# Prevention and control strategies for ticks and pathogen transmission

J. de la Fuente [1, 2]*, K.M. Kocan [2] & M. Contreras [1]

(1) SaBio, Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ronda de Toledo s/n, 13005 Ciudad Real, Spain
(2) Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, United States of America
*Corresponding author: jose_delafuente@yahoo.com

**Summary**

Ticks and tick-borne pathogens have evolved together, resulting in a complex relationship in which the pathogen's life cycle is perfectly coordinated with the tick's feeding cycle, and the tick can harbour high pathogen levels without affecting its biology. Tick-borne diseases (TBDs) continue to emerge and/or spread, and pose an increasing threat to human and animal health. The disruptive impacts of global change have resulted in ecosystem instability and the future outcomes of management and control programmes for ticks and TBDs are difficult to predict. In particular, the selection of acaricide-resistant ticks has reduced the value of acaricides as a sole means of tick control. Vaccines provide an alternative control method, but the use of tick vaccines has not advanced since the first vaccines were registered in the early 1990s. An understanding of the complex molecular relationship between hosts, ticks and pathogens and the use of systems biology and vaccinomics approaches are needed to discover proteins with the relevant biological function in tick feeding, reproduction, development, immune response, the subversion of host immunity and pathogen transmission, all of which mediate tick and pathogen success. The same approaches will also be required to characterise candidate protective antigens and to validate vaccine formulations. Tick vaccines with a dual effect on tick infestations and pathogen transmission could reduce both tick infestations and their vector capacity for humans, animals and reservoir hosts. The development of integrated tick control strategies, including vaccines and synthetic and botanical acaricides, in combination with managing drug resistance and educating producers, should lead to the sustainable control of ticks and TBDs.

**Keywords**

Acaricide resistance – Cattle – Integrated control – Tick – Tick-borne disease – Tick vaccine – Vector-borne disease.

## Overview of ticks and their impact

Ticks are the oldest and most successful group of arthropods and have survived over millions of years with minimal change (1, 2). Ticks parasitise every class of terrestrial vertebrates worldwide (3). While the two major taxonomic families, Ixodidae (hard ticks) and Argasidae (soft ticks), contain approximately 850 species, the majority of ticks that are important as pests and/or vectors of pathogens are found in five subfamilies and six genera, including *Ixodes*, *Dermacentor*, *Amblyomma*, *Haemophysalis*, *Hyalomma* and *Rhipicephalus* (3, 4, 5, 6).

Even though ticks are dependent on blood as their sole food source and require a blood meal at each stage of their development, they not only continue to survive but prosper. The range and population size of several important tick species are increasing, thanks to their ability to adapt and disperse (7). In addition to the impact of tick infestations on vertebrate hosts, ticks transmit a greater variety of pathogens than any other arthropod group. The pathogens they transmit include fungi, viruses, bacteria (including rickettsiae) and protozoa, some of which are zoonotic. Furthermore, the presence of large tick populations has an impact upon recreational parks and renders them unsuitable for use during the tick season.

The economic loss due to ticks and tick-borne diseases (TBDs) is estimated to be billions of dollars annually (8). Throughout the complex tick life cycle, from the egg to the larva, nymph and adult stages, the quantity of blood required increases at each stage and the duration of feeding required by females lengthens from 7 to 14 days. These prolonged feeding periods contribute to a wide range of host impacts, and tick infestations alone can cause tick paralysis, allergic reactions and tick-based toxicosis, damage to hides, secondary infections, septicaemia, reductions in weight gain, decreased milk production and/or abortion (9, 10, 11). Despite the use of modern medical and veterinary treatments, the widespread use of acaricides and the development of new methods of tick control, long-term attempts to limit the geographic expansion of ticks or to reduce their populations in specific areas have been remarkably unsuccessful. The enormous reproductive capacity of ticks, in which one fully engorged female can produce 5,000 to 10,000 eggs, allows tick populations to rapidly rebound.

## Ticks as vectors of pathogens

Ticks were the first arthropods to be clearly established as vectors of disease-causing pathogens, and they transmit a wider variety of pathogenic microbes to humans and animals than any other arthropod (12). In the historic discovery by Smith and Kilbourne (13), *Boophilus annulatus* (recently reclassified as *Rhipicephalus [Boophilus] annulatus*) was shown to be the vector of *Babesia bigemina*, the protozoan that causes Texas cattle fever. Thereafter, other arthropods were found to act as vectors for pathogens. They include fleas, which carry *Yersinia pestis*, the bacterial agent of plague, and mosquitoes, which transmit yellow fever virus, filariosis and *Plasmodium* sp., the aetiologic agent of malaria.

Since these early discoveries of TBDs, a growing list of others has unfolded, including rickettsial spotted fevers, viral encephalitis and haemorrhagic fevers, Colorado tick fever and Kyasanur Forest disease, Q fever, ehrlichiosis, anaplasmosis and babesiosis (14). Lyme borreliosis was a notable TBD discovered in the 1970s, which was followed by the emergence of *Babesia microti,* the aetiologic agent of human babesiosis, in the 1980s. Since 2000, the emergence of human granulocytic anaplasmosis caused by *Anaplasma phagocytophilum* has been reported in the United States (USA) and is currently also an emerging disease of humans in Europe and other areas of the world. The latest emerging TBD, Bourbon virus, was reported in Kansas in 2014 (15). More than 16 specific TBDs of humans and 19 TBDs of livestock and companion animals have been described (16, 17) and this trend of the emerging TBD will most likely continue.

Ticks and the pathogens that they transmit have co-evolved, resulting in a complex relationship which is complementary to both the tick host and pathogen. The infection and transmission cycle of tick-borne pathogens is perfectly coordinated with the tick's feeding cycle. Ticks can harbour high pathogen levels without adverse effects on their biology and so pathogen infections do not affect their population densities.

## Impact of global change on ticks and tick-borne pathogens

While the increase in tick populations and the emergence of TBDs is not fully understood, global change has increased the vulnerability of human and animal populations to emerging and re-emerging TBDs (18, 19). Global change, defined as the environmental and climatic changes ascribed to human activity, includes disruptive processes that result in the instability of ecosystems and contribute to the uncertainty of future outcomes of management and control strategies for ticks and TBDs. Such disruptive processes affect the epidemiology of TBDs and may result in the emergence and re-emergence of ticks and TBDs (19, 20). The array of impacts of global change include the alteration of ecosystems and agro-ecosystems, incursions into natural habitats by humans and animals, shifts in land use, the movement of people and animals, the unrestricted importation of plants and animals, shifts in bird migration patterns, climate change, the selection of acaricide-resistant ticks, increased international trade and travel, civil unrest, the expansion of tick and TBD host ranges and governmental and/or management failure (21, 22, 23). In addition, tick control efforts have been disrupted by a deterioration in public safety, such as the illegal drug activities which have interrupted the control efforts of the Cattle Fever Tick Eradication Program (CFTEP) in and around quarantine areas in south Texas that border on Mexico (24). These impacts, compounded by the zoonotic nature of some TBDs, have presented public health and central veterinary authorities with the unexpected challenges and consequences of global change (24). Climate change alone has transformed some areas into habitats suitable for ticks and wildlife hosts. An underappreciated factor that has contributed to the increase of tick species and TBDs is the accidental importation of ticks on exotic animals during the international trade of live animals and reptiles, and the movements of travellers and their baggage, animal products, plant material and migratory birds. One study (25) recently detailed the unrestricted introduction of 100 non-native ticks into the USA over the past 50 years. Some of these imported ticks have the potential to transmit TBDs that could have an enormous impact on humans and food animal production.

# Tick eradication campaigns: successes, failures and lessons learned

Controlling ticks is difficult because they have few natural enemies and are not susceptible to immediate 'knock-down' effects. Control efforts are compounded by the complex tick life cycle, their enormous reproductive capacity and the number of hosts (one, two or three) that are required to complete the life cycle. Long-term attempts to limit geographic expansion or to decrease the size of tick populations responsible for human disease have been remarkably unsuccessful, with the exception of the widespread use of acaricides for the eradication of *R.* (*Boophilus) microplus* during the CFTEP campaign in the southern USA during the early 1900s, and of *Ixodes persulcatus* and tick-borne encephalitis in the former Soviet Union from 1965 to 1971 (26). However, the limitations of chemical control have now been realised since the intensive and repeated use of acaricides, the centrepiece of these control attempts, has resulted in the selection of acaricide-resistant ticks.

Insight into the challenges of tick control was provided by two large-scale tick eradication campaigns: the CFTEP to eradicate *R. (Boophilus) microplus* and the programme to eradicate *Amblyomma variegatum* in the French West Indies and Puerto Rico.

## The Cattle Fever Tick Eradication Program

The CFTEP tick control campaign was begun in the early 1900s in the southern USA to eradicate *R. (Boophilus) microplus*. This tick species is the vector of *B. bigemina* and *B. bovis*, the aetiologic agents of Texas cattle fever, and *Anaplasma marginale*, which causes bovine anaplasmosis. In addition to the damage caused by tick infestations, these two diseases have also had a severe impact on cattle production and resulted in enormous economic losses (9, 10, 11).

## The programme to eradicate *Amblyomma variegatum*

The tropical bont tick, *Amblyomma variegatum,* was introduced onto the Caribbean island of Guadeloupe in the early 1800s. The French West Indies and Puerto Rico were targeted in the 1990s because of this species' impact on local livestock production and the threat of its introduction into the USA. *Amblyomma variegatum* is the vector of *Ehrlichia rumiantium,* the causative agent of heartwater disease in ruminants (also known as cowdriosis), and the threat of introduction of this tick/disease into the cattle population in the USA continues to be of great concern.

## Lessons learned

Tick control by the application of acaricides was the centrepiece of both these tick campaigns because of its immediate effect and because this is a cost-effective means of reducing tick populations. The Cattle Fever Tick Eradication Program had the advantage that *R.* (*Boophilus*) spp. are one-host ticks that parasitise and complete their life cycle on a single host, with cattle as that preferred host, whereas the life cycle of the three-host tick *A. variegatum* involves multiple host species. Therefore, CFTEP could be focused wholly on the treatment of cattle. The bont tick campaign fell short of its goal because the systematic programme and supply of acaricides were interrupted when donor countries withdrew funding. More recently, slow-release tags impregnated with an aggregation-attachment pheromone and acaricide have been tested and show promise if the issues raised by this control method can be addressed (27).

The intensely orchestrated CFTEP campaign resulted in the declared eradication of *R. microplus* in most southern states by 1943. However, complete eradication was not possible along the border of Texas and Mexico, and small outbreaks continued to occur. In recent years these outbreaks have dramatically increased due to the complex series of impacts of global change (28), the most notable of which are the selection of ticks resistant to multiple acaricides (29), the role of white-tailed deer (*Odocoileus virginianus*) as alternative hosts for the cattle fever tick (30), changes in human populations and land use, climate change, and the impact of cartel violence on control efforts (24).

The overriding problem of the selection of acaricide-resistant ticks has also been reported in tick control efforts worldwide, including those in Australia, Africa, Mexico, South America and the Middle East (29, 31, 32). The continued use of acaricides in tick control campaigns is now dependent on the identification of resistance markers in ticks and the periodic monitoring of tick populations for resistance against multiple acaricides. Clearly, all of these attempts to control or eradicate ticks have demonstrated that long-term and consistent commitment is required for tick control and that the eradication of any tick species is not likely to be a realistic goal.

# Development of vaccines for control of tick infestations

New approaches for tick control depend upon defining the molecular interactions between hosts, ticks and pathogens, to enable the discovery of key molecules that could be tested for their ability to intervene in tick–pathogen cycles (33). Tick vaccines offer the important advantage of being a cost-

effective and environmentally friendly alternative, with a dual effect of reducing tick infestations and preventing ticks from transmitting disease-causing pathogens. Tick antigens studied thus far have demonstrated multiple tick impacts when used in a vaccine format. These include reductions in:

– tick infestations and fertility
– pathogen infection
– vector capacity for pathogen transmission
– tick response to pathogen infection.

Importantly, the limited use of cattle tick vaccines has demonstrated our greatly reduced dependence on acaricides, which lowers the risk of the selection of acaricide-resistant populations (34, 35). Characterisation of the molecular mechanisms that mediate tick–pathogen and tick–host interactions will probably provide new targets for vaccines to reduce tick infestations and tick vector competency for pathogens (33, 36, 37). Recent research has provided evidence that the co-evolution and long-term survival of ticks and TBDs involve the genetic traits of both ticks and pathogens in which pathogen ligands and tick receptors enable the pathogenic infection of ticks (38, 39).

# The historical perspective

Allen and Humphreys (40) demonstrated the feasibility of controlling tick infestations by immunising hosts with tick antigens. Following their preliminary results, Willadsen and his group at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, discovered the tick protective antigen Bm86 from *R.* (*Boophilus*) *microplus* (41, 42, 43, 44), which subsequently led to the development of tick vaccines for cattle in the early 1990s that induced immunological protection against tick infestations (35, 42, 44, 45). Between 1993 and 1997, two vaccines using recombinant Bm86 were registered in Latin American countries (Gavac) and Australia (TickGARD) (34, 35, 46, 47, 48). These tick vaccines, which have similar efficacy for the control of cattle tick infestations (35, 49), are the only commercial vaccines for the control of ectoparasites and they have led the way for research on the development of vaccines for arthropod vectors of pathogens that affect human and animal health. Bm86 vaccines have continued to be used for over a decade since their registration and commercialisation, demonstrating the importance and potential impact of tick vaccines (35).

The use of Bm86-based tick vaccines resulted in a reduction in the number, weight and reproductive capacity of engorging female ticks, and so the greatest effect of these vaccines is their reduction of tick infestations in subsequent generations (35). Experimental data and model systems have shown that a vaccine efficacy greater than 50% is sufficient to reduce larval infestations in subsequent generations without eradicating ticks (48, 50, 51, 52, 53, 54).

# Discovery and characterisation of new tick protective antigens

The experience with Bm86-based vaccines revealed the need for the development of improved tick vaccines. However, the identification of tick protective antigens has been the limiting factor in this approach. The critical step of the identification and characterisation of novel tick protective antigens requires the implementation of high-throughput methods. Tick protective antigens have been identified by:

– the evaluation of proteins after host immunisation and tick challenge that were derived from the progressive fractionation of crude tick extracts (41, 55)

– immunomapping of tick antigens that elicit an antibody response in an infested host, or with antibodies against tick tissue extracts (56, 57, 58, 59, 60, 61)

– reverse genetics and testing tick proteins that were considered important for tick feeding and reproduction and for the parasite's function and/or survival (33, 54).

Recently, new and exciting possibilities for predicting, screening and identifying protective antigens against tick infestations (62) have been provided through complementary DNA (cDNA), expressed sequence tags and genome and proteome databases (e.g. those of the National Center for Biotechnology Information, VectorBase, and the Exon distribution server of the National Institute of Allergy and Infectious Diseases) of different tick tissues, their developmental stages, and the genes expressed in response to various stimuli (i.e. tick feeding or infection with pathogens). The prediction of tick protective antigens can now also be carried out *in silico*, using defined selection criteria and bioinformatics tools (63, 64, 65, 66). Screening platforms used to identify tick protective antigens include expression library immunisation (67), suppression subtractive hybridisation (68), *in vitro* tick feeding systems such as capillary feeding (69, 70, 71), and RNA interference (RNAi) (72, 73, 74). RNA interference has become the most important tool for genetic manipulation in ticks (75, 76), and this approach might also enable the identification of antigens that interfere with pathogen development and transmission (77, 78).

Since the 1980s, many tick protein extracts and subunits have been proposed as protective antigens for the control of tick infestations (79). However, only some of these antigens have been evaluated in vaccination experiments, which is necessary for their validation as candidate tick protective antigens. Some putative vaccine antigens failed to protect hosts against tick infestation (80, 81, 82), possibly because they were expressed at low levels or were inaccessible to host antibodies. Over the course of their evolution, ticks have most probably developed mechanisms to counteract the effects of the host immune response, particularly

for exposed antigens. These mechanisms might include immunosuppressive molecules secreted during tick feeding and multi-gene families with redundant biological functions (83, 84, 85). However, it was recently demonstrated that tick feeding affects the host immune response by inhibiting the differentiation of mature B cells into plasma cells at the feeding site, but does not alter the formation of memory B cells required for vaccine efficacy (85). Apart from Bm86 and Bm95, examples of tick antigens that have shown promising results in vaccination trials are the 64P cement protein, subolesin and ferritin 2. Recently, Nijhof *et al.* (86) identified a novel protein, ATAQ (named after a part of its signature peptide), in metastriate ticks that has structural similarities to Bm86. Sequence analysis of ATAQ suggests that this gene might be a paralogue of Bm86. Although its function is unknown, ATAQ is expressed in both tick midguts and Malpighian tubules, whereas Bm86 is expressed only in midguts. The vaccine efficacy of recombinant ATAQ proteins against tick infestations has not yet been evaluated, but they might be good candidates for use in vaccines against tick infestations.

New research approaches for identifying vaccine candidate antigens involve functional genomics and reverse genetics (87, 88, 89, 90) that could be synthesised in the new vaccinology (33). Furthermore, recent results have challenged the paradigm that protective antigens for tick vaccines must be extracellular proteins and thus have greatly expanded the repertoire of candidate protective antigens (89, 91, 92, 93, 94).

# Dual vaccines for controlling tick infestations and the transmission of tick-borne pathogens

Vaccine trials with Bm86-based vaccines resulted in a reduction in the incidence of babesiosis and the transmission of *Babesia* spp. where the target ticks were the main vectors. The reduction of bovine anaplasmosis was not as notable, most probably because this pathogen is also transmitted mechanically by the blood-contaminated mouthparts of biting insects and fomites (34).

Tick–pathogen co-evolution involves the genetic traits of both the vector and the pathogen (39). Recent reports have confirmed the presence of tick receptors for tick-borne pathogens. Pal *et al.* (38) identified a tick receptor (TROSPA) for the OspA bacterial ligand that is required for *B. burgdorferi* colonisation of *I. scapularis*. Furthermore, genetic factors have been associated with intraspecific variation in tick competence for tick-borne pathogens such as *Anaplasma* spp., *Babesia* spp. and *Borrelia* spp. (95, 96, 97, 98, 99).

Therefore, focusing on the characterisation of the molecular events at the tick–pathogen interface will likely result in the identification of candidate tick antigens directed towards the control of both ticks and pathogen transmission (33, 88, 100). For example, recent experiments demonstrated that *A. marginale* and *A. phagocytophilum* modulate gene expression in infected ticks and tick cells (77, 78, 101, 102, 103, 104, 105, 106, 107, 108, 109). Genes differentially expressed in response to pathogen infection were used for RNAi studies to discover their function during infection. Some genes were shown to encode for proteins involved in tick defences to limit pathogen infection, while the expression of other genes was manipulated by the pathogen to increase infectivity (77, 78, 101). Differential gene expression in response to microbial infection has also been shown for *I. ricinus* and *I. scapularis* with *B. burgdorferi* (110, 111); *D. variabilis* with *Rickettsia montanensis* (112, 113); *R. appendiculatus* with *Theileria parva* (114), and *R. microplus* with *B. bovis* (115). The genes involved in tick–pathogen interactions are good candidates for the development of vaccines to control pathogen transmission (39). In addition, suppressing the gene products required for pathogen infection, multiplication and transmission should reduce the tick vectorial capacity, and suppressing the tick antimicrobial response might result in higher pathogen infection levels that would be detrimental to the tick.

Recent experiments have illustrated how vaccination with tick antigens also affects tick vector capacity. Labuda *et al.* (116) and de la Fuente *et al.* (117) reported that the vaccination of mice with 64P and subolesin antigens prevented the transmission of tick-borne encephalitis virus by *I. ricinus* and decreased *I. scapularis* infection by *A. phagocytophilum*, respectively. Jeyabal *et al.* (118) provided evidence that cattle vaccination with the Bm86 orthologue of *Hyalomma anatolicum anatolicum*, Haa86, might reduce tick transmission of *T. annulata*. Antibodies against the *I. scapularis* salivary protein Salp15 protected mice from the Lyme disease agent *B. burgdorferi* that binds to this protein to facilitate tick transmission and host infection (119). Cattle vaccination with recombinant subolesin reduced *R. microplus* infection levels with *A. marginale* and *B. bigemina* (92, 120). Recently, a vaccine trial on cattle and sheep farms in Sicily demonstrated the effect of subolesin on tick infestations and weight and on the prevalence of some tick-borne pathogens and pathogen genotypes (121). These experiments demonstrated that tick vaccines might contribute to the control of tick-borne pathogens by decreasing the exposure of susceptible hosts to ticks and by reducing tick vectorial capacity (35, 54, 92, 122, 123). Therefore, the development of dual-target recombinant vaccines could be directed towards the control of both ticks and tick-borne pathogens.

## New vaccine formulations

Although this field is less advanced than other research areas, the development of new expression systems and cost-effective processes to produce recombinant antigens is essential for the successful and economical production of tick vaccines (124). Research in this area should be directed towards reducing production costs while increasing the yield and efficacy of vaccine antigens (124, 125, 126, 127, 128, 129).

The identification of protective antigens conserved across tick species provides an opportunity to develop vaccines to protect hosts against infestations by multiple tick species (91). As discussed previously (91, 130), tick antigens such as subolesin/akirin conserved among arthropod vectors may also be conserved in vertebrate hosts, raising the question of safety because of the potential for inducing autoimmune responses that might be damaging to the host. However, it is expected that the antibody response would be primarily directed against non-self epitopes, thereby reducing the possibility of detrimental effects to the host.

Research on sugar epitopes in tick proteins and their potential use for controlling tick infestations and pathogen transmission is still in its infancy. However, carbohydrate targets might lead to a universal transmission-blocking vaccine for the control of multiple pathogens (131, 132).

Other possibilities for improving the efficacy of vaccine formulations are the development of improved adjuvants and rational antigen design. Adjuvant research for tick vaccines is needed to improve vaccine efficacy, in order to extend the duration of the antibody response and thus the period of protection in vaccinated hosts (133, 134). An example of rational antigen design is the evaluation of chimeric antigens containing protective epitopes from Bm86 (125, 128, 135) and from tick subolesin and mosquito akirin (93, 94, 130, 136). The subolesin/akirin chimeric antigen might be useful for developing vaccines to control multiple arthropod vector infestations (91, 94). The possible drawbacks of this approach include the induction of antibodies with specificities different from those induced by the native protein and the selection of escape mutants by oligoclonal antibodies produced in vaccinated hosts, although this might not be a problem if mutation is constrained by the functional requirements of the protein (91).

## Additional approaches considered for tick control

Wildlife has been shown to play an essential role in sustaining and expanding tick populations (137, 138). Thus, tick control interventions that could efficiently be applied to wildlife may be essential for tick control in some regions. These interventions could include the application of acaricides (139), wildlife host management (140), autocidal control (141, 142) and vaccination (143), which have been proven to effectively control tick populations under field and/or controlled conditions. However, additional developments are required before tick control can effectively be applied to wildlife to reduce the impact of ticks on human health and animal health and production. For example, the development of oral vaccine formulations, similar to those under investigation for tuberculosis control in wildlife, could contribute to the application of vaccination programmes to deer and other wild tick hosts (144, 145).

Biological control of ticks using entomopathogenic fungi and nematodes has been demonstrated under laboratory conditions but further research is needed to prove its efficacy and practical application under field conditions (146, 147, 148, 149). Although the presence of some insect, spider and plant species has been associated with lower or higher levels of tick infestation (see, for example, 150, 151), the use of these organisms for tick control has not been proven and is unlikely to succeed, due to the fact that ticks have very few natural enemies and can still reach a high prevalence in their presence if the appropriate hosts are available.

Recently, research into the discovery and characterisation of botanical acaricides has begun to promise possibilities for more effective and environmentally friendly acaricides for tick control (152).

## Future strategies for tick control

New tick control strategies are urgently needed because ticks and TBDs will most likely continue to emerge and/or spread to new areas of the world, posing an ever-increasing threat to human and animal health. Complete tick eradication does not appear to be a realistic goal. Moreover, tick eradication remains a controversial issue because it might result in the elimination of enzootic stability for tick-borne pathogens, which would result in highly susceptible host populations for tick-borne pathogens and their associated diseases. Additionally, tick eradication might be difficult to sustain during conditions favourable for tick population expansion (21, 153, 154, 155, 156). It would also reduce biodiversity and the arsenal of bioactive compounds that might be isolated from ticks (83–157).

However, the control and reduction of tick populations is possible by employing control programmes with integrated and consistent approaches (158, 159). The most successful tick control programmes are those that have been mandated and carried out by governments, involving regimented programmes with careful monitoring of tick populations. This integrated approach could include the use of vaccines,

synthetic and botanical acaricides, producer education and the monitoring and management of drug resistance as well as of tick and host populations. Reducing our dependence on acaricides will also reduce their drawbacks – the selection of resistant ticks, environmental contamination and toxic reactions in animals and humans – as well as reducing the expense of treatment. The complicated nature of current and future problems driven by global change requires that societal, environmental, scientific and policy considerations be integrated to develop the most effective and sustainable strategies and goals to prevent increases in tick populations and outbreaks of TBDs (29).

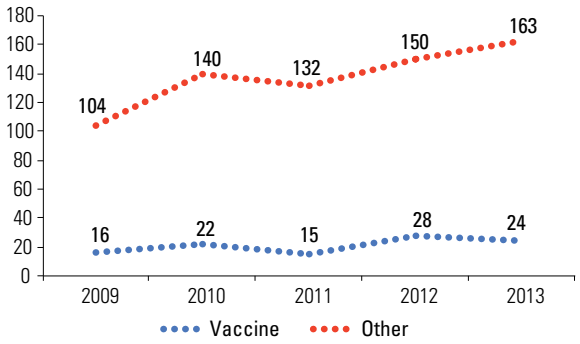
Tick vaccines have been shown to be a cost-effective and environmentally sound approach to tick control. After more than a decade of commercial tick vaccine use in the field, effective control of cattle tick populations has been achieved, accompanied by improved cattle production and reduced dependence on acaricides. The long-term benefits of reduced acaricide use are considerable and include environmental and health considerations and reduced selection of resistant ticks from repeated acaricide treatments (160). If the rate of selection of acaricide-resistant ticks can be decreased, the useful life of an acaricide will be prolonged, providing a further control option until more effective tick vaccines are developed. Additionally, although it might be possible for ticks to develop resistance to tick vaccines, it is highly unlikely because of the polyclonal nature of the protective antibody response (161). Together, these results support the importance of tick vaccines in integrated control strategies in regions where acaricide-resistant ticks have already or may become a major problem for the cattle industry.

The development of improved vaccines in the future will be greatly enhanced by molecular technologies and systems biology approaches that are already contributing to the characterisation of tick–pathogen and tick–host interactions (33). The discovery of new candidate vaccine antigens for the control of tick infestations and pathogen transmission requires the development of effective screening platforms and algorithms that allow the analysis and validation of data produced through systems biology approaches to tick research. The combination of tick-derived and pathogen-derived antigens may result in more effective vaccines to control TBDs. Tick vaccines that reduce both tick infestations and pathogen transmission could be used to vaccinate human and animal populations at risk, so as to reduce host exposure to ticks while also reducing the number of infected ticks and their vectorial capacity for pathogens that affect human and animal health worldwide.

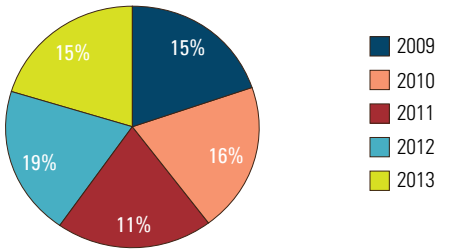
In the future, transgenic or paratransgenic ticks (36), genetically modified tick-borne pathogens (162) and vertebrate hosts which have been genetically modified to confer resistance to pathogen infection may be produced and combined with other tick control interventions.

Effective integrated pest management programmes and modelling programs that take vaccines into account should be developed and implemented, with the aid of government intervention in some countries, as needed. Recent publications and patents show a growing trend in tick control research with about one-quarter of this effort devoted to tick vaccines (Fig. 1). Although the lack of funding and commercial interest might be a limiting factor for the development of new tick control tools, the growing need for improved, effective and environmentally sound methods for tick control should motivate producers, stakeholders, scientists and the general public to achieve this goal.

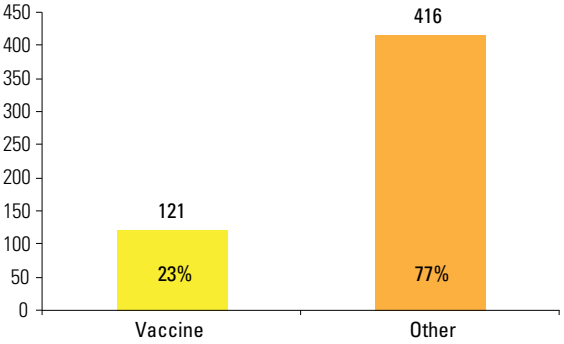
***a)* Number of publications**




***b)* Percentage of publications addressing tick vaccines***




***c)* Number of patents****




* Data obtained by searching the PubMed database (www.ncbi.nlm.nih.gov) for publications on 'tick control' AND/NOT 'vaccine'

** Data obtained by searching the World Intellectual Property Organization, the United States Patent and Trademark Office, the European Patent Office and the Free Patents Online databases, using Biowebspin (www.biowebspin.com/publications-patents/) in May 2014.

**Fig. 1**
**Research trends in tick control: vaccines and other methods**

## Acknowledgements

The authors thank the members of their laboratories for fruitful discussions. The preparation of this paper was supported by Grant BFU2011-23896, European Union FP7 ANTIGONE Project Number 278976 and the Walter R. Sitlington Endowed Chair for Food Animal Research to K.M. Kocan.

# Stratégies de prévention et de contrôle des tiques et de la transmission d'agents pathogènes par les tiques

J. de la Fuente, K.M. Kocan & M. Contreras

**Résumé**

Les tiques et les agents pathogènes transmis par les tiques ont évolué ensemble et noué des liens complexes ; ainsi, le cycle évolutif des agents pathogènes est parfaitement en phase avec le cycle alimentaire des tiques et ces dernières peuvent être porteuses d'une abondante charge pathogène sans que cela affecte leur propre biologie. Nous assistons à une émergence et/ou à une propagation continue des maladies transmises par les tiques, qui représentent une menace croissante pour la santé humaine et animale. L'instabilité induite au sein des écosystèmes par les effets perturbateurs des changements planétaires ne permet pas d'anticiper les résultats futurs des programmes de gestion et de contrôle des tiques et des maladies transmises par les tiques. En particulier, la sélection de tiques devenues résistantes aux acaricides a réduit l'intérêt de ces produits en tant que seuls outils de lutte contre les tiques. Les vaccins constituent une solution alternative, mais l'utilisation de vaccins contre les tiques n'a guère évolué depuis l'enregistrement des premiers vaccins de ce type au début des années 90. Une bonne connaissance de la complexité des liens moléculaires entre les hôtes, les tiques et les agents pathogènes et le recours aux systèmes biologiques et aux méthodes de la vaccinomique permettent d'identifier les protéines dotées de fonctions biologiques pertinentes chez les tiques en termes d'alimentation, de reproduction, de croissance, de réponse immune, de subversion de l'immunité chez l'hôte et de transmission des agents pathogènes, autant d'aspects qui participent au succès des tiques et des agents pathogènes dont elles sont porteuses. Il faudra recourir aux mêmes méthodes pour caractériser des antigènes protecteurs candidats et pour valider la composition de vaccins. Des vaccins anti-tiques à double effet (contre l'infestation par les tiques et contre la transmission d'agents pathogènes) pourraient contribuer à réduire ces infestations et à limiter la capacité vectorielle des tiques chez l'homme, les animaux et les hôtes réservoirs. La mise au point de stratégies intégrées de lutte contre les tiques, dont les vaccins et les acaricides de synthèse et botaniques, associée à la gestion de la résistance aux médicaments et à une sensibilisation des producteurs devrait aboutir à un contrôle durable des tiques et des maladies transmises par les tiques.

**Mots-clés**

Bovins – Lutte intégrée – Maladie à transmission vectorielle – Maladie transmise par les tiques – Résistance aux acaricides – Tique – Vaccin contre les tiques.

# Estrategias de prevención y control de las garrapatas y la transmisión de patógenos

J. de la Fuente, K.M. Kocan & M. Contreras

**Resumen**

Las garrapatas y los patógenos que transmiten han evolucionado conjuntamente, lo que ha desembocado en una compleja relación en la cual el ciclo vital del patógeno está perfectamente coordinado con el ciclo de alimentación de la garrapata, que puede albergar un elevado nivel de patógenos sin que su biología resulte afectada. Las enfermedades transmitidas por garrapatas siguen surgiendo y/o propagándose, hecho que entraña una creciente amenaza para la salud de personas y animales. Los perturbadores efectos del cambio planetario han generado inestabilidad en los ecosistemas, por lo que resulta difícil predecir los resultados que van a deparar en el futuro los programas de gestión y control de las garrapatas y las enfermedades que transmiten. En particular, la selección de garrapatas resistentes a los acaricidas ha restado valor a estas sustancias como medio único de control. Las vacunas constituyen un método de lucha alternativo, aunque su utilización no ha avanzado desde que a principios de los años 90 se registraron las primeras vacunas contra garrapatas. Es preciso entender la compleja relación molecular entre anfitriones, garrapatas y patógenos, utilizar la biología de sistemas y aplicar métodos de vacunómica para descubrir proteínas dotadas de la función biológica pertinente en la alimentación, reproducción, desarrollo y respuesta inmunitaria de la garrapata, la alteración de la inmunidad del anfitrión y la transmisión del patógeno, factores todos ellos que determinan el éxito de la garrapata y el patógeno. También serán necesarios tales métodos para caracterizar posibles antígenos protectores y para validar formulaciones de vacuna. Las vacunas contra garrapatas que tienen un efecto dual (sobre las infestaciones por el parásito y la transmisión de patógenos) podrían reducir a la vez las infestaciones por garrapatas y su capacidad para transmitir enfermedades a personas, animales y anfitriones que constituyan un reservorio. La elaboración de estrategias integradas de lucha contra las garrapatas, que incluyan el uso de vacunas y el de acaricidas sintéticos y botánicos, junto con la gestión de la farmacorresistencia y una labor pedagógica dirigida a los productores, debería redundar en un control duradero de las garrapatas y las enfermedades que transmiten.

**Palabras clave**

Control integrado – Enfermedad transmitida por garrapatas – Enfermedad transmitida por vectores – Ganado vacuno – Garrapata – Resistencia a los acaricidas – Vacuna contra garrapatas.

## References

1. Dennis D.T. & Piesman J.F. (1991). – Tick-borne diseases of humans (J.L. Goodman, D.T. Dennis & D.E. Sonenshine, eds). ASM Press, Washington, DC, 3–11.

2. De la Fuente J. (2003). – The fossil record and the origin of ticks (Acari: Parasitiformes: Ixodida). *Experim. Appl. Acarol.*, **29** (3–4), 331–344.

3. Sonenshine D.E. (1991). – Introduction. *In* Biology of ticks (D.E. Sonenshine, ed.), Vol. I. Oxford University Press, New York, 3–12.

4. Barker S.C. & Murrell A. (2008). – Systematics and evolution of ticks with a list of valid genus and species names. *In* Ticks: biology, disease and control (A.S. Bowman & P.A. Nuttall, eds). Cambridge University Press, Cambridge, 1–39.

5. Sonenshine D.E., Lane R.S. & Nicholson W.L. (2002). – Ticks (Ixodida). *In* Medical and veterinary entomology (G. Mullen & L. Durden, eds). Academic Press, New York, 517–558.

6. De la Fuente J., Estrada-Peña A., Venzal J.M., Kocan K.M. & Sonenshine D.E. (2008). – Overview: ticks as vectors of pathogens that cause disease in humans and animals. *Front. Biosci.*, **13**, 6938–6946.

7. Korch G.W. Jr (1991). – Geographic dissemination of tick-borne zoonoses. *In* Biology of ticks (D.E. Sonenshine, ed.), Vol. I. Oxford University Press, New York, 139–197.

8. Jonejan F. & Uilenberg G. (2004). – The global importance of ticks. *Parasitol.*, **129**, S3–S14.

9. De Castro J.J. (1997). – Sustainable tick and tick-borne disease control in livestock improvement in developing countries. *Vet Parasitol.*, **71** (2–3), 77–97.

10. Alim M.A., Das S., Roy K., Masuduzzaman M., Sikder S., Hassan M.M., Siddiki A.Z. & Hossain M.A. (2012). – Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. *Pakistan Vet. J.*, **32** (2), 221–224.

11. Valente P.P., Amorim J.M., Castilho R.O., Leite R.C. & Ribeiro M.F. (2014). – *In vitro* acaricidal efficacy of plant extracts from Brazilian flora and isolated substances against *Rhipicephalus microplus* (Acari: Ixodidae). *Parasitol. Res.*, **113** (1), 17–23.

12. Munderloh U.G., Jauron S.D. & Kurtti T.J. (2005). – The tick: a different kind of host for human pathogens. *In* Tick-borne diseases of humans (J.L. Goodman, D.T. Dennis & D.E. Sonenshine, eds). ASM Press, Washington, DC, 37–64.

13. Smith T. & Kilbourne F.L. (1893). – Investigations into the nature, causation, and prevention of Texas or southern cattle fever. *Bur. Anim. Ind. Bull.*, **1**, 177–304.

14. Parola P. & Raoult D. (2001). – Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat. *Clin. Infect. Dis.*, **32** (6), 897–928.

15. Lowes R. (2014). – Discovery of Bourbon virus raises many questions. *Medscape Med. News*, 24 December. Available at: www.medscape.com/viewarticle/837229 (accessed on 22 January 2015).

16. Nicholson W., Sonenshine D.E., Lane R.S. & Uilenberg G. (2009). – Ticks (Ixodida). *In* Tick-borne diseases of humans (L.A. Durden & G. Mullen, eds). ASM Press, Washington, DC, 150–163.

17. Sonenshine D.E. & Roe R.M. (eds) (2014). – The biology of ticks, 2nd Ed., Vol. I. Oxford University Press, New York, 560 pp.

18. Sutherst R. (2001). – The vulnerability of animal and human health to parasites under global change. *Int. J. Parasitol.*, **31** (9), 933–948.

19. Camill P. (2010). – Global change. *Nat. Educ. Knowl.*, **2**, 49.

20. Estrada-Peña A., Ayllón N. & de la Fuente J. (2012). – Impact of climate trends on tick-borne pathogen transmission. *Front. Physiol.,* **3**, 64. doi:10.3389/fphys.2012.00064.

21. Pérez de León A.A., Strickman D.A., Knowles D.P., Fish D., Thacker E., de la Fuente J., Krause P., Wike S.K., Miller R.S., Wagner G.G., Almazán C., Hillman R., Messenger M.T., Ugstad P.O., Duhaime R.A., Teel P.D., Ortega-Santos A., Hewitt D.G., Bowers E.J., Bent S., Cochran M.H., McElwain T.F., Scoles G.A., Suarez C.E., Davey R., Howell Freeman J.M., Lohmeyer K., Li A., Guerrero F., Kammlah D., Phillips P. & Pound J.M. (2010). – One Health approach to identify research needs in bovine and human babesioses: workshop report. *Parasit. Vectors,* **3** (1), 36.

22. Estrada-Peña A., Ostfeld R.S., Peterson A.T., Poulin R. & de la Fuente J. (2014). – Effects of environmental change on zoonotic disease risk: an ecological primer. *Trends Parasitol.*, **30** (4), 205–214.

23. Gortazar C., Reperant L.A., Kuiken T., de la Fuente J., Boadella M., Martínez B., Ruiz-Fons J.F., Estrada-Peña A., Drosten C., Medley G., Ostfeld R., Peterson T., VerCauteren K., Menge C., Artois M., Schultsz C., Delahay R., Serra-Cobo J., Poulin R., Keck F., Aguirre A.A., Henttonen H., Dobson A.P., Kutz S. & Lubroth J. (2014). – Crossing the interspecies barrier: opening the door to zoonotic pathogens. *PLoS Pathog.*, **10** (6), e1004129. doi:10.1371/journal.ppat.1004129.

24. Aguilar J. (2011). – Cartel violence complicates tick eradication plan. *NY Times*, 16 April 2011. Available at: www.nytimes.com/2011/04/17/us/17ttticks.html?_r=0 (accessed on 9 January 2015).

25. Burridge M.J. (2011). – Non-native and invasive ticks. Threats to human and animal health in the United States. University Press of Florida, Gainesville, Florida, 292 pp.

26. Korenberg E.I. & Kovalevskii Y.V. (1999). – Main features of tick-borne encephalitis epidemiology in Russia. *Zentralbl. Bakteriol.*, **289** (5–7), 525–539.

27. Kelly P.J., Lucas H.M., Randolph C.M., Ackerson K., Blackburn J.K. & Dark M.J. (2014). – Efficacy of slow-release impregnated tags with aggregation-attachment pheromone and deltamethrin for control of *Amblyomma variegatum* on St. Kitts, West Indies. *Parasit. Vectors,* **7**, 182.

28. Pérez de León A.A., Teel P.D., Auclair A.N., Messenger M.T., Guerrero F.D., Schuster G. & Miller R.J. (2012). – Integrated strategy for sustainable cattle fever tick eradication in USA is required to mitigate the impact of global change. *Front. Physiol.*, **3**, 195–211.

29. Abbas R.Z., Zaman M.A., Colwell D.D., Gilleard J. & Iqbal Z. (2014). – Acaricide resistance in cattle ticks and approaches to its management: the state of play. *Vet. Parasitol.*, **203** (1–2), 6–20.

30. Busch J.D., Stone N.E., Nottingham R., Araya-Anchetta A., Lewis J., Hochhalter C., Giles J.R., Gruendike J., Freeman J., Buckmeier G., Bodine D., Duhaime R., Miller R.J., Davey R.B., Olafson P.U., Scoles G.A. & Wagner D.M. (2014). – Widespread movement of invasive cattle fever ticks (*Rhipicephalus microplus*) in southern Texas leads to shared local infestations on cattle and deer. *Parasit. Vectors*, **7**, 188.

31. Angus B.M. (1996). – The history of the cattle tick *Boophilus microplus* in Australia and achievements in its control. *Int. J. Parasitol.*, **26** (12), 1341–1355.

32. Mendes M.C., Pereira J.R. & Prado A.P. (2007). – Sensitivity of *Boophilus microplus* (Acari: Ixodidae) to pyrethroids and organophosphate in farms in the Vale do Paraíba region, São Paulo, Brazil. *Arq. Inst. Biol.*, **74**, 81–85.

33. De la Fuente J. & Merino O. (2013). – Vaccinomics, the new road to tick vaccines. *Vaccine*, **31** (50), 5923–5929.

34. De la Fuente J., Rodríguez M., Redondo M., Montero C., García-García J.C., Méndez L., Serrano E., Valdés M., Enriquez A., Canales M., Ramos E., Boué O., Machado H., Lleonart R., de Armas C.A., Rey S., Rodríguez J.L., Artiles M. & García L. (1998). – Field studies and cost-effectiveness analysis of vaccination with Gavac™ against the cattle tick *Boophilus microplus*. *Vaccine*, **16** (4), 366–373.

35. De la Fuente J., Almazán C., Canales M., Pérez de la Lastra J.M., Kocan K.M. & Willadsen P. (2007). – A ten-year review of commercial vaccine performance for control of tick infestations on cattle. *Ann. Hlth Res. Rev.*, **8** (1), 23–28.

36. De la Fuente J., Kocan K.M., Almazán C. & Blouin E.F. (2008). – Targeting the tick–pathogen interface for novel control strategies. *Front. Biosci.*, **13**, 6947–6956.

37. Kocan K.M., de la Fuente J. & Blouin E.F. (2008). – Advances toward understanding the molecular biology of the *Anaplasma*–tick interface. *Front. Biosci.*, **13**, 7032–7045.

38. Pal U., Li X., Wang T., Montgomery R.R., Ramamoorthi N., Desilva A.M., Bao F., Yang X., Pypaert M., Pradhan D., Kantor F.S., Telford S., Anderson J.F. & Fikrig E. (2004). – TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. *Cell,* **119** (4), 457–468.

39. De la Fuente J., Kocan K.M., Blouin E.F., Zivkovic Z., Naranjo V., Almazán C., Esteves E., Jongejan J., Daffre S. & Mangold A.J. (2010). – Functional genomics and evolution of tick–*Anaplasma* interactions and vaccine development. *Vet. Parasitol.*, **167** (2–4), 175–186.

40. Allen J.R. & Humphreys S.J. (1979). – Immunization of guinea pigs and cattle against ticks. *Nature,* **280** (5722), 491–493.

41. Willadsen P., McKenna R.V. & Riding G.A. (1988). – Isolation from the cattle tick, *Boophilus microplus*, of antigenic material capable of eliciting a protective immunological response in the bovine host. *Int. J. Parasitol.*, **18** (2), 183–189.

42. Willadsen P., Riding G.A., McKenna R.V., Kemp D.H., Tellam R.L., Nielsen J.N., Lahstein J., Cobon G.S. & Gough J.M. (1989). – Immunological control of a parasitic arthropod: identification of a protective antigen from *Boophilus microplus*. *J. Immunol.*, **143** (4), 1346–1351.

43. Willadsen P. & Kemp D.H. (1988). – Vaccination with 'concealed' antigens for tick control. *Parasitol. Today,* **4** (7), 196–198.

44. Rand K.N., Moore T., Sriskantha A., Spring K., Tellam R., Willadsen P. & Cobon G. (1989). – Cloning and expression of a protective antigen from the cattle tick *Boophilus microplus*. *Proc. Natl Acad. Sci. USA*, **86** (24), 9657–9661.

45. Rodríguez M., Rubiera R., Penichet M., Montesino R., Cremata J., Falcón V., Sánchez G., Bringas R., Cordovéz C., Valdés M., Leonart R., Herrera L. & de la Fuente J. (1994). – High level expression of the *Boophilus microplus* Bm86 antigen in the yeast *Pichia pastoris* forming highly immunogenic particles for cattle. *J. Biotechnol.*, **33** (2), 135–146.

46. De la Fuente J., Rodríguez J., Fragoso H., Ortiz M., Massard C.L., García O., García-García J.C. & Lleonart R. (1995). – Efficacy of vaccination with Gavac™ in the control of *Boophilus microplus* infestations. *In* Recombinant vaccines for the control of cattle ticks (J. de la Fuente, ed.). Elfos Scintiae, Havana, Cuba, 177–186.

47. Willadsen P., Bird P., Cobon G.S. & Hungerford J. (1995). – Commercialization of a recombinant vaccine against *Boophilus microplus*. *Parasitology*, **110**, S43–S50.

48. Canales M., Enríquez A., Ramos E., Cabrera D., Dandie H., Soto A., Falcón V., Rodríguez M. & de la Fuente J. (1997). – Large-scale production in *Pichia pastoris* of the recombinant vaccine Gavac™ against cattle tick. *Vaccine*, **15** (4), 414–422. doi:10.1016/S0264-410X(96)00192-2.

49. Andreotti R. (2006). – Performance of two Bm86 antigen vaccine formulations against ticks using crossbreed bovines in stall test. *Rev. Bras. Parasitol. Vet.*, **15** (3), 97–100.

50. Floyd R.B., Sutherst R.W. & Hungerford J. (1995). – Modelling the field efficacy of a genetically engineered vaccine against the cattle tick, *Boophilus microplus*. *Int. J. Parasitol.*, **25** (3), 285–291.

51. Labarta V., Rodríguez M., Penichet M., Lleonart R., Luaces L.L. & de la Fuente J. (1996). – Simulation of control strategies for the cattle tick *Boophilus microplus* employing vaccination with a recombinant Bm86 antigen preparation. *Vet. Parasitol.*, **63** (1–2), 131–160.

52. Lodos J., Ochogavía M.E., Rodríguez M. & de la Fuente J. (1999). – A simulation study of the effects of acaricides and vaccination on *Boophilus* cattle-tick populations. *Prev. Vet. Med.*, **38** (1), 47–63.

53. Lodos J., Boué O. & de la Fuente J. (2000). – A model to simulate the effect of vaccination against *Boophilus* ticks on cattle. *Vet. Parasitol.*, **87** (4), 315–326.

54. De la Fuente J. & Kocan K.M. (2003). – Advances in the identification and characterization of protective antigens for recombinant vaccines against tick infestations. *Expert Rev. Vaccines*, **2** (4), 583–593.

55. Ghosh S., Ray D.D., Vanlahmuaka Das G., Singh N.K., Sharma J.K. & Azhahianambi P. (2008). – Progress in development of vaccine against *Hyalomma anatolicum anatolicum* – Indian scenario. *Vaccine,* **26**, G40–G47.

56. Wikel S.K. & Whelen A.C. (1986). – Ixodid-host immune interaction. Identification and characterization of relevant antigens and tick-induced host immunosuppression. *Vet. Parasitol.,* **20** (1–3), 149–174.

57. Lee R.P. & Opdebeeck J.P. (1991). – Isolation of protective antigens from the gut of *Boophilus microplus* using monoclonal antibodies. *Immunology*, **72** (1), 121–126.

58. Nakajima M., Kodama M., Yanase H., Iwanaga T., Mulenga A., Ohashi K. & Onuma M. (2003). – Production and characterization of monoclonal antibodies against midgut of ixodid tick, *Haemaphysalis longicornis*. *Vet. Parasitol*., **115** (4), 355–363.

59. Manzano-Román R., García-Varas S., Encinas-Grandes A. & Pérez-Sánchez R. (2007). – Purification and characterization of a 45-kDa concealed antigen from the midgut membranes of *Ornithodoros erraticus* that induces lethal anti-tick immune responses in pigs. *Vet. Parasitol*., **145** (3–4), 314–325.

60. Gao J., Luo J., Fan R., Schulte-Spechtel U.C., Fingerle V., Guan G., Zhao H., Li Y., Ren Q., Ma M., Liu Z., Liu A., Dang Z., Sugimoto C. & Yin H. (2009). – Characterization of a concealed antigen Hq05 from the hard tick *Haemaphysalis qinghaiensis* and its effect as a vaccine against tick infestation in sheep. *Vaccine*, **27** (3), 483–490.

61. Schuijt T.J., Narasimhan S., Daffre S., DePonte K., Hovius J.W., Van't Veer C., van der Poll T., Bakhtiari K., Meijers J.C., Boder E.T., van Dam A.P. & Fikrig E. (2011). – Identification and characterization of *Ixodes scapularis* antigens that elicit tick immunity using yeast surface display. *PLoS ONE,* **6** (1), e15926.

62. Jongejan F., Nene V., de la Fuente J., Pain A. & Willadsen P. (2007). – Advances in the genomics of ticks and tick-borne pathogens. *Trends Parasitol.*, **23** (9), 391–396.

63. Tarleton R.L. & Kissinger J. (2001). – Parasite genomics: current status and future prospects. *Curr. Opin. Immunol*., **13** (4), 395–402.

64. Valenzuela J.G. (2004). – Exploring tick saliva: from biochemistry to 'sialomes' and functional genomics. *Parasitology,* **129**, S83–S94.

65. Francischetti I.M., My Pham V., Mans B.J., Andersen J.F., Mather T.N., Lane R.S. & Ribeiro J.M. (2005). – The transcriptome of the salivary glands of the female western black-legged tick *Ixodes pacificus* (Acari: Ixodidae). *Insect Biochem. Molec. Biol.*, **35** (10), 1142–1161.

66. Kongsuwan K., Josh P., Zhu Y., Pearson R., Gough J. & Colgrave M.L. (2010). – Exploring the midgut proteome of partially fed female cattle tick (*Rhipicephalus [Boophilus] microplus*). *J. Insect Physiol.,* **56** (2), 212–226. doi:10.1016/j.jinsphys.2009.10.003.

67. Almazán C., Kocan K.M., Bergman D.K., García-García J.C., Blouin E.F. & de la Fuente J. (2003). – Identification of protective antigens for the control of *Ixodes scapularis* infestations using cDNA expression library immunization. *Vaccine*, **21** (13–14), 1492–1501.

68. Xu Y., Bruno J.F. & Luft B.J. (2005). – Identification of novel tick salivary gland proteins for vaccine development. *Biochem. Biophys. Res. Commun.*, **326** (4), 901–904.

69. Kröber T. & Guerin P.M. (2007). – *In vitro* feeding assays for hard ticks. *Trends Parasitol.,* **23** (9), 445–449.

70. Antunes S., Merino O., Mosqueda J., Moreno-Cid J.A., Bell-Sakyi L., Fragkoudis R., Weisheit S., Pérez de la Lastra J.M., Alberdi P., Domingos A. & de la Fuente J. (2014). – Tick capillary feeding for the study of proteins involved in tick–pathogen interactions as potential antigens for the control of tick infestation and pathogen infection. *Parasit. Vectors*, **7**, 42.

71. Lew-Tabor A.E., Bruyeres A.G., Zhang B. & Rodríguez Valle M. (2014). – *Rhipicephalus (Boophilus) microplus* tick *in vitro* feeding methods for functional (dsRNA) and vaccine candidate (antibody) screening. *Ticks Tick-borne Dis.*, **5** (5), 500–510.

72. De la Fuente J., Almazán C., Blouin E.F., Naranjo V. & Kocan K.M. (2005). – RNA interference screening in ticks for identification of protective antigens. *Parasitol. Res.*, **96** (3), 137–141.

73. De la Fuente J., Manzano-Roman R., Naranjo V., Kocan K.M., Zivkovic Z., Blouin E.F., Canales M., Almazán C., Galindo R.C., Step D.L. & Villar M. (2010). – Identification of protective antigens by RNA interference for control of the lone star tick, *Amblyomma americanum*. *Vaccine,* **28** (7), 1786–1795.

74. Almazán C., Lagunes R., Villar M., Canales M., Rosario-Cruz R., Jongejan F. & de la Fuente J. (2010). – Identification and characterization of *Rhipicephalus (Boophilus) microplus* candidate protective antigens for the control of cattle tick infestations. *Parasitol. Res.*, **106** (2), 471–479.

75. De la Fuente J., Kocan K.M., Almazán C. & Blouin E.F. (2007). – RNA interference for the study and genetic manipulation of ticks. *Trends Parasitol.*, **23** (9), 427–433.

76. Kocan K.M., Blouin E.F. & de la Fuente J. (2011). – RNA interference in ticks. *J. Visual. Exp.,* **47**, 2474. doi:10.3791/2474.

77. De la Fuente J., Blouin E.F., Manzano-Roman R., Naranjo V., Almazán C., Pérez de la Lastra J.M., Zivkovic Z., Jongejan F. & Kocan K.M. (2007). – Functional genomic studies of tick cells in response to infection with the cattle pathogen, *Anaplasma marginale*. *Genomics*, **90** (6), 712–722.

78. Zivkovic Z., Esteves E., Almazán C., Daffre S., Nijhof A.M., Kocan K.M., Jongejan F. & de la Fuente J. (2010). – Differential expression of genes in salivary glands of male *Rhipicephalus (Boophilus) microplus* in response to infection with *Anaplasma marginale*. *BMC Genomics*, **11**, 186.

79. Guerrero F.D., Miller R.J. & Pérez de León A.A. (2012). – Cattle tick vaccines: many candidate antigens, but will a commercially viable product emerge? *Int. J. Parasitol.*, **42** (5), 421–427.

80. Gao J., Luo J., Li Y., Fan R., Zhao H., Guan G., Liu J., Wiske B., Sugimoto C. & Yin H. (2007). – Cloning and characterization of a ribosomal protein L23a from *Haemaphysalis qinghaiensis* eggs by immuno screening of a cDNA expression library. *Experim. Appl. Acarol.*, **41** (4), 289–303.

81. Gao J., Luo J., Fan R., Guan G., Fingerle V., Sugimoto C., Inoue N. & Yin H. (2008). – Cloning and characterization of a cDNA clone encoding troponin T from tick *Haemaphysalis qinghaiensis* (Acari: Ixodidae). *Comp. Biochem. Physiol., B, Biochem. Molec. Biol.*, **151** (3), 323–329.

82. Tian Z., Zhang P., Luo J., Yin H., Luo J., Xie J., Zhang L. & Liu G. (2010). – Cloning and characterization of a ribosomal protein L24 from *Haemaphysalis longicornis* eggs. *Parasitol. Res.*, **107** (5), 1213–1220.

83. Ribeiro J.M. & Francischetti I.M. (2003). – Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives. *Annu. Rev. Entomol.*, **48**, 73–88.

84. Menten-Dedoyart C., Couvreur B., Thellin O., Drion P.V., Herry M., Jolois O. & Heinen E. (2008). – Influence of the *Ixodes ricinus* tick blood-feeding on the antigen-specific antibody response *in vivo*. *Vaccine*, **26** (52), 6956–6964.

85. Menten-Dedoyart C., Couvreur B., Jolois O., Van Lerberghe P.B., Duwez L., Drion P. & Heinen E. (2011). – Kinetic study of the antibody response during the blood meal of *Ixodes ricinus*: implication on plasma cell maturation *in vivo* and for anti-*Ixodes* vaccination. *Vaccine*, **29** (11), 2044–2050.

86. Nijhof A.M., Balk J.A., Postigo M., Rhebergen A.M., Taoufik A. & Jongejan F. (2010). – Bm86 homologues and novel ATAQ proteins with multiple epidermal growth factor (EGF)-like domains from hard and soft ticks. *Int. J. Parasitol.*, **40** (14), 1587–1597.

87. Maritz-Olivier C., van Zyl W. & Stutzer C. (2012). – A systematic, functional genomics and reverse vaccinology approach to the identification of vaccine candidates in the cattle tick, *Rhipicephalus microplus*. *Ticks Tick-borne Dis.*, **3**, 179–187.

88. Marcelino I., Martinho de Almeida A., Ventosa M., Pruneau L., Meyer D.F., Martínez D., Lefrançois T., Vachiéry N. & Varela Coelho A. (2012). – Tick-borne diseases in cattle: applications of proteomics to develop new generation vaccines. *J. Proteomics*, **75** (14), 4232–4250.

89. De la Fuente J. & Kocan K.M. (2014). – Development of vaccines for control of tick infestations and interruption of pathogen transmission. *In* Biology of ticks (D. Sonenshine & M. Roe, eds), 2nd Ed. Oxford University Press, New York, 333–352.

90. Parizi L.F., Githaka N.W., Logullo C., Konnai S., Masuda A., Ohashi K., da Silva Vaz I. Jr. (2012). – The quest for a universal vaccine against ticks: cross-immunity insights. *Vet. J.*, **194** (2), 158–165.

91. De la Fuente J., Moreno-Cid J.A., Canales M., Villar M., Pérez de la Lastra J.M., Kocan K.M., Galindo R.C., Almazán C. & Blouin E.F. (2011). – Targeting arthropod subolesin/akirin for the development of a universal vaccine for control of vector infestations and pathogen transmission. *Vet. Parasitol.*, **181** (1), 17–22.

92. Merino O., Alberdi P., Pérez de la Lastra J.M. & de la Fuente J. (2013). – Tick vaccines and the control of tick-borne pathogens. *Front. Cell. Infect. Microbiol.*, **3**, 30.

93. Merino M., Antunes S., Mosqueda J., Moreno-Cid J.A., Pérez de la Lastra J.M., Rosario-Cruz R., Rodríguez S., Domingos A. & de la Fuente J. (2013). – Vaccination with proteins involved in tick–pathogen interactions reduces vector infestations and pathogen infection. *Vaccine*, **31** (49), 5889–5896.

94. Moreno-Cid J.A., Pérez de la Lastra J.M., Villar M., Jiménez M., Pinal R., Estrada-Peña A., Alarcón P., Delacour S., Oropeza V., Ruiz I., Molina R., Lucientes J., Prudencio C.R., Galindo R.C., Almazán C., Nijhof A.M., Mangold A.J., Gortázar C. & de la Fuente J. (2013). – Control of multiple arthropod vector infestations with subolesin/akirin vaccines. *Vaccine*, **31** (8), 1187–1196.

95. Futse J.E., Ueti M.W., Knowles D.P. Jr & Palmer G.H. (2003). – Transmission of *Anaplasma marginale* by *Boophilus microplus*: retention of vector competence in the absence of vector–pathogen interaction. *J. Clin. Microbiol.*, **41** (8), 3829–3834.

96. Scoles G.A., Ueti M.W. & Palmer G.H. (2005). – Variation among geographically separated populations of *Dermacentor andersoni* (Acari: Ixodidae) in midgut susceptibility to *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). *J. Med. Entomol.*, **42** (2), 153–162.

97. Teglas M.B. & Foley J. (2006). – Differences in the transmissibility of two *Anaplasma phagocytophilum* strains by the North American tick vector species, *Ixodes pacificus* and *Ixodes scapularis* (Acari: Ixodidae). *Experim. Appl. Acarol.*, **38** (1), 47–58.

98. Estrada-Peña A., Naranjo V., Acevedo-Whitehouse K., Mangold A.J., Kocan K.M. & de la Fuente J. (2009). – Phylogeographic analysis reveals association of tick-borne pathogen, *Anaplasma marginale*, MSP1a sequences with ecological traits affecting tick vector performance. *BMC Biology,* **7**, 57.

99. Hajdusek O., Sojka D., Kopacek P., Buresova V., Franta Z., Sauman I., Winzerling J. & Grubhoffer L. (2009). – Knockdown of proteins involved in iron metabolism limits tick reproduction and development. *Proc. Natl Acad. Sci. USA*, **106** (4), 1033–1038.

100. Titus R.G., Bishop J.V. & Mejia J.S. (2006). – The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission. *Parasite Immunol.*, **28** (4), 131–141.

101. Zivkovic Z., Blouin E.F., Manzano-Roman R., Almazán C., Naranjo V., Massung R.F., Jongejan F., Kocan K.M. & de la Fuente J. (2009). – *Anaplasma phagocytophilum* and *A. marginale* elicit different gene expression responses in ticks and cultured tick cells. *Comp. Funct. Genomics*, 705034. doi:10.1155/2009/705034.

102. Sukumaran B., Narasimhan S., Anderson J.F., DePonte K., Marcantonio N., Krishnan M.N., Fish D., Telford S.R., Kantor F.S. & Fikrig E. (2006). – An *Ixodes scapularis* protein required for survival of *Anaplasma phagocytophilum* in tick salivary glands. *J. Exp. Med.,* **203** (6), 1507–1517.

103. Pedra J.H., Narasimhan S., Rendic D., DePonte K., Bell-Sakyi L., Wilson I.B. & Fikrig E. (2010). – Fucosylation enhances colonization of ticks by *Anaplasma phagocytophilum*. *Cell. Microbiol.*, **12** (9), 1222–1234.

104. Severo M.S., Choy A., Stephens K.D., Sakhon O.S., Chen G., Chung D.-W., Le Roch K.G., Blaha G. & Pedra J.H.F. (2013). – The E3 ubiquitin ligase XIAP restricts *Anaplasma phagocytophilum* colonization of *Ixodes scapularis* ticks. *J. Infect. Dis.*, **208** (11), 1830–1840. doi:10.1093/infdis/jit380.

105. Sultana H., Neelakanta G., Kantor F.S., Malawista S.E., Fish D., Montgomery R.R. & Fikrig E. (2010). – *Anaplasma phagocytophilum* induces actin phosphorylation to selectively regulate gene transcription in *Ixodes scapularis* ticks. *J. Exp. Med.,* **207** (8), 1727–1743.

106. Busby A.T., Ayllón N., Kocan K.M., Blouin E.F., de la Fuente G., Galindo R.C., Villar M. & de la Fuente J. (2012). – Expression of heat-shock proteins and subolesin affects stress responses, *Anaplasma phagocytophilum* infection and questing behavior in the tick, *Ixodes scapularis*. *Med. Vet. Entomol.*, **26** (1), 92–102.

107. Ayllón N., Villar M., Busby A.T., Kocan K.M., Blouin E.F., Bonzón-Kulichenko E., Galindo R.C., Mangold A.J., Alberdi P., Pérez de la Lastra J.M., Vázquez J. & de la Fuente J. (2013). – *Anaplasma phagocytophilum* inhibits apoptosis and promotes cytoskeleton rearrangement for infection of tick cells. *Infect. Immun.*, **81** (7), 2415–2425.

108. Naranjo N., Ayllón N., Pérez de la Lastra J.M., Galindo R.C., Kocan K.M., Blouin E.F., Mitra R., Alberdi P., Villar M. & de la Fuente J. (2013). – Reciprocal regulation of NF-kB (Relish) and subolesin in the tick vector, *Ixodes scapularis*. *PLoS ONE*, **8** (6), e65915.

109. Hajdušek O., Šíma R., Ayllón N., Jalovecká M., Perner J., de la Fuente J. & Kopáček P. (2013). – Interaction of the tick immune system with transmitted pathogens. *Front. Cell. Infect. Microbiol.*, **3**, 26.

110. Rudenko N., Golovchenko M., Edwards M.J. & Grubhoffer L. (2005). – Differential expression of *Ixodes ricinus* tick genes induced by blood feeding or *Borrelia burgdorferi* infection. *J. Med. Entomol.,* **42** (1), 36–41.

111. Ribeiro J.M., Alarcon-Chaidez F., Francischetti I.M., Mans B.J. & Mather T.N. (2006). – An annotated catalog of salivary gland transcripts from *Ixodes scapularis* ticks. *Insect Biochem. Molec. Biol.,* **36** (2), 111–129.

112. Macaluso K.R., Mulenga A., Simser J.A. & Azad A.F. (2003). – Differential expression of genes in uninfected and rickettsia-infected *Dermacentor variabilis* ticks as assessed by differential-display PCR. *Infect. Immun.,* **71** (11), 6165–6170.

113. Mulenga A., Macaluso K.R., Simser J.A. & Azad A.F. (2003). – Dynamics of *Rickettsia*-tick interactions: identification and characterization of differentially expressed mRNAs in uninfected and infected *Dermacentor variabilis*. *Insect Molec. Biol.*, **12** (2), 185–193.

114. Nene V., Lee D., Kang'a S., Skilton R. & Shah T. (2004). – Genes transcribed in the salivary glands of female *Rhipicephalus appendiculatus* ticks infected with *Theileria parva*. *Insect Biochem. Molec. Biol.*, **34** (10), 1117–1128. doi:10.1016/j.ibmb.2004.07.002.

115. Rachinsky A., Guerrero F.D. & Scoles G.A. (2008). – Proteomic profiling of *Rhipicephalus (Boophilus) microplus* midgut responses to infection with *Babesia bovis*. *Vet. Parasitol.*, **152** (3–4), 294–313.

116. Labuda M., Trimnell A.R., Licková M., Kazimírová M., Davies G.M., Lissina O., Hails R.S. & Nuttall P.A. (2006). – An antivector vaccine protects against a lethal vector-borne pathogen. *PLoS Pathog.*, **2** (4), e27.

117. De la Fuente J., Almazán C., Blouin E.F., Naranjo V. & Kocan K.M. (2006). – Reduction of tick infections with *Anaplasma marginale* and *A. phagocytophilum* by targeting the tick protective antigen subolesin. *Parasitol. Res.*, **100** (1), 85–91.

118. Jeyabal L., Azhahianambi P., Susitha K., Ray D.D., Chaudhuri P., Vanlahmuaka V. & Ghosh S. (2010). – Efficacy of rHaa86, an orthologue of Bm86, against challenge infestations of *Hyalomma anatolicum anatolicum*. *Transbound. Emerg. Dis.*, **57** (1–2), 96–102.

119. Dai J., Wang P., Adusumilli S., Booth C.J., Narasimhan S., Anguita J. & Fikrig E. (2009). – Antibodies against a tick protein, Salp15, protect mice from the Lyme disease agent. *Cell Host Microbe*, **6** (5), 482–492.

120. Merino O., Almazán C., Canales M., Villar M., Moreno-Cid J.A., Galindo R.C. & de la Fuente J. (2011). – Targeting the tick protective antigen subolesin reduces vector infestations and pathogen infection by *Anaplasma marginale* and *Babesia bigemina*. *Vaccine*, **29** (47), 8575–8579.

121. Torina A., Moreno-Cid J.A., Blanda V., Fernández de Mera I.G., Pérez de la Lastra J.M., Scimeca S., Blanda M., Scariano M.E., Briganò S., Disclafani R., Piazza A., Vicente J., Gortázar C., Caracappa S., Lelli R.C. & de la Fuente J. (2014). – Control of tick infestations and pathogen prevalence in cattle and sheep farms vaccinated with the recombinant subolesin-major surface protein 1a chimeric antigen. *Parasit. Vectors*, **7**, 10.

122. De la Fuente J., Kocan K.M. & Blouin E.F. (2007). – Tick vaccines and the transmission of tick-borne pathogens. *Vet. Res. Commun.*, **31** (Suppl. 1), 85–90.

123. Willadsen P. (2006). – Tick control: thoughts on a research agenda. *Vet. Parasitol.*, **138** (1–2),161–168.

124. Canales M., Moreno-Cid J.A., Almazán C., Villar M. & de la Fuente J. (2010). – Bioprocess design and economics of recombinant BM86/BM95 antigen production for anti-tick vaccines. *Biochem. Engin. J.*, **52**, 79–90.

125. Canales M., Almazán C., Pérez de la Lastra J.M. & de la Fuente J. (2008). – *Anaplasma marginale* major surface protein 1a directs cell surface display of tick BM95 immunogenic peptides on *Escherichia coli*. *J. Biotechnol.,* **135** (4), 326–332.

126. Canales M., Pérez de la Lastra J.M., Naranjo V., Nijhof A.M., Hope M., Jongejan F. & de la Fuente J. (2008). – Expression of recombinant *Rhipicephalus (Boophilus) microplus, R. annulatus* and *R. decoloratus* Bm86 orthologs as secreted proteins in *Pichia pastoris*. *BMC Biotechnol.*, **8**, 14.

127. Canales M., Ballesteros C., Moreno-Cid J.A., Espinosa A.M., Villar M. & de la Fuente J. (2009). – Extractive bioconversion to produce the *Aedes albopictus* akirin in an aqueous two-phase system supporting *Pichia pastoris* growth and protein secretion. *Biochem. Engin. J.*, **46**, 105–114.

128. Canales M., Labruna M.B., Soares J.F., Prudencio C.R. & de la Fuente J. (2009). – Protective efficacy of bacterial membranes containing surface-exposed BM95 antigenic peptides for the control of cattle tick infestations. *Vaccine,* **27** (52), 7244–7248.

129. Moreno-Cid J.A., Canales M. & de la Fuente J. (2012). – Production of recombinant *Aedes albopictus* akirin in *Pichia pastoris* using an aqueous two-phase semicontinuous fermentation process. *Biochem. Engin. J.*, **68**, 114–119.

130. Canales M., Naranjo V., Almazán C., Molina R., Tsuruta S.A., Szabó M.P.J., Manzano-Roman R., Pérez de la Lastra J.M., Kocan K.M., Jiménez M.I., Lucientes J., Villar M. & de la Fuente J. (2009). – Conservation and immunogenicity of the mosquito ortholog of the tick protective antigen, subolesin. *Parasitol. Res.*, **105** (1), 97–111.

131. Dinglasan R.R., Valenzuela J.G. & Azad A.F. (2005). – Sugar epitopes as potential universal disease transmission blocking targets. *Insect Biochem. Molec. Biol.*, **35** (1), 1–10.

132. De la Fuente J., Canales M. & Kocan K.M. (2006). – The importance of protein glycosylation in development of novel tick vaccine strategies. *Parasite Immunol.*, **28** (12), 687–688.

133. Jackson L.A. & Opdebeeck J.P. (1994). – Quil A and ISCOMs as adjuvants for midgut membrane antigens of *Boophilus microplus*. *Appl. Parasitol.*, **35** (2), 87–98.

134. Rodríguez Valle M., Montero C., Machado H., Joglar M., de la Fuente J. & García-García J.C. (2001). – The evaluation of yeast derivatives as adjuvants for the immune response to the Bm86 antigen in cattle. *BMC Biotechnol.*, **1** (1), 3.

135. Patarroyo J.H., Portela R.W., De Castro R.O., Pimentel J.C., Guzman F., Patarroyo M.E., Vargas M.I., Prates A.A. & Mendes M.A. (2002). – Immunization of cattle with synthetic peptides derived from the *Boophilus microplus* gut protein (Bm86). *Vet. Immunol. Immunopathol.*, **88** (3–4), 63–72.

136. Prudencio C.R., Pérez de la Lastra J.M., Canales M., Villar M. & de la Fuente J. (2010). – Mapping protective epitopes in the tick and mosquito subolesin ortholog proteins. *Vaccine*, **28** (33), 5398–5406.

137. Marshall C.M., Seaman G.A. & Hayes G.A. (1993). – A critique on the tropical cattle fever ticks controversy and its relationship to white-tailed deer. *In* Transactions of the 28th North American Wildlife and Natural Resources Conference. Wildlife Management Institute, Washington, DC, 226–232.

138. Lohmeyer K.H., Pound J.M., May M.A., Kammlah D.M. & Davey R.B. (2011). – Distribution of *Rhipicephalus (Boophilus) microplus* and *Rhipicephalus (Boophilus) annulatus* (Acari: Ixodidae) infestations detected in the United States along the Texas/Mexico border. *J. Med. Entomol.,* **48** (4), 770–774.

139. Pound J.M., George J.E., Lohmeyer K.H. & Davey R.B. (2010). – Evidence for role of white-tailed deer (Artiodactyla: Cervidae) in epizootiology of cattle ticks and southern cattle ticks (Acari: Ixodidae) in reinfestations along the Texas/Mexico border in South Texas: a review and update. *J. Econ. Entomol.,* **103** (2), 211–218. doi:10.1603/EC09359.

140. Porter R., Norman R.A. & Gilbert L. (2013). – An alternative to killing? Treatment of reservoir hosts to control a vector and pathogen in a susceptible species. *Parasitology*, **140** (2), 247–257.

141. De la Fuente J., Almazán C., Naranjo V., Blouin E.F., Meyer J.M. & Kocan K.M. (2006). – Autocidal control of ticks by silencing of a single gene by RNA interference. *Biochem. Biophys. Res. Commun.*, **344** (1), 332–338.

142. Merino O., Almazán C., Canales M., Villar M., Moreno-Cid J.A., Estrada-Peña A., Kocan K.M. & de la Fuente J. (2011). – Control of *Rhipicephalus (Boophilus) microplus* infestations by the combination of subolesin vaccination and tick autocidal control after subolesin gene knockdown in ticks fed on cattle. *Vaccine*, **29** (12), 2248–2254.

143. Carreón D., Pérez de la Lastra J.M., Almazán C., Canales M., Ruiz-Fons F., Boadella M., Moreno-Cid J.A., Villar M., Gortázar C., Reglero M., Villarreal R. & de la Fuente J. (2012). – Vaccination with BM86, subolesin and akirin protective antigens for the control of tick infestations in white tailed deer and red deer. *Vaccine*, **30** (2), 273–279. doi:10.1016/j.vaccine.2011.10.099.

144. Ballesteros C., Pérez de la Lastra J.M. & de la Fuente J. (2007). – Recent developments in oral bait vaccines for wildlife. *Recent Pat. Drug Deliv. Formul.*, **1** (3), 230–235.

145. Ballesteros C., Garrido J.M., Vicente J., Romero B., Galindo R.C., Minguijón E., Villar M., Martín-Hernando M.P., Sevilla I., Juste R., Aranaz A., de la Fuente J. & Gortázar C. (2009). – First data on Eurasian wild boar response to oral immunization with BCG and challenge with a *Mycobacterium bovis* field strain. *Vaccine,* **27** (48), 6662–6668.

146. Kocan K.M., Blouin E.F., Pidherney M.S., Claypool P.L., Samish M. & Glazer I. (1998). – Entomopathogenic nematodes as a potential biological control method for ticks. *Ann. N.Y. Acad. Sci.*, **849**, 355–364.

147. Kocan K.M., Pidherney M.S., Blouin E.F., Claypool P.L., Samish M. & Glazer I. (1998). – Interaction of entomopathogenic nematodes (Steinernematidae) with selected species of ixodid ticks (Acarina: Ixodidae). *J. Med. Entomol.*, **35** (4), 514–520.

148. Fernandes E.K., Bittencourt V.R. & Roberts D.W. (2012). – Perspectives on the potential of entomopathogenic fungi in biological control of ticks. *Experim. Parasitol.*, **130** (3), 300–305.

149. Monteiro C.M., Matos R.D., Araújo L.X., Campos R., Bittencourt V.R., Dolinski C., Furlong J. & Prata M.C. (2014). – Entomopathogenic nematodes in insect cadaver formulations for the control of *Rhipicephalus microplus* (Acari: Ixodidae). *Vet. Parasitol.*, **203** (3–4), 310–317 doi:10.1016/j.vetpar.2014.04.003.

150. Samish M. & Alekseev E. (2001). – Arthropods as predators of ticks (Ixodoidea). *J. Med. Entomol.*, **38** (1), 1–11. doi:10.1603/0022-2585-38.1.1.

151. Nava S., Mastropaolo M., Guglielmone A.A. & Mangold A.J. (2013). – Effect of deforestation and introduction of exotic grasses as livestock forage on the population dynamics of the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in northern Argentina. *Res. Vet. Sci.*, **95** (3), 1046–1054.

152. Borges L.M., Sousa L.A. & da Silva Barbosa C. (2011). – Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus (Boophilus) microplus*. *Rev. Bras. Parasitol. Vet.*, **20** (2), 89–96.

153. Barker S.C. & Murrell A. (2004). – Systematics and evolution of ticks with a list of valid genus and species names. *Parasitology,* **129**, S15–S36.

154. Estrada-Peña A., Bouattour A., Camicas J.L., Guglielmone A., Horak I., Jongejan F., Latif A., Pegram R. & Walker A.R. (2006). – The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. *Experim. Appl. Acarol.*, **38** (2–3), 219–235.

155. Olwoch J.M., Van Jaarsveld A.S., Scholtz C.H. & Horak I.G. (2007). – Climate change and the genus *Rhipicephalus* (Acari: Ixodidae) in Africa. *Onderstepoort J. Vet. Res.*, **74** (1), 45–72.

156. Madder M., Thys E., Achi L., Touré A. & De Deken R. (2011). – *Rhipicephalus (Boophilus) microplus*: a most successful invasive tick species in West-Africa. *Experim. Appl. Acarol.*, **53** (2), 139–145. doi:10.1007/s10493-010-9390-8.

157. Verstraete M. (1995). – New developments in antiplatelet and antithrombotic therapy. *Eur. Heart J.*, **16**, 16–23.

158. Kiss T., Cadar D. & Spînu M. (2012). – Tick prevention at a crossroad: new and renewed solutions. *Vet. Parasitol.*, **187** (3–4), 357–366.

159. Mondal D.B., Sarma K. & Saravanan M. (2013). – Upcoming of the integrated tick control program of ruminants with special emphasis on livestock farming system in India. *Ticks Tick-borne Dis.*, **4** (1–2), 1–10. doi:10.1016/j.ttbdis.2012.05.006.

160. Jonsson N.N., Mayer D.G. & Green P.E. (2000). – Possible risk factors on Queensland dairy farms for acaricide resistance in cattle tick (*Boophilus microplus*). *Vet. Parasitol.*, **88** (1–2), 79–92. doi:10.1016/S0304-4017(99)00189-2.

161. García-García J.C., González I.L., González D.M., Valdés M., Méndez L., Lamberti J., D'Agostino B., Citroni D., Fragoso H., Ortiz M., Rodríguez M. & de la Fuente J. (1999). – Sequence variations in the *Boophilus microplus* Bm86 locus and implications for immunoprotection in cattle vaccinated with this antigen. *Experim. Appl. Acarol.*, **23** (11), 883–895.

162. Laughery J.M., Knowles D.P., Schneider D.A., Bastos R.G., McElwain T.F. & Suarez C.E. (2014). – Targeted surface expression of an exogenous antigen in stably transfected *Babesia bovis*. *PLoS ONE*, **9** (5), e97890.