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Advances toward understanding the molecular biology of the *Anaplasma*-tick interface

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1. ABSTRACT

The genus *Anaplasma* includes a diverse group of tick-borne pathogens found exclusively within membrane-bound vacuoles in host cells. While *A. marginale*, *A. centrale* and *A. ovis*, vectored by *Dermacentor* and *Rhipicephalu*s ticks, are host-specific for ruminants, *A. phagocytophilum*, vectored by *Ixodes* spp., infects a wide range of hosts. In ticks *Anaplasma* undergoes a developmental cycle that is coordinated with the tick feeding cycle. Although research at the tick/*Anaplasma* interface is in its infancy, recent studies have provided evidence that *Anaplasma* infection and transmission is mediated by a molecular mechanism involving both tick cell and pathogen genes. Application of a growing array of molecular approaches, such as RNA interference, genomics and proteomics, are rapidly expanding our knowledge of the tick/pathogen interface. Targeting key tick cell molecules required for pathogen development in vaccine strategies may compromise the vector capacity of ticks for *Anaplasma,* thus reducing transmission and infection of vertebrates. Collectively, this information will likely lead to the development of dual target vaccines designed to protect vertebrates against tick infestations and prevent the transmission of pathogens.

2. INTRODUCTION

Pathogens classified within the family Anaplasmataceae develop in two very different hosts, vertebrates and ticks, and the co-evolution of pathogens with both of these hosts has insured their mutual survival. While pathogens remain dormant in ticks that are off the host, their multiplication, development and subsequent transmission to vertebrate hosts are perfectly coordinated with the tick feeding cycle.

The family Anaplasmataceae is one of two families in the Order Rickettsiales, along with the family Rickettsiaceae (1). Organisms within the family Anaplasmataceae are obligate intracellular organisms that are found exclusively within membrane-bound vacuoles in the vertebrate or tick host cell cytoplasm. Within the family Anaplasmataceae, phylogenetic analyses consistently supported the formation of four distinct genetic groups of the organisms: (1) *Anaplasma* with a 96.1% similarity, (2) *Ehrlichia* with a 97.7% similarity, (3) *Wolbachia* with a minimum of 95.6% similarity and (4) *Neorickettsia* with a minimum of 94.9% similarity (1). After a recent reclassification, the genus *Anaplasma* was expanded from three pathogens of ruminants, *A. marginale*, *A. centrale* an

Table 1. Current Classification of the Genus *Anaplasma*

Order Rickettsiales	
Family Rickettsiaceae	
Family Anaplasmataceae	Obligate intracellular bacteria that replicate with membrane-derived vacuoles in the cytoplasm of eukaryotic host cells
Genus *Anaplasma*	*Anaplasma marginale* (type species)
	Anaplasma centrale
	Anaplasma ovis
	Anaplasma phagocytophilum (formerly Ehrlichia phagocytophilum, E. equi
	HGE agent)
	*Anaplasma bovis (*formerly *Ehrlichia bovis*)
	*Anaplasma platys (*formerly *Ehrlichia platys*)
	*Aegyptianella (*genus incertae sedis due to lack of sequence information)

A. ovis, to also include *A. bovis* (formerly *Ehrlichia bovis*), *A. phagocytophilum (*formerly *E. phagocytophila*, *E. equi* and the HGE agent), and *A. platys (*formerly *E. platys*). *Aegyptianella,* also included in this genus*,* was retained as a *genus incertae sedis* due to lack of sequence information (Table 1).

A. marginale, A. centrale and *A. ovis* are host-specific for ruminants (primarily cattle, sheep, goats and deer) and the main tick vectors are *Dermacentor* and *Rhipicephalus (Boophilus)*. The type species of the genus *Anaplasma*, *A. marginale*, is distributed worldwide in tropical and subtropical regions of the New World, Europe, Africa, Asia and Australia where it causes bovine anaplasmosis (2). Many strains of *A. marginale* have been identified worldwide which differ in morphology, protein sequence, antigenic characteristics and their ability to infect and be transmitted by ticks (3, 4, 5, 6, 7, 8, 9, 10). While some strains have been shown not to be transmissible by ticks (Illinois, Okeechobee, Florida, Brazil; Mississippi, Canadian) (3, 4, 11, 12, 13, 14), ticks appear to have maintained vector competence for most other strains despite the diverse geographic locations of tick species and *A. marginale* strains (15, 16, 17). The closely related *A. ovis* infects sheep, goats and wild ruminants (18, 19, 20, 21, 22, 23, 24, 25) and was recently reported in mule deer in the U.S. (26). The less pathogenic *A. centrale* for cattle is used as a live cattle vaccine in Europe, Australia and Africa (27, 28, 29).

A. phagocytophilum infects a wide range of host animals including rodents, ruminants, birds, felids, horses and donkeys, dogs and humans and is transmitted by ticks of the *Ixodes ricinus* complex (1, 30). *A. phagocytophilum* is the causative agent of tick-borne fever (TBF) in ruminants and human, equine and canine granulocytic anaplasmosis (1, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40). *A. phagocytophilum* causes an emerging disease of public health concern in many areas of the world (1, 40) and was recently listed as the most widespread tick-borne infection of animals in Europe (30). Clinical manifestations of human granulocytic anaplasmosis (HGA) include fever, headache, myalgia, anemia, leukopenia, thrombocytopenia, and elevated liver enzymes (41, 42, 43, 44, 45, 46). The white-footed mouse, *Peromyscus leucopus*, is considered to be the main reservoir host of *A. phagocytphilum* in North America, but raccoons, gray squirrels, and cottontail rabbits become naturally infected with the pathogen and may also serve as reservoir hosts, thus contributing to the maintenance of the natural cycle of infection (47, 48). The list of wildlife hosts that may serve as reservoir hosts continues to expand (49, 50, 51, 52). The wide and expanding host range of this pathogen will most likely ensure increasing distribution of *A. phagocytophilum* worldwide and the continued emergence of granulocytic anaplasmosis in domestic animals and humans (30).

Anaplasma spp. share many common features. Vertebrate hosts develop persistent infections with *Anaplasma* spp. which, in turn, allows them to serve as reservoirs of infection. *Anaplasma* are transmitted horizontally by ixodid ticks while transovarial transmission does not appear to occur. Transstadial transmission occurs from stage to stage (larvae-nymphs, nymphs-adults and larvae-adults) and therefore every tick generation must obtain infection by feeding on reservoir hosts. For the ruminant host-specific *Anaplasma*, intrastadial transmission is effected by male ticks which can both acquire infection and, during a second feeding, transmit the pathogen (53, 54, 55), while intrastadial transmission of *A. phagocytophilum* by male ticks has not been well characterized. The developmental cycles of *A. ovis* and *A. centrale* and most extensively *A. marginale* have been described and shown to be coordinated with the tick feeding cycle (13, 55, 56, 57, 58). The midgut is the first site of infection in which large membrane-bound vacuoles or colonies first contain reticulated forms that divide by binary fission and then subsequently transform into dense forms. After attaching and feeding on a second host, the salivary glands become infected and the pathogen undergoes development similar to the midgut cycle (2, 54, 56). In addition to midgut and salivary gland sites, other tick tissues can become infected. Male ticks develop persistent *A. marginale* infections and can serve as a reservoir of infection (57). While *A. phagocytophilum* has been detected in ticks by molecular technologies and more recently by microscopy (Figure 2 a, b; unpublished data), the tick cycle has not been described by use of microscopy and the role of male ticks, similar to that in the transmission of *A. marginale*, has not been reported. Characterization of the tick development cycle in ticks would be a fundamental contribution toward advancing research on the molecular mechanism of *A. phagocytophilum* in tick-pathogen interactions.

Limited information has historically been available on the molecular interactions of *Anaplasma* and ticks, due at least in part to the recent availability of research tools, including a tick cell culture system, molecular technologies and genomic information. The development of tick cell culture system has provided *in vitro* model systems for both *A. marginale* and *A. phagocytophilum* tick cell-pathogen interactions. In addition, molecular technologies in ticks, such as the use of RNA interference (RNAi) for the study gene function by silencing expression (59), genomics and proteomics are rapidly expanding the scope of tick/pathogen interface research. The recent report of the transfection of *A.*

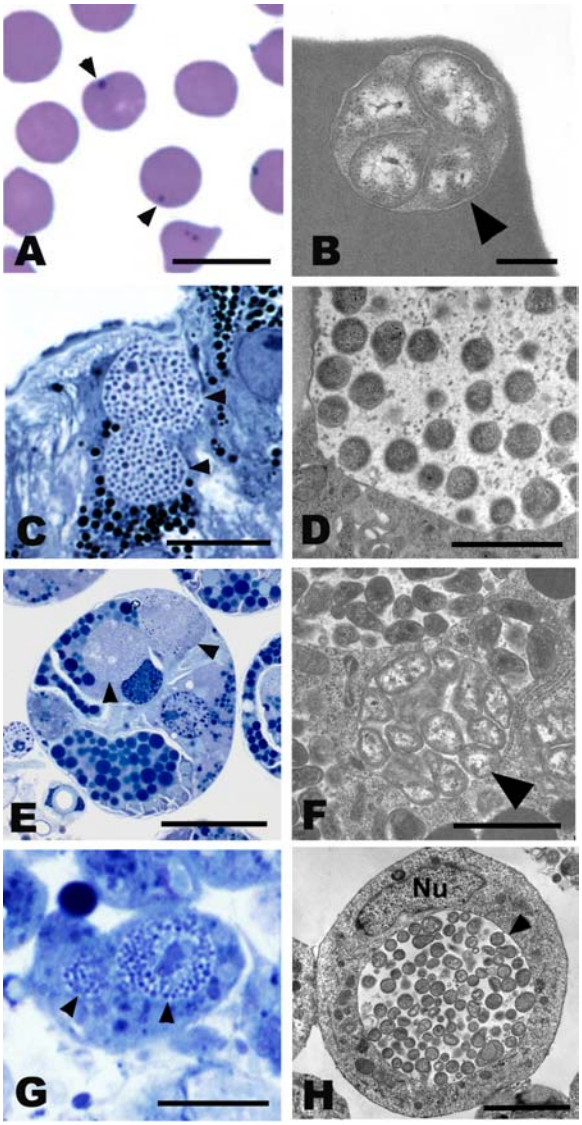

Figure 1. Light and electron micrographs of *Anaplasma marginale* in bovine erythrocytes and native and cultured tick cells. (A) Light and (B) electron micrographs of *A. marginale* inclusion bodies (arrows) in bovine erythrocytes; (C) Light and (D) electron micrographs of *A. marginale* colonies (arrows) in gut cells of *Dermacentor variabilis*; (E) Light and (F) electron micrographs of *A. marginale* (arrows) in tick salivary gland cells; (G) Light and (H) electron micrographs of *A. marginale* colonies (arrows) in cultured IDE8 tick cells. Bars: A, C, E, & G, 10 µm; B, 0.5 µm; D, F, 1 µm; H, 5 µm.

phagocytophilum and other intracellular pathogens shows promise for the molecular manipulation of key pathogen genes (60). Importantly, the rapidly increasing data bases of genomic information from the sequencing of pathogen and tick genomes, in concert with these molecular technologies, are rapidly creating new and exciting research opportunities.

Although research on the molecular interactions between *Anaplasma* spp. and their tick vectors is in its infancy, definition of these interactions is fundamental to the formulation of novel control measures. The dual approach of reducing the vector capacity of ticks by interrupting the pathogen development cycle and transmission and by interfering with tick biology, feeding and reproduction would be an approach that would most certainly contribute to reduction of both tick infestations and tick-borne infections in vertebrates (61, 62). With the exception of *A. marginale,* which is frequently transmitted mechanically by any means of transferring infective blood from infected to susceptible animals, transmission of the other *Anaplasma* spp. are dependent on the respective tick vectors.

Herein, the *Anaplasma* host-specific for ruminants, namely *A. marginale*, *A. ovis* and *A. centrale*, will be reviewed together because of their similarities, while *A. phagocytophilum* will be considered separately because of its differences when compared to the other species. *A. bovis, A. platys,* and *Aegyptianella* will not be covered because information about these organisms in the tick vector (s) is minimal. The authors hope that this review will point to the importance of focusing research and the development of control measures on the tick-pathogen interface and thus stimulate interest in conducting research on the molecular mechanisms of the tick-pathogen interaction.

3. MOLECULAR INTERACTION OF *ANAPLASMA* SPP. HOST-SPECIFIC FOR RUMINANTS AND THE TICK VECTOR

A characteristic of the ruminant-specific *Anaplasma* spp, is that these pathogens are all found exclusively within membrane-bound vacuoles in the host erythrocytes and in the cytoplasm of tick cells. Because of the extensive research conducted on *A. marginale* over the years, more information is currently available for *A. marginale* than the other two species.

3.1. Tick vectors of ruminant host-specific *Anaplasma*

In the United States, *A. marginale* and *A. ovis* are vectored primarily by *Dermacentor* ticks (*D. andersoni*, *D. variaibilis* and *D. ablipictus*) (1, 2), while the one-host cattle ticks, *Rhipicephalus (Boophilus) microplus* and *R. annulatus*, are the main vectors of *A. marginale* in tropical and subtropical regions of the world. *R. simus* was also identified as a tick vector of *A. marginale* in South Africa (63). Recent research has demonstrated that although the *A. centrale* vaccine strain infects and develops in ticks, this species is not transmitted by ticks (Kocan, unpublished data; 13). In general, *Anaplasma* tick infections are higher when acquired from acutely infected cattle, but ticks that feed on persistently infected cattle also become infected and the biological replication of the organism in ticks appears to make up for initial differences in the infecting dose (64, 65, 66).

3.2. *Anaplasma* tick developmental cycle

The tick life cycle of the ruminant-specific *Anaplasma* is complex and coordinated with tick feeding cycle (2, 54, 56, 57). Bovine erythrocytes infected with *A. marginale* (Figure 1 a, b) are ingested by ticks in the

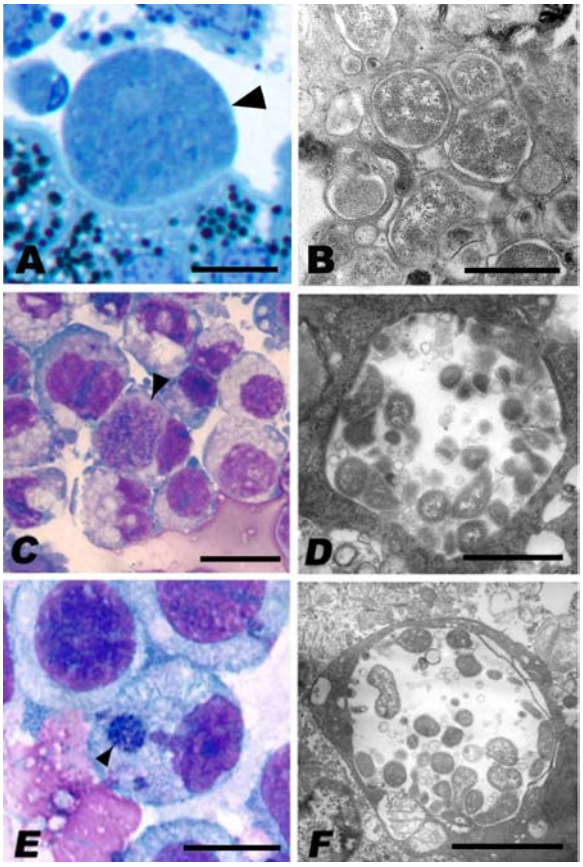

Figure 2. Light and electron micrographs of *Anaplasma phagocytophilum* native and cultured tick cells. (A) Light micrograph of an *A. phagocytophilum* colony (arrow) in a gut muscle cell of *Ixodes scapularis*; (B) Electron micrograph of *A. phagocytophilum* organisms in a tick gut muscle cell; Light and electron micrographs of *A. phagocytophilum* (arrows) in cultured IDE8 cells (C & D) and cultured ISE6 cells (E & F). Bars: A, C, & E, 10 µm; B, 0.5 µm; D, F, 1 µm.

bloodmeal and the first site of infection in ticks occurs in gut cells (Figure 1, c, d), after which many other tick tissues become infected. Infection of salivary glands occurs during a subsequent feeding from where transmission is effected (Figure 1 e, f). The first form of *A. marginale* seen within colonies at each site of development is the reticulated (vegetative) form that divides by binary fission and results in formation of large colonies that may contain hundreds of organisms. The reticulated form then transforms into the dense or infective form which can survive for a short time outside of cells. The tick cell line, IDE8, supports the growth and propagation of *A. marginale* and the large colonies that form within these cells is similar to those described in naturally infected ticks (Figure 1 g, h).

3.3. Phylogenetic diversity of *A. marginale* and tick-pathogen co-evolution

The worldwide phylogenetic diversity of *Anaplasma* strains in cattle, as characterized by study of the major surface proteins (MSPs) MSP1a and MSP4, is quite remarkable and most likely has increased as a result of the long-term movement of cattle (67, 68, 69). This genetic heterogeneity has been observed among *A. marginale* strains within endemic regions such as (70), Oklahoma (11) and Kansas (70) in the United States, Mexico (67, 71), Argentina (29, 67, 72), Minas Gerais and Paraná in Brasil (73), Castilla – La Mancha in Spain (74), and Sicily, Italy (75, 76, 77). This strain diversity could be explained by cattle movement and maintenance of different genotypes by independent transmission events, due to the mechanism of infection exclusion of *A. marginale* in cattle (7, 70, 78). Recent studies on *A. marginale*-infected cattle in endemic areas demonstrated that multiple *msp1α* genotypes were present, but that generally only one genotype was found per individual bovine (7, 78). In contrast, sequences of Australian *A. marginale* strains were the only ones to cluster together, further supporting a more genetically homogeneous *A. marginale* population in this region in which minimal cattle introductions had occurred (68, 79, 80). Recently, Futse *et al.* (81) showed that cattle superinfection may be a driver of genomic diversification in *A. marginale*. These results provide additional support for the generation of genetic diversity in endemic areas and advance the question of whether a similar mechanism also exists in ticks where MSP2 antigenic variants arise during pathogen multiplication (82).

The genetic variation of *A. ovis* MSP4 has been analyzed in the United States for sheep, bighorn sheep and mule deer strains, and in Sicily, Italy, for sheep strains (24, 25, 67, 76). The results of these studies demonstrated that, while *A. ovis msp4* genotypes may vary among geographic regions, the variation observed thus far is less than that observed with *A. marginale* and *A. phagocytophilum*. This finding may have resulted from the restricted movement of infected animals. Additionally, the limited host range of *A. ovis* as compared with *A. phagocytophilum* may have also contributed to the lack of genetic diversity of this pathogen.

The genetic diversity of MSP1a sequences complicates the study of tick-pathogen co-evolution. Analysis of MSP1a repeated sequences provided evidence of the presence of common sequences in strains from different geographic regions, a finding consistent with the existence of the same vector tick species in these regions (80). Furthermore, as suggested by sequence analysis of MSP1a repeats (80), tick-pathogen interactions could influence the presence of unique MSP1a repeats in strains of *A. marginale* from particular geographic regions. However, mechanical transmission of *A. marginale* strains that are not transmissible by ticks could also play an important role in the evolution of *A. marginale* (2).

3.4. *A. marginale* molecules involved in interactions at the tick/pathogen interface

Most studies of molecular events currently described to be involved in *Anaplasma* interactions with host cells have been on *A. marginale* (2, 83), with a greater emphasis on vertebrate host-pathogen interactions than at the tick-pathogen interface. Several major surface proteins (MSPs) have been identified in *Anaplasma* spp., which

have been characterized most extensively in *A. marginale* (2, 9, 77, 84, 85). These MSPs are likely to evolve more rapidly than other genes because they are subjected to selective pressures exerted by host immune systems. Limited information is available for *A. centrale* and *A. ovis* MSPs, and reports have not been published for *A. bovis* and *A. platys* (reviewed by 1, 77).

One of the MSPs studied extensively in tick/*Anaplasma* interactions is the MSP1 complex (5, 82, 86, 87). The MSP1 complex described in *A. marginale* is a heterodimer of two structurally unrelated polypeptides, MSP1a and MSP1b, which are both encoded by genes that have proved to be stable genetic markers of geographic strains of *A. marginale* throughout the developmental cycle in cattle and ticks (88, 89). MSP1a, encoded by the gene *msp1α*, has only been identified thus far in *A. marginale* despite attempts to clone this gene from *A. centrale* and *A. ovis (*90, de la Fuente unpublished data). MSP1b, encoded by two genes, *msp1β1* and *msp1β2*, has also only been identified in *A. marginale* (88, 89). However, only a single MSP1b protein, MSP1b1, was identified within the MSP1 complex (89). The MSP1a of *A. marginale* geographic strains differs in molecular weight because of a variable number of these tandem 23-31 amino acid repeats, and has been used for identification of geographic strains (5, 10, 11, 79).

MSP1a was shown to participate in infection and transmission of *A. marginale* by *Dermacentor* spp. ticks (8), and to be involved in immunity to *A. marginale* infection in cattle (91, 92). Additional studies demonstrated the critical role of the 20th amino acid of the repeat in the interaction of MSP1a with host cell receptors (10, 11). However, analysis of tandemly repeated MSP1a peptides of several geographic isolates of *A. marginale* revealed a complex relationship between the *msp1α* genotype and the tick-transmissible phenotype of the isolate and suggested that both the sequence and conformation of the repeated peptides influenced the adhesive properties of MSP1a (11). The adhesive properties of MSP1a may also involve the glycosylated portion of the protein as described by Garcia-Garcia *et al.* (93). Notably, MSP1a was found to be differentially regulated in bovine erythrocytes and tick cells (94). Down regulation of MSP1a was observed in *A. marginale* in tick salivary glands and cultured ticks cells while this MSP was upregulated by *A. marginale* in bovine erythrocytes.

The MSP2 protein superfamily of *A. marginale* contains immunodominant MSPs with orthologues in all *Anaplasma* spp. studied thus far (95). The *A. marginale* (strain St. Maries) genome is reported to have 56 genes belonging to this superfamily, including eight *msp2*, eight *msp3*, two *msp3* remnants, one *msp4*, three *opag*, 15 *omp-1*, 12 *orfX* and seven *orfY* (83). The most completely characterized representative of this superfamily, MSP2, is encoded by several genes in *A. marginale*, *A. centrale* and *A. ovis* (83, 96, 97). Antigenic variation of MSP2 occurs during persistent *A. marginale* infections in cattle and ticks (82, 83). This mechanism of antigenic variation has been posited to allow *A. marginale* to evade the host immune response, thus contributing to the maintenance of persistent infections (83). Multiple antigenic variants of MSP2, MSP3 of *A. marginale* arise during the multiplication of the pathogen in mammals and ticks as the result of combinatorial gene conversion into expression sites (83). However, differences between ruminant-specific *Anaplasma* (*A. marginale*, *A. central*, and *A. ovis*) and the *A. phagocytophilum* MSP2 suggest that the function(s) of MSP2 may vary within *Anaplasma* spp. *A. marginale omp-1* genes are differentially regulated in bovine erythrocytes and tick cells but show a high degree of conservation during pathogen life cycle in the mammalian host and the tick vector (87). Although the function of MSP4 is presently unknown, the sequence of this gene is genetically stable during the multiplication of *A. marginale* in mammalian and tick cells (72, 88). The *A. marginale* MSP2s are capable of inducing strong T-cell responses and contain antigenically variable B-cell epitopes in the hypervariable region that are recognized by the immune system of *A. marginale*-infected cattle, which results in the selection of new variants that most likely allow the pathogen to establish persistent infections (77, 98). The role of this molecule in establishing persistent infection in the tick is unknown but may be involved in pathogen infection of tick cells.

3.5. Tick molecules involved in interactions at the tick/pathogen interface

Tick-pathogen co-evolution likely involves genetic traits of the vector, as well as those of the pathogen. While recent reports have confirmed the presence of tick receptors for tick-borne pathogens (99), the tick receptor for *A. marginale* and the other ruminant-specific *Anaplasma* spp. has not been identified. In addition, expression of tick genes/proteins that facilitate the infection and multiplication of these pathogens have not been well described (61).

Clearly, isolates of *A. marginale* have been shown to vary in their infectivity for ticks. As mentioned previously, some isolates are refractive to establishing infections in ticks, some appear to infect midguts, while *A. centrale* was recently shown to infect both midguts and salivary glands but was unable to be transmitted by ticks to cattle (13). While Scoles *et al.* (16) demonstrated significant variation in *D. andersoni* midgut susceptibility to the pathogen, Futse *et al.* (15) took a different approach to study tick-*A. marginale* evolutionary adaptations and demonstrated that ticks and *A. marginale* strains retain competence for tick transmission in the absence of vector-pathogen interaction.

Recently, the role of the tick protective protein, subolesin, in the infection and transmission of *A. marginale* was characterized (62). Subolesin was shown in vaccine and RNAi studies to protect against tick infestations and to affect tick feeding, reproduction, and development, as well as infection of host cells by *Anaplasma marginale* and *A. phagocytophilum* (62, 100, 101, 102, 103). Additional experiments provided evidence that infection of tick cells with these two pathogens modified subolesin gene expression and suggested a role for subolesin in

Anaplasma-tick interactions (104). These experiments presented evidence of differential expression of subolesin in *A. marginale* and *A. phagocytophilum* infected cells (62). 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 gland cells but not in the gut cells. 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.

The global tick cell response to infection with *A. marginale* was characterized at the mRNA and protein levels, and RNAi was used to study the function of these molecules during *A. marginale* infection and multiplication in ticks and cultured IDE8 tick cells (105). In these experiments, four genes (encoding for putative GST, salivary selenoprotein M, vATPase and ubiquitin) coincided with significantly lower *A. marginale* infection levels after RNAi in *D. variabilis* guts and/or salivary glands. Six genes (encoding for putative selenoprotein W2a, hematopoietic stem/progenitor cells protein-like, proteasome 26S subunit, ferritin, GST and subolesin control) affected *A. marginale* infection levels in IDE8 tick cells after RNAi. These experiments demonstrated that some of tick genes had different expression patterns in tick guts and salivary glands and affected the *A. marginale* life cycle at different sites in the tick, thus providing additional evidence of the distinct role that guts and salivary glands play on *Anaplasma* infection and transmission by ticks (13), Collectively these data support the hypothesis that *A. marginale* traffics through ticks by means of a molecular mechanism, and the pathogen's subsequent transmission is mediated by tick cell gene expression.

Another influence on pathogen development in ticks is the tick innate immune system. Tick-borne pathogens have apparently co-evolved with ticks for their mutual survival because, while pathogens undergo considerable multiplication in ticks, these infections do not appear to be detrimental to tick feeding or biology (57, 106, 107). Antimicrobial peptides are a component of the innate immune system in ticks that have been shown to provide immunity against both gram-negative and gram-positive bacteria. One component of innate immune systems of eukaryotic organisms are small cationic peptides, defensins, which have been identified in a wide range of species ranging from the simplest invertebrates to mammals, as well as in plants (108). Among invertebrates, the most completely characterized defensins contain 6 cysteines and provide immunity against gram-positive bacteria (109, 110). Defensins have also been identified in a variety of ixodid ticks, including *D. variabilis (*111, 112), *Ixodes scapularis* (113), *Amblyomma americanum* (114), *A. hebraeum* (115) and *R. microplus* (116, 117). Upregulation of defensin occurred in response to challenge-exposure of *D. variabilis* with the gram-negative rickettsia, *Rickettsia montanensis,* fed to ticks via capillary tubes (118). A defensin varisin, identified in *Dermacentor variabilis,* was shown to be expressed primarily in hemocytes but transcript levels were also upregulated in midguts and other tick cells.

We recently studied the role of varisin in the immunity of ticks to *A. marginale (*119). Expression of the varisin gene was silenced by RNAi in which male ticks were injected with varisin dsRNA and then allowed to feed and acquire *A. marginale* infection on an experimentally-infected calf. Silencing of varisin occurred in hemocytes, midguts and salivary glands after RNAi. While we expected that silencing of varisin would increase *A. marginale* infections in ticks, the results demonstrated that tick infections, as determined by an *A. marginale msp4* quantitative PCR, were significantly reduced in the varisin-silenced ticks. Furthermore, colonies of *A. marginale* in ticks used for RNAi were morphologically abnormal from those seen in buffer injected control ticks. The colony shape was irregular and in some cases the *A. marginale* appeared to be free in the cytoplasm of midgut cells. Some ticks were found to be systemically infected with a microbe that may have been affected by the silencing of varisin. Surprisingly, silencing of this defensin in ticks resulted either directly or indirectly in reduced rather than increased pathogen infections in ticks and therefore defensin may be used by the pathogen during its developmental cycle. In addition, this unexpected effect of defensin silencing on *A. marginale* may have resulted because of its intracellular location and development within parasitophorous vacuoles. However, defensin may play a yet unknown function during *A. marginale* infection and may not be active against this pathogen in the natural tick vector but rather acts to limit the infection of other pathogens such as those discovered in tick with varisin gene knockdown.

4. MOLECULAR INTERACTIONS OF *A. PHAGOCYTOPHILUM* AND THE TICK VECTOR

While ticks are known to become infected with *A. phagocytophilum* and transmit the pathogen, the developmental cycle has not been described morphologically in ticks. Furthermore, little is known about the pathogen and tick molecules involved in tick-pathogen interactions.

4.1. Tick vectors and developmental cycle

Tick vectors of *A. phagocytophilum*, *Ixodes* spp. of the *I. ricinus* complex, are commonly found in regions of the United States, Europe, and Asia (120, 121, 122, 123, 124, 125, 126). *I. scapularis* and *I. pacificus* are the main vectors of *A. phagocytophilum* in North America (127). While *I. ricinus* appears to be the main vector of *A. phagocytophilum* in Europe (128), several other ticks may also be vectors, including *Haemaphysalis punctata*, *I. persulcatus*, *I. trianguliceps* and *Rhipicephalus sanguineus* (as reviewed by 30). Although the developmental cycle of *A. phagocytophilum* has not been described in ticks, the pathogen was recently described in ticks (Reichard and Kocan, unpulished data; Figure 2 a, b). In addition, *A. phagocytophilum* has been propagated in the ISE6 and IDE8 tick cell lines derived originally form embryos of *I. scapularis* (Figure 2 c-f), and the basic developmental cycle in these cell lines is similar to that described for *A. marginale* (129, 130, 131). Large colonies form within the cell cytoplasm and the first form seen within the colonies were reticulated forms which subsequently transforms into

dense forms. However, the morphology of *A. phagocytophilum* in cultured tick cells was found to be considerably more diverse than *A. marginale* (Figure 2 d, f) (129).

4.2. Evidence of tick-*A. phagocytophilum* co-evolution

The tick-pathogen co-evolution has not been as well characterized in *A. phagocytophilum* as compared with *A. marginale*. However, initial evidence suggests a process of co-evolution between *A. phagocytophilum* and the tick vector. For example, as with *A. marginale*, differences in vector competence have been reported among tick species and strains of *A. phagocytophilum* (132, 133). However, it is not known if these differences in vector competence are regulated at the tick-pathogen interface.

4.3. *A. phagocytophilum* molecules involved in interactions at the tick/pathogen interface

A comparative genomic analysis revealed differences between *A. marginale* and *A. phagocytophilum* with important evolutionary implications (95). For example, genes for cell wall biosynthesis are present in *A. marginale* but absent in *A. phagocytophilum*, which may represent an evolutionary adaptation to be important in the ability of *A. phagocytophilum* to infect vertebrate immune cells without the activation of leukocytes which occurs after binding of peptidoglycan to Toll-like receptor 2. Dunning Hotopp *et al.* (95) also identified one ortholog gene cluster containing conserved hypothetical proteins with homology to the patatin family of phospholipases that is present in rickettsia such as *A. marginale* that infect animals but has been lost from *A. phagocytophilum* and other human pathogens, possibly being related to establishment of infection in humans. However, *A. phagocytophilum* shares some common genes and MSPs with other *Anaplasma* spp. The *A. phagocytophilum* (strain HZ) genome has one *msp2*, two *msp2* homologs, one *msp4*, 113 *p44* and three *omp-1*, for a total of 121 genes that belong to the MSP2 superfamily (95).

Gene differential expression has been less characterized in *A. phagocytophilum* than in *A. marginale*. In *A. phagocytophilum*, diverse *p44* paralogs are expressed in mammals and ticks, a regulatory mechanism that confers environmental adaptations especially during tick transmission (95). Multiple antigenic variants of P44 arise during the multiplication of the pathogen in mammals and ticks as the result of combinatorial gene conversion into expression sites (83, 95). These results indicate that p44 genes and apxR are specifically up-regulated in the mammalian host environment and suggest that ApxR not only is positively regulated but also acts as a transcriptional regulator f p44E (134). Other surface proteins have been identified in *A. phagocytophilum* but their interaction with tick cells has not been reported (95, 135).

4.4. Tick molecules involved in interactions at the tick/pathogen interface

An excellent example of the molecular tick/pathogen interface was reported by Sukumaran et al. (136, 137) for the *I. scapularis* salp16 protein which is expressed during feeding by tick salivary glands in response to infection with *A. phagocytophilum*. While several salp proteins were identified that affect tick feeding, the salp16 was the only one that was upregulated in response to *A. phagocytohilum* infection. When salp16 was silenced in ticks by RNAi, the infection levels in tick salivary gland were greatly reduced. Therefore, there is a molecular relationship between the tick and *A. phagocytophilum* and the pathogen uses this protein to enhance its uptake from the host and infect salivary glands (136, 137). Other proteins of the same family such as *I. scapularis* Salp14 affect tick feeding but not pathogen infection (138).

Additional evidence of tick-pathogen interactions were provided in experiments that demonstrated the effect of pathogen infection on the molting success of *I. scapularis* larvae (139). On the other hand, some results discussed above with *A. marginale* suggest the evolution of mechanisms to limit pathogen infection levels in ticks and thus prevent deleterious effects of infection.

5. SUMMARY AND PERSPECTIVE

This review of the molecular interactions of *Anaplasma* and tick cells points to the complexity of tick-pathogen co-evolution relationships and suggested that genetic loci of both the vector and the pathogen are required infection and transmission of pathogens by ticks (140). Importantly, recent research has demonstrated that a molecular mechanism occurs by which *Anaplasma* infects tick midgut cells, establish infection, traffic to salivary glands, establish a second major site of infection and then are transmitted to the vertebrate host during a second feeding. This concept is supported by the finding that infection of ticks may develop only midguts or that infection of salivary glands does not always infer that the pathogen will be transmitted to vertebrates during feeding.

Genetic factors have been associated with intraspecific variation in vector competence for *A. marginale* (10, 15, 16). These results illustrate the complexity of tick-pathogen co-evolution relationships and suggested that genetic loci of both the vector and the pathogen are required infection and transmission of pathogens by ticks (140).

Although there are notable differences between the ruminant host-specific *Anaplasma* and *A. phagocytophilum* which are infective for a wide host range, tick infections of both pathogens are maintained from the interaction between pathogen tick cell molecules. The overall results are that pathogens are only infective for the tick species with which they have co-evolved and the ticks have a evolved a mechanism to limit infections which ensures survival of both tick and pathogens. *Anaplasma* spp are unique among tick-borne pathogens because they develop persistent infections in both the tick and vertebrate hosts which both serve as reservoirs of infection along. Targeting research on both key tick and pathogen antigens will likely contribute to control strategies through reduction of the vector capacity of ticks and may even prevent transmission of *Anaplasma* pathogens to the vertebrate

hosts, thus reducing the challenge-exposure of vertebrate hosts immunized against the pathogen. However, use of pathogens harvested from tick cell lines as vaccine antigens may be less effective because of the differential expression of key surface antigens as demonstrated by the differential expression of the *A. marginale* adhesion protein, MSP1a. Molecular characterization of the tick/pathogen interface will be fundamental toward the design of dual target vaccines that will be protective against tick infestations and prevent or reduce the transmission of pathogens.

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7. REFERENCES

1. Dumler, J.S., Barbet, A.C., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y. & Rurangirwa, F.R.: Reorganization of the genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions subjective synonyms of *Ehrlichia phagocytophila*. *Int J Sys Evol Microbiol* 51, 2145-2165 (2001)

2. Kocan, K.M, de la Fuente, J., Blouin, E.F. & Garcia-Garcia J.C.: *Anaplasma marginale* (Rickettsiales : Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. *Parasitol* 129, S285-S300 (2004)

3. Smith, R., Levy, M.G., Kuhlenschmidt, M.S., Adams, J..H, Rzechula, D.G., Hardt, T.Z. & Kocan, K.M.: Isolate of *Anaplasma marginale* not transmitted by ticks. *Am J Vet Res* 47, 127-129 (1986)

4. Wickwire, K.B., Kocan, K.M., Barron, S.J., Ewing, S.A., Smith, R.D. & Hair, J.A.: Infectivity of three isolates of *Anaplasma marginale* for *Dermacentor andersoni*. *Am J Vet Res* 48, 96-99 (1987)

5. Allred, D.R., McGuire, T.C., Palmer, G.H., Leib, S.R., Harkins, T.M., McElwain, T.F. & Barbet, A.F.: Molecular basis for surface antigen size polymorphisms and conservation of a neutralization-sensitive epitope in *Anaplasma marginale*. *Proc Natl Acad Sci USA* 87, 3220-3224 (1990)

6. Rodríguez, S.D., Garcîa Ortiz, M.A., Hernández Salgado, G., Santos Cerda, N.A., Aboytes Torre, R. & Cantó Alarcón, G.J.: *Anaplasma marginale* inactivated vaccine: dose titration against a homologous challenge. *Comp Immunol Microbiol Infect Di*s 23, 239-252 *(*2000)

7. Palmer, G.H., Rurangirwa, F.R. & McElwain, T.F.: Strain composition of the ehrlichia *Anaplasma marginale* within persistently infected cattle, a mammalian reservoir for tick transmission. *J Clin Microbiol* 39, 631-635 (2001)

8. de la Fuente, J., Garcia-Garcia, J.C., Blouin, E.F. & Kocan, K.M.: Major surface protein 1a effects tick infection and transmission of the ehrlichial pathogen *Anaplasma marginale*. *Int J Parasitol* 31, 1705-1714 (2001)

9. de la Fuente, J., Van Den Bussche, R.A. & Kocan, K.M.: Molecular phylogeny and biogeography of North American isolates of *Anaplasma marginale* (Rickettsiaceae: Ehrlichieae). *Vet Parasitol* 97, 65-76 (2001)

10. de la Fuente, J., Garcia-Garcia, J.C., Blouin, E.F., Rodriguez, S.D., Garcia, M.A. & Kocan, K.M.: Evolution and function of tandem repeats in the major surface protein 1a of the ehrlichial pathogen *Anaplasma marginale*. *Animal Health Res Rev* 2, 163-173 (2001)

11. de la Fuente, J., Garcia-Garcia, J.C., Blouin, E.F. & Kocan, K.M.: Characterization of the functional domain of the major surface protein 1a involved in adhesion of *Anaplasma marginale* to host cells. *Vet Microbiol* 91, 265-283 (2003)

12. Gonçalves Ruis, P.M., Friche Passos, L.M. & Barbosa Ribeiro, M.F.: Lack of infectivity of a Brazilian *Anaplasma marginale* isolate for *Boophilus microplus* ticks. *Vet Parasitol* 128, 325-331 (2005)

13. Ueti, M.W., Reagan, J.O., Jr., Knowles, D.P. Jr., Scoles, G.A. Shkap, V. & Palmer, G.H.: Identification of midgut and salivary glands as specific and distinct barriers to efficient tick-borne transmission of *Anaplasma marginale*. *Infect Immun* 75, 2959-2964 (2007)

14. Scoles, G.A., McElwain, T.F., Rurangirwa, F.R., Knowles, D.P. & Lysyk, T.J.: A Canadian bison isolate of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) is not transmissible by *Dermacentor andersoni* (Acari: Ixodidae), whereas ticks from two Canadian *D. andersoni* populations are competent vectors of a U.S. strain. *J Med Entomol* 43, 971-975 (2006)

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

16. Scoles, G.A., Broce, A.B., Lysyk, T.J. & Palmer, G.H.: Relative efficiency of biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae)

by *Dermacentor andersoni* (Acari: Ixodidae) compared with mechanical transmission by *Stomoxys calcitrans* (Diptera: Muscidae). *J Med Entomol* 42, 668-675 (2005)

17. Scoles, G.A., Ueti, M.W., Noh, S.M., Knowles, D.P. & Palmer, G.H.: Conservation of transmission phenotype of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) strains among *Dermacentor* and *Rhipicephalus* ticks (Acari: Ixodidae). *J Med Entomol* 44, 484-491 (2007)

18. Krier, J.P. & Ristic, M.: Anaplasmosis. VII. Experimental *Anaplasma ovis* infection in white-tailed deer (*Dama virginiana*). *Am J Vet Res* 24, 567-572 (1963)

19. Kuttler, K.L.: *Anaplasma* infections in wild and domestic ruminants: a review. *J Wildl Dis* 20, 12-20 (1984)

20. Zaugg, J.L.: Experimental infections of *Anaplasma ovis* in pronghorn antelope. *J Wildl Dis* 23, 205-210 (1987).

21. Zaugg, J.L.: Experimental anaplasmosis in mule deer: persistence of infection of *Anaplasma marginale* and susceptibility to *A. ovis*. *J Wildl Dis* 24, 120-126 (1988)

22. Zaugg, J.L., Goff, W.L., Foreyt, W. & Hunter, D.L.: Susceptibility of elk (*Cervus elaphus*) to experimental infection with *Anaplasma marginale* and *A. ovis*. *J Wildl Dis* 32, 62-66 (1996)

23. Friedhoff, K.T.: Tick-borne diseases of sheep and goats caused by *Babesia*, *Theileria* or *Anaplasma* spp. *Parassitologia* 39, 99-109 (1997)

24. Yabsley, M.J., Davidson, W.R., Stallknecht, D.E., Varela, A.S., Swift, P.K., Devos, J.C. Jr. & Dubay, S.A.: Evidence of tick-borne organisms in mule deer (*Odocoileus hemionus*) from the western United States. *Vector Borne Zoonotic Dis* 5, 351-362 (2005)

25. de la Fuente, J., Atkinson, M.W., Hogg, J.T., Miller, D.S., Naranjo V., Almazán, C., Anderson, N. & Kocan, K.M.: Genetic characterization of *Anaplasma ovis* strains from bighorn sheep in Montana. *J Wildlife Dis* 42, 381-385 (2006)

26. de la Fuente, J., Atkinson, M.W., Naranjo, V., Fernández de Mera, I.G., Mangold A.J., Keating, K.A. & Kocan, K.M.: Sequence analysis of the *msp4* gene of *Anplasma ovis* strains. *Vet Microbiol* 119, 375-381 (2007)

27. Pipano, E., Krigel, Y., Frank, M., Marcovics, A. & Mayer, E.: Frozen *Anaplasma centrale* vaccine against anaplasmosis in cattle. *Br Vet J* 142: 553-556 (1986)

28. Turton, J.A., Katsande, T.C., Matingo, M.B., Jorgensen, W.K., Ushewokunze-Obatolu, U. & Dalgliesh, R.J.: Observations on the use of *Anaplasma centrale* for immunization of cattle against anaplasmosis in Zimbabwe. *Onderstepoort J Vet Res* 65, 81-86 (1998)

29. Shkap, V., Molad, T., Fish, L. & Palmer, G.H.: Detection of the *Anaplasma centrale* vaccine strain and specific differentiation from *Anaplasma marginale* in vaccinated and infected cattle. *Parasitol Res* 88, 546-552 (2002).

30. Stuen, S.: *Anaplasma phagocytophilum* – the most widespread tick-borne infection in animals in Europe. *Vet Res Com* 31, 79-84 (2007)

31. Gordon, W.S., Brownlee, A., Wilson, D.R. & MacLeod, J.: Tickborne fever. *J Comp Pathol Ther* 45**,** 301–307 (1932)

32. MacLeod, J.R. & Gordon, W.S.: Studies of tick-borne fever of sheep. 1. Transmission by the tick *Ixodes ricinus*, with a description of the disease produced. *Parasitol* 25, 273–285 (1933)

33. Brodie, T.A., Holmes, P.J. & Urquhart, G.M.: Some aspects of tick-borne disease of British sheep. *Vet Res* 118, 415-418 (1986)

34. Madigan, J.E. & Gribble, D.: Equine ehrlichiosis in northern California: 49 cases (1968-1981). *J Am Vet Med Assoc* 190,445–448 (1987)

35. Gribble, D.H.: Equine ehrlichiosis. J Am Vet Med Assoc 155, 462–469 (1969)

36. Björsdorff, A., Johnsson, A., Sjostrom, A. & Madigan, J.E.: *Ehrlichia equi*-infektion diagnostiserat hos hast. *Svensk Vet Tidning* 42, 357–360 (1990)

37. Madigan, J.E., Barlough, J.E., Dumler, J.S., Schankman, N.S. & DeRock, E.: Equine granulocytic ehrlichiosis in Connecticut caused by an agent resembling the human granulocytotropic ehrlichia. *J Clin Microbiol* 34, 434-435 (1996)

38. Pusterla, N., Chae, J.S., Kimsey, R.B., Berger Pusterla, J., DeRock, E., Dumler, J.S. & Madigan, J.E.: Transmission of *Anaplasma phagocytophila* (human granulocytic ehrlichiosis agent) in horses using experimentally infected ticks (*Ixodes scapularis*). *J Vet Med B Infect Dis Vet Public Health* 49, 484-488 (2002)

39. von Loewenich, F.D., Stumpf, G., Baumgarten, B.U., Röllinghoff, M., Dumler, J.S. & Bogdan, C.: A Case of Equine Granulocytic Ehrlichiosis Provides Molecular Evidence for the Presence of Pathogenic *Anaplasma phagocytophilum* (HGE Agent) in Germany. *Eur J Clin Microbiol Infect Dis* 22, 303-305 (2003)

40. Dumler, J.S., Madigan, J.E., Pusteria, N. & Bakken, J.S.: Ehrlichioses in humans: epidemiology, clinical presentations, diagnosis, and treatment. *Clin. Infect. Dis.* 45 (Suppl. 1), S45-S51 (2007)

41. Chen, S.M., Dumler, J.S., Bakken, J.S. & Walker, D.H.: Identification of a granulocytotropic *Ehrlichia* species as

the etiologic agent of human disease. *J Clin Microbiol* 32, 589-595 (1994)

42. Bakken, J.S., Dumler, J.S., Chen, S.M., Eckman, M.R., Van Etta, L.L. & Walker, D.H.: Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? *JAMA* 272, 212-218 (1994)

43. Dumler, J.S. & Bakken, J.S.: Human ehrlichioses: newly recognized infections transmitted by ticks. *Annu Rev Med* 49, 201-213 (1998)

44. Bakken, J.S & Dumler, J.S.: Human granulocytic ehrlichiosis. *Clin Infect Dis* 3, 554-560 (2000)

45. Blanco, J.R. & Oteo, J.A.: Human granulocytic ehrlichiosis in Europe. *Clin Microbiol Infect* 8, 763-772 (2002)

46. Gardner, S.L., Holman, R.C., Krebs, J.W., Berkelman, R. & Childs, J.E.: National surveillance for the human ehrlichioses in the United States, 1997-2001, and proposed methods for evaluation of data quality. *Ann N Y Acad Sci* 990, 80-89 (2003)

47. Levin, M.L., Nicholson, W.L., Massung, R.F., Sumner, J.W. & Fish, D.: Comparison of the reservoir competence of medium-sized mammals and *Peromyscus leucopus* for *Anaplasma phagocytophilum* in Connecticut. *Vector Borne Zoonotic Dis* 2, 125-136 (2002)

48. Goethert, H.K. & Telford 3rd, S.R.: Enzootic transmission of the agent of human granulocytic ehrlichiosis among cottontail rabbits. *Am J Trop Med Hyg* 68, 633-637 (2003)

49. Nieto, N.C., Dabritz, H., Foley, P., Drazenovich, N., Calder, L., Adjemain, J., Conrad, P.A. & Foley, J.E.: Ectoparasite diversity and exposure to vector-borne disease agents in wild rodents in central coastal California. *J Med Ent* 44, 328-335 (2007)

50. Foley, J. E., Nieto, N.C., Bernadette Clueit, S., Foley, P., Nicholson, W.N. & Brown, R.N.: Survey for zoonotic rickettsial pathogens in northern flying squirrels, *Glaucomys sabrinus*, in California. *J Wild Dis* 43, 684-689 (2007)

51. Adamska, M. & Skotarczak, B.: Wild game as a reservoir of *Anaplasma phagocytophilum* in north-western Poland. *Wiad Parazytol* 53, 103-107 (2007)

52. Ebani, V.V., Cerri, D., Fratini, F., Ampola, M. & Andreani, E.: *Anaplasma phagocytophilum* infection in a fallow deer (*Dama dama*) population in a preserve of central Italy. *New Microbiol* 30, 161-165 (2007)

53. Kocan, K.M., Hair, J.A. & Ewing, S.A.: Ultrastructure of *Anaplasma marginale* Theiler in *Dermacentor andersoni* Stiles and *Dermacentor variabilis* (Say). *Am J Vet Res* 41, 1966-1976 (1980)

54. Kocan, K.M.: Development of *Anaplasma marginale* in ixodid ticks: coordinated development of a rickettsial organism and its tick host. In: Sauer, J.R., Hair, J.A. (Eds.), Morphology, Physiology and Behavioral Ecology of Ticks. Ellis Horwood Ltd., England, pp. 472-505 (1986)

55. Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F. & Ewing, S.A.: The Natural History of *Anaplasma marginale*. *Vet Parasitol* in press (2008).

56. Kocan, K.M., Stiller, D., Goff, W.L., Claypool, P.L., Edwards, W., Ewing, S.A., McGuire, T.C., Hair, J.A. & Barron, S.J.: Development of *Anaplasma marginale* in male *Dermacentor andersoni* transferred from infected to susceptible cattle. Am. J. Vet. Res. 5, 499-507 (1992)

57. Kocan, K.M., Goff, W.L., Stiller, D., Claypool, P.L., Edwards, W., Ewing, S.A., Hair, J.A. & Barron, S.J.: Persistence of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) in male *Dermacentor andersoni* (Acari:Ixodidae) transferred successively from infected to susceptible cattle. *J Med Ent* 29, 657-668 (1992)

58. Kocan, K.M. & Stiller, D.: Development of *Anaplasma ovis* (Rickettsiales: Anaplasmataceae) in male *Dermacentor andersoni* (Acari:Ixodidae) transferred from infected to susceptible sheep. *J Med Ent* 29, 98-107 (1992)

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

60. 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)

61. 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)

62. 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. *Parasitology Research* 100, 85-91 (2006)

63. Potgeiter, F. T. & van Rensberug, L.: Isolation of *Anaplasma marginale* from *Rhipicephalus simus* males. *Onderstepoort J Vet Res* 47, 285-286 (1980)

64. Kocan, K.M., Holbert, D., Ewing, S.A., Hair, J.A. & Barron, S.J.: Influence of parasitemia level at feeding on development of *Anaplasma marginale* Theiler in *Dermacentor andersoni* Stiles. *Am J Vet Res* 44, 554-557 (1983).

65. Aguirre, D.H., Gaido, A.B., Vinabal, A.E., De Eshaide, S.T. & Guglielmone, A.A.: Transmission of *Anaplasma marginale* with adult *Boophilus microplus* ticks fed as

nymphs on calves with different level of rickettsaemia. Parasite 1, 405-407 (1994)

66. Eriks, I.S., Stiller, D. & Palmer, G.H.: Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. *J Clin Microbiol* 31, 2091-2096 (1993)

67. de la Fuente, J., Van Den Bussche, R.A., Garcia-Garcia, J.C., Rodríguez, S.D., García, M.A., Guglielmone, A.A., Mangold, A.J., Friche Passos, L.M., Barbosa Ribeiro, M.F., Blouin, E.F. & Kocan, K.M.: Phylogeography of New World isolates of *Anaplasma marginale* based on major surface protein sequences. *Vet. Microbiol.* 88, 275-285 (2002)

68. de la Fuente, J., Ruybal, P., Mtshali, M.S., Naranjo, V.. Li Shuqing, L., Mangold, A.J., Rodríguez, S.D., Jiménez, R., Vicente, J., Moretta, R., Torina, A., Almazán, C., Mbati, P.M., Torioni de Echaide, S., Farber, M., Gortazar, C. & Kocan, K.M.: Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences. *Veterinary Microbiology*, 119, 382-390 (2007)

69. Mtshali, M.S., de la Fuente, J., Ruybal, P., Kocan, K.M., Vicente, J., Mbati, P.A., Shkap, V., Blouin, E.F., Mohale, N.E., Spickett, A.M. & Latif, A.A.: Prevalence and genetic diversity of *Anaplasma marginale* strains in cattle in South Africa. *Zoonoses and Public Health* 54, 23-30 (2007)

70. Palmer, G.H., Knowles, D. P., Rodriquez, J.L., Gnad, D.P. Hollis, L.C., Marston, T. & Brayton, K.A.: Stochastic transmission of multiple genotypically distinct *Anaplasma marginale* strains in a herd with high prevalence of *Anaplasma* infection. *J Clin Microbiol* 42, 5381-5384 (2004)

71. de la Fuente, J., Kocan, K.M., Mangold, A.J., Blouin, E.F., Rodríguez, R.D., Naranjo, V., Alamazán, V. & Jiménez, R.: Biogeography and molecular evolution of *Anaplasma* species. *Vet Parasitol* In press (2007)

72. de la Fuente, J., Massung, R.F., Wong, S., Chu, F.K., Lutz, H., Meli, M., von Loewenich, F.D., Grzeszczuk, A., Torina, A., Caracappa, S., Mangold, A.J., Naranjo, V., Stuen, S. & Kocan, K.M.: Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. *J Clin Microbiol* 43, 1309-1317 (2005)

73. de la Fuente, J., Passos, L.M.F., Van Den Bussche, R.A., Ribeiro, M.F.B., Facury-Filho, E.F. & Kocan, K.M.: Genetic diversity and molecular phylogeny of *Anaplasma marginale* isolates from Minas Gerais, Brazil, *Vet Parasitol* 121, 307-316 (2004)

74. de la Fuente, J., Vicente, J., Höfle, U., Ruiz-Fons, F., Fernández de Mera, I.G., Van Den Bussche, R.A., Kocan, K.M. & Gortázar, C.: *Anaplasma marginale* infection in free-ranging Iberian red deer in the region of Castilla La Mancha, Spain. *Vet Micro* 100, 163-173 (2004)

75. de la Fuente, J., Torina, A., Caracappa, S., Tumino, G., Furlá, R., Almazán, C. & Kocan, K.M.: Serologic and molecular characterization of *Anaplasma* species infection in farm animals and ticks from Sicily. *Vet Parasitol* 133, 357-362 (2005)

76. de la Fuente, J., Torina, A., Naranjo, V., Caracappa, S., Vicente, J., Mangold, A.J., Vicari, D., Alongó, A., Scimeca, S. & Kocan, K.M.: Genetic diversity of *Anaplasma marginale* strains from cattle farms with different husbandry systems in the Province of Palermo, Sicily. *Journal of Veterinary Medicine B* 52, 226-229 (2005)

77. de la Fuente, J., Lew, A., Lutz, H., Meli, M.L., Hofmann-Lehmann, R., Shkap, V., Molad, T., Mangold, A.J., Almazán, C., Naranjo, V., Gortázar, C., Torina, A., Caracappa, S., García-Pérez, A.L., Barral, M., Oporto, B., Ceci, L., Carelli, G., Blouin, E.F. & Kocan, K.M.: Genetic diversity of *Anaplasma* species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. *Animal Health Res Rev* 6, 75-89 (2005)

78. de la Fuente, J., Blouin, E.F. & Kocan, K.M.: Infection exclusion of the rickettsial pathogen *Anaplasma marginale* in the tick vector *Dermacentor variabilis*. *Clin Diagn Lab Immunol* 10, 182-184 (2003)

79. de la Fuente, J., Lew, A., Lutz, H., Meli, M.L., Hofmann-Lehmann, R., Shkap, V., Molad, T., Mangold, A.J., Almazán, C., Naranjo, V., Gortázar, C., Torina, A., Caracappa, S., García-Pérez, A.L., Barral, M., Oporto, B., Ceci, L., Carelli, G., Blouin, E.F. & Kocan, K.M.: Genetic diversity of *Anaplasma* species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. *Animal Health Research Reviews* 6:75-89 (2005)

80. de la Fuente, J., Kocan, K.M., Mangold, A.J., Blouin, E.F., Rodríguez, R.D., Naranjo, V., Alamazán, V. & Jiménez, R.: Biogeography and molecular evolution of *Anaplasma* species. *Vet Parasitol*, in press (2008)

81. Futse, J.E., Brayton, K.A., Dark, M.J., Knowles, D.P. Jr. & Palmer, G.H.: Superinfection as a driver of genomic diversification in antigenically variant pathogens.
Proc Natl Acad Sci USA 105, 2123-2127 (2008)

82. de la Fuente, J. & Kocan, K.M.: Expression of *Anaplasma marginale* major surface protein 2 variants in persistently infected ticks. *Infect Immun* 69, 5151-5156 (2001)

83. Brayton, K.A., Kappmeyer, L.S., Herndon, D.R., Dark, M.J., Tibbals, D.L., Palmer, G.H., McGuire, T.C. & Knowles, D.P. Jr.: Complete genome sequencing of

Anaplasma marginale reveals that the surface is skewed to two superfamilies of outer membrane proteins. *Proc Natl Acad Sci USA* 102, 844-849 (2005)

84. Palmer, G.H., Kocan, K.M., Barron, S.J., Hair, J.A., Barbet, A.F., Davis, W.C. & McGuire, T.C.: Presence of common antigens, including major surface protein epitopes, between the cattle (intraerythrocytic) and tick stages of *Anaplasma marginale*. *Infect Immun* 50, 881-886 (1985)

85. Kocan, K. M., de la Fuente, J., Guglielmone, A. A. & Melendéz, R. D.: Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. *Clin Microbiol Rev* 16, 698-712 (2003)

86. Barbet, A.S., Yi, J., Lundgren, A., McEwen, B.R., Blouin, E.F. & Kocan, K.M.: Antigenic variation of *Anaplasma marginale*: MSP2 diversity during cyclic transmission between ticks and cattle. *Infect Immun* 69, 3057-3066 (2001)

87. Noh, S. M., Brayton, K. A., Knowles, D. P, Agnes, J. T., Dark, M. J., Brown, W. C.L., Baszler, T. V. & Palmer, G. H.: Differential expression and sequence conservation of the *Anaplasma marginale msp2* gene superfamily outer membrane proteins. *Infect Immun* 74, 3471-9 (2006)

88. Bowie, M.V., de la Fuente, J., Kocan, K.M., Blouin, E.F. & Barbet, A.F.: Conservation of major surface protein 1 genes of the ehrlichial pathogen *Anaplasma marginale* during cyclic transmission between ticks and cattle. *Gene* 282, 95-102 (2002)

89. Macmillan, H., Brayton, K.A., Palmer, G.H., McGuire, T.C., Munske, G., Siems, W.F. & Brown, W.C.: Analysis of the *Anaplasma marginale* major surface protein 1 complex protein composition by tandem mass spectrometry. *J Bacteriol* 188, 4983-4991 (2006)

90. Lew, A.E., Bock, R.E., Minchin, C.M. & Masaka, S.: A *msp1α* polymerase chain reaction assay for specific detection and differentiation of *Anaplasma marginale* isolates. *Vet Microbiol* 86, 325-35 (2002).

91. Palmer, G.H.,, Waghela, S.D., Barbet, A.F., Davis, W. C. & McGuire, T.C.: Characterization of a neutralization-sensitive epitope on the Am 105 surface protein of *Anaplasma marginale*. *Int J Parasitol* 17, 1279-85 (1987)

92. Brown, W.C., Palmer, G.H., Lewin, H.A. & McGuire, T.C.: CD4 (+) T lymphocytes from calves immunized with *Anaplasma marginale* major surface protein 1 (MSP1), a heteromeric complex of MSP1a and MSP1b, preferentially recognize the MSP1a carboxyl terminus that is conserved among strains. *Infect Immun* 69, 6853-6862 (2001)

93. Garcia-Garcia, J.C., de la Fuente, J., Kocan, K.M., Blouin, E.F., Halbur, T., Onet, V. C. & Saliki, J.T.: Mapping of B-cell epitopes in the N-terminal repeated peptides of the *Anaplasma marginale* major surface protein 1a and characterization of the humoral immune response of cattle immunized with recombinant and whole organism antigens. *Vet Immunol Immunopath* 98, 137-151 (2004)

94. Garcia-Garcia, J.C., de la Fuente, J., Blouin, E.F., Halbur, T., Onet, V., Saliki, J.T. & Kocan, K.M.: Differential expression of the *msp1α* gene of *Anaplasma marginale* occurs in bovine erythrocytes and tick cells. *Vet Microbiol* 98, 261-272 (2004)

95. Dunning Hotopp, J.C., Lin, M., Madupu, R., Crabtree, J., Angiuolt, S.V., Eisen, J., Seshadri, R, Ren, Q., Wu, M., Utterback, T.R., Smith, S.,Lewis, M., Khouri, H., Zhang, C., Niu, H., Lin, Q., Ohashi, N., Zhi, N., Nelson, W., Brinkac, L.M., Dodson, R.J. Rosovitz, M.J., Sundaram, J., Daugherty, S.C., Davidsen, T., Durkin, A.S., Gwinnm M., Haft, D.H., Selengut, J.D., Sullivan, S.A., Zafar, N., Zhou, L., Benahmed, F., Forberger, H., Halpin, R., Mulligan, S., Robinson, J., White, O., Rikihisa. Y. & Tettelin. H.: Comparative genomics of emerging human ehrlichiosis agents. *PloS Genet 2* e21 (2006)

96. Palmer, G.H., Abbott, J.R., French, D.M. & McElwain, T.F.: Persistence of *Anaplasma ovis* infection and conservation of the *msp-2* and *msp-3* multigene families within the genus *Anaplasma*. *Infect Immun* 66, 6035-6039 (1998)

97. Shkap, V., Molad, T., Brayton, K.A., Brown, W.C. & Palmer, G.H.: Expression of major surface protein 2 variants with conserved T-cell epitopes in *Anaplasma centrale* vaccinates. *Infect Immun* 70, 642-648 (2002)

98. Abbott, J.R., Palmer, G.H., Howard, C.J., Hope, J.C. & Brown, W.C.:
Anaplasma marginale major surface protein 2 CD4+-T-cell epitopes are evenly distributed in conserved and hypervariable regions (HVR), whereas linear B-cell epitopes are predominantly located in the HVR. *Infect Immun* 72, 7360-7366 (2004)

99. 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).

100. 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)

101. 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 digestion and reproduction *Vaccine* 24, 4082-4095 (2006)

102. 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 Comm* 344, 332-338 (2006).

103. 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)

104. 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. 2008. Expression of the tick protective antigen, subolesin, in *Anaplasma marginale* and *A. phagocytophilum* infected cells. *NY Acad Sci* in press (2008)

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

106. Kocan, K.M., Yoshioka, J., Sonenshine, D.E., de la Fuente, J., Ceraul, S.M., Blouin, E.F. & Almazán, C.: Capillary tube feeding system for studying tick/pathogen interactions of *Dermacentor variabilis* (Acari: Ixodidae) and *Anaplasma marginale (*Rickettisales: Anaplasmataceae). *J Med Entomol* 42, 864-874 (2005)

107. Sonenshine, D.E., Hynes, W.L., Ceraul, S.M., Mitchell, R. & Benzine, T.: Host blood proteins and peptides in the midgut of the tick *Dermacentor variabilis* contribute to bacterial control. *Exp Appl Acarol* 36, 207-223 (2005)

108. Gillespie, J.P., Kanost, M.R. & Trenczek, T.: Biological mediators of insect immunity. *Ann Rev Entomol* 42, 611-643 (1997)

109. Ganz, T., Lherer, R. I.: Defensins. *Curr Opin Immunol* 6:584-589 (1994)

110. Fogaca, A.C., Lorenzini, D.M., Kaku, L.M., Esteves, E., Bulet, P. & Daffre, S.: Cysteine-rich antimicrobial peptides of the cattle tick *Boophilus microplus*: isolation, structural characterization and tissue expression profile. *Develop Comp Immunol* 28, 191-200 (2004)

111. Johns, R., Sonenshine, D.E. & Hynes, W.L.: Identification of a defensin from the hemolymph of the American dog tick, *Dermacentor variabilis*. *Insect Biochem Mol Biol* 31, 857-865 (2001)

112. Ceraul, S.M., Sonenshine, D.E., Ratzlaff, R.E. & Hynes,W.L.: An arthropod defensin expressed by the hemocytes of the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae). *Insect Biochem Mol Biol* 33, 1099 –1103 (2003)

113. Hynes, W.L., Ceraul, S.M., Todd, S.M., Seguin, K.C. & Sonenshine, D.E.: A defensin-like gene expressed in the black-legged tick, *Ixodes scapularis*. *Med Vet Entomol* 19, 339-344 (2005)

114. Todd, S.M., Sonenshine, D.E. & Hynes, W.L.: Tissue and life-stage distribution of a defensin gene in the Lone Star Tick, *Amblyomma americanum*. *Med Vet Entomol* 21, 141–147 (2007)

115. Lai, R., Lomas, L.O., Jonczy, J., Turner, P.C. & Rees, H.H.: Two novel non-cationic defensin-like antimicrobial peptides from haemolymph of the female tick, *Amblyomma hebraeum*. *Biochem J* 379, 681-685 (2004)

116. Fogaca, A.C., da Silva Jr., P.I., Miranda, M.T., Bianchi, A.G., Miranda, A., Ribolla, P.E. & Daffre, S.: Antimicrobial activity of a bovine hemoglobin fragment in the tick *Boophilus microplus*. *J Biol Chem* 274, 25330-25334 (1999)

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

118. Ceraul, S.M., Dreher-Lesnick, S., Gillespie, J.J., Sayeedur Rahman, M. & Azad, A.F.: A new tick defensin isoform and antimicrobial gene expression in response to *Rickettsia montanensis* challenge. *Infect Immun* 75**,** 1973-19 (2007)

119. Kocan, K.M., de la Fuente, J., Manzano-Roman, R., Naranjo, V., Sonenshine, D.E. & Hynes, W.L.: Silencing of the defensin, varisin, in male *Dermacentor variabilis* by RNA interference results in reduced *Anaplasma marginale* infections. *Exp Appl Acarol* in press (2008).

120. MacLeod, J. R. & Gordon, W. S.: Studies in tick-borne fever of sheep. I. Transmission by the tick*, Ixodes ricinus*, with a description of the disease produced. *Parasitol* 25, 273-285 (1933)

121. Magnarelli, L.A., Stafford, K.C., Mather, T.N., Yeh, M.T., Horn, K.D. & Dumler, J.S.: Hemocytic rickettsia-like organisms in ticks: serologic reactivity with antisera to ehrlichiae and detection of DNA of agent of human granulocytic ehrlichiosis by PCR. *J Clin Microbiol* 33, 2710-2714 (1995)

122. Pancholi, P., Kolbert, C.P., Mitchell, P.D., Reed, K.D. Jr., Dumler, J.S., Bakken, J.S., Telford, S.R .3rd, & Persing, D.H.: *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. *J Infect Dis* 172, 1007-1012 (1995)

123. Des Vignes, F. & Fish, D.: Transmission of the agent of human granulocytic ehrlichiosis by host-seeking *Ixodes scapularis (*Acari: Ixodidae) in southern New York state. *J Med Entomol* 34**,** 379-382 (1997)

124. Reubel, G.H., Kimsey, R.B., Barlough, J.E. & Madigan, J.E.: Experimental transmission of *Ehrlichia equi* to horses through naturally infected ticks (*Ixodes pacificus*) from northern California. *J Clin Microbiol* 36, 2131–2134 (1998)

125. Cao, W.C., Zhao, Q.M., Zhang, P.H., Dumler, J.S, Zhang ,X.T., Fang, L.Q. & Yang, H.: *Granulocytic ehrlichiae* in *Ixodes persulcatus* ticks from an area in China where Lyme disease is endemic. *J Clin Microbiol* 38**,** 4208–4210 (2000).

126. Levin, M.L. & Ross, D.E.: Acquisition of different isolates of *Anaplasma phagocytophilum* by *Ixodes scapularis* from a model animal. *Vector Borne Zoonotic Dis* 4, 53-59 (2004)

127. Swanson, S.J., Neitzel, D., Reed, K.D. & Belongia, E.A.: Coinfection acquired from *Ixodes* ticks. *Clin Microbiol Rev* 19, 708-727 (2006)

128. Strle F.: Human granulocytic ehrlichiosis in Europe. *Int J Med Microbiol* 293, S37, 27–35 (2004)

129. Munderloh, U.G., Lynch, M.J., Herron, M.J., Palmer, A.T., Jurtti, T.J., Nelson, R.D. & Goodman, J.L.: Infection of endothelial cells with *Anaplasma marginale* and *A. phagocytophilum*. *Vet Microbiol* 101, 53-64 (2004)

130. Massung, R.F., Levin, M.L., Munderloh, U.G., Silverman, D.J., Lynch, M.J. & Kurtti, T.J.: Isolation of *Anaplasma phagocytophilum* strain Ap-variant 1 in a tick-derived cell line. *Ann NY Acad Sci* 1078, 541-544 (2006)

131. Blouin, E.F, de la Fuente, J., Garcia-Garcia, J.C., Sauer, J.R., Saliki, J.T. & Kocan, K.M.: Applications of a cell culture system for studying the interaction of *Anaplasma marginale* with tick cells. *Animal Health Res Rev* 3, 57-68 (2002)

132. Levin, M.L. & Ross, D.E.: Acquisition of different isolates of *Anaplasma phagocytophilum* by *Ixodes scapularis* from a model animal. *Vector Borne Zoonotic Dis* 4, 53-59 (2004)

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

134. Wang, X., Cheng, Z., Zhang, C., Kikuchi, T. & Rikihisa, Y.: *Anaplasma phagocytophilum* p44 mRNA expression is differentially regulated in mammalian and tick host cells: involvement of the DNA binding protein ApxR. *J Bacteriol* 189, 8651-8659 (2007)

135. Ge, Y. & Rikihisa, Y.: Identification of novel surface proteins of *Anaplasma phagocytophilum* by affinity purification and proteomics. *J Bacteriol* 189, 7819-7828 (2007)

136. Sukumaran, B., Narasimhan, S., Anderson, J.F., DePonte, K., Marc Antonio, 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)

137. Foley, J. & Nieto, N.: *Anaplasma phagocytophilum* subverts tick salivary gland proteins. *Trends Parasitol* 23, 3-5 (2007)

138. 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)

139. Ross, D.E. & Levin, M.L.: Effects of *Anaplasma phagocytophilum* infection on the molting success of *Ixodes scapularis* (Acari: Ixodidae) larvae. *J Med Entomol* 41, 476-483 (2004)

140. de la Fuente, J., Kocan, K.M. & Blouin, E.F.: Tick vaccines and the transmission of tick-borne pathogens. *Veterinary Research Communications*, 31 (Suppl.1), 85-90, (2008)

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