banerjee 1992a, b; Thakur et al. 1992). But the results obtained in preliminary studies have not been confirmed in the natural hosts. Kumar and a group of scientists from Haryana Agriculture University, Hissar reported cross-protective efficacy of midgut extracts of *H. dromedarii* following immunization of rabbits (Kumar and Kumar 1995, 1996). However, the challenge dose ( $n = 10$  pairs of ticks) used for the cross-protection study was not sufficient to establish the cross-protective potentiality of the antigen tested. Common cross-reactive proteins of 66 kDa were detected in the salivary gland extracts of *H. a. anatolicum* and in *B. microplus* (Parmar and Grewal 1996; Parmar et al. 1996). Similarly, Ghosh et al. (1998) reported six immunodominant proteins of 97.4, 85, 66, 47.3, 42 and 31 kDa in all the stages of *H. a. anatolicum*. Subsequently, Ghosh and Khan (2000) reported common proteins of 68, 57.5, 50.8, 47.3, 43 and < 43 kDa in all the stages of *B. microplus* and *H. a. anatolicum*. However, further work using the identified common proteins for raising cross-protective immunogen has not been reported.



Banerjee et al. (1990) prepared three extracts of salivary glands and tested in cattle and concluded that the protective antigens are present in the sediment collected after centrifugation. A comparatively higher level of immunity in calves immunized by whole extracts of salivary gland in combination with ascaris extract as immunomodulator was reported by Sran et al. (1996). Sangwan et al. (1998) prepared whole nymphal extract, nymphal membrane antigen and nymphal soluble antigens of *H. a. anatolicum* and used these for immunization of cattle. They were of the opinion that whole nymphal extracts are more suitable than the soluble and membrane antigens but none of the above studies have been carried further to attain the ultimate goal.

In comparison to the work done on three host ticks, experiments conducted for immunization of cattle against one host tick, *B. microplus*, are very limited. For example, in four separate experiments the team of the Indian Veterinary Research Institute (IVRI) used crude extracts of partially fed adults and unfed larvae of *B. microplus* for the immunization of cross-bred calves and proteins of 105.4 and 92.2 kDa were recognized as immunodominant proteins present in adults and in larvae of the tick species (Ghosh and Khan 1996, 1997a,b, c). However, further work for exploitation of the identified antigens has not been done.

#### *Purification of antigens and its testing as vaccine candidates*

Under the priority research program of IVRI, as an empirical method of identification of target antigen, crude larval and nymphal extracts of *H. a. anatolicum* were used for the immunization of New Zealand white rabbits (Ghosh et al. 1998) and significant reduction in the engorged weight and egg masses of adults fed on immunized animals were recorded. Getting the impetus from the preliminary positive results, Ghosh and Khan (1999) immunized cross-bred calves with the larval antigens and challenged them with larvae and nymphs. A significant rejection, 57.25% of larvae and 45.75% of nymphs, was recorded. A significant percentage of abnormally fed larvae ( $11.4 \pm 0.8$ ) and nymphs ( $8.25 \pm 1.2$ ) was also recorded. The larval antigens were further purified, a protein of 39 kDa was isolated and the purification level of 93.3% was achieved. The antigen was found effective in conferring protection by reducing 71.6% of larvae and 77.3% of nymphs (Ghosh et al. 1999). In continuation, the nymphal antigens were strategically purified and a protein of the same molecular weight was isolated and tested against experimental challenge infestations and found protective (Sharma et al. 2001). Later, the antigen has been included in the list of identified tick vaccine candidate (Willadsen 2001).

As a concealed antigen approach, larval gut antigens were isolated as three proteins of 100, 59.4 and 37 kDa. Immunization of animals with the isolated antigens conferred protection by reducing larval, nymphal and adult infestations by 70.6%, 54.5% and 61.9%, respectively (Das et al. 2000). Simultaneously, adult extracts of *H. a. anatolicum* were purified by immunoaffinity chromatography using anti-gut IgG as legends. A protein of 68 kDa was identified as a candidate molecule for conferring protection against tick challenge (Das et al. 2003). Further, Singh and Ghosh (2003) specifically isolated two glycoproteins of 34 and 29 kDa from the larvae of *H. a. anatolicum* and *B. microplus*, respectively. The cumulative effect of the isolated glycoproteins was tested by immunization of calves with both antigens

and challenged with the stages of both species of ticks. The direct effect of immunization on the stages of the challenged ticks (% DT) was calculated as 69% and 52% against larvae and adults of *H. a. anatolicum* and 60% against *B. microplus* adults. The duration of immunity conferred by the isolated glycoproteins was studied in two tick active seasons and significant protection against both the species of ticks was recorded up to 30 weeks (Ghosh et al. 2005). The antigens were also tested in IPM format and a significant reduction of 35% in the frequency of application of insecticides was recorded (Ghosh, personal communication).

In an another study, of the three earlier identified gut origin larval antigens, a 37 kDa protein was specifically isolated and tested for its protective as well as pathogen transmission blocking efficacy (Das et al. 2005). Besides the direct effect on tick stages, a partial reduction in the growth rate of *T. annulata* in ticks fed on immunized cattle was evidenced in comparison to the ticks fed on control calves. As an important component of vaccine formulation, the comparative immunopotentiating properties of incomplete Freund's adjuvant and saponin in combination with the 39 kDa larval antigen of *H. a. anatolicum* was compared and it was found that IFA could be replaced by the surfactant adjuvant, saponin, for immunization of cross-bred cattle against infestation of *H. a. anatolicum* (Ghosh et al. 2001).

The evaluation of immunoprotective and pathogen transmission limiting potential of the 34 kDa larval glycoprotein in combination with saponin is underway. Work is also underway to evaluate the cross-protective potential of individual antigens and to clone and express the most effective protein-encoding genes in a suitable vector.

As evidenced from several experiments, Bm86 or its homologues are clearly present in other tick species (reviewed by de la Fuente and Kocan 2003). A homologue of Bm86 has been found in other tick species such as *H. a. anatolicum* (Willadsen and Jongejan 1999) and *R. appendiculatus* (Saimo et al. 2005). A significant level of cross-protection between the *B. microplus* vaccine and *H. a. anatolicum* has been found (de la Fuente et al. 1999). With these informations as a lead, the IVRI team has cloned and expressed the Bm86 homologue gene of *H. a. anatolicum* (Izatnagar isolate) in *Pichia pastoris* system. The Bm86 homologue of *H. a. anatolicum* vaccine would probably have a greater efficacy against *H. a. anatolicum* than the Bm86 commercial vaccines and it would be expected to reduce the transmission of the tropical bovine theileriosis. Besides the expected level of protection against homologous species there is also hope for obtaining cross-protection against *B. microplus* and *H. dromedarii*, the important tick species of India.

### Host resistance

Host resistance is stable, heritable, long lasting and the single most important factor affecting the economics of tick control. It is a low cost permanent solution requiring no extra resources and incurring no additional costs. High host resistance is advantageous in any tick control programme. However, its improvement has been almost entirely neglected in Asia (Frisch 1999) including India.

In India, cattle breeds of Ongole/Nellore, Sahiwal, Ponwar hill cattle and buffaloes are recorded in literature as resistance to tick infestation (Gaur et al. 2002). Most of the times, the tick resistance status of these animals was attributed to the physical characters and behaviour of these animals. The genetic or molecular basis of host resistance to tick and tick borne diseases is unknown.

### *Ongole/Nellore cattle breed*

A dual-purpose, large sized breed with loosely knit frames. The breed tract comprised between 15°00' to 16°10' east latitude and 79°04' to 80°02' north longitude, Andhra Pradesh State and is found to have high level of resistance against *Boophilus microplus*. The physical characteristic of the skin coat is highly unfavorable for the tick attachment and feeding (Joshi and Phillips 1953; Gaur et al. 2002). The dense skin texture, reflecting and glistening skin coat and the presence of large number of sweat glands per unit area of skin made the skin coat hostile for the tick attachment. Flexible tail tip, having cartilage in place of last three or four vertebrae helps as a brush to repel vectors and the presence of well developed subcutaneous panniculus carnosus muscle repels vectors by twitching. The skin being more vascular and with large number of sweat glands, more heat is dissipated through the skin surface which maintains the lower skin temperature, confers higher tick resistance (Schleger et al. 1981; O'Kelly and Spiers 1983). The sebum secretion of the Nellore cattle was reported to have fly repellent activity and the tick repellent activity of the sebum is unknown.

### *Sahiwal cattle breed*

The Sahiwal is a milk breed originated in the dry Punjab region which lies along the India–Pakistan border. It is noted for its high resistance to parasites, both internal and external and heat-tolerant.

### *Ponwar cattle breed*

The Ponwar is a draught cattle breed and is native to the Pilibhit District of Uttar Pradesh. The breeding tract lies between latitude 28°4' and 28°8' North and between longitude 79°0' and 80°4' east. The body is small, compact and non-fleshy and the skin is tight. The tail is long and reaches to below the hock and it is highly helpful in removing the vector attachment (Gaur et al. 2004). This cattle breed was reported to be highly resistant to tick and tick borne diseases. Since these hill cattle were being maintained on grazing in the forest area, they might have acquired the resistance towards ticks and other diseases due to continuous natural challenge.

### *Buffalo*

Indian buffaloes are notably resistant to the tick species of India. Healthy buffaloes are not commonly affected by tick borne diseases nor are the hides damaged by their bites. Though the buffaloes are equally susceptible to the tick borne diseases as cattle breeds, the fewer occurrences of tick borne diseases could be linked to the tick resistance nature. The thick skin coat and wallowing and rubbing habits of the buffaloes could be attributed to the tick resistance. In a recent survey it was observed that the buffalo population in the village level are rarely infested with tick species (Ghosh, personal observation).

Selection of dual purpose Nellore breed and buffalo for breeding has tremendous scope in developing stock of animals having high level of tick resistance in India. Selection will be most efficient in improving productivity if a multi-trait index is developed by incorporating breeding values for both production and resistance

traits. Since there is a zero correlation between tick resistance and growth (Jonsson et al. 2000), simultaneous selection for both traits is expected to result in improvement of both traits (Mackinnon et al. 1991). Milk yield did not decrease under intense selection for tick resistance (Utech and Wharton 1982), which suggests that both traits can be improved simultaneously.

### Pasture management

Although pasture management is a sustainable method to control ticks, its application is difficult in India where many livestock farmers have only small land holdings. But still this method is a viable option that can be adapted in organized livestock farms (India has 257 government controlled livestock farms and many dairy cooperatives) or in cooperative farming where pasture land is available for animals. This approach only requires good fencing and management skills. In India, *Stylosanthes* is regarded as the most important range legume for the humid to semi-arid tropics. It is extensively utilized in pastoral, agro-pastoral and silvi-pastoral systems for animal production (Chandra et al. 2006). Apart from their tick-trapping properties, livestock nutrition will also be improved. Government policies favouring the cultivation of this type of grasses by providing seeds at lower cost and by motivating the farmers through extension education would have some positive impact on TTBDs control. Simply by pasture management viz., pasture spelling, rotational grazing and cultivation of tick-trapping grasses that incurs no additional costs to generate an environment with reduced tick population/tick free environment and by the additional effect of the reduced use of acaricides, a livestock farmer can produce milk and meat of high quality (without acaricide residues) with lower prices.

### Botanical acaricides

The ethno-veterinary and medical knowledge offers a range of herbs to be evaluated for their insecticidal and acaricidal property. Certain plants and herbs are known to possess insecticidal, growth inhibiting, anti-moulting and repellent activities. Khudrathulla and Jagannath (2000) studied the effect of methonolic extract of *Stylosanthes scabra* on Ixodid ticks. The leaves of tobacco (*Nicotiana tabacum*) were found effective against *R. haemophysaloides* (Choudhury et al. 2004). Methonolic extracts of neem (*Azadirachta indica*) leaves and bark, nochi (*Vitex negundo*) leaves, vashambu (*Acorus calamus*) rhizome and Pungu (*Pongamia glabra*) leaves were tested for the acaricidal effects. Neem bark proved to be the most effective followed by vashamu (Pathak et al. 2004).

In our laboratory the alcoholic extract of sitaphal (*Annona squamosa*) seed is being evaluated for its acaricidal property against different life stages of *H. a. anaticum* and *B. microplus* and the initial results are highly encouraging. The evaluation of botanical acaricides in crude extract form and subsequent isolation and characterization of the key active components, have a great scope for commercialization. India possesses 45,000 plant species of which 15,000–20,000 have proven medicinal value and have lands not suitable for agriculture activities. The tested/yet to be tested locally available plant extracts prepared from the perennial plants can easily be grown in these unutilized lands. Once the active principle of the effective components is known the same can be commercially produced and marketed. Besides the cost effectiveness,

the environment friendly products will have low mammalian toxicity, less residual effect in animal products and have the potential to reduce or replace the chemical acaricide use. Besides, since botanical acaricides consist of number of active molecules against ticks, the chances of selection of resistance tick population is expected to be slower than commonly used acaricides where single point mutation is rendering an insecticide ineffective.

### Newer generation acaricides

In the current system of livestock production in developing countries like India, present tick control can not be imagined without the use of acaricides despite the increasing resistant tick populations. This is entirely because of the lack of effective alternative tick control methods practically compatible with the farming practices. Newer generation acaricides targeting previously unexplored metabolic pathways or bio-molecules synthesis pathways should be generated and these acaricides should be kept in reserve to meet any emergency situations expected to arrive by the multi-acaricide resistant tick population in future. Beside development of new generation acaricides, on farm sensitive monitoring system of acaricide resistance is required to develop. Till the molecular test is available for field use, the FAO larval Packet Test—LPT (Stone and Haydock 1962), modified for Amitraz also, can be effectively utilized for the detection of resistance in the field. Early detection of the resistance, subsequent withdrawal and replacement with another acaricide maintain the susceptibility of the tick population in the field. In India, serious efforts need to be taken to assess the resistance level which is underreported so far.

### Broad spectrum anti-tick vaccines

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 TickGARD (Hoechst Animal Health, Australia) and Gavac (Heber Biotec S. A., Havana, Cuba) confers partial protection against phylogenetically related *Hyalomma* and *Rhipicephalus* tick genera (de Vos et al. 2001). However, immunization with BM86 failed to protect against the more phylogenetically distant *Amblyomma* spp. (de Vos et al. 2001). 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 (Almazán et al. 2003a, b, 2005a, b; de la Fuente and Kocan 2006). 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 (Almazán et al. 2005a; de la Fuente and Kocan 2006). The 4D8 molecule is expected to present in important tick species of India.

## Cocktail vaccines against ticks and tick borne pathogens

The control of tick-borne diseases mainly depends on the control of vectors. Often integrated tick control minimizes the tick population significantly, but the complete elimination of the vector population is not realistic due to biological constraints and it may be undesirable if it merely produced a vacuum in ecological niche that might be readily filled up by new immigrants (Curtis 1994). Although reduction in disease transmission potential of ticks is achieved by vaccination against ticks (de la Fuente et al. 1998; Labuda et al. 2002; Das et al. 2005; de la Fuente and Kocan 2006), a cocktail vaccine against both tick and pathogen/parasite is expected to give an even higher level of protection against tick-borne diseases.

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 (Kaba et al. 2005; Musoke et al. 2005). 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 (Ghosh, personal communication). A cocktail vaccine consisting of antigens Tams 1 and rHAA86 is expected to protect Indian cattle population against *H. a. anatolicum* and *T. annulata*.

An important component in a cocktail vaccine against TTBDs is the adjuvant. Adjuvant choice is crucial in increasing the response to vaccines against both tick and tick-borne diseases. It is hoped that a multivalent chimeric antigen consisting of the immunoprotective epitopes of vector and pathogen can be produced using the recombinant DNA technology. Delivery of this multivalent chimeric antigen along with appropriate adjuvants / immunomodulators would give protection against TTBDs.

## Concluding remarks

Due to high costs involved in treating animals with insecticides most of the small and marginal farmers are utilizing indigenous method of keeping their animals tick free (including hand pick method). The multi-tick infestations pattern on animals are completely different in different agro-climatic regions of India (India has 15 agro-climatic regions) and so there is an urgent need to develop strategic region specific schedule of treatment of animals. The future control strategies should involve the use of tick vaccine in simulation with utilization of botanical acaricides and minimum use of chemical acaricides. Since multi-tick infestation is common in India, the future tick vaccine should contain molecules which are effective against the economically most important tick species (*H. a. anatolicum*, *H. m. isaaci*, *B. microplus*, *R. haemaphysaloides*, *H. bispinosa*) infesting cattle, buffaloes, sheep and goats. Many of the indigenous *B. indicus* breeds of cattle are low producer but significantly resistant to many diseases including those caused by TTBDs. Concerted efforts need to be directed to identify the gene(s) for tick resistance in the promising indigenous breeds which can later be incorporated into the strategic breeding programme to develop animal stocks with high genetic resistance to TTBDs. Government and

private funding towards the research on TTBDs and its control is to be strengthened to involve more working groups in this field of research. Additional research initiatives are to be taken by Indian Council of Medical Research to work on the development of vaccine against *H. spinigera* infestations on animals, which is expected to reduce the transmission of Kyasanur Forest Disease virus to human beings.

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