Rev. Sci. Tech. Off. Int. Epiz., 2015, **34** (2), 577-586

The genus *Anaplasma*: new challenges after reclassification

K.M. Kocan [(1)*], J. de la Fuente [(1, 2)] & A. Cabezas-Cruz [(2, 3)]

(1) Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, United States of America
(2) SaBio, Instituto de Investigación en Recursos Cinegéticos (IREC)–Consejo Superior de Investigaciones Científicas (CSIC)–Universidad de Castilla–La Mancha (UCLM)-Junta de Comunidades de Castilla–La Mancha (JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain
(3) Centre for Infection and Immunity of Lille (CIIL), Institut national de la santé et de la recherche médicale (INSERM) U1019 – Centre national de la recherche scientifique (CNRS) UMR 8204, Université Lille Nord de France, Institut Pasteur de Lille, Lille, France
*Corresponding author: Katherine.Kocan@okstate.edu

Summary

The genus *Anaplasma* is one of four distinct genera in the family Anaplasmataceae, which are obligate intracellular pathogens vectored by ticks and found exclusively within parasitophorous vacuoles in the host cell cytoplasm. The 2001 reclassification of the order Rickettsiales expanded the genus *Anaplasma*, which previously contained pathogens that were host specific for ruminants (*A. marginale, A. centrale* and *A. ovis*), by adding *A. phagocytophilum*, a unification of three organisms previously classified as *Ehrlichia* (*E. equi, E. phagocytophila* and the unnamed agent of human granulocytic ehrlichiosis). Also included in the genus *Anaplasma* were *A. bovis* (formerly *E. bovis*), *A. platys* (formerly *E. platys*) and *Aegyptianella pullorum.* Despite the genomic relatedness of the regrouped organisms, many aspects of their biology are diverse, including their host specificity, host cell preferences, major surface proteins (MSPs) and tick vectors. This review focuses on the two most important pathogens: *A. marginale*, which causes bovine anaplasmosis, and *A. phagocytophilum*, the aetiologic agent of tick-borne fever in sheep and human granulocytic anaplasmosis, an emerging tick-borne disease of humans. For both pathogens, strain diversity is much greater than previously recognised. While MSPs were found to be useful in phylogenetic studies and strain identification, highly conserved MSPs were found to affect the specificity of serologic tests. Comparison of these two important pathogens highlights the challenges and insight derived from reclassification and molecular analysis, both of which have implications for the development and evaluation of diagnosis and control strategies.

Keywords

Anaplasma marginale – *Anaplasma phagocytophilum* – Anaplasmataceae – Anaplasmosis – Tick-borne.

Introduction and current classification

The genus *Anaplasma* is one of four distinct genera within the family Anaplasmataceae, alongside *Ehrlichia*, *Wolbachia* and *Neorickettsia* (1). The organisms within this family are Gram-negative, obligate intracellular bacteria vectored primarily by ticks. A unique feature of these organisms is that they reside and replicate within parasitophorous vacuoles in the host cell cytoplasm of their tick and vertebrate hosts.

The genus *Anaplasma* was reorganised by Dumler *et al.* (1), based upon comprehensive genetic analyses of the *16S rRNA*, *groESL* and surface protein genes. This reclassification expanded the genus, which previously contained pathogens that were host specific for ruminants (*A. marginale* and *A. centrale*, both cattle pathogens, and *A. ovis*, which is infective for sheep and goats). Three organisms previously classified as *Ehrlichia* (*E. equi*, *E. phagocytophila* and the unnamed agent of human granulocytic ehrlichiosis) were unified into one taxon, *A. phagocytophilum*, and added to the genus *Anaplasma*, along with *A. bovis* (formerly *E. bovis*), *A. platys* (formerly *E. platys*) and *Aegyptianella pullorum.*

Despite the genomic relatedness of these regrouped organisms, many aspects of their biology are quite diverse. Overall, molecular analyses of organisms in the reorganised *Anaplasma* spp. have advanced our understanding of pathogen infection and developmental cycles in vertebrate and tick hosts, vertebrate host immune responses, the role and dynamics of the parasitophorous vacuole, and the establishment mechanism of persistent infections.

The most important disease-producing pathogens in the genus *Anaplasma* are *A. marginale*, which causes bovine anaplasmosis, and *A. phagocytophilum*, the aetiologic agent of tick-borne fever in sheep and human granulocytic anaplasmosis (HGA), an emerging tick-borne disease of humans. Erythrocytes are the host cell for *A. marginale, A. centrale* and *A. ovis* and the inclusions within erythrocytes are called 'marginal' or 'initial bodies', while granulocytes are the host cell for *A. phagocytophilum* and the inclusions are referred to as 'morula' (2, 3). Clearly, these two important pathogens are completely distinct, are vectored by different ticks and cause different diseases. Since *A. marginale* is only infective for ruminants, acutely or persistently infected cattle are not a threat to human health. However, if *A. phagocytophilum,* clearly a human pathogen, emerges as a pathogen of cattle or other ruminants, these infected animals would serve as hosts for infection of ticks and could therefore also contribute to human exposure to infection. Recent studies have confirmed the infection of sheep and ticks with a United States human isolate without evidence of clinical signs (4, 5, 6). If clinical signs are absent, then the emergence of *A. phagocytophilum* in animals would not be apparent.

This review focuses on the common and unique characteristics of the biology of *A. marginale* and *A. phagocytophilum* and current issues of their emergence, diagnosis and control. For further information on *A. marginale* and *A. phagocytophilum,* too extensive for this review, the reader is directed towards the following current comprehensive reviews: *A. marginale* (2, 7, 8, 9, 10) and *A. phagocytophilum* (3, 11, 12).

Distribution and importance as pathogens

Anaplasma marginale

Anaplasma marginale causes the disease bovine anaplasmosis, which is endemic in tropical and subtropical areas and results in considerable economic losses to both beef and dairy industries worldwide, including those in the Americas, Europe, Australia, Asia and Africa. This organism infects erythrocytes and causes mild-to-severe haemolytic disease as a result of the removal of infected erythrocytes by the reticuloendothelial cells. Mild-to-severe anaemia with icterus subsequently develops, without haemoglobinaemia and haemoglobinuria. Clinical signs may include fever, weight loss, abortion, lethargy, icterus, and often death in animals over two years old (as reviewed by 2); younger cattle are resistant to clinical disease but become persistently infected. Cattle that survive acute infection also develop persistent infection, characterised by cyclic, low-level rickettsaemia (as reviewed by 2, 9). Persistently infected or 'carrier' cattle have life-long immunity and, upon challenge exposure, are resistant to clinical disease. The losses due to anaplasmosis are measured through several parameters: low weight gain, reduction in milk production, abortion, the cost of anaplasmosis treatments, and mortality.

Anaplasma phagocytophilum

Anaplasma phagocytophilum, recognised as the most common tick-borne disease of animals in Europe and, more recently, as an emerging disease of humans in the United States (USA) and Europe, has a wide host range and has been reported in mammals and ticks throughout Europe, the USA and other areas of the world. The distribution of the organism is determined by the population densities of tick vectors, hosts and reservoir host species (3, 11, 12, 13). The severity of the disease depends upon the variant and the respective host's susceptibility to infection. In contrast to *A. marginale*, *A. phagocytophilum* has a broad host range, including rodents, hedgehogs, birds, cats, deer, horses, cattle, dogs, sheep and humans (3, 12, 14). *Anaplasma phagocytophilum* was first identified as the aetiologic agent of tick-borne fever in sheep and other ruminants, a disease which impacts upon sheep production in Europe. Young animals are most affected and clinical signs include high fever, anorexia, sudden drops in milk yield and abortion in ewes. Reduced fertility in rams has also been reported (12). More recently, *A. phagocytophilum* has been recognised as the cause of a febrile illness in humans (human granulocytic anaplasmosis: HGA); horses (equine granulocytic anaplasmosis: EGA); and dogs (canine granulocytic anaplasmosis: CGA) (1). HGA is an emerging tick-borne disease in the USA and Europe and is now considered to be one of the most common tick-borne pathogens in these areas (as reviewed by 12). In humans, HGA is usually self-limiting, and is characterised by fever, headache, myalgia and malaise, but disease may be more severe when infection occurs in immunocompromised individuals or concurrently with other haemoparasite infections.

Antigenic diversity and epidemiology

Molecular analyses have demonstrated a much greater diversity of genotypes of both *A. marginale* and

A. phagocytophilum than was previously recognised. Although *A. marginale* is host specific for cattle, research conducted since 2000 has demonstrated a worldwide diversity of *A. marginale* strains, in part because molecular tools are now available for strain identification and sequence analyses of the *A. marginale* major surface proteins (MSPs) and other gene sequences (15, 16, 17, 18). While MSP1a is a stable marker of strain identity, phylogenetic analysis of MSP1a does not provide information about the geographical origin. However, MSP4 sequences have provided strain identity and phylogeographic information (as reviewed by 17). The diversity of *A. marginale* strains was found to be extensive, especially in areas of intense cattle movement. In contrast, this strain diversity has not been reported in Australia, where the importation of cattle has been restricted. This increased strain diversity complicates control strategies because widely diverse strains may not be cross-protective if used for vaccine development, and *A. marginale* strains may not have uniform susceptibility to antimicrobials (H. Coetzee, unpublished data). In addition to strain diversity, recent research has demonstrated that different genotypes can be maintained in nature because of an 'infection-exclusion' mechanism of *A. marginale* in cattle and ticks. In infection exclusion, the establishment of one *A. marginale* genotype prevents a second genotype from becoming established after challenge exposure (19, 20, 21). However, subsequent research demonstrated that a low percentage of cattle could become infected with more than one *A. marginale* strain if the strains were not closely related (22). Overall, the importance of these findings helps to explain how multiple *A. marginale* strains can occur and be maintained within cattle herds or geographical regions.

A growing number of *A. phagocytophilum* variants continues to be reported worldwide. A recent investigation of sequence variation in the *msp4* gene of *A. phagocytophilum* in 50 samples from the USA, Germany, Poland, Norway, Italy and Switzerland, and four samples from white-tailed deer (*Odocoileus virginianus*) in the USA, revealed greater sequence variation in *A. phagocytophilum* strains than in those of *A. marginale* (13). These study results also differentiated between strains of *A. phagocytophilum* from ruminants, horses and dogs, and the strains isolated from white-tailed deer were found to be more diverse than other variants. These findings have been supported by similar studies using sequence analysis of the *msp2* genes. Overall, sequence analysis studies have provided evidence that the human strains differ from ruminant ones and may be maintained in nature within different reservoir hosts (13). *Anaplasma phagocytophilum* has been identified in feral ruminant populations in the United Kingdom, being reported in feral goats and red, fallow and roe deer (12, 23, 24). This organism has also been isolated from cervids, moose and chamois in Norway, Slovenia, Switzerland and Austria (as reviewed by 3). Other species known to become infected with *A. phagocytophilum* include wild rabbits, birds and cats (11, 12, 13, 25). This wide range of hosts that are susceptible to infection and serve as reservoir hosts probably contributes greatly to the establishment and spread of *A. phagocytophilum*.

Transmission, tick vectors and developmental cycle

Mechanical and transplacental transmission

The transmission dynamics of *A. marginale* are more complex than those of *A. phagocytophilum* because mechanical transmission can be effected by any means of transferring infective erythrocytes, such as by blood-contaminated mouthparts of biting flies and by instruments frequently used in veterinary practice (i.e. needles, ear-tag applicators, and castration and dehorning equipment) (2, 8). In geographical areas where tick vectors are absent or unable to transmit local *A. marginale* strains, such as in Florida where isolates of *A. marginale* have so far proven to be non-infective for ticks, mechanical transmission may be the only way of transmitting *A. marginale*. In general, mechanical transmission has been under-appreciated as a means of spreading bovine anaplasmosis. Persistently infected cattle are the most important reservoir hosts for this pathogen and serve as a source of infection for both mechanical transmission and biological transmission by ticks. In contrast, mechanical transmission, other than by blood transfusion, does not appear to play a role in *A. phagocytophilum* transmission.

Transplacental transmission of *A. marginale* occurs in cattle, resulting in healthy but persistently infected calves. More recently, transplacental transmission of *A. phagocytophilum* has been reported in experimentally and naturally infected cattle (26), sheep (5) and humans (as reviewed by 27), and while the entire impact of this mode of transmission has not yet been determined, transplacental transmission is not likely to contribute markedly to the overall epidemiology of *A. phagocytophilum*.

The role of ticks in transmission

Although both *A. marginale* and *A. phagocytophilum* are transmitted biologically by ticks, the tick species involved are different. Many species of ticks are reported to serve as vectors of *A. marginale* (as reviewed by 7), but the most common vectors throughout tropical and subtropical areas of the world are *Dermacentor* spp. (*D. andersoni*, *D. variabilis* and *D. albipictus*) and *Rhipicephalus* (*Boophilus*) spp. (*R. microplus* and *R. annulatus*). *Anaplasma phagocytophilum* is transmitted primarily by ticks of the *Ixodes persulcatus* complex, which are distributed mainly in the Northern Hemisphere (3, 12). *Ixodes scapularis* and *I. pacificus* are

vectors in the USA and *I. ricinus* is the main vector of *A. phagocytophilum* in Europe (12), but other ticks may also be vectors, including *Haemaphysalis punctata*, *I. persulcatus*, *I. trianguliceps* and *R. sanguineus* (as reviewed by 11, 12). Interestingly, molecular assays have provided evidence that other tick species (e.g. *D. marginatus*, *Haemaphysalis concinna*, *R. bursa* and *Hyalomma marginatum*) are also infected with this pathogen and may be involved in its transmission (28, 29). *Anaplasma phagocytophilum* variants may prove to have differing tick vectors, transmission patterns and target hosts (12, 13, 29).

Development of *Anaplasma* in ticks

The development of both *A. marginale* and *A. phagocytophilum* is complex, and is coordinated with the tick feeding cycle. Infection and colonisation of ticks occurs first in gut cells and subsequently in other tissues, including the salivary glands from where transmission occurs during feeding (as reviewed by 7, 30). While infection of salivary glands and transmission of *A. marginale* require a second feeding, ticks rapidly develop a generalised *A. phagocytophilum* infection and the salivary glands become infected after a short feeding period on an infected host (4, 6). The developmental cycle of *A. marginale* is more completely described (as reviewed by 7) than that of *A. phagocytophilum,* but both *Anaplasma* spp. are transmitted interstadially from one stage to the next (from larva to nymph, nymph to adult or larva to adult). Transovarial transmission is not generally thought to occur in either *Anaplasma* sp.; however, Baldridge *et al.* (31) demonstrated transovarial transmission of *A. phagocytophilum* variants in *D. albipictus*. Transovarial transmission of *A. phagocytophilum* would reduce its dependence on mammalian reservoirs. Studies on tick transmission patterns of *A. phagocytophilum* strains and variants are needed to more fully define the role of ticks in the epidemiology of this pathogen. Male ticks develop persistent *A. marginale* infections and, since they are intermittent feeders and transfer among cattle, are likely to be the main source of transmission by one-host ticks, such as *D. albipictus* or *Rhipicephalus* (*Boophilus*) spp. (7). In contrast, *Ixodes* males do not require a blood meal, rarely feed and mate off the host, and because they do not feed, they are not likely to contribute to the pathogen transmission cycle.

Epidemiology and wildlife hosts

Anaplasma marginale

The epidemiology of anaplasmosis is complex and not well defined. The seroprevalence rates of *A. marginale* in cattle vary widely and the variability of these rates has contributed to the development of geographically stable and unstable enzootic regions. Asymptomatic but persistently infected cattle are the major reservoir host and the unrestricted transport of these cattle has contributed to both the spread of anaplasmosis and the increase in strain diversity. Persistently infected cattle serve as a source of infection both for mechanical transmission and of the infection of ticks for biological transmission. While many species of wildlife are susceptible to infection with *A. marginale*, potential reservoir hosts can only be definitively demonstrated by molecular diagnostic tests (as reviewed by 8). For example, mule deer, previously considered to be a reservoir host for *A. marginale*, were found to be infected with *A. ovis*, a pathogen which is not infective for cattle (as reviewed by 9).

Anaplasma phagocytophilum

Several species of wild ruminants have been shown to be reservoirs of *A. phagocytophilum* in the USA, Europe and Asia (as reviewed by 3 and 12). Deer are the main hosts for adult ticks, a fact which contributes to their role as reservoir hosts for *A. phagocytophilum*. Many molecular studies in Europe have confirmed the prevalence of *A. phagocytophilum* in questing ticks (12). However, variants of *A. phagocytophilum* reported in deer and other animals may differ in pathogenicity (32, 33). A variety of small mammals, which also serve as hosts for ticks, have been found to be infected with *A. phagocytophilum* (as reviewed by 12). However, the epidemiology of *A. phagocytophilum* is complex and variants carried by these hosts and their impact on disease transmission have not been well defined. The emergence of *A. phagocytophilum* in the USA is directly related to spreading populations of *I. scapularis*, which also transmit *Borrelia burgdorferi*, the aetiologic agent of Lyme disease. The geographical expansion of *I. ricinus*, the European tick vector of *A. phagocytophilum*, is contributing to the emergence of HGA in several countries.

Diagnosis

Anaplasma spp. infections are diagnosed by serologic tests or, more recently, through a variety of molecular diagnostic assays, but serologic tests are currently the most practical for testing large numbers of animals (12, 34, 35). The analysis of genome sequences and molecular characterisation of the MSPs of these closely related *Anaplasma* sp. organisms are rapidly advancing (36). In addition, discovery of MSPs among these organisms, which includes species-specific and highly conserved MSPs throughout the genus, has impacted on the development and specificity of serologic tests. For bovine anaplasmosis, a tentative diagnosis can be made based on a combination of the geographical location, season, clinical signs and/or necropsy findings (as reviewed by 2). Demonstration of marginal bodies in stained blood smears may not be reliable when parasitaemia levels are low and before clinical signs appear, or in persistently infected animals. During these periods, *A. marginale* inclusion bodies can be easily confused with Howell-Jolly

bodies, basophilic stippling of immature erythrocytes, and stain contamination. The serologic tests used previously for diagnosis of anaplasmosis (complement fixation and card agglutination tests) lacked the sensitivity for diagnosis of cattle in the incubation period and during persistent infection (37, 38). A competitive enzyme-linked immunosorbent assay (cELISA), based on the *A. marginale* MSP5 surface-exposed protein, proved to have high sensitivity and specificity, but was subsequently found to cross-react with antibodies to other organisms of the genus *Anaplasma* because of the conservation of the *msp5* gene (as reviewed by 9). This cross-reaction renders the MSP cELISA accurate only to the genus *Anaplasma* but not to the species level, and this fact should be taken into consideration when evaluating the research literature published before 2000. The MSP5 cELISA also cross-reacted in sheep that had been experimentally infected with *A. phagocytophilum* (as reviewed by 9). As a result of the lack of specificity of the MSP5 cELISA, the emergence of *A. phagocytophilum* in cattle may not be recognised. While polymerase chain reaction (PCR) assays allow determination of the species because they are based on organism-specific DNA or RNA, these assays are not practical for large-scale surveillance. However, PCR should be used as a definitive diagnostic tool when serologic test results require confirmation of the organism and strain identity. For example, the MSP5 cELISA demonstrated false positives for *A. marginale* in a herd of cattle in British Columbia, Canada, where the disease had not been reported previously (39). PCR studies using the *A. marginale msp5* and rickettsial *16S rRNA* gene sequences subsequently demonstrated that these cattle were infected with a novel *Ehrlichia*.

Molecular interactions (ligands and host cell receptors)

Anaplasma marginale

The discovery of ligands and receptors involved in host–pathogen interactions provides relevant information for the development of control measures. Several MSPs (MSP1a, MSP1b and MSP2) of *A. marginale* have been identified as possible adhesins (as reviewed by 17). However, one of the best-characterised molecules involved in *A. marginale*–host (cattle and tick) interactions is MSP1a (40, 41). This protein was found to be an adhesin that binds to bovine erythrocytes (41), tick cell extracts (41) and the gut cells of vector ticks (40). The binding domains of MSP1a are located in the tandem repeat region of this protein (42). The host cell receptors that bind with MSP1a on tick cells and erythrocytes have not been described. However, previous studies of erythrocytes and *A. marginale* initial bodies have shown that the bovine receptor may be comprised of both protein and carbohydrate (as reviewed by 17).

Anaplasma phagocytophilum

Host–pathogen interactions for *A. phagocytophilum* are more completely characterised than those of *A. marginale*. *Anaplasma phagocytophilum* adhesion and invasion are complex processes that involve interactions between host membrane-associated structures, such as glypiated- (GPI) anchored proteins, Caveolin-1, lipid raft, β2-Integrin (CD18), P-selectin glycoprotein ligand-1 (PSGL-1) and outer membrane proteins (OMPs) from the bacterium (43). The most well-characterised *A. phagocytophilum* receptor is PSGL-1. *Anaplasma phagocytophilum* uptake is mediated by PSGL-1 expressed on human neutrophils, bone marrow progenitor and human promyelocytic leukaemia (HL-60) cells (44, 45). The bacterium cooperatively binds the N-terminal peptide of PSGL-1 and two carbohydrate determinants of this molecule: α1,3-fucose, and α2,3-sialic acid (46). At least three *A. phagocytophilum* OMPs interact with PSGL-1: OmpA, which binds the α2,3-sialic acid; MSP2 (p44), which possibly binds the N-terminal peptide; and an unknown molecule which binds α1,3-fucose (43). Nevertheless, adhesion and invasion may also occur through PSGL-1-independent ways that involve β2-Integrin and lipid rafts (47).

Control measures, including vaccine strategies and chemotherapy

Anaplasma marginale

Control options for bovine anaplasmosis vary depending upon whether outbreaks occur in a known endemic area or as focal isolated events in non-endemic areas, and include:

– maintaining an *A. marginale*-free herd
– vaccination
– treatment with tetracycline during acute disease
– administration of low-level tetracycline for prevention of clinical disease (as reviewed by 2 and 9).

Importantly, it should be noted that the goal of incorporating vaccines or antimicrobials in health protocols is the prevention of clinical anaplasmosis, and that neither treatment prevents infection of cattle after challenge exposure. Cattle used as stock (including cows that are used only as embryo donors and bulls used for semen collection) or which are exported to non-endemic areas should be completely free of *A. marginale* infection. In areas where persistently infected cattle are common and contribute to endemic stability, it may be important to maintain this situation, rather than to introduce susceptible cattle which will most likely increase the risk of acute disease. In some

areas, especially in southern regions where tick vectors are frequently active during warm winters, control strategies may be required year round; while in other areas, control may be required primarily during the vector season plus one month to account for the incubation period. While tetracycline antimicrobials are approved by the USA Food and Drug Administration for the prevention or treatment of acute anaplasmosis, no antimicrobials are labelled for the elimination of persistent *A. marginale* infection (9).

Two major vaccine types, killed and live, have been used to control anaplasmosis, both of which rely on *A. marginale*-infected blood as the antigen/infection source (as reviewed by 2 and 8). Killed vaccines were marketed in the USA but are not currently available. Live vaccines, which involve infecting cattle with the less pathogenic *A. centrale*, are used in some European and African countries. Cattle infected with the *A. centrale* live vaccine develop life-long persistent infection, which provides protection against clinical disease when cattle are challenge-exposed with *A. marginale*. Recent studies demonstrated that *A. centrale* was not infective for *H. excavatum*, *R. sanguineus* or *R. annulatus* (48). However, live vaccines depend on donor cattle as a source of infective blood, which could result in the transmission of other haemoparasites, including mycoplasmas, rickettsiae and viruses which are carried silently by cattle. Overall, the challenges of vaccine development include the antigenic variation of *A. marginale* that occurs during persistent infections and the increased diversity of *A. marginale* strains, especially in areas of continual cattle movement.

Anaplasma phagocytophilum

While *A. phagocytophilum* infection may resolve without antibiotic therapy, the pathogen is susceptible to tetracycline antibiotics (49). Current disease prevention strategies in domestic animals are based on the reduction of tick infestations by application of acaricides. As with *A. marginale*, long-acting tetracycline is used as a prophylactic measure before animals are moved to tick-infested pastures (12, 49). While several candidate vaccine antigens have been suggested, vaccines for *A. phagocytophilum* have not yet been developed (12).

Future vaccine development directions

The limitations of acaricide control for ticks and antimicrobial therapy for *Anaplasma* spp. are being realised because of the selection of resistant ticks and restrictions on the use of antimicrobials (50). Tick vaccines offer important advantages by being a cost-effective and environmentally friendly control alternative with the dual effect of reducing tick infestations and preventing ticks from transmitting disease-causing pathogens. Tick antigens studied thus far have demonstrated multiple effects when used in a vaccine format, including reductions in:

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

Development of vaccines for ticks and *Anaplasma* spp. will be dependent on further definition of the molecular interactions between hosts, ticks and pathogens, which will allow for discovery of key molecules that could be tested in vaccine formats for intervention in tick–pathogen cycles. The general approach for developing *Anaplasma* spp. vaccines is a vaccinomics approach through which key molecules can be discovered that mediate tick and pathogen success, including those which are involved in key biological functions, such as feeding, reproduction, development, immune response, subversion of host immunity and pathogen infection, multiplication and transmission.

Acknowledgements

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

Les bactéries du genre *Anaplasma* : nouveaux défis suite à la reclassification

K.M. Kocan, J. de la Fuente & A. Cabezas-Cruz

Résumé

Le genre *Anaplasma* est l'un des quatre genres distincts de la famille des Anaplasmataceae, bactéries pathogènes intracellulaires obligatoires vectorisées par des tiques et présentes exclusivement dans les vacuoles parasitophores du cytoplasme des cellules hôtes. Suite à la reclassification de l'ordre des Rickettsiales en 2001, le genre *Anaplasma*, qui ne contenait précédemment que des pathogènes ayant une spécificité d'hôte pour les ruminants (*A. marginale, A. centrale* et *A. ovis*) a été reconsidéré et intègre désormais *A. phagocytophilum*, taxon regroupant trois microorganismes précédemment classés dans le genre *Ehrlichia* (*E. equi, E. phagocytophila* et l'agent de l'ehrlichiose granulocytaire humaine). Le genre *Anaplasma* contient également *A. bovis* (anciennement *E. bovis*), *A. platys* (anciennement *E. platys*) et *Aegyptianella pullorum*. En dépit de leur parenté génomique, les microorganismes ainsi regroupés se distinguent par de nombreux aspects de leur biologie, en particulier leur spécificité d'hôte, le tropisme des cellules hôtes, les protéines majeures de surface et la transmission par des tiques vectrices.

Les auteurs examinent tout particulièrement les deux principaux agents pathogènes de ce genre, à savoir *A. marginale*, l'agent de l'anaplasmose bovine, et *A. phagocytophilum*, l'agent étiologique de fièvres transmises par les tiques chez les ovins et de l'anaplasmose granulocytaire humaine, une maladie humaine émergente transmise par les tiques. La diversité des souches de ces deux agents est bien plus importante qu'on ne l'avait estimé précédemment. Les protéines majeures de surface jouent un rôle précieux lors des études phylogénétiques et de l'identification des souches, mais il a été constaté qu'une conservation trop longue altérait la spécificité des tests sérologiques. La comparaison entre ces deux agents pathogènes majeurs souligne les défis et les éclairages nouveaux induits par la reclassification et par l'analyse moléculaire ainsi que leurs répercussions, dans chaque cas, sur la conception et la mise en œuvre dans le temps de stratégies de diagnostic et de contrôle appropriées.

Mots-clés

Agent pathogène transmis par les tiques – *Anaplasma marginale* – *Anaplasma phagocytophilum* – Anaplasmataceae – Anaplasmose.

Nuevas dificultades tras la reclasificación del género *Anaplasma*

K.M. Kocan, J. de la Fuente & A. Cabezas-Cruz

Resumen

El género *Anaplasma* es uno de los cuatro que componen la familia Anaplasmataceae, integrada por patógenos intracelulares obligados que se transmiten por garrapatas y residen exclusivamente en vacuolas parasitóforas dentro del citoplasma de las células del hospedador. En 2001, al reclasificar el orden Rickettsiales, se amplió el género *Anaplasma*, que hasta entonces contenía patógenos específicos de distintos hospedadores rumiantes (*A. marginale, A. centrale* y *A. ovis*), para dar cabida en él a *A. phagocytophilum*, especie

resultante de la unificación de tres microorganismos anteriormente clasificados como *Ehrlichia* (*E. equi, E. phagocytophila* y el agente innominado de la erliquiosis granulocítica humana). También se incluyeron en el género *Anaplasma* las especies *A. bovis* (anteriormente *E. bovis*), *A. platys* (anteriormente *E. platys*) y *Aegyptianella pullorum.* A pesar del parentesco genómico que existe entre los microorganismos reagrupados, estos difieren en muchos aspectos de su biología, en particular su especificidad por uno u otro hospedador, su tropismo por distintos tipos celulares, sus principales proteínas de superficie (PPS), y las garrapatas que ejercen de vector.
Los autores se centran en los dos patógenos más importantes: *A. marginale*, causante de la anaplasmosis bovina, y *A. phagocytophilum*, causante en los ovinos de una fiebre transmitida por garrapatas y en el hombre de anaplasmosis granulocítica, enfermedad humana emergente que también se transmite por garrapatas. En ambos patógenos, la diversidad entre cepas es mucho mayor de lo que anteriormente se había observado. Se ha descubierto que las PPS son útiles para los estudios filogenéticos y la caracterización de las cepas, y también se ha observado que las PPS muy conservadas afectan a la especificidad de las técnicas de prueba serológica. La comparación entre estos dos importantes patógenos pone de manifiesto las dificultades y los nuevos conocimientos que han traído consigo la reclasificación y el análisis molecular, que a su vez tienen repercusiones en la concepción y evaluación de estrategias de diagnóstico y control.

Palabras clave
Anaplasma marginale – *Anaplasma phagocytophilum* – Anaplasmataceae – Anaplasmosis – Patógeno transmitido por garrapatas.

References

1. Dumler J.S., Barbet A.F., Bekker C.P.J., Dasch G.A., Palmer G.H., Ray S.C., Rikihisa Y. & Rurangirwa F.R. (2001). – Reorganization of 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 of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int. J. Syst. Evolut. Microbiol.*, **51** (6), 2145–2165.

2. Kocan K.M., de la Fuente J., Guglielmone A.A. & Meléndez R.D. (2003). – Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. *Clin. Microbiol. Rev.*, **16** (4), 698–712.

3. Woldehiwet Z. (2010). – The natural history of *Anaplasma phagocytophilum*. *Vet. Parasitol.*, **167** (2–4), 108–122.

4. Kocan K.M., Busby A.T., Allison R.W., Breshears M.A., Coburn L., Galindo R.C., Ayllón N., Blouin E.F. & de la Fuente J. (2012). – Sheep experimentally infected with a human isolate of *Anaplasma phagocytophilum* serve as a host for infection of *Ixodes scapularis* ticks. *Ticks Tick Borne Dis.*, **3** (3), 147–153. doi:10.1016/j.ttbdis.2012.01.004.

5. Reppert E., Galindo R.C., Breshears M.A., Kocan K.M., Blouin E.F. & de la Fuente J. (2012). – Demonstration of transplacental transmission of a human isolate of *Anaplasma phagocytophilum* in an experimentally infected sheep. *Transbound. Emerg. Dis.*, **60** (Suppl. 1), 93–96.

6. Reppert E., Galindo R.C., Ayllón N., Breshears M.A., Kocan K.M., Blouin E.F. & de la Fuente J. (2014). – Studies of *Anaplasma phagocytophilum* in sheep experimentally infected with the human NY-18 isolate: characterization of tick feeding sites. *Ticks Tick Borne Dis.*, **5** (6), 744–752. doi:10.1016/j.ttbdis.2014.05.014.

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

8. Kocan K.M., de la Fuente J., Step D.L., Blouin E.F., Coetzee J.F., Simpson K.M., Genova S.G. & Boileau M.J. (2010). – Current challenges of the management and epidemiology of bovine anaplasmosis. *Bov. Practit.*, **44** (2), 93–102.

9. Kocan K.M., Coetzee J.F., Step D.L., de la Fuente J., Blouin E.F., Reppert E., Simpson K.M. & Boileau M.J. (2012). – Current challenges in the diagnosis and control of bovine anaplasmosis. *Bov. Practit.*, **46**, 67–77.

10. Aubry P. & Geale D.W. (2011). – A review of bovine anaplasmosis. *Transbound. Emerg. Dis.*, **58** (1), 1–30.

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

12. Stuen S., Granquist E.G. & Silaghi C. (2013). – *Anaplasma phagocytophilum*: a widespread multi-host pathogen with highly adaptive strategies. *Front. Cell. Infect. Microbiol.*, **3**, 31. doi:10.3389/fcimb.2013.00031.

13. de la Fuente J., Naranjo V., Ruiz-Fons F., Höfle U., Fernández de Mera I.G., Villanúa D., Almazán C., Torina A., Caracappa S., Kocan K.M. & Gortázar C. (2005). – Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in central Spain. *Vector Borne Zoonotic Dis.*, **5** (4), 390–401.

14. Dumitrache M.O., Paştiu I.A., Kalmár Z., Mircean V., Sándor A.D., Gherman C.M., Peştean C., Mihalca A.D. & Cozma V. (2013). – Northern white-breasted hedgehogs *Erinaceus roumanicus* as hosts for ticks infected with *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in Romania. *Ticks Tick Borne Dis.*, **4** (3), 214–217.

15. 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. (2007). – Prevalence and genetic diversity of *Anaplasma marginale* strains in cattle in South Africa. *Zoonoses Public Hlth*, **54** (1), 23–30.

16. Ruybal P., Moretta R., Perez A., Petrigh R., Zimmer P., Alcaraz E., Echaide I., Torioni de Echaide S., Kocan K.M., de la Fuente J. & Farber M. (2009). – Genetic diversity of *Anaplasma marginale* in Argentina. *Vet. Parasitol.*, **162** (1–2), 176–180.

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

18. Awad H., Antunes S., Galindo R.C., do Rosário V.E., de la Fuente J., Domingos A. & El Hussein A.M. (2011). – Prevalence and genetic diversity of *Babesia* and *Anaplasma* species in cattle in Sudan. *Vet. Parasitol.*, **181** (2–4), 146–152.

19. Bastos C.V., Passos L.M.F., Facury-Filho E.J., Rabelo E.M.L., de la Fuente J. & Ribeiro M.F.B. (2010). – Protection in the absence of exclusion between two Brazilian isolates of *Anaplasma marginale* in experimentally infected calves. *Vet. J.*, **186** (3), 374–378.

20. de la Fuente J., Garcia-Garcia J.C., Blouin E.F. & Kocan K.M. (2002). – Infection of tick cells and bovine erythrocytes with one genotype of the intracellular ehrlichia *Anaplasma marginale* excludes infection with other genotypes. *J. Clin. Diagn. Lab. Immunol.*, **9** (3), 658–668.

21. de la Fuente J., Blouin E.F. & Kocan K.M. (2003). – Infection exclusion of the rickettsial pathogen, *Anaplasma marginale*, in the tick vector, *Dermacentor variabilis*. *Clin. Diagn. Lab. Immunol.*, **10** (1), 182–184.

22. Palmer G.H., Knowles D.P. Jr, Rodriguez J.L., Gnad D.P., Hollis L.C., Marston T. & Brayton K.A. (2004). – Stochastic transmission of multiple genotypically distinct *Anaplasma marginale* strains in a herd with high prevalence of *Anaplasma* infection. *J. Clin. Microbiol.*, **42** (11), 5381–5384.

23. Foster W.N.M. & Greig J.C. (1969). – Isolation of tick-borne fever from feral goats in New Galloway. *Vet. Rec.*, **85** (21), 585–586.

24. McDiarmid A. (1965). – Modern trends in animal health and husbandry: some infectious diseases of free-living wildlife. *Br. Vet. J.*, **121**, 245–257.

25. Lappin M.R., Breitschwerdt E.B., Jensen W.A., Dunnigan B., Rha J.Y., Williams C.R., Brewer M. & Fall M. (2004). – Molecular and serologic evidence of *Anaplasma phagocytophilum* infection in cats in North America. *JAMA*, **225** (6), 893–896.

26. Henniger T., Henniger P., Grossmann T., Distl O., Ganter M. & von Leowenich F.D. (2013). – Congenital infection with *Anaplasma phagocytophilum* in a calf in northern Germany. *Acta Vet. Scand.*, **55**, 38.

27. Dhand A., Nadelman R.B., Aguero-Rosenfeld M., Haddad F.A., Stokes D.P. & Horowitz H.W. (2007). – Human granulocytic anaplasmosis during pregnancy: case series and literature review. *Clin. Infect. Dis.*, **45** (5), 589–593.

28. Barandika J.F., Hurtado A., García-Sanmartín J., Juste R.A., Anda P. & García-Pérez A.L. (2008). – Prevalence of tick-borne zoonotic bacteria in questing adult ticks from northern Spain. *Vector Borne Zoonotic Dis.*, **8** (6), 829–836.

29. Naranjo V., Ruiz-Fons F., Höfle U., Fernández de Mera I.G., Villanúa D., Almazán C., Torina A., Caracappa S., Kocan K.M., Gortázar C. & de la Fuente J. (2006). – Molecular epidemiology of human and bovine anaplasmosis in southern Europe. *Ann. N.Y. Acad. Sci.*, **1078**, 95–99.

30. Reichard M.V., Manzano Roman R., Kocan K.M., Blouin E.F., de la Fuente J., Snider T.A., Heinz R.E., Massung R.F., West M.D. & Little S.E. (2009). – Inoculation of white-tailed deer (*Odocoileus virginianus*) with Ap-V1 or NY-18 strains of *Anaplasma phagocytophilum* and microscopic demonstration of Ap-V1 in *Ixodes scapularis* adults that acquired infection from deer as nymphs. *Vector Borne Zoonotic Dis.*, **9** (5), 565–568.

31. Baldridge G.D., Scoles G.A., Burkhardt N.Y., Schloeder B., Kurtti T.J. & Muderloh U.G. (2009). – Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (Acari: Ixodidae). *J. Med. Entomol.*, **46** (3), 625–632. doi:10.1603/033.046.0330.

32. Overzier E., Pfister K., Herb I., Mahling M., Böck G. Jr & Silaghi C. (2013). – Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), in questing ticks (*Ixodes ricinus*), and in ticks questing roe deer in southern Germany. *Ticks Tick Borne Dis.*, **4** (4), 320–328.

33. Overzier E., Pfister K., Thiel C., Herb I., Mahling M. & Silaghi C. (2013). – *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks: comparison of prevalences and partial *16S rRNA* gene variants in urban, pasture, and natural habitats. *Appl. Environ. Microbiol.*, **79** (5), 1730–1734.

34. Massung R.F. & Slater K.G. (2003). – Comparison of PCR assays for detection of the agent of human granulocytic ehrlichiosis, *Anaplasma phagocytophilum*. *J. Clin. Microbiol.*, **41** (2), 717–722.

35. Coetzee J.F., Schmidt P.L., Apley M.D., Reinbold J.B. & Kocan K.M. (2007). – Comparison of the complement fixation test and competitive ELISA for serodiagnosis of *Anaplasma marginale* infection in experimentally infected steers. *Am. J. Vet. Res.*, **68** (8), 872–878.

36. Brayton K.A., Kappmeyer L.S., Herndon D.R., Dark M.J., Tibbals D.L., Palmer G.H., McGuire T.C. & Knowles Jr D.P. (2005). – 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** (3), 844–849.

37. Bradway D.S., Torioni de Echaide S., Knowles D.P., Hennager S.G. & McElwain T.F. (2001). – Sensitivity and specificity of the complement fixation test for detection of cattle persistently infected with *Anaplasma marginale*. *J. Vet. Diagn. Invest.,* **13** (1), 79–81.

38. Coetzee J.F., Apley M.D., Kocan K.M., Rurangirwa F.R. & Van Donkersgoed J. (2005). – Comparison of three oxytetracycline regimes for the treatment of persistent *Anaplasma marginale* infections in beef cattle. *Vet. Parasitol.*, **127** (1), 61–73.

39. Howden K.J., Geale D.W., Paré J., Golsteyn-Thomas E.J. & Gajadhar A.A. (2010). – An update on bovine anaplasmosis (*Anaplasma marginale*) in Canada. *Can. Vet. J.*, **51** (8), 830–837.

40. de la Fuente J., Garcia-Garcia J., Blouin E., McEwen B., Clawson D. & Kocan K. (2001). – Major surface protein 1a effects tick infection and transmission of *Anaplasma marginale*. *Int. J. Parasitol.*, **31** (14), 1705–1714.

41. de la Fuente J., Garcia-Garcia J., Blouin E. & Kocan K. (2001). – Differential adhesion of major surface proteins 1a and 1b of the ehrlichial cattle pathogen *Anaplasma marginale* to bovine erythrocytes and tick cells. *Int. J. Parasitol.*, **31** (2), 145–153.

42. de la Fuente J., Garcia-Garcia J., Blouin E. & Kocan K. (2003). – Characterization of the functional domain of major surface protein 1a involved in adhesion of the rickettsia *Anaplasma marginale* to host cells. *Vet. Microbiol.*, **91** (2–3), 265–283.

43. Truchan H.K., Seidman D. & Carlyon J.A. (2013). – Breaking in and grabbing a meal: *Anaplasma phagocytophilum* cellular invasion, nutrient acquisition, and promising tools for their study. *Microbes Infect.*, **15** (14–15), 1017–1025.

44. Goodman J.L., Nelson C.M., Klein M.B., Hayes S.F. & Weston B.W. (1999). – Leukocyte infection by the granulocytic ehrlichiosis agent is linked to expression of a selectin ligand. *J. Clin. Invest.*, **103** (3), 407–412.

45. Herron M.J., Nelson C.M., Larson J., Snapp K.R., Kansas G.S. & Goodman J.L. (2000). – Intracellular parasitism by the human granulocytic ehrlichiosis bacterium through the P-selectin ligand, PSGL-1. *Science*, **288** (5471), 1653–1656.

46. Yago T., Leppänen A., Carlyon J.A., Akkoyunlu M., Karmakar S., Fikrig E., Cummings R.D. & McEver R.P. (2003). – Structurally distinct requirements for binding of P-selectin glycoprotein ligand-1 and sialyl Lewis x to *Anaplasma phagocytophilum* and P-selectin. *J. Biol. Chem.*, **278** (39), 37987–37997. E–pub.: 7 July 2003.

47. Schaff U.Y., Trott K.A., Chase S., Tam K., Johns J.L., Carlyon J.A., Genetos D.C., Walker N.J., Simon S.I. & Borjesson D.L. (2010). – Neutrophils exposed to *A. phagocytophilum* under shear stress fail to fully activate, polarize, and transmigrate across inflamed endothelium. *Am. J. Physiol. Cell. Physiol.*, **299** (1), 87–96. doi:10.1152/ajpcell.00165.2009.

48. Shkap V., Kocan K., Molad T., Mazuz M., Leibovich B., Krigel Y., Michoytchenko A., Blouin E., de la Fuente J., Samish M., Mtshali M., Zweygarth E., Fleiderovich E.L. & Fish L. (2009). – Experimental transmission of field *Anaplasma marginale* and the *A. centrale* vaccine strain by *Hyalomma excavatum*, *Rhipicephalus sanguineus* and *Rhipicephalus* (*Boophilus*) *annulatus* ticks. *Vet. Microbiol.*, **134** (3–4), 254–260.

49. Stuen S., Enemark J.M., Artursson K. & Nielsen B. (2012). – Prophylactic treatment with flumethrin, a pyrethroid (Bayticol®, Bayer), against *Anaplasma phagocytophilum* infection in lambs. *Acta Vet. Scand.*, **54**, 31. doi:10.1186/1751-0147-54-31.

50. de la Fuente J., Kocan K.M. & Contreras M. (2015). – Prevention and control strategies for ticks and pathogen transmission. *In* New developments in major vector-borne diseases. Part I: An overview (S. Zientara, D. Verwoerd & P.-P. Pastoret, eds). *Rev. Sci. Tech. Off. Int. Epiz.,* **34** (1), 249–264.