[Frontiers in Bioscience 13, 6947-6956, May 1, 2008]

Targeting the tick-pathogen interface for novel control strategies

Jose de la Fuente[1,2], Katherine M. Kocan[1], Consuelo Almazan[3], Edmour F. Blouin[1]

[1]Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA, [2]Instituto de Investigacion en Recursos Cinegeticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain, [3]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Tamaulipas, Km. 5 carretera Victoria-Mante, CP 87000 Victoria, Tamaulipas, Mexico

TABLE OF CONTENTS

1. ABSTRACT

Ticks are ectoparasites of wild and domestic animals and humans that most notably impact global health by transmitting disease-causing pathogens. While information on the molecular interactions between ticks and pathogens that facilitate pathogen infection, development and transmission is limited, a comprehensive understanding of the tick-pathogen interface would be fundamental toward development of new and novel measures for control of both tick infestations and tick-borne pathogens. Recently, vaccine studies using key tick antigens and characterization of tick gene function by RNA interference (RNAi) have provided new information on genes that impact the tick-pathogen interface. In this review we summarize current research and prospects of tick vaccines and genetic manipulation of ticks targeted to the tick-pathogen interface. The knowledge gained from these collective studies will be fundamental toward understanding of tick-pathogen interactions and for formulation of control methods targeted at both ticks and tick-borne pathogens. Use of these molecular approaches will likely contribute to control measures that will notably reduce tick populations and tick-borne diseases in the future.

2. INTRODUCTION

Ticks are obligate hematophagous ectoparasites of wild and domestic animals and humans that are classified in the subclass Acari, order Parasitiformes, suborder Ixodida and distributed from Arctic to tropical regions of the world (1, 2). Despite efforts to implement measures to control tick infestations, ticks and the pathogens they transmit continue to be a serious constraint to human and animal health (3, 4).

Ticks are considered to be second worldwide to mosquitoes as vectors of human diseases (5) and the most important vector of diseases that affect the cattle industry worldwide (6). For example, *Ixodes* spp. vector several human pathogens, including *Borrelia burgdorferi* (Lyme disease), the closely related *B. afzelii*, *B. lusitaniae*, *B. valaisiana* and *B. garinii*, *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), *Coxiella burnetii* (Q fever), *Francisella tularensis* (tularemia), *Rickettsia helvetica*, *R. japonica* and *R. australis*, *Babesia divergens*, as well as tick-borne encephalitis virus (TBEV), a causative agent of tick-borne encephalitis. *Boophilus* spp. are major tick pests of veterinary importance in tropical regions. Infestations with the cattle tick, *B. microplus*, economically

impact cattle production by causing reductions in weight gains and milk production, and by transmitting pathogens that cause babesiosis (*Babesia bovis* and *B. bigemina*) and anaplasmosis *(A. marginale*).

New strategies are needed for the control of ticks and tick-borne pathogens. Characterization of the molecular mechanisms that mediate tick-pathogen interactions will likely provide new targets for vaccines and genetic manipulation of ticks. Evidence that co-evolution and long term survival of ticks and tick-borne pathogens undoubtedly involves genetic traits from both organisms includes reports of pathogen ligands and tick receptors that enable pathogen infection of ticks (7, 8).

Recent advancements in tick vaccine development and RNA interference (RNAi) have been reviewed in several publications (9-15). Herein, we will extend these reviews by focusing on the application of molecular technologies targeted to the tick-pathogen interface. These technologies, in concert with other emerging ones, are needed to advance our understanding of tick-pathogen interactions and will likely provide new and novel strategies for development of improved vaccines for the simultaneous control of ticks and tick-borne pathogens.

3. TICK VACCINES AND THE TICK-PATHOGEN INTERFACE

3.1. Tick vaccines for the control of tick infestations

The feasibility of controlling tick infestations through immunization of hosts with selected tick antigens was demonstrated following the pioneering work of Allen and Humphreys (16) with the development of vaccines that reduced *Boophilus* spp. infestations on cattle (recently reviewed by 3, 4, 13, 14). This vaccine, based on the recombinant *B. microplus* Bm86 gut antigen, caused reductions in the number, weight and reproductive capacity of female ticks that fed on immunized cattle. Overall, the most notable vaccine effect was the decline of larval infestations in subsequent tick generations. Controlled field trials using Bm86-based vaccines in cattle resulted in the control of *B. microplus* and *B. annulatus* infestations and a correlation was found between the antibody titers of cattle immunized with Bm86 and tick fecundity (17). Use of the integrated approach of acaricide application and vaccination in relation to seasonal tick populations resulted in a more cost-effective and environmentally sound mean of controlling tick infestations by reducing acaricides applications (13, 14, 17, 18).

3.2. Discovery of tick vaccine antigens

The Bm86 tick vaccine antigen that was first used in commercial cattle tick vaccines was discovered from proteins in crude tick extracts after fractionation, immunization and tick challenge in cattle (19). Subsequent approaches for antigen identification included immunomapping tick antigens that elicited an antibody response in the infested hosts and the testing in vaccination experiments of tick proteins that were considered to be important for the parasite function and/or survival (3, 13).

Recently, advances in molecular biology and systems biology have provided new avenues for efficient discovery of tick vaccine antigens. The initial tick antigens selected for study were based on the assumption that cell membrane-exposed or secreted proteins would be required for antibody recognition in ticks fed on vaccinated cattle. However, tick protective antigens identified by expression library immunization and RNAi included those intracellular proteins that play important roles in tick physiology and reproduction and may therefore also be candidate vaccine antigens (20, 21). Although the mechanism by which antibodies are transported across tick cell membranes and complex with antigens is not completely understood, antibodies are specifically transported inside tick cells to interact and neutralize intracellular proteins (22, 23). Therefore, these results challenge the paradigm that vaccine antigens must be extracellular proteins and thus expand the repertoire of candidate tick vaccine antigens.

In the near future, the application of high throughput functional genomics screening will likely result in the discovery of tick vaccine antigens that include both extracellular and intracellular proteins.

3.3. Tick vaccines and the control of tick-borne pathogens

While reduction of tick infestations has been a foremost goal of tick vaccine development, an equally important impact is reduction of the transmission of medically and economically important pathogens by ticks. Immunization of cattle with Bm86 vaccines resulted in lower infestations as well as a reduction in the incidence of babesiosis and anaplasmosis in some regions (13, 14, 17, 18). Tick vaccines may therefore have a dual impact of targeting tick infestations and the incidence of tick-borne diseases by decreasing the exposure of susceptible hosts to ticks (Figure 1).

3.4. Feasibility of targeting tick proteins to reduce tick vector capacity

Recent research results demonstrated that tick vaccines also reduce tick vector capacity. The candidate antigens involved in vector capacity were those at the tick-pathogen interface found to be necessary for the infection, development and transmission of pathogens. Three proteins have been shown thus far to be involved in tick vector capacity: the tick receptor for OspA (TROSPA) that is required for colonization of *I. scapularis* with *B. burgdorferi* (8); the *R. sanguineus* P64 putative cement protein (24); and the tick protective antigen subolesin (25). Use of these antigens in vaccine formulations has resulted in reduced vector capacity of ticks for infection with *B. burgdorferi*, TBEV and *A. phagocytophilum*/*A. marginale*, respectively. Recently, Narasimhan *et al.* (26) demonstrated that immunity against *I. scapularis* salivary proteins that are expressed within 24 hours of tick attachment impaired transmission of *B. burgdorferi* by ticks to guinea pigs.

The feasibility of vaccinating animal host populations to decrease the prevalence of tick-borne pathogens in ticks, thus reducing the risk for pathogen

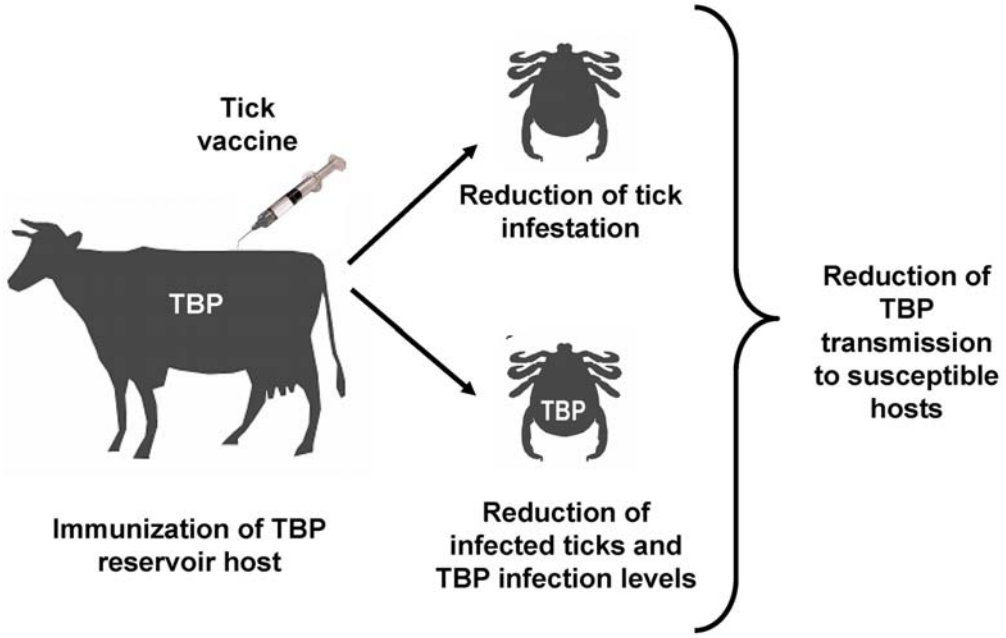

Figure 1. Tick vaccines could be used to immunize animal host populations to decrease tick infestations and the prevalence of tick-borne pathogens (TBP) in ticks, thus reducing the risk for pathogen transmission to susceptible animals and humans.

transmission to humans and animals, was recently demonstrated by Gomes-Solecki *et al.* (27) and Tsao *et al.* (28). In this research, *B. burgdorferi* infections in *I. scapularis* were reduced when ticks fed on mice immunized with the bacterial protein OspA, possibly by blocking bacterial adhesion to the tick receptor.

Collectively, these results demonstrated the feasibility of developing vaccines by targeting pathogen and tick-derived key proteins at the tick-pathogen interface for the control of tick infestations and the transmission of tick-borne pathogens (29-31) (Figure 1).

4. GENETIC MANIPULATION OF TICKS TO TARGET THE TICK-PATHOGEN INTERFACE

4.1. Methods and mechanism of RNAi in ticks

RNAi is a nucleic acid-based reverse genetic approach that results in the silencing of gene expression and allows for study of the impact of gene function on metabolic pathways (32). Small interfering RNAs (siRNAs) are the effector molecules of the RNAi pathway that is initiated by double-stranded RNA (dsRNA) and results in a potent sequence-specific degradation of cytoplasmic mRNAs containing the same sequence as the dsRNA trigger (33). Post-transcriptional gene silencing mechanisms initiated by dsRNA have been reported in all eukaryotes studied thus far, and RNAi has rapidly become and important molecular tool for functional genomics studies on a variety of organisms, as well as other applications (34).

RNAi is currently the most widely used gene-silencing technique in ticks because use of other methods of genetic manipulations has been limited. As recently reviewed by de la Fuente *et al.* (15), RNAi has rapidly proved to be a valuable tool for studying tick gene function, for characterization of the tick-pathogen interface and for the screening and characterization of tick protective antigens.

Four methods have been used for RNAi in ticks: (i) injection or microinjection of dsRNA into unfed or fed ticks, (ii) soaking or incubation of dsRNA with ticks, tick tissues or cells, (iii) feeding of dsRNA to ticks and (iv) virus production of dsRNA (15). The application of these methods for RNAi in ticks depends on the experimental design and objectives. Injection of dsRNA into adult ticks is the most universal method for *in vivo* RNAi in ticks, particularly with the possibility of generating a high number of treated individuals through inherited RNAi (35, 36). Inherited RNAi and feeding of dsRNA solutions to ticks are relatively easy ways to deliver dsRNA into immature tick stages for the study of tick-pathogen interactions (35-37). However, incubation with dsRNA solutions is probably the best approach for *ex vivo* and *in vitro* studies of gene expression and pathogen infection and multiplication in isolated tick organs and cell lines (38, 39). Virus production of dsRNA may impact the generation of transgenic ticks resistant to tick-borne pathogens (see section 4.3).

The mechanism of RNAi in ticks has not been characterized and the proteins involved in the process of RNAi have not been identified, with the exception of a *B. microplus* sequence that was shown to be similar to Argonaute (Ago)-2 (15). We recently proposed a model for RNAi in ticks based on current information on RNAi in *Drosophila melanogaster* and mosquitoes (15). Exogenous or viral dsRNA enter the cytoplasm, where it is first

Table 1. Summary of RNA interference experiments targeting the tick-pathogen interface

Tick species	Target gene	Delivery of dsRNA	Phenotype at the tick-pathogen interface	Refs
Ixodes scapularis	TROSPA	Microinjection into nymphal ticks	Reduction in infection and transmission of *B. burgdorferi*	8
Ixodes scapularis	Salp15	Microinjection into nymphal ticks	Reduction in the capacity of tick-borne *B. burgdorferi* to infect mice	43
Ixodes scapularis	Salp16	Microinjection into nymphal ticks	Reduction of *A. phagocytophilum* survival in ticks	44
Ixodes scapularis	Salp14	Microinjection into nymphal ticks	Reduction in mRNA levels but no effect on acquisition of *A. phagocytophilum* and *B. burgdorferi*	45
Ixodes scapularis	Isac	Nymph capillary feeding	Reduction in tick infection by *B. burgdorferi*	37
Dermacentor variabilis	Subolesin (4D8)	Injection into adult ticks	Reduction in tick infection by *A. marginale*	25, 42, 51
Haemaphysalis longicornis	Longicin	Microinjection into adult ticks	Inhibition of *B. gibsoni* killing by ticks	46
Dermacentor variabilis	GST	Injection into adult ticks	Reduction in *A. marginale* levels in tick guts after AF and salivary glands after TF	42
Dermacentor variabilis	Ubiquitin vATPase	Injection into adult ticks	Reduction in *A. marginale* levels in tick guts after AF	42
Dermacentor variabilis	Salivary selenoprotein M	Injection into adult ticks	Reduction in *A. marginale* levels in salivary glands after TF	42
Ixodes scapularis IDE8 cells	Proteasome Ferritin GST	Incubation with dsRNA solution	Reduction in *A. marginale* levels	42
Ixodes scapularis IDE8 cells	Subolesin (4D8)	Incubation with dsRNA solution	Reduction in *A. marginale* levels	42, 51
Ixodes scapularis IDE8 cells	Selenoprotein W2a HSC	Incubation with dsRNA solution	Increase in *A. marginale* levels	42

Data updated after (15). Abbreviations: TROSPA, tick receptor for *B. burgdorferi* OspA; Salp, salivary gland protein; Isac, *I. scapularis* anticomplement; GST, glutathione-S transferase; AF, acquisition feeding; TF, transmission feeding; HSC, hematopoietic stem/progenitor cells protein-like.

processed into double stranded siRNAs about 21–23 nucleotides in length. The key protein for this specific degradation in *D. melanogaster* is Dicer-1. This RNase III-like dsRNA-specific ribonuclease contains RNase III, helicase, and PAZ (Piwi/Argonaute/Zwille) domains that are involved in protein–protein interactions, a dsRNA binding domain and a DEAD-box helicase domain. Dicer-1 then presents the siRNAs to the RNA-induced silencing complex (RISC), which incorporates the siRNAs and targets and degrades any mRNA with cognate sequences. Other protein components of RISC, such as the Ago protein family, may be required for RNAi. However, a more complete understanding of the mechanism of dsRNA-induced RNAi in ticks would facilitate a better use of this genetic approach.

4.2. RNAi for the characterization of the tick-pathogen interface

Molecular characterization of the tick-pathogen interface is an emerging research area. Differential gene expression has been characterized in *Dermacentor variabilis* ovaries in response to rickettsial infection (40), in salivary glands of female *R. appendiculatus* infected with *Theileria parva* (41) and in *I. scapularis* IDE8 cells, *D. variabilis* and *B. microplus* ticks in response to infection with *A. marginale* (42). While the function of many tick genes at the tick-pathogen interface is unknown, results thus far have provided evidence that pathogens modify expression of tick genes involved in defense mechanisms, thus facilitating tick vector capacity.

While RNAi has been used primarily for study of tick gene function (15), it was also used recently to study the role of tick gene expression in infection and transmission of *B. burgdorferi* (8, 37, 43), *A. phagocytophilum* (44), and *A. marginale* (25). Silencing of key genes in the respective tick vectors resulted in reduction of the tick vector capacity for these pathogens (Table 1). However, in other RNAi experiments, gene knockdown did not affect pathogen acquisition by ticks (45) or facilitated *B. gibsoni* transmission by targeting the *Haemaphysalis longicornis* antimicrobial peptide, longicin (46) (Table 1).

Studies of the tick protective antigen, subolesin, provided evidence that tick gene expression may be targeted by RNAi to test the role of encoded proteins in tick biology and the vector capacity of ticks (Figure 2). Subolesin was recently shown by both RNAi gene silencing and immunization trials using the recombinant protein to protect hosts against tick infestations, reduce tick survival and reproduction, and cause degeneration of gut, salivary gland, reproductive tissues and embryos (35, 36, 47-50). The silencing of subolesin expression by RNAi also decreased vector capacity of ticks for A. marginale (25). In addition, subolesin mRNA levels were determined by real-time RT-PCR in uninfected and A. marginale-infected D. variabilis guts and salivary glands and IDE8 cultured tick cells, as well as in uninfected and A. phagocytophilum-infected I. scapularis nymphs and ISE6 cultured tick cells (51). In these experiments, subolesin was differentially expressed in A. marginale-infected ticks in a tissue-specific manner in which mRNA levels increased in response to *A. marginale* infection in tick salivary glands but not in the guts. Subolesin knockdown by RNAi reduced *Anaplasma* infection/multiplication only in cells in which infection increased subolesin expression, i.e. in *A. marginale*-infected *D. variabilis* salivary glands and IDE8 cells (51).

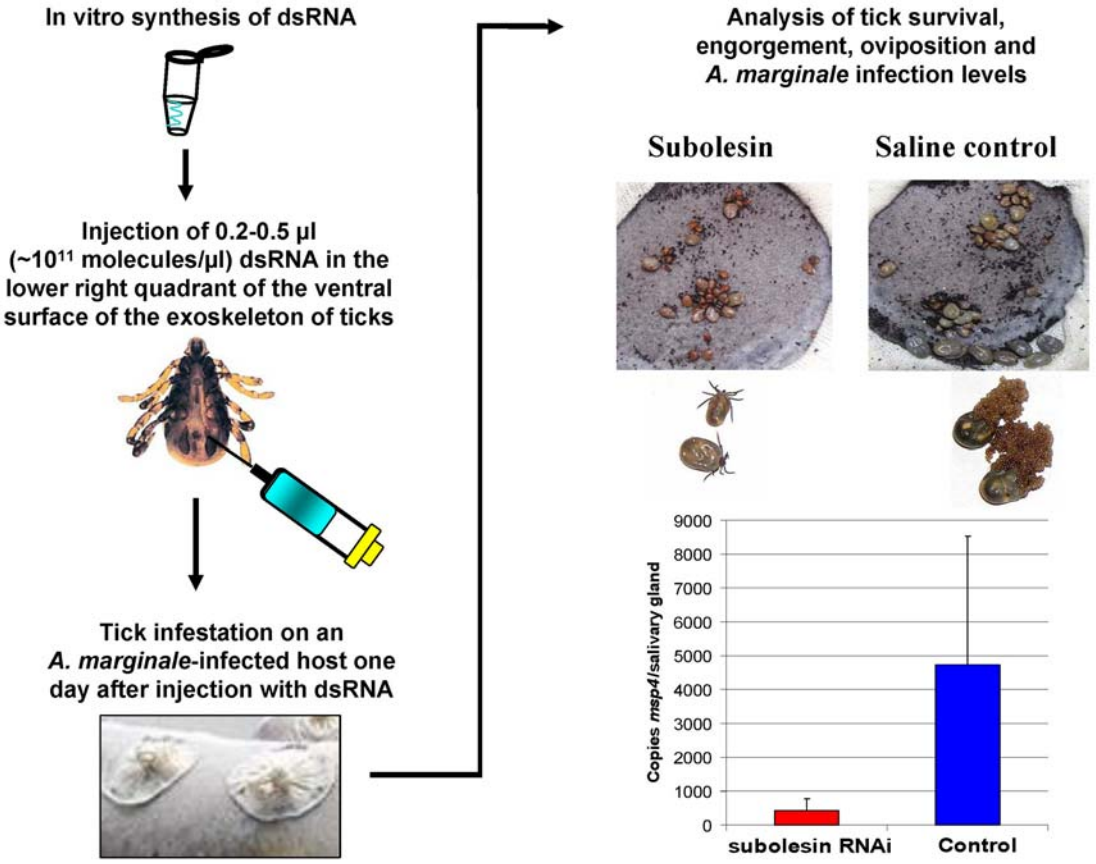

Figure 2. The tick protective antigen subolesin provided evidence that tick gene expression may be targeted by RNAi to test the role of encoded proteins in tick biology and the vectorial capacity of ticks. Experimental results were derived from de la Fuente *et al.* (25, 91).

These studies demonstrated a role of subolesin in *Anaplasma*-tick interactions, suggesting that this protein may be useful in a vaccine formulation for the control of tick infestations and pathogen infection/multiplication in ticks.

Recently, a functional genomics approach to characterize tick genes regulated in response to *A. marginale* infection in cultured IDE8 tick cells and ticks was reported by de la Fuente *et al.* (42). RNAi in *D. variabilis* ticks and IDE8 tick cells was used for functional studies of differentially expressed genes/proteins that were identified by suppression-subtractive hybridization and differential in-gel electrophoresis analyses. Through these studies molecules were identified that affected *A. marginale* infection in IDE8 tick cells and at different sites of development in ticks (Table 1). Importantly, the results revealed that a molecular mechanism occurs by which tick gene expression mediates the *A. marginale* developmental cycle and trafficking through ticks.

Collectively, these studies demonstrated that RNAi constitutes an important tool for the study of the tick-pathogen interface which will likely contribute to the rapid identification and characterization of candidate antigens for use in pathogen transmission-blocking tick vaccines.

4.3. Other potential approaches for the genetic manipulation of ticks and the characterization of the tick-pathogen interface

Recombinant bacteria, parasites and viruses that naturally infect ticks may be useful for developing novel methods for the study of tick-pathogen interactions and the manipulation of the tick-pathogen interface. However, the genetic transformation of intracellular bacteria that infect ticks has been difficult to attain because of their intracellular location, small size and complex growth conditions. Transformation and tick infection with recombinant bacteria has recently been reported for *Escherichia coli* (52, 53), *B. burgdorferi* (54-57), *B. afzelii* (58), and *R. monacensis* (59). Methods have been developed for the production and detection of recombinant organisms in cultured cells for the tick-borne rickettsial pathogens, *R. prowazekii* (60-62), *C. burnetii* (63, 64), *A. phagocytophilum* (65), *Ehrlichia muris* (66), and *F. tularensis* (67-69). Transformation of the protozoan parasite, *B. bovis,* with potential applications for other *Babesia* species, has also been reported (70).

Therefore, paratransgenesis is an alternative approach for the manipulation of ticks by production of infective recombinant tick symbionts such as *Wolbachia* and other related organisms (71-73). Although these experiments have not been reported in ticks, recent developments suggest that transformation of *Wolbachia* may be feasible (74, 75). Additionally, the sequencing of the *I. scapularis* genome has revealed the presence of sequences with identity to *Rickettsia* spp., which are possible tick symbionts with a long co-evolutionary history (90).

These preliminary studies suggest the possibility of developing paratransgenic ticks with transformed pathogens for study of tick-pathogen interactions by expression of recombinant proteins and other molecules in the tick. Use of fluorescent bacteria and parasites may allow researchers to document pathogen infection, multiplication and trafficking in ticks (57). Antibiotic-resistant bacteria are an important tool for *in vitro* studies in cell culture. Recombinant bacteria and parasites could serve as a vehicle for delivery of recombinant proteins and dsRNAs for use in functional studies of tick biology and tick-pathogen interactions.

The infection of tick cells with vector-borne RNA viruses such as Semliki Forest virus was found to trigger the RNAi pathway and may be useful in characterizing dsRNA-mediated gene silencing in ticks (76). Recombinant viruses engineered to produce dsRNA may allow for induced and/or enhanced RNAi *in vivo* in ticks. This approach has been used to render mosquitoes resistant to arboviruses (77-80) and *Plasmodium* parasites (81), thus confirming the potential of this approach for targeting the vector-pathogen interface to control the transmission of vector-borne pathogens.

Finally, although remotely possible at this time, the production of transgenic ticks through embryo microinjection, electroporation or transfection may be feasible. While transformation methods with transposable elements similar to those developed for *Drosophila* (82) and mosquitoes (83) have not been established in ticks, electroporation and transfection of foreign DNA has been accomplished in shrimp embryos (84-86). Recently, Sunter *et al.* (91) discovered a short interspersed repetitive element (SINE; designed Ruka) that may be mobile in the genome of *R. appendiculatus*, *B. microplus*, *A. variegatum* and *I. scapularis*. Although at its infancy, this and other similar sequences present in tick genomes may be used to develop transformation methods in ticks. Transgenesis is being investigated as a means of engineering mosquitoes with a genetic trait that confers resistance to malaria or causes population suppression while driving the new trait through field populations (87). Recently, a promising result was reported in which transient sterile ticks were produced by RNAi of subolesin and may be used in the future development of methods for autocidal control of ticks (48). Development of techniques for production of transgenic ticks for autocidal control and resistance to pathogen transmission would enhance research in this area.

5. SUMMARY AND PERSPECTIVE

Tick vaccines and RNAi have proven useful for studying tick-pathogen interactions and for targeting the tick-pathogen interface with the goal of interrupting the transmission of tick-borne pathogens. Advances in the use and applications of RNAi in ticks, as well as development of new approaches for the genetic manipulation of ticks, will greatly enhance future opportunities to study the tick-pathogen interface. The combination of tick and pathogen genomics (88-90) with high throughput screening platforms using RNAi (92) and other forthcoming technologies will increase our knowledge of the tick-pathogen interface and will likely provide novel and improved vaccines targeted both at control of tick infestations and the transmission of tick-borne pathogens.

6. ACKNOWLEDGEMENTS

We thank members of our laboratories for fruitful discussions and technical assistance. This work was supported by the project No. 1669 of the Oklahoma Agricultural Experiment Station and the Endowed Chair for Food Animal Research (K.M. Kocan), the Instituto de Ciencias de la Salud, Spain (ICS-JCCM) (project 06036-00), the Consejería de Educación y Ciencia, JCCM, Spain (project PAI06-0046-5285), and the Wellcome Trust under the Animal Health in the Developing World initiative through project 0757990 entitled **"**Adapting recombinant anti-tick vaccines to livestock in Africa".

7. REFERENCES

1. Camicas J.L., Hervy, J.P., Adam, F., & Morel, P.C.: The ticks of the world (Acarida, Ixodida). Orstom editions, Paris, France (1998)

2. Barker, S.C. & Murrell, A.: Systematics and evolution of ticks with a list of valid genus and species names. *Parasitol* 129, S15-S36 (2004)

3. Willadsen, P.: Tick control: Thoughts on a research agenda. *Vet Parasitol* 138, 161-168 (2006)

4. de la Fuente, J. & Kocan, K.M.: Strategies for development of vaccines for control of ixodid tick species. *Parasite Immunol* 28, 275-283 (2006)

5. Parola, P. & Raoult, D.: Tick-borne bacterial diseases emerging in Europe. *Clin Microbiol Infect* 7, 80-83 (2001)

6. Peter, R.J., Van den Bossche, P., Penzhorn, B.L. & Sharp, B.: Tick, fly, and mosquito control-Lessons from the past, solutions for the future. *Vet Parasitol* 132, 205-215 (2005)

7. de la Fuente, J., Kocan, K.M., Mangold, A.J., Blouin, E.F., Naranjo, V. & Almazán, C.: Biogeography and molecular evolution of *Anaplasma* species. *Vet Parasitol*, in press (2007)

8. 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.: TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. *Cell* 119, 457-468 (2004)

9. Nuttall, P.A., Trimnell, A.R., Kazimirova, M. & Labuda, M.: Exposed and concealed antigens as vaccine targets for controlling ticks and tick-borne diseases. *Parasite Immunol* 28, 155-163 (2006)

10. Willadsen, P.: Anti-tick vaccines. *Parasitol* 129, S367-387 (2004)

11. Sonenshine, D.E., Kocan, K.M. & de la Fuente, J.: Tick control: further thoughts on a research agenda. *Trends Parasitol* 22, 550-551 (2006)

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

13. de la Fuente, J. & Kocan, KM.: Advances in the identification and characterization of protective antigens for development of recombinant vaccines against tick infestations. *Expert Rev Vaccines* 2, 583-593 (2003)

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

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

16. Allen, J.R. & Humphreys, S.J.: Immunisation of guinea pigs and cattle against ticks. *Nature* 280, 491-493 (1979)

17. 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.: Field studies and cost-effectiveness analysis of vaccination with Gavac™ against the cattle tick *Boophilus microplus*. *Vaccine* 16, 366-373 (1998)

18. Valle, M.R., Méndez, L., Valdez, M., Redondo, M., Espinosa, C.M., Vargas, M., Cruz, R.L., Barrios, H.P., Seoane, G., Ramírez, E.S., Boué, O., Vigil, J.L., Machado, H., Nordelo, C.B. & Piñeiro, M.J.: Integrated control of *Boophilus microplus* ticks in Cuba based on vaccination with the anti-tick vaccine Gavac. *Exp Appl Acarol* 34, 375-382 (2004)

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

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

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

22. Vaz Junior Ida, S., Martinez, R.H., Oliveira, A., Heck, A., Logullo, C., Gonzales, J.C., Dewes, H. & Masuda, A.: Functional bovine immunoglobulins in *Boophilus microplus* hemolymph. *Vet Parasitol* 62, 155-60 (1996)

23. Jasinskas, A., Jaworski, D.C., Barbour, A.G.: *Amblyomma americanum*: specific uptake of immunoglobulins into tick hemolymph during feeding. *Exp Parasitol* 96, 213-21 (2000)

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

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

26. Narasimhan, S., Deponte, K., Marcantonio, N., Liang, X., Royce, T.E., Nelson, K.F., Booth, C.J., Koski, B., Anderson, J.F., Kantor, F. & Fikrig, E.: Immunity against *Ixodes scapularis* salivary proteins expressed within 24 hours of attachment thwarts tick feeding and impairs *Borrelia* transmission. *PLoS ONE* 2:e451 (2007)

27. Gomes-Solecki, M.J.C., Brisson, D.R. & Dattwyler, R.J.: Oral vaccine that breaks the transmission cycle of the Lyme disease spirochete can be delivered via bait. *Vaccine* 24, 4440-4449 (2006)

28. Tsao, J.I., Wootton, J.T., Bunikis, J., Luna, M.G., Fish, D. & Barbour, A.G.: An ecological approach to preventing human infection: vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. *Proc Natl Acad Sci U S A* 101, 18159-18164 (2004)

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

30. de la Fuente, J., Ayoubi, P., Blouin, E.F., Almazán, C., Naranjo, V. & Kocan, K.M.: Anaplasmosis: focusing on host-vector-pathogen interactions for vaccine development. *Ann N Y Acad Sci* 1078, 416-423 (2006)

31. Kocan, K.M., de la Fuente, J. & Blouin, E.F.: Targeting the tick/pathogen interface for developing new anaplasmosis vaccine strategies. *Vet Res Commun* 31 (Suppl. 1), 91-96 (2007)

32. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E. & Mello, C.C.: Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806-811 (1998)

33.Mello, C.C. & Conte, D. Jr.: Revealing the world of RNA interference. *Nature* 431, 338-342 (2004)

34. Zhou, D., He, Q.S., Wang, C., Zhang, J. & Wong-Staal, F.: RNA interference and potential applications. *Curr Top Med Chem* 6, 901-911 (2006)

35. Nijhof, A.M., Taoufik, A., de la Fuente, J., Kocan, K.M., de Vries, E. & Jongejan, F.: Gene silencing of the tick protective antigens, *Bm86*, *Bm91* and *subolesin,* in the one-host tick *Boophilus microplus* by RNA interference. *Int J Parasitol* 37, 653-662 (2007)

36. Kocan, K.M., Manzano-Roman, R. & de la Fuente, J.: Transovarial silencing of the subolesin gene in three-host ixodid tick species after injection of replete females with subolesin dsRNA. *Parasitol Res* 100, 1411-1415 (2007)

37. Soares, C.A., Lima, C.M., Dolan, M.C., Piesman, J., Beard, C.B. & Zeidner, N.S.: Capillary feeding of specific dsRNA induces silencing of the isac gene in nymphal *Ixodes scapularis* ticks. *Insect Mol Biol* 14, 443-452 (2005)

38. Ramakrishnan, V.G., Aljamali, M.N., Sauer, J.R. & Essenberg, R.C.: Application of RNA interference in tick salivary gland research. *J Biomol Tech* 16, 297-305 (2005)

39. Blouin, E.F., Manzano-Roman, R., de la Fuente, J. & Kocan, K.M.: Defining the role of subolesin in tick cell culture by use of RNA interference. *Ann N Y Acad Sci*, in press (2008)

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

41. Nene, V., Lee, D., Kang'a, S., Skilton, R., Shah, T., de Villiers, E., Mwaura, S., Taylor, D., Quackenbush, J. & Bishop, R.: Genes transcribed in the salivary glands of female *Rhipicephalus appendiculatus* ticks infected with *Theileria parva*. *Insect Biochem Mol Biol* 34, 1117-1128 (2004)

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

43. Ramamoorthi, N., Narasimhan, S., Pal, U., Bao, F., Yang, X.F., Fish, D., Anguita, J., Norgard, M.V., Kantor, F.S., Anderson, J.F., Koski, R.A. & Fikrig, E.: The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature* 436, 573-577 (2005)

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

45. Pedra, J.H., Narasimhan, S., Deponte, K., Marcantonio, N., Kantor, F.S. & Fikrig, E.: Disruption of the salivary protein 14 in *Ixodes scapularis* nymphs and impact on pathogen acquisition. *Am J Trop Med Hyg* 75, 677-682 (2006)

46. Tsuji, N., Battsetseg, B., Boldbaatar, D., Miyoshi, T., Xuan, X., Oliver, J.H.Jr. & Fujisaki, K.: Babesial vector tick defensin against *Babesia* sp. parasites. *Infect Immun* 75, 3633-3640 (2007)

47. de la Fuente, J., Almazán, C., Blas-Machado, U., Naranjo, V., Mangold, A.J., Blouin, E.F., Gortazar, C. & Kocan, K.M.: The tick protective antigen, 4D8, is a conserved protein involved in modulation of tick blood ingestion and reproduction. *Vaccine* 24, 4082-4095 (2006)

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

49. Almazán, C., Blas-Machado, U., Kocan, K. M., Yoshioka, J. H., Blouin, E. F., Mangold, A. J. & de la Fuente, J.: Characterization of three *Ixodes scapularis* cDNAs protective against tick infestations. *Vaccine* 23, 4403-4416 (2005)

50. Almazán, C., Kocan, K. M., Blouin, E. F. & de la Fuente, J.: Vaccination with recombinant tick antigens for the control of *Ixodes scapularis* adult infestations. *Vaccine* 23, 5294-5298 (2005)

51. de la Fuente, J., Blouin, E.F., Manzano-Roman, R., Naranjo, V., Almazán, C., Pérez de la Lastra, J.M., Zivkovic, Z., Massung, R.F., Jongejan, F. & Kocan, K.M.: Differential expression of the tick protective antigen, subolesin, in *Anaplasma marginale* and *A. phagocytophilum* infected host cells. *Ann N Y Acad Sci*, in press (2008)

52. Ceraul, S.M., Sonenshine, D.E. & Hynes, W.L.: Resistance of the tick *Dermacentor variabilis* (Acari: Ixodidae) following challenge with the bacterium *Escherichia coli* (Enterobacteriales: Enterobacteriaceae). *J Med Entomol* 39, 376-383 (2002)

53. Matsuo, T., Okoda, Y., Badgar, B., Inoue, N., Xuan, X., Taylor, D. & Fujisaki, K.: Fate of GFP-expressing

Escherichia coli in the midgut and response to ingestion in a tick, *Ornithodoros moubata* (Acari: Argasidae). *Exp Parasitol* 108, 67-73 (2004)

54. Kawabata, H., Norris, S.J. & Watanabe, H.: BBE02 disruption mutants of *Borrelia burgdorferi* B31 have a highly transformable, infectious phenotype. *Infect Immun* 72, 7147-7154 (2004)

55. Jacobs, M.B., Norris, S.J., Phillippi-Falkenstein, K.M., & Philipp, M.T.: Infectivity of the highly transformable BBE02- lp56- mutant of *Borrelia burgdorferi*, the Lyme disease spirochete, via ticks. *Infect Immun* 74, 3678-3681 (2006)

56. Li, X., Pal, U., Ramamoorthi, N., Liu, X., Desrosiers, D.C., Eggers, C.H., Anderson, J.F., Radolf, J.D. & Fikrig, E.: The Lyme disease agent *Borrelia burgdorferi* requires BB0690, a *Dps* homologue, to persist within ticks. *Mol Microbiol* 63, 694-710 (2007)

57. Revel, A.T., Blevins, J.S., Almazán, C., Kocan, K.M., de la Fuente, J., Hagman, K.E. & Norgard, M.V.: *bptA* (*bbe16*) is essential for the persistente of the Lyme disease spirochete, *Borrelia burgdorferi*, in its natural tick vector. *Proc Natl Acad Sci U.S.A* 102, 6972-6977 (2005)

58. Fingerle, V., Goettner, G., Gern, L., Wilske, B. & Schulte-Spechtel, U.: Complementation of a *Borrelia afzelii* OspC mutant highlights the crucial role of OspC for dissemination of *Borrelia afzelii* in *Ixodes ricinus*. *Int J Med Microbiol* 297, 97-107 (2007)

59. Baldridge, G.D., Kurtti, T.J., Burkhardt, N., Baldridge, A.S., Nelson, C.M., Oliva, A.S. & Munderloh, U.G.: Infection of *Ixodes scapularis* ticks with *Rickettsia monacensis* expressing green fluorescent protein: a model system. *J Invertebr Pathol* 94, 163-174 (2007)

60. Rachek, L.I., Tucker, A.M., Winkler, H.H. & Wood, D.O.: Transformation of *Rickettsia prowazekii* to rifampin resistance. *J Bacteriol* 180, 2118-2124 (1998). Erratum in: *J Bacteriol* 180, 4015 (1998)

61. Rachek, L.I., Hines, A., Tucker, A.M., Winkler, H.H. & Wood, D.O.: Transformation of *Rickettsia prowazekii* to erythromycin resistance encoded by the *Escherichia coli ereB* gene. *J Bacteriol* 182, 3289-3291 (2000)

62. Qin, A., Tucker, A.M., Hines, A. & Wood, D.O.: Transposon mutagenesis of the obligate intracellular pathogen *Rickettsia prowazekii*. *Appl Environ Microbiol* 70, 2816-2822 (2004)

63. Lukácová, M., Valková, D., Quevedo Diaz, M., Perecko, D. & Barák, I.: Green fluorescent protein as a detection marker for *Coxiella burnetii* transformation. *FEMS Microbiol Lett* 175, 255-260 (1999)

64. Suhan, M.L. & Thompson, H.A.: Expression of beta-lactamase in *Coxiella burnetii* transformants. *FEMS Microbiol Lett.* 184, 303-306 (2000)

65. Felsheim, R.F., Herron, M.J., Nelson, C.M., Burkhardt, N.Y., Barbet, A.F., Kurtti, T.J. & Munderloh, U.G.: Transformation of *Anaplasma phagocytophilum*. *BMC Biotechnol* 6, 42 (2006)

66. Long, S.W., Whitworth, T.J., Walker, D.H. & Yu, X.J.: Overcoming barriers to the transformation of the genus *Ehrlichia*. *Ann N Y Acad Sci* 1063, 403-410 (2005)

67. Baron, G.S., Myltseva, S.V. & Nano, F.E.: Electroporation of *Francisella tularensis*. *Methods Mol Biol* 47, 149-154 (1995)

68. Maier, T.M., Havig, A., Casey, M., Nano, F.E., Frank, D.W. & Zahrt, T.C.: Construction and characterization of a highly efficient *Francisella* shuttle plasmid. *Appl Environ Microbiol* 70, 7511-7519 (2004)

69. Kawula, T.H., Hall, J.D., Fuller, J.R. & Craven, R.R.: Use of transposon-transposase complexes to create stable insertion mutant strains of *Francisella tularensis* LVS. *Appl Environ Microbiol* 70, 6901-6904 (2004)

70. Suarez, C.E., Lacy, P., Laughery, J., Gonzalez, M.G. and McElwain, T.: Optimization of *Babesia bovis* transfection methods. *Parassitologia* 49 (Suppl 1), 67-70 (2007)

71. Noda, H., Munderloh, U.G., Kurtti, T.J.: Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl Environ Microbiol* 63, 3926-3932 (1997)

72. Rymaszewska, A.: Symbiotic bacteria in oocyte and ovarian cell mitochondria of the tick *Ixodes ricinus*: biology and phylogenetic position. *Parasitol Res* 100, 917-920 (2007)

73. Fenn, K., Conlon, C., Jones, M., Quail, M.A., Holroyd, N.E., Parkhill, J. & Blaxter, M.: Phylogenetic relationships of the Wolbachia of nematodes and arthropods. *PLoS Pathog* 2, e94 (2006)

74. Cheng, Q. & Aksoy, S.: Tissue tropism, transmission and expression of foreign genes *in vivo* in midgut symbionts of tsetse flies. *Insect Mol Biol* 8, 125-132 (1999)

75. Fujii, Y., Kubo, T., Ishikawa, H. & Sasaki, T.: Isolation and characterization of the bacteriophage WO from *Wolbachia*, an arthropod endosymbiont. *Biochem Biophys Res Commun* 317, 1183-1188 (2004)

76. Garcia, S., Billecocq, A., Crance, J.M., Prins, M., Garin, D. & Bouloy, M.: Viral suppressors of RNA interference impair RNA silencing induced by a Semliki Forest virus replicon in tick cells. *J Gen Virol* 87, 1985-1989 (2006)

77. Blair, S.D., Sanchez-Vargas, I., Franz, A.W.E. & Olson, K.E.: Rendering mosquitoes resistant to arboviruses through RNA interference. *Microbe* 1, 466-470 (2006)

78. Franz, A.W., Sanchez-Vargas, I., Adelman, Z.N., Blair, C.D., Beaty, B.J., James, A.A. & Olson, KE.: Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proc Natl Acad Sci U S A* 103, 4198-4203 (2006)

79. Olson, K.E., Higgs, S., Gaines, P.J., Powers, A.M., Davis, B.S., Kamrud, K.I., Carlson, J.O., Blair, C.D. & Beaty, B.J.: Genetically engineered resistance to dengue-2 virus transmission in mosquitoes. *Science* 272, 884-886 (1996)

80. Olson, K.E., Adelman, Z.N., Travanty, E.A., Sanchez-Vargas, I., Beaty, B.J. & Blair, C.D.: Developing arbovirus resistance in mosquitoes. *Insect Biochem Mol Biol* 32, 1333-1343 (2002)

81. Brown, A.E. & Catteruccia, F.: Toward silencing the burden of malaria: progress and prospects for RNAi-based approaches. *Biotechniques* Suppl, 38-44 (2006)

82. Ryder, E. & Russell, S.: Transposable elements as tools for genomics and genetics in *Drosophila*. *Brief Funct Genomic Proteomic* 2, 57-71 (2003)

83. Marrelli, M.T., Moreira, C.K., Kelly, D., Alphey, L. & Jacobs-Lorena, M.: Mosquito transgenesis: what is the fitness cost? *Trends Parasitol* 22,197-202 (2006)

84. Cabrera, E., Pimentel, R., Abad, Z., Piña, J. C., Hernández, O., Lleonart, R. & de la Fuente, J.: Transient transformation of shrimp (*Penaeus schmitti*) embryos by DNA microinjection. *Theriogenology* 43, 180 (1995)

85. Tseng, F.S., Tsai, H.J., Liao, I.C. & Song, Y.L.: Introducing foreign DNA into tiger shrimp (*Penaeus monodon*) by electroporation. *Theriogenology* 54, 1421-1432 (2000)

86. Lu, Y. & Sun, P.S.: Viral resistance in shrimp that express an antisense Taura syndrome virus coat protein gene. *Antiviral Res* 67, 141-146 (2005)

87. Christophides, G.K.: Transgenic mosquitoes and malaria transmission. *Cell Microbiol* 7, 325-333 (2005)

88. Jensen, K., de Miranda Santos, I.K. & Glass, E.J.: Using genomic approaches to unravel livestock (host)-tick-pathogen interactions. *Trends Parasitol* 23, 439-444 (2007)

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

90. Pagel Van Zee, J., Geraci, N.S., Guerrero, F.D., Wikel, S.K., Stuart, J.J., Nene, V.M. & Hill, C.A.: Tick genomics: The *Ixodes* genome project and beyond. *Int J Parasitol* 37, 1297-1305 (2007)

91. Sunter, J.D., Patel, S.P., Skilton, R.A., Githaka, N., Knowles, D.P., Scoles, G.A., Nene, V., de Villiers, E., Bishop, R.P. A novel SINE family occurs frequently in both genomic DNA and transcribed sequences in ixodid ticks of the arthropod sub-phylum Chelicerata. Gene, in press (2008) doi:10.1016/j.gene.2008.01.026

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

Abbreviations: RNAi, RNA interference; TBEV, tick-borne encephalitis virus; siRNA, small interfering RNA; dsRNA, double-stranded RNA; Ago, Argonaute; PAZ, Piwi/Argonaute/Zwille; DEAD, i.e. the single letter code for amino acids Asp-Glu-Ala-Asp; RISC, RNA-induced silencing complex; TROSPA, tick receptor for *B. burgdorferi* OspA; Salp, salivary gland protein; Isac, *I. scapularis* anticomplement; GST, glutathione-S transferase; AF, acquisition feeding; TF, transmission feeding; HSC, hematopoietic stem/progenitor cells protein-like.

Key Words: Tick, Vaccine, RNA interference, Tick-pathogen interface, Evolution, Control, Paratransgenesis, Rickettsia, Anaplasma, Borrelia, Ehrlichia, Wolbachia, Virus, Review

Send correspondence to: Jose de la Fuente, Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, 250 McElroy Hall, Oklahoma State University, Stillwater, OK 74078. Tel: 405-744-0372. Fax: 405-744-5275, E-mail: jose.de_la_fuente@okstate.edu

http://www.bioscience.org/current/vol13.htm