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Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: A review

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ABSTRACT

Ticks, as vectors of several zoonotic diseases, are ranked second only to mosquitoes as vectors. The diseases spread by ticks are a major constraint to animal productivity while causing morbidity and mortality in both animals and humans. A number of tick species have been recognised since long as vectors of lethal pathogens, *viz.* Crimean-Congo haemorrhagic fever virus (CCHFV), Kyasanur forest disease virus (KFDV), *Babesia* spp, *Theileria*, *Rickettsia conorii*, *Anaplasma marginale, etc.* and the damages caused by them are well-recognised. There is a need to reassess the renewed threat posed by the tick vectors and to prioritize the tick control research programme. This review is focused on the major tick-borne human and animal diseases in India and the progress in vector control research with emphasis on acaricide resistance, tick vaccine and the development of potential phytoacaricides as an integral part of integrated tick control programme.

Key words Acaricides; phytoacaricides; resistance; tick; tick-borne diseases; vaccine

INTRODUCTION

Ticks are highly specialized obligate haematophagous ectoparasites of mammals, birds and reptiles, distributed worldwide and are of enormous medical and veterinary relevance owing to the direct damage they cause to their hosts and as vectors of a large variety of human and animal pathogens. Today, most emerging infectious diseases arise from zoonotic pathogens, and many of them are transmitted by tick vectors. Ticks are among the most competent and versatile vectors of pathogens and are second to mosquitoes as vectors of a number of human pathogens, like viruses, bacteria, rickettsia, spirochetes, *etc*, and the most important vector of pathogens affecting cattle worldwide[1]. Several characteristics of ticks make them outstanding vectors of pathogenic agents—the wide host range and tendency to feed on several hosts during life cycle ensures ample opportunity to acquire and transmit pathogens; hardiness and longevity enable them to survive long periods in unfavourable environmental conditions; high reproductive potential, ensuring maintenance of a large populations and a high frequency of host-vector contact; slow feeding habit and in the case of ixodids, attachment with hosts for relatively longer periods, which allow sufficient time for pathogen acquisition and transmission, as well as tick dispersal by migrating or wandering hosts. In humans, tick infestations typically involve few specimens and the greatest risk for people bitten by a tick lies in infection due to a tick-borne pathogen[2]. In animals, tick infestations are much more severe than in humans. Animals can be parasitized by thousands of ticks, which multiply the effect on the host, either by direct injuries or disease transmission. Direct injuries to animals can be very serious, especially in tropical climates, and are mainly observed in infestations with ixodid ticks. In India, cattle and buffaloes are frequently heavily infested with multi-species of ticks, which apart from transmitting diseases such as theileriosis, babesiosis and anaplasmosis, also cause extensive damage to the livestock health and production. The global loss due to ticks and tick-borne diseases (TTBDs) was estimated to be between US$ 13.9 and 18.7 billion annually[3] while in India the cost of controlling TTBDs has been estimated as US$ 498.7 million/annum[4]. Tick-borne infectious diseases are growing steadily partly due to the establishment of the tick vector in urban areas/new areas and posing serious threat to the world health problem[5]. The outbreaks of KFD (Kyasanur forest disease) in Karnataka, India despite ongoing vaccinations and the 2011 Crimean-Congo haemorrhagic fever (CCHF) outbreak in Gujarat, India underlines the importance of monitoring the vector activities and checking human interference in natural habitat of ticks and their wild hosts. The number of pathogens transmitted by ticks (Table 1) and its consequences to human and animal health signifies the involvement of interdisciplinary research team in the area of study. The complex triangular interactions between pathogen-host-vectors complicate the subject and multiple pathways are being targeted to control TTBDs.

Table 1. Important tick-borne diseases of man and livestock

Vector	Vector-borne diseases	Parasite/Pathogen
Viral diseases		
Haemophysalis spinigera	Kyasanur forest disease	Group B Toganvirus (Flavidiridae)
Hyalomma anatolicum	Crimean-Congo haemorragic fever	Nirovirus (Bunyaviridae)
Hyalomma dromedarii	African horse sickness	Reoviridae (African horse sickness virus)
Ornithodorus mobuta	African swine fever	African swine fever virus
Rhipicephalus appendiculatus	Nairobi sheep disease	Bunyaviridae
Rickettsial diseases		
Rhipicephalus sanguines	Ehrlichiosis	*Ehrlichia canis, E. equi*
	Human monocytic ehrlichiosis	*E. senetsu, E. chaffeensis, E. phagocytophilia*
Amblyoma variegatum	Cowdriosis	*Cowdria ruminantium*
	Anaplasmosis	*Anaplasma marginale*
R. sanguineus, Dermacenter andersoni, R. (B.) decoloratus	Indian tick typhus (ITT)	*Rickettsia conorii*
Spirochete diseases		
Ixodes ricinus	Lyme disease	*Borrelia burgdorferi*
Bacterial diseases		
Dermacentor spp.	Tularemia	*Francisella tularensis*
Protozoan diseases		
H. anatolicum, R. appenticulatus	Theileriosis	*Theileria annulata, T. parva, T. hirci*
R. (B.) microplus	Babesiosis	*B. bigemina, B. ovis*
Haemaphysalis spp.		*B. motasi*
H. anatolicum		*B. equi*
Ixodes spp.	Human babesiosis	*B. microti, B. divergens*

Distribution of important tick vectors in India

India is predominantly an agricultural country with about 70% of its population dependent on income from agriculture. Farmers are keeping animals for milk, meat, wool, hide and for various farm operations. India accounts for a significant share of the world's livestock resources with approximately 199 million cattle and 105 million buffaloes[6], most of which are suffering from multi-species tick infestations[7] with an estimated control cost of US$ 498.7 million/annum[4]. Amongst the 106 tick species reported from India[8], a few of them have been experimentally established as the principal vectors of pathogens, their distribution and status of vaccine development against the disease has been highlighted in Table 2. The genera *Rhipicephalus* and *Hyalomma* are most widely distributed in India. *Rhipicephalus (Boophilus) microplus, R. sanguineus* and *Hyalomma anatolicum* species are reported in 24, 21 and 20 states of India, respectively (Table 2).

Major TBDs prevalent in India

Indian tick typhus (ITT)

A type of rickettsial spotted fever similar to rocky mountain spotted fever (SF) and is caused by *Rickettsia conorii.* The disease is reported from Maharashtra, Tamil Nadu, Karnataka, Kerala, Jammu and Kashmir, Uttarakhand, Himachal Pradesh, Rajasthan, Assam and West Bengal[9–11]. The dog tick, *R. sanguineus,* is the principal vector of ITT although some species of *Haemaphysalis* and *Hyalomma* may also transmit the infection. The ITT in India has been recognised clinically but cases have not been documented frequently possibly due to the lack of efficient diagnostic tools. Between 1996 and 1998, serological testing amongst residents of southern India confirmed that spotted fever continues to occur[9]. An extensive study on tick-borne rickettsiosis in Pune district of Maharashtra revealed that Indian tick typhus exists as zoonosis[12]. Subsequently, ITT has been reported in Mumbai[13], Himachal Pradesh[11], in a French traveller returning from India[14] and in Haryana[15]. Recently, Kumar *et al*[16] reported a case of ITT with gangrene in a 10-year old boy from Delhi.

No rapid laboratory tests are available to diagnose rickettsial infection early in the course of the disease. Indirect immunofluorescence assay (IFA) is the preferred method for detection of infection but its availability and cost are major constraints in India and other developing countries. The ELISA technique, particularly immuno-

Table 2. Important tick-borne diseases in India, distribution of vectors and status of vaccine development

Tick vector	Pathogen/Parasite	Distribution of vectors†	Host	Vaccine status
Haemophysalis spinigera *H. turturis*	KFD virus	1, 2, 6, 8, 14–17, 19, 22, 26	Man	Chick embryo tissue culture vaccine*
Rhipicephalus sanguineus	*Rickettsia conorii*	2–6, 9–17, 20, 22–24, 26, 29, 30	Man	Nil
Hyalomma anatolicum	*Theileria annulata* CCHF virus	2, 4–12, 14, 16, 17, 22–24, 26, 28–30	Cattle Man	Attenuated macroschizont infected lymphoblast vaccine** Nil
R. (B.) microplus	*B. bigemina*	2–14, 16, 19, 20, 22–26, 28–30	Cattle, Buffalo	Nil
Haemaphysalis spp.	*B. motasi*	(–)	Goat	
R. sanguineus	*B. canis*	2–6, 9–17, 20, 22, 24, 26, 29, 30	Dog	
Rhipicephalus spp.	*B. ovis*	2–6, 9–17, 20, 22, 4, 26, 29, 30	Sheep	
H. anatolicum	*B. equi*	2, 4–12, 14, 16, 17, 22–24, 26, 28–30	Horse	
R. (B). microplus	*Anaplasma marginale*	2–14, 16, 19,20, 22–26, 28–30	Cattle, Bufallo, Sheep	Nil
Hyalomma spp. *R. sanguineus*	*Ehrlichia bovis* *E. canis*	2, 4–12, 14, 16, 17, 22–24, 26, 28–30 2–6, 9–17, 20, 22, 4, 26, 29, 30	Cattle Dog	Nil

*Not fully effective in field situation; **Effective but not in large-scale use; (–) Unknown; †1: Andaman and Nicobar Islands; 2: Andhra Pradesh; 3: Arunachal Pradesh; 4: Assam; 5: Bihar; 6: Chhattisgarh; 7: Delhi; 8: Goa; 9: Gujarat; 10: Haryana; 11: Himachal Pradesh; 12: Jammu and Kashmir; 13: Jharkhand ; 14: Karnataka; 15: Kerala; 16: Madhya Pradesh; 17: Maharashtra; 18: Manipur; 19: Meghalaya; 20: Mizoram; 21: Nagaland; 22: Odisha; 23: Punjab; 24: Rajasthan; 25: Sikkim; 26: Tamil Nadu; 27: Tripura; 28: Uttarakhand; 29: Uttar Pradesh; 30: West Bengal.

globulin M (IgM) capture assay, is probably the most sensitive test available for diagnosis, and the presence of IgM antibodies indicates recent infection with rickettsial diseases. The Weil Felix test is a cheap and easily available when other means of diagnosis are not available but the downside is its poor reliability and specificity. Rickettsial diseases can be easily confused with a variety of viral (measles, enteroviral exanthema, dengue and infectious mononucleosis), protozoal (malaria), bacterial (meningococcemia, typhoid, leptospirosis, toxic shock syndrome, scarlet fever, *etc*), collagen vascular (Kawasaki disease, other vasculitis) diseases, and adverse drug reactions. As the incidence of TBDs increases and the geographic areas in which they are found expanded, it is of utmost importance that health workers should be able to distinguish the diverse and overlapping clinical symptoms of these diseases. The treatment of rickettsial infection is relatively easy after diagnosis and the commonly used antibiotics for treatment are tetracyclines, chloramphenicol, macrolides and rifampicin. Among tetracyclines, doxycycline is considered as a drug of choice for SF rickettsioses.

Crimean-Congo haemorrhagic fever (CCHF)

Crimean-Congo haemorrhagic fever was described in the Crimea in 1944 during an outbreak and was called Crimean hemorrhagic fever. Later, the same virus was isolated from Congo and the nomenclature was changed to CCHFV[17]. The CCHFV of *Nairovirus* group circulates in an enzootic tick-vertebrate-tick cycle. Although there is no evidence that the virus causes disease in animals, a wide range of domestic and wild animals may get CCHFV infection[18].

The CCHFV is mainly seen in the Middle East and Asia and parts of Europe including southern portions of the former Union of Soviet Socialist Republics (USSR). In the recent past, most cases have been reported from Pakistan[19], Iran[20], Sudan[21], Bulgaria[22] and Turkey[23]. In India, the CCHF had not been reported before the outbreak in Gujarat in January 2011[24–25]. Since, its discovery in 1960s, nearly 140 outbreaks involving >5000 cases have been reported all over the world. The wide distribution of tick vector, *Hyalomma anatolicum* contributed significantly in spreading the disease.

Diagnosis of infection is possible with serological and molecular assays. A one-step real-time RT-PCR assay using primers to the nucleoprotein gene and another real-time RT-PCR assay using TaqMan-Minor Groove Binding Protein (MGB) probe, which had higher specificity

and a shorter probe length, were developed and used for diagnosis. A CCHFV recombinant nucleoprotein (rNP) based IgG ELISA and IgM-capture ELISA have also been found to be useful for diagnosis of CCHFV infections[26]. Treatment options are limited as there is currently no specific antiviral therapy approved for use in humans by the FDA. Although nucleoside analog ribavirin is shown to be effective for inhibiting CCHFV *in vitro* but its efficacy is not very well-documented in randomized control study. However, Tasdelen *et al*[27] have shown the beneficial effect of ribavirin if given at an early phase of infection. According to the World Health Organization (WHO), ribavirin is the antiviral medication of choice and the recommended dose is an initial dose of 30 mg/kg followed by 15 mg/kg for four days and then 7.5 mg/kg for six days for a total of 10 days. A vaccine has been in use in Bulgaria for many years but its efficacy and safety is not well-quantified.

Kyasanur forest disease (Monkey disease)

The disease is caused by Kyasanur forest disease virus (KFDV), a member of the family Flaviviridae. The KFDV was identified in 1957 when it was isolated from a sick monkey from the Kyasanur forest in Karnataka state, India. The disease is localized in five districts (Shimoga, Chikamagalur, Uttar Kannada, Dakshina Kannada and Udupi) of the state and occurs as seasonal outbreaks during December to May when the nymphal activity of the vector ticks in the forest is maximum[28]. As per the compiled data from 2003 to March 2012, 3263 suspected cases, 823 confirmed cases and 28 deaths due to KFD have been reported[29]. The two major vectors of KFD are *Haemaphysalis spinigera* and *H. turturis.* Besides the two major tick vectors, other species of *Haemaphysalis, Ixodes, Hyalomma, Dermacentor* and *Rhipicephalus* are capable of transmitting the pathogen. The main hosts of KFDV are small rodents, but shrews, bats and monkeys may also carry the virus and transmitted through the bite of an infected tick while the transmission to humans is through the bites of nymphs or by contact with an infected animal.

Diagnosis can be made by serological testing during the convalescent phase using haemagglutination inhibition, complement fixation, and through mass tag polymerase chain reaction. There is no specific treatment, but supportive therapy is important. Supportive therapy includes the maintenance of hydration and the usual precautions for patients with bleeding disorders. Due to lack of specific treatments, prophylaxis by vaccination is advised. National Institute of Virology, Pune (India) developed a formalin inactivated chick embryo tissue culture vaccine which evokes neutralizing antibodies response in about 70% of the vaccinated persons. The technology has been transferred to Karnataka Public Health Department for production and vaccination. Regular vaccination and booster campaigns are run by the State authorities in the affected areas but there are reports of the reduced efficacy of vaccine in recent years[28, 30] that necessitates a review of the current vaccination protocol including the storage and administration. The antigenic variations in the current strains of virus and the strain used for vaccine preparation (isolated in 1950s) have to be determined and a new vaccine with current strain of virus needs to be developed.

Theileriosis

Theileriosis caused by *T. annulata* and *T. orientalis* is the most important tick (*H. anatolicum*) borne disease affecting cattle and buffaloes and has a significant adverse effect on the productivity and also proves to be fatal if left untreated. Approximately, 33 million cross-bred cattle and 105 million buffaloes[6] in India are at risk to this disease with an estimated annual loss of US$ 239.5 million[4]. Several reports of subclinical infections and severe outbreaks of theileriosis have been documented. Serological screening of cattle maintained in unorganized cattle farms all over India found that 30–60% of the cattle harbour antibodies to *T. annulata* piroplasms[31]. In a six year survey in project area of cooperative milk producers union, Mysore, a district of southern India, a total of 17.7% cattle showed *T. annulata* infection in blood smears[32]. In Bareilly district (Uttar Pradesh), 20 out of 466 buffaloes were found to have antibodies against *T. annulata* while seven of them had piroplams/schizonts[33]. A study of 388 samples in Punjab revealed that *T. annulata* was the most prevalent blood protozoan in buffaloes[34]. In a theileriosis outbreak at Babugarh (U.P.), high parasitemia (40–50%) was detected in the blood smears of the affected animals and 17 cross-bred calves died due to the infection. The authors also detected 18.9% carrier cattle showing low leve1 of *T. annulata* infection in 148 smears from the states of Punjab, Uttar Pradesh, Rajasthan and Odisha[35]. Haque *et al*[36] carried out a prevalence study of *T. annulata* in *H. anatolicum* ticks in Punjab and detected 15.45% prevalence of *T. annulata* in female ticks.

Diagnosis of theileriosis is mainly dependent on clinical signs as well as on microscopic examination of Giemsa-stained lymph nodes and blood smears. ELISA using *T. annulata* surface protein (TaSP) and *T. annulata* merozoite surface antigen 1 (TamS1) antigens is being used to detect antibodies in infected animals. The PCR amplification of small subunit ribosomal RNA (SSU rRNA) gene can be used to detect infection even in car-

rier cattle. Anti-parasitic drugs are effective in animals with clinical signs, but in most of the cases the animals may remain carriers. Parvaquone is effective against theileriosis at a dose rate of 20 mg/kg body weight. Buparvaquone, a second-generation hydroxynaphthoquinone related to parvaquone, is more effective in the treatment of both experimental and natural infections of *T. annulata* in cattle and buffalo. Milk and meat of the treated animals are recommended as unfit for human consumption for 48 and 42 days, respectively, post-treatment.

The live attenuated vaccine of *T. annulata* was produced by prolonged *in vitro* cultivation of lymphoblasts of cattle infected with macroschizonts[37]. The protection engendered by the attenuated schizont vaccine has been evaluated by laboratory challenge with live infected ticks or with ground up tick sporozoites (GUTS) inoculated through syringe passage. The results of such challenge ranged from no clinical response to mild transient clinical reactions with low parasitaemia to death from acute theileriosis. In endemic areas, single vaccination appeared to be adequate for protection when the animals receive continuous challenge from natural tick infestations[38]. The attenuated schizont vaccine of *T. annulata* was commercialized under the trade name of "Raksha Vac-T" and is produced and marketed by Indian Immunologicals, Hyderabad, India.

Babesiosis

Babesiosis or tick fever, is a febrile disease of domestic and wild animals characterized by extensive erythrocytic lysis leading to anaemia, icterus and haemoglobinuria. The disease is caused by protozoan parasites of the genus *Babesia,* an intraerythrocytic protozoan parasite transmitted mainly by *R. (B.) microplus*. In cattle, *B. bigemina* is the primary pathogen and its incidence in indigenous, cross-bred cattle and in buffaloes has been reported frequently since long. Depending on the sensitivity of the serological tests, different authors reported up to 86% seroprevalence of the pathogen in Indian dairy animals[39–40].

Diagnosis of babesiosis usually includes examination of stained blood smears as well as serologic evaluation with indirect fluorescent antibody tests (IFATs) and also by PCR. Because of improved sensitivity, PCR has become the test of choice for confirmation of actual infection in antibody-reactive individuals and for monitoring therapeutic responses. Live, attenuated strains of *B. bovis, B. bigemina* or *B. divergens* are used to vaccinate cattle in some countries but some safety issues including the potential for virulence in adult animals, possible contamination with other pathogens, and hypersensitivity reactions to blood proteins have been noted. Efficacy of anti-parasitic drugs (diminazene diaceturate, imidocarb, amicarbalide) depends on early detection of the disease.

Human babesiosis mostly occurs in USA, but cases have also been reported in several European countries. Human babesiosis is caused by one of the several babesial species that have distinct geographical distributions based on the presence of competent hosts. In North America, babesiosis is caused predominantly by *B. microti*[41] while in Europe, babesiosis is considerably rare but more lethal and it is caused by *B. divergens*[42]. In Indian situation, there is only one documented report of a *B. microti* infection in a 51-yr-old male patient[43]. However, as babesiosis in humans can be confused with *Plasmodium* infection on examination of blood smears, the actual incidence of babesiosis in humans needs to be worked out. Careful examination of peripheral smears and surveillance studies are necessary to know the true prevalence of human babesiosis in India.

Bovine anaplasmosis

Anaplasmosis is considered as one of the top 10 economically important rickettsial diseases affecting ruminants in India[44] and is principally transmitted by *R. (B.) microplus*. It is an infectious, non-contagious haemotropic disease characterized in acute form by fever, anaemia, weakness, constipation, yellowing of the mucous membranes, lack of appetite, depression, dehydration and laboured breathing. Animals surviving with an acute attack often make a slow recovery, resulting in loss of milk or meat production. Generally, mortality is between 5 and 40% but may reach up to 70% during a severe outbreak. The epizootiology of anaplasmosis is complicated by the life-long carrier state which occurs in animals that have recovered from the clinical disease.

Clinical anaplasmosis was first recorded in Indian cattle from the State of Odisha[45]. Subsequently, *A. marginale* infection was detected in livestock of Uttar Pradesh, Punjab, Haryana, Tamil Nadu, Karnataka, Jammu and from parts of north and central India[46–48]. In recent times, anaplasmosis has been recorded in Jammu[49], Karnataka[50], Haryana[51] and Tamil Nadu[52].

With the advent of molecular tools, the sensitivity of detection of infection in diseased and carrier animals has been improved. PCR and semi-nested PCR assays are more sensitive and have been used to detect infection where microscopic examination could not detect infection. Oxytetracycline at 20 mg/kg body weight is the drug of choice. It was believed earlier that repeated treatments with oxytetracycline eliminated the carrier status of treated

animals but with higher sensitivity of PCR it has been proved to be wrong.

Although research to develop control measure for TBDs has been focused on the diagnosis and treatment of the disease and development of a suitable prophylaxis system for protection against the disease, the target has not been achieved due to widespread distribution of vectors and vector control has not been suitably addressed. Large-scale vector control has been shown to be possible in USA with eradication of *R.(B.) microplus* and *R.(B.) annulatus*, the vectors of babesiosis. But in India, the large-scale campaign for tick control is debatable for both financial and practical reasons because livestock rearing is basically an unorganized sector with limited resources available to deal with the problem. Therefore, there is need to develop tick control methods that can be applied by individuals and by communities. The progress in this area of research is discussed in the following sections.

Chemical control

Chemical control with acaricides can be directed against ticks parasitizing the host or the tick stages living in the environment. The more preferred and commonly used method is the application of acaricides on the host to kill parasitic stages. The four classes of chemical acaricides which are the mainstay of tick control programme in India are organophosphates, pyrethroids, formamidines and macrocyclic lactones. Organophosphates and pyrethroids have been widely used all over the country. Use of formamidines like amitraz and macrocylic lactones like ivermectin is comparatively recent and is rising due to the inefficiency of OP and SP acaricides to control tick infestations. The drawback of using acaricides inconsistently and indiscriminately is the selection of acaricide resistant ticks which makes existing acaricides ineffective and thereby limiting the efficacy of existing tick control methods. Another potential problem associated with use of acaricides is the environmental contamination and the contamination of milk and meat products with chemical residues.

Globally, there have been frequent reports of acaricide resistance in ticks. Cases of *R. (B.) microplus* developing resistance to organophosphates[53] and synthetic pyrethroids[54] are well-documented. Till now, acaricide resistance in India was not well-documented even though possibility of widespread resistance was reported in a FAO questionnaire survey. In a comprehensive study spanning six agro-climatic regions, using laboratory standardized resistance monitoring tools, *R. (B.) microplus* populations from the States of Bihar, Punjab, Haryana, Rajasthan, Uttar Pradesh and West Bengal were characterized and found resistant to OP and SP compounds at the level varied from low (level-I) to high (level-IV)[55–56]. The suitability of the monitoring tools has been validated by other workers[57–59]. A similar study on the multi-host tick, *H. anatolicum* detected comparatively less resistant (Level I–II) against SP and OP compounds[60–61].

The development of resistance against OP and SP acaricides has driven the farmers to rely on formamidines (amitraz) and macrocyclic lactones (ivermectin). Consequently, resistance to amitraz against *R.(B.) microplus* from various parts of the world has been reported[62–63]. Recently, Singh *et al*[64] detected amitraz resistance in Gujarat state and subsequently, Kumar *et al*[65] reported resistance ranging from level I to level III in 11 isolates of north India. In the same study, it was also observed that multi-acaricide resistance has developed at many places which renders SP, OP and amitraz ineffective to the prevalent tick population. These reports imply that the use of amitraz should be regulated and carefully monitored to avoid the development of widespread resistance to amitraz. Ivermectin is conveniently used these days against SP and OP resistant tick populations. Currently, there has been no report of resistance against ivermectin in India but as resistance to ivermectin has already been reported from Brazil[66] and Mexico[67] care should be taken to avoid indiscriminate use of ivermectin so that its utility as a potent acaricide is not short-lived.

For effective implementation of chemical control measures there is a need to develop resistance monitoring tools which should be robust and efficient. Bioassays can be used along with molecular assays which allow assessment of resistance without knowledge of the underlying mechanism. *In vitro* assay, such as the adult immersion test (AIT) and the larval packet test (LPT) recommended by FAO[68] can be used effectively to identify resistant phenotypes. The AIT has been generally preferred assay for detection of resistance in India. In the Entomology laboratory of IVRI, AIT has been effectively standardized using technical grade insecticides and discriminating concentration (DC) for deltamethrin, cypermethrin, malathion, diazinon, fipronil, coumaphos, fenvalerate and amitraz were worked out with repeatability. The AIT method has been successfully adopted in India as evident in published reports[55–56, 59]. The LPT has also been used to determine the resistant status of tick populations in India[60–61] and found repeatable.

Allele-specific PCR assay (AS-PCR) has been developed as a tool to detect single nucleotide changes that result in target site insensitivity in the resistant population. Guerrero *et al*[69] developed an AS-PCR assay utilizing the T2134A mutation site in domain III S6 fragment

of sodium channel gene. Later, this mutation was found to be localized in North American tick only[70]. Morgan *et al*[71] developed another AS-PCR assay using the C190A mutation site in domain II S4-5 linker region of sodium channel gene. The C190A mutation has also been identified recently in Brazil[72] and in India[73]. In India, Vatsya *et al*[74] employed AS-PCR using T2134A mutation to determine resistant allele frequency in tick populations. However, the authors didn't present any comparative sequence data to justify the results using domain IIIS6 mutation site for AS-PCR. A separate study also failed to detect the domain IIIS6 mutation even in highly resistant isolates of Indian ticks[73]. Therefore, the C190A mutation site should be utilized in India to develop AS-PCR assay as a pyrethroid resistance monitoring tool.

Tick vaccine

As vector control through chemicals has many drawbacks and so development of vaccine against vector is considered as one of the important options. Vaccination is a cost-effective, environment friendly that allows control of several VBDs by targeting their common vectors. Vaccination can reduce vector capacity to transmit pathogens, *viz.* prevention of transmission of *B. bigemina* and reduced transmission of *B. bovis* using the Bm86-based vaccine against *B. annulatus*[75], reduced mortality due to tick-borne encephalitis virus transmitted by *Ixodes ricinus* using a recombinant antigen derived from *R. appendiculatus*[76] as well as reduced incidence of babesiosis and anaplasmosis after extensive use of a Bm86-based vaccine in Cuba[77].

Due to concerted research efforts in this field, two recombinant vaccines (Gavac™ and TickGARD^{PLUS}) against *R. (B.) microplus* are available commercially[78]. Both the vaccines are based on the concealed tick midgut protein, Bm86. Early experiment with Bm86-based vaccines demonstrated cross-protection against *R. (B.) annulatus* and *R. (B.) decoloratus* infestations and conferred partial protection against *Hyalomma* and *Rhipicephalus* spp[79–81]. However, immunization with Bm86 failed to protect animals against *Amblyomma* spp[80] and against some geographical strains of *R. (B.) microplus*[82]. In India, much of the earlier work was focused on immunization of animals using crude and partially purified antigens to develop a protective immune response against ticks[83–85]. Several immunodominant antigens were identified from the crude larval and nymphal extracts of *H. anatolicun* and *R. (B.) microplus* with varied efficacy against challenge infestations[86–87]. However, none of the studies have reached to the development of immunoprophylactic measure against the target tick species. With the success story of Bm86 based vaccine, research efforts were directed for identification of Bm86 homologue in other tick species. In the entomology laboratory of Indian Veterinary Research Institute, Azhahianambi *et al*[88] cloned and expressed the Bm86 homologue gene of *H. anatolicum* in *Pichia pastoris* expression vector. The recombinant yeast expressed Haa86 was purified but significant loss in the recovery of protein was reported. The *Bm86* gene was further expressed in *E. coli* pET 32 system and the expressed protein was tested against homologous challenge infestations and found protective[89–91]. The protective efficacy of rBm86 against *R. (B.) microplus* (IVRI-1 line) and *H. anatolicum* (IVRI-II line) was evaluated and the results indicated moderate efficacy of commercially available rBm86 based vaccine against *R. (B.) microplus* and low efficacy against *H. anatolicum* and recommended identification of more protective antigen for development of vaccine suitable to Indian condition. The vaccine potential of recombinant antigens of *T. annulata* (rTaSP) and *H. anatolicum* (rHaa86) was evaluated by Jeyabal *et al*[92] with the outcome that the animals immunized with rHaa86 antigen partially protected calves against lethal challenge of *T. annulata*. As compared to earlier studies with rBm86 against *H. anatolicum,* this study indicated that a vaccine developed from a homologous antigen has better efficacy than the vaccine from a heterologous one.

One of the most important requirements for a commercially viable vaccine is that it should be cross-protective and more so in a country like India with diverse tick species. Therefore, new antigens are to be identified for development of a cross-protective effective vaccine. The identification of suitable antigens for a cattle tick vaccine and its development has become the subject of research around the world. Several molecules were identified, like Bm95[93], vitellin[94], 64P[95], trypsin inhibitors[96], SBm7462[97], ferritin 2[98] and subolesin[99–100]. Few of these molecules have shown promising results as vaccine candidates. Recently, the vaccine efficacy of recombinant subolesin against both homologous and heterologous challenge infestations[99–101] created renewed enthusiasm for the development of a broad-spectrum vaccine against different tick species. However, in India a study with recombinant subolesin vaccine using montanide 888 as adjuvant was found 44% effective against challenge infestation with *R. (B.) microplus*[102]. Further study is warranted with different dose and adjuvant combinations to validate the findings.

Phytoacaricides

To address the problems associated with the application of chemical acaricides, focus has been directed to-

wards the development of herbal acaricides (phytoacaricides) which are safe for animal use and there will be less chance of development of resistance to herbal formulations. In reality, however, botanical products have certain advantages but an equal number of drawbacks in practical use. The advantages of phytoacaricides lie in their rapid degradation and lack of persistence and bioaccumulation in the environment, which have been the major problems in synthetic chemical use.

Acaricidal property of plant extracts can provide a potential substitute to synthetic acaricides currently used for tick control as has been reported through testing of some plant extracts against *R.(B.) microplus*[103]. Acaricidal activity was reported from essential oils from leaves and flowers of *Ageratum houstonianum*, *Origanum onites* and *O. minutiflorum* against *R. (B.) annulatus* and *R. turanicus*[104–105]. The root and stem extracts of the *Petiveria alliacea* containing benzyltrisulfide (BTS) and benzyldisulfide (BDS) metabolites were found to have potent acaricidal activity[106]. The Cadina-4, 10 (15)-dien-3-one isolated from the leaves and stems of *Hyptis verticillata* disrupted the oviposition and hatching of *R. (B.) microplus* eggs[107]. But none of these compounds were tested against ticks resistant to chemical acaricides.

India is one of the 12 mega biodiversity centres having 45,000 plant species; its diversity is unmatched due to the 16 different agro-climatic zones, 10 vegetative zones, and 15 biotic provinces. However, little attention has been paid in India to explore the huge potentiality of the medicinal plants as acaricides. Khudrathulla and Jagannath[108] studied the effect of a methanolic extract of *Styloxanthes scabra* on ixodid ticks. The leaves of tobacco (*N. tabacum*) were found to be effective against *R. haemaphysaloides*[109] while the ethanolic extracts of *Annona squamosa* seed and *Azadirachta indica* leaves, bark and seed were found to have high efficacy of 70.8% and 80%, respectively, against *R.(B.) microplus*[110]. However, further progress on development of suitable formulation for the control of acaricide resistant ticks has not been made.

In the last few years, some credible information has been generated in an initiative of Indian Council of Agricultural Research through World Bank funded National Agricultural Innovation Project. The rhizome extract of *Acorus calamus* was characterized and evaluated for its acaricidal effect. It proved highly efficacious and 100% final mortality within 14 days post-treatment was recorded. *In vivo* experiments confirmed the efficacy of the extract up to 42%[111]. In a comprehensive study, 95% ethanolic extract of *Ricinus communis* was tested *in vitro* against cattle ticks. The extract significantly affected the mortality rate of ticks with an additional effect on reproductive physiology by inhibiting oviposition. The leaf extract was found effective in killing 48, 56.7 and 60% diazinon, deltamethrin and multi-acaricide resistant ticks, respectively. The HPTLC finger printing profile showed presence of quercetin, gallic acid, flavone and kaempferol indicating a synergistic acaricidal action. The authors postulated that 95% ethanolic extract of *R. communis* leaves can be used effectively in integrated format for the control of acaricide resistant ticks[112]. Ravindran *et al*[113] tested crude ethanolic extract of aerial parts of *Leucas aspera* for its acaricidal properties against *R. (B.) annulatus*. Adult tick mortality was significant at the concentration of 100 mg/ml and also inhibits eclosion of eggs from the treated ticks even at lower dilutions of the extract. Shyma *et al*[114] reported significant anti-tick activity of the crude methanolic extracts of leaves of *Datura stramonium*, *Azardirachta indica* and seeds of *Allium sativum* and *Carica papaya*. Amongst the different extracts tested, the extract prepared from the seeds of *C. papaya* was found most effective.

Despite many advantages, the phytoacaricide market has a number of major challenges and although there has been growth, it has not grown in a comparable way to botanical medicine market in the recent years. There has been considerable progress in the recent past in phytoacaricide research. However, most of the encouraging findings have been limited to *in vitro* studies only. The loss of efficiency of plant extracts when used in *in vivo* is a hindrance in the development of phytoacaricides. There is a need to conduct pharmacokinetic investigations and identification of marker compound in order to ensure that standard extracts are used. The effects of geographical and climatic variations on the chemical constituents within the same species need to be studied for better quality control. One more hurdle is expensive toxicology testing for new products which may have limited intellectual property (IP) protection and a relatively small market size. Other challenges include economical supply of plant product, biased perception regarding chemical acaricide *vis-a-vis* phytoformulation, quality control and lack of stability under sunlight.

CONCLUSION

The impact of TTBDs will continue to increase in many parts of the world including Indian subcontinent. Long-term use of hazardous chemicals is leading to the development of many societal, governmental and environmental issues. Amongst the different components of integrated vector management system, continuous moni-

toring of resistance using robust tools, development of vaccine against vector and formulation of eco-friendly phytoacaricides are showing a lot of promise. Recent advances in vector biology open new possibilities in target identification and vaccine development. The efforts to characterize the genomes of *I. scapularis* and *B. microplus* will impact positively 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. 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. 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 forced to look into strategic control of tick vectors in an integrated format. Globally two tick research groups are trying to develop an effective vaccine against tick vectors to reduce the transmission of TBD virus to man. In the same line, immunological control of *H. spinigera*, tick vector of KFD and other wild reservoirs of KFD virus in the endemic areas is expected to reduce the transmission of KFD to man. An oral vaccination strategy using baits could be an option to immunize monkeys, the amplifier host of KFD virus. The endemic potential of CCHF in India is huge given the ecological suitability of the virus and the regular outbreaks in the neighbouring country, Pakistan. The CCHF outbreak in Gujarat in 2011 calls for active surveillance using molecular tools to prevent or minimize further outbreaks in the country. The availability of better diagnostic tools is the need of the hour for CCHFV and rickettsial disease like ITT. Specific and sensitive tests like IFA to be made available inexpensively and the clinical staff to be trained in distinguishing ITT from similar viral and bacterial diseases. The development of a suitable prophylaxis system will be a great achievement for managing the future outbreaks of CCHFV and ITT.

Natural products have shown immense potential in controlling many disease conditions if used in scientific way. Although many reports are poring on possible effects of plant extracts against different pathogens including tick vector, the final product has not yet come. Recently, two promising formulations are developed for the control of ticks including chemical acaricide resistant tick populations and vigorous efforts are on to commercialize the same in Indian market. The products are expected to reduce the use of chemical acaricides for the control of ticks[115].

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