

# Hemotrophic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential

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**Abstract:** The red cell parasites formerly known as *Haemobartonella* and *Eperythrozoon* spp have been reclassified as hemotrophic mycoplasmas (hemoplasmas) based on strong phylogenetic evidence and 16S ribosomal RNA gene sequences. The latter form the basis for polymerase chain reaction assays used to detect infection. *Candidatus* designation was given to incompletely characterized species. Like other mycoplasmas, hemoplasmas are small epicellular parasites that lack a cell wall and are susceptible to tetracyclines; their circular, double-stranded DNA encodes only those gene products essential for life. Diseases caused by infection with hemoplasmas range from overt life-threatening hemolytic anemia to subtle chronic anemia, ill-thrift, and infertility. In addition, the organisms may act as cofactors in the progression of retroviral, neoplastic, and immune-mediated diseases. Intimate contact of hemoplasma organisms with RBCs leads to cell injury through immune-mediated and other mechanisms that have not yet been defined. Despite an intense immune response and even with antibiotic treatment, infected animals probably remain chronic carriers after clinical signs have resolved. (*Vet Clin Pathol.* 2004;33:2-13)

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**Key Words:** *Eperythrozoon*, erythrocyte, *Haemobartonella*, *Mycoplasma*, parasite, red blood cell

I. Historical Perspective . . . . .	2
A. <i>Haemobartonella</i> and <i>Eperythrozoon</i> spp . . . . .	2
B. Ultrastructural morphology . . . . .	3
II. Molecular Perspective . . . . .	4
A. Reclassification as <i>Mycoplasma</i> spp . . . . .	4
B. PCR diagnosis of hemoplasma infection . . . . .	5
III. Clinicopathologic Findings in Hemoplasma	
Infections . . . . .	6
A. Acute disease . . . . .	6
B. Chronic disease . . . . .	7
IV. Properties and Pathogenicity of Hemoplasmas and	
Other Mycoplasmas . . . . .	7
A. The genomic basis of mycoplasma survival . . . . .	8
B. Association with retroviral and immune-mediated diseases . . . . .	8
C. Mechanisms of pathogenicity . . . . .	8
D. Mechanisms of adherence to host cells . . . . .	9
V. Summary and Future Directions . . . . .	10
VI. References . . . . .	10

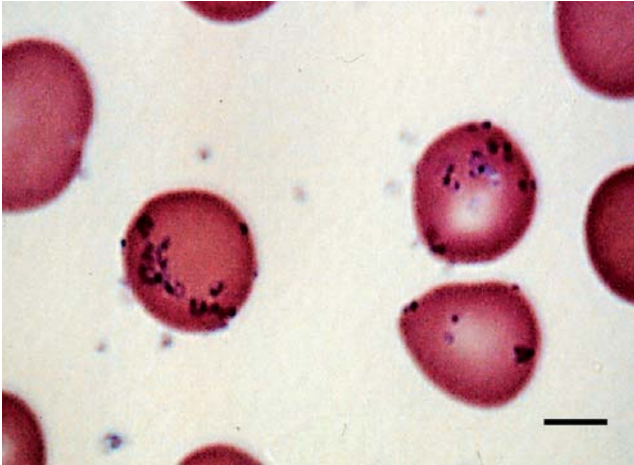
## Historical Perspective

### *Haemobartonella* and *Eperythrozoon* spp

The organisms formerly known as *Haemobartonella* and *Eperythrozoon* spp are small, pleomorphic bacteria that parasitize red blood cells (RBCs) of a wide range of vertebrate animals. The organisms are gram-negative, obligate red cell parasites that have not been grown successfully in culture. They may be rod-shaped, spherical, or ring-shaped and are found individually or in chains across the red cell surface (Figure 1). Morphologic differentiation of the 2 genera has been based on the more frequent occurrence of ring forms and of organisms free in the plasma for *Eperythrozoon* spp compared with *Haemobartonella* spp.<sup>2</sup> *Eperythrozoon* organisms have been described in pigs (*E suis* and *E parvum*),<sup>3-5</sup> sheep and goats (*E ovis*),<sup>6,7</sup> cattle (*E wenyonii*, *E tejanodes*, and *E tumoi*),<sup>8-11</sup> llamas and alpacas,<sup>12-14</sup> mice (*E coccoides*),<sup>15</sup> and a flying fox (*E mariboi*).<sup>16</sup> Two species of *Haemobartonella* have been described in cats; the Ohio organism or large form of *H felis* is the cause of feline infectious anemia,<sup>17</sup> whereas the California organism or small form appears to have low virulence.<sup>18</sup> *Haemobartonella* infections also have been reported in dogs (*H canis*),<sup>19,20</sup> rats (*H muris*),<sup>21</sup> raccoons (*H procyoni*),<sup>22</sup> an opossum,<sup>1</sup> monkeys,<sup>23-25</sup>

*Could it be that the mycoplasmas behave as opportunistic organisms, cofactors in the pathogenesis of AIDS (retroviral diseases), or pathogenic agents in their own right? None of these possibilities can be excluded.*

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**Figure 1.** Light micrograph of hemoplasma parasites (*Candidatus Mycoplasma haemodidelphi*) found individually and in chains across the surface of red cells. Note the rod-shaped, spherical, and ring-shaped forms. Wright's-Giemsa, 0.8 cm (bar) = 5.0  $\mu\text{m}$ . Reprinted with permission from Messick et al, *J Zoo Wildl Med*.<sup>1</sup>



**Figure 2.** Scanning electron micrograph of several hemoplasma parasites (*Mycoplasma haemosuis*) within shallow depressions on the surface of a RBC. 1 cm = 1  $\mu\text{m}$ . Photograph kindly provided by Dr James Zachary, University of Illinois.

and human beings.<sup>26,27</sup> In laboratory and domestic animals, naturally-occurring diseases caused by *Eperythrozoon* and *Haemobartonella* spp have been documented in the United States, England, Ireland, Germany, Belgium, Africa, Mexico, Brazil, and many other countries.<sup>28-36</sup>

Blood parasites in mice (*E coccoides*) and dogs (*H canis*) were first observed in Germany in 1928.<sup>33</sup> Adler and Ellenbogen<sup>34</sup> reported finding similar parasites in anemic cattle in Palestine about 6 years later. Also in the early 1930s, *Eperythrozoon* infection in pigs, characterized by icterus and anemia, was first recognized in the United States.<sup>2,3</sup> In 1941, Lotze and Yiengst<sup>37</sup> documented *E wenyonii* infection in cattle in the United States, and soon thereafter, Jensen<sup>38</sup> found that *E ovis* was a common blood parasite of native Louisiana sheep. More than a decade later, Flint and Moss<sup>39</sup> recognized *H felis* as the cause of feline infectious anemia, a contagious disease of cats. A report by Benjamin and Lumb<sup>18</sup> in 1959 described a similar disease in dogs; however, in the United States, sporadic cases of hemobartonellosis in dogs were recognized as early as 1935.<sup>40,41</sup>

### Ultrastructural morphology

Transmission electron microscopic features of *H muris* were compared with *E coccoides* and *Mycoplasma pulmonis* in 1965.<sup>42</sup> Despite the divergence of their taxonomic positions, the fine ultrastructural details of

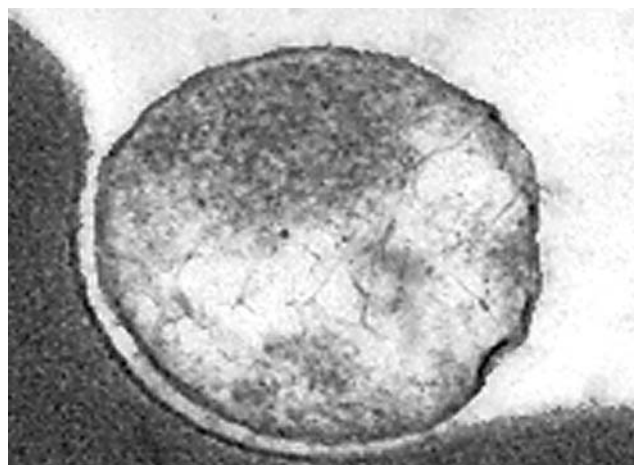
*H muris* and *E coccoides* are fundamentally similar. Both are spherical, 350 to 700 nm in diameter, devoid of nuclear structure, and lack a cell wall. There is evidence that they multiply through binary fission, but no life cycle was described. *M pulmonis*, on the other hand, is remarkably pleomorphic in size and shape; however, other ultrastructural features are similar to those of *H muris* and *E coccoides*. To distinguish these 3 organisms, emphasis has been placed on the growth of *M pulmonis* versus the failure of *H muris* and *E coccoides* to propagate in cell-free media. The ultrastructural morphology of *H felis*,<sup>43-46</sup> *H canis*,<sup>47</sup> *E ovis*,<sup>48</sup> *E wenyonii*,<sup>49</sup> *E suis*,<sup>50,51</sup> and a red cell parasite in naturally infected llamas<sup>13</sup> was described by various groups over the next 20 years. More recently, the clinicopathologic and light and transmission electron microscopic features of red cell parasites in a naturally infected alpaca<sup>14</sup> and in an opossum<sup>1</sup> were described. Collectively, the ultrastructural findings show striking similarity among these red cell parasites. The organisms are round to elongate, 0.3 to 3  $\mu\text{m}$  in diameter, and enclosed by a single limiting membrane. Although they have no nucleus, small granules and a few filamentous structures are found in the cytoplasm. The parasites adhere to, but do not penetrate, the RBC surface (Figure 2). They are found in shallow depressions and deep infoldings on the surface, with a 15- to 25-nm clear zone separating the parasite from the RBC membrane. Delicate fibrils from the parasite appear to extend through this clear zone, attaching the organism to its host cell (Figure 3).

**Molecular Perspective**

Reclassification as *Mycoplasma* spp

Considerable confusion about the true nature of *Haemobartonella* and *Eperythrozoon* spp has persisted over the past 50 years. Until 1993, the order Rickettsiales contained 3 families: Rickettsiaceae, Bartonellaceae, and Anaplasmataceae. The hemotropic bacteria, *Haemobartonella* and *Eperythrozoon*, were classified as members of the family Anaplasmataceae based on biologic and phenotypic characteristics.<sup>2</sup> Bartonellaceae are parasites of human RBCs and have morphologic features and growth characteristics of bacteria, whereas *Haemobartonella* and *Eperythrozoon* spp have not been cultivated in vitro and their ultrastructural features are not typical of bacteria. The proposed transmission of *Haemobartonella* and *Eperythrozoon* spp by arthropod vectors was also consistent with their classification in the family Rickettsiaceae. There was, nevertheless, a long-held suspicion that *Eperythrozoon* and *Haemobartonella* spp were not rickettsial parasites, but rather were more closely related to members of the class Mollicutes.<sup>42</sup> This suspicion was based on their lack of intracellular parasitism, small size, lack of a cell wall, lack of flagellae, resistance to penicillin and its analogues, and susceptibility to tetracyclines. The Mollicutes are phylogenetically diverse and include more than 150 species in 8 genera (*Mycoplasma*, *Ureaplasma*, *Spiroplasma*, *Acholeplasma*, *Anaeroplasmata*, *Asteroleplasma*, *Mesoplasma*, and *Entomoplasma*).<sup>52</sup> The term mycoplasma commonly is used to describe any member of the class Mollicutes (*mollis*, soft; *cutis*, skin).

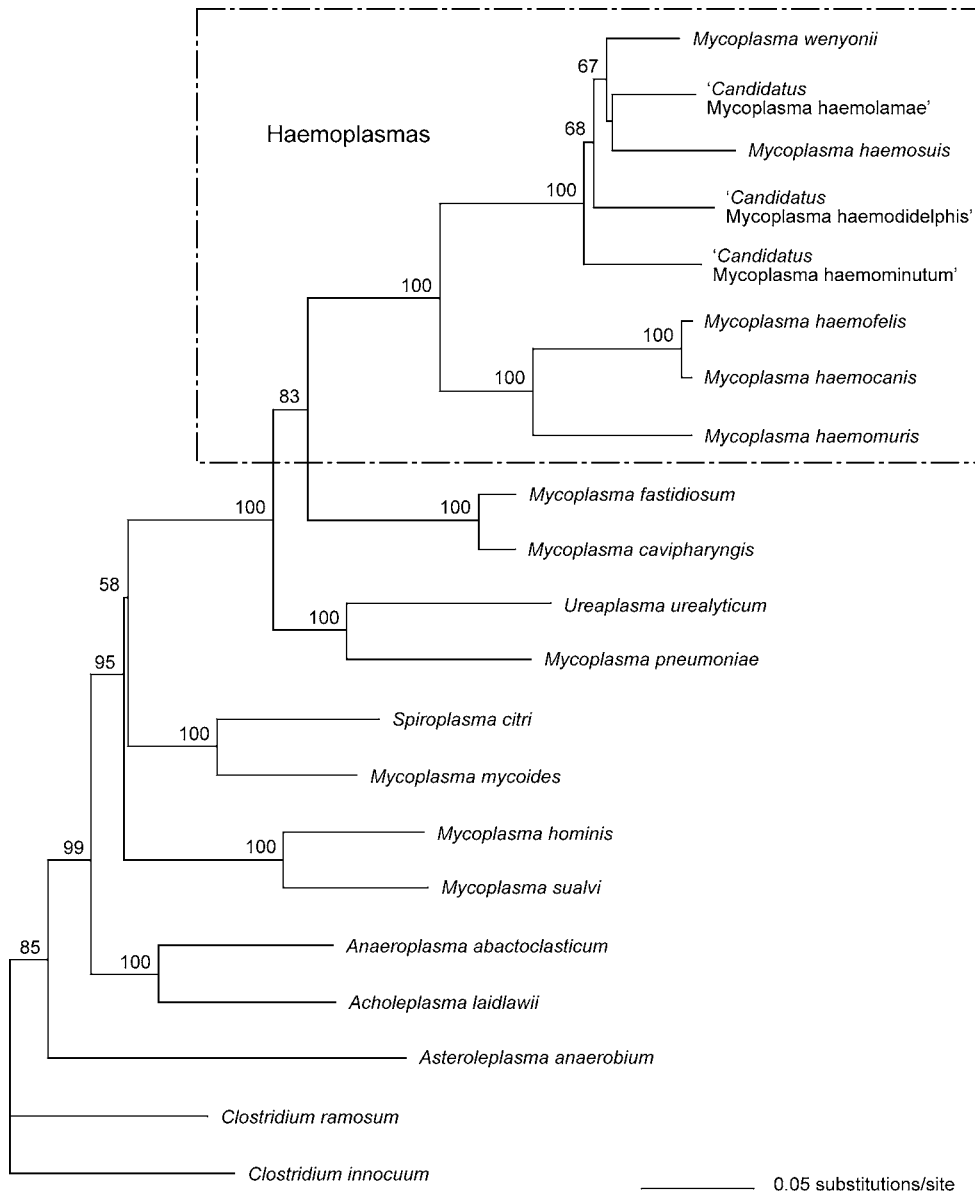
An objective and precise means of phylogenetic classification<sup>53,54</sup> of bacteria was made possible recently by sequence analysis of the 16S ribosomal RNA gene. Rikihisa et al<sup>55</sup> first reported 16S rRNA gene sequences from *Haemobartonella* and *Eperythrozoon* spp in 1997. By using the polymerase chain reaction (PCR), the 16S rRNA gene from *Haemobartonella* and *Eperythrozoon* organisms was amplified, sequenced, and compared with sequences of known bacteria. The 16S rRNA sequences that were generated bore little similarity to those of other Rickettsial organisms; rather, they indicated a closer phylogenetic relation with *Mycoplasma* spp. In the same year, Neimark and Kocan<sup>56</sup> sequenced the 16S rRNA gene of *E wenyonii* from 2 splenectomized calves and also concluded that the bacterium was a mycoplasma. Messick and colleagues<sup>14</sup> subsequently sequenced the 16S rRNA genes from 3 other hemotropic parasites, including a newly identified species from an opossum, an organism isolated from an alpaca, and an organism isolated from



**Figure 3.** Transmission electron micrograph of a hemoplasma parasite (*Mycoplasma haemofelis*) illustrating a single limiting membrane separating the cytoplasm of the organism from the host RBC. Note the amorphous nature of the cytoplasm, which contains ribosomes and some fibrous components. Delicate fibrils attach the organism to the host cell. 1 cm = 0.25 μm.

a splenectomized dog that showed 99.7% sequence similarity to the large form of *H felis*.

Recently, it was proposed that taxonomic classifications should be changed to reflect the newly recognized phylogenetic affiliation of *Haemobartonella* and *Eperythrozoon* spp with the genus *Mycoplasma* and that a *Candidatus* designation should be appended to those taxa that were newly and incompletely described.<sup>57,58</sup> Thus, *H felis* (Ohio organism or large form), *H canis*, and *H muris* were transferred to the genus *Mycoplasma* as *M haemofelis*,<sup>55</sup> *M haemocanis*,<sup>14</sup> and *M haemomuris*,<sup>55</sup> respectively. *E suis* and *E wenyonii* were transferred to the genus *Mycoplasma* as *M suis*<sup>55</sup> and *M wenyonii*,<sup>56</sup> respectively. Hemotropic mycoplasmas of the cat (California organism or small form of *H felis*), opossum, and alpaca, which are new and incompletely characterized species, were designated ‘*Candidatus Mycoplasma haemominutum*,’ ‘*Candidatus Mycoplasma haemodidelphis*,’ and ‘*Candidatus Mycoplasma haemolamae*,’ respectively.<sup>14,59</sup> There is a close phylogenetic relationship between this group of red cell pathogens and organisms belonging to the *pneumoniae* group of mycoplasmas (Figure 4). However, despite this close relationship, hemotropic mycoplasmas represent a distinct new cluster within the genus *Mycoplasma* and have been given the trivial name of “hemoplasmas.” On the basis of their molecular relatedness and phenotypic characteristics, the genera *Haemobartonella* and *Eperythrozoon* therefore have been removed from the order Rickettsiales and placed in the family Mycoplasmataceae.<sup>57,58</sup>



**Figure 4.** Mycoplasma phylogenetic tree reconstructed from 16S ribosomal RNA sequence comparisons. The cluster of hemoplasmas (*Haemobartonella* and *Eperythrozoon* spp) is enclosed within a box to highlight its position on the tree. Branch lengths are proportional to evolutionary distance. The scale at the bottom denotes the branch distance corresponding to 10 base changes per 100 nucleotides. Reprinted with permission from Messick et al, *Int J Syst Evol Microbiol.*<sup>14</sup>

### PCR diagnosis of hemoplasma infection

The recent development of PCR-based assays has provided a more efficient means of diagnosing hemoplasma infections in cats,<sup>18,60-62</sup> pigs,<sup>63</sup> llamas,<sup>64</sup> and cattle.<sup>65</sup> PCR is an exquisitely sensitive molecular technique that amplifies a particular fragment of organismal DNA in vitro. The 16S rRNA gene is the basis for all hemoplasma PCR assays to date, with several different primer pairs reported. Two separate

PCR-based assays have been developed at the University of Illinois, one for detection of *M haemofelis*<sup>60,61</sup> in cats and another for detection of *M suis* infection in pigs.<sup>63</sup> Foley et al<sup>18</sup> developed a PCR assay at the University of California-Davis for detection of 'Candidatus Mycoplasma haemominutum' infection in cats. During the past 3 years, more than 400 cats have been tested in the author's laboratory with both the Illinois and the California PCR assays for *M haemofelis* and 'Candidatus Mycoplasma haemominutum' infection,

respectively (unpublished observations). About 12% of anemic cats were infected with *M haemofelis* compared with only 1.5% of nonanemic cats. However, 7.2% of anemic cats and 5.3% of nonanemic cats were infected with 'Candidatus Mycoplasma haemominutum.' Jensen and colleagues<sup>62</sup> developed a single PCR assay for the concurrent detection of both feline hemoplasma species. Using this assay, they found that 28% of blood samples from cats in which infection was suspected on the basis of fever, anemia, or microscopic evidence of parasitemia were positive for one or both feline hemoplasmas. *M haemofelis*, alone or in combination with 'Candidatus Mycoplasma haemominutum' accounted for infection in 17.1% of the suspect cats; whereas, none of the cats without clinical signs of infection (control cats) were infected with *M haemofelis*. However, 11% of suspect cats and 13.7% of control cats were infected with 'Candidatus Mycoplasma haemominutum.' The overall prevalence among suspect and control cats in that study was 19.5%. In a recent study in the United Kingdom using the same PCR assay, a prevalence of 18.5% for hemoplasma infection in cats was reported; however, 92% of the infections were due to 'Candidatus Mycoplasma haemominutum.'<sup>66</sup> Thus, although the overall prevalence of hemoplasma infection in cats was similar in these 3 studies, there were notable geographic differences in the prevalence of specific parasites, particularly between the United States and the United Kingdom. A recently developed real-time quantitative PCR assay may provide additional information about the significance of a positive PCR result and could be a useful method for assessing response to treatment with antibiotics.<sup>67</sup>

To date, there are no published studies using PCR in which the overall prevalence of *M suis* infection in pigs and 'Candidatus Mycoplasma haemolamae' infection in llamas or alpacas has been evaluated. However, almost 29% of blood samples from 60 pigs tested at the University of Illinois over the past 3 years were positive by PCR for *M suis* infection (Messick J, unpublished observations). PCR testing also has been used to better characterize the disease caused by 'Candidatus Mycoplasma haemolamae' in camelids; the parasite is not cleared by a standard tetracycline regime, and, once infected, many of these animals become chronic carriers.<sup>64</sup>

### Clinicopathologic Findings in Hemoplasma Infections

Hemoplasmas can cause acute hemolytic anemia and various chronic diseases in vertebrate hosts. The clinical spectrum of infection ranges from asymptomatic to life-threatening, depending partially on host susceptibility.

Animals may be predisposed to acute infection by age, concurrent disease, immunosuppression, or splenectomy. In chronically-infected animals clinical disease may be occult or poorly defined. Chronic infections typically occur in apparently healthy, immunocompetent animals that have not undergone splenectomy. For example, *M haemocanis* infection frequently is latent and remains subclinical unless a dog's spleen is removed, after which acute hemolytic anemia develops. In contrast, *M haemofelis* causes acute hemolytic anemia in nonsplenectomized cats. Other factors appear to influence the susceptibility of pigs, llamas, mice, and primates to hemoplasma infection, because both acute and chronic forms of the disease occur in the presence or absence of a spleen. The inherent pathogenicity of certain strains or species of hemoplasmas also likely plays a key role in the development of disease. Further, the route of infection and dose of inoculum may influence the severity of an infection.

### Acute disease

The clinical features of acute disease have been studied extensively in several animal species. In cats, acute infection with *M haemofelis* is associated with massive parasitemia of RBCs, causing severe and sometimes fatal hemolytic anemia. Clinical signs of disease in both naturally and experimentally infected cats include lethargy, anorexia, fever, and anemia. Recently, PCR was used to correlate the presence of bacteremia with the severity of clinical disease, thereby fulfilling the molecular criteria for disease causation by *M haemofelis*.<sup>17,68</sup> However, healthy cats experimentally infected with the smaller hemoplasma, 'Candidatus Mycoplasma haemominutum' developed only minimal clinical signs of acute disease.<sup>18</sup> Although differences in the severity of clinical disease may represent differing pathogenicity of feline hemoplasmas, it also is possible they reflect dose-dependent effects as suggested by Westfall et al.<sup>69</sup> Additional studies are needed to better define differences in virulence between these 2 parasites.

*M suis* can cause acute hemolytic disease and sometimes death in young piglets, pregnant sows immediately prepartum and at the time of weaning, and feeder pigs under stress.<sup>3,4</sup> More commonly, mild anemia and poor growth rates are seen in infected nursery and feeder pigs. *M suis* infection in sows may result in pyrexia, anorexia, depression, decreased milk production, and poor maternal behavior. The common use of tetracyclines in hog feed has led to a marked decrease in incidence of the acute form of the disease; however, pigs still may become persistently infected, asymptomatic carriers.<sup>4,70,71</sup> An indirect hemagglutina-

tion assay (IHA) for detection of antibodies to *M suis* may be used to identify infection on a herd basis.<sup>72</sup> Approximately 20% of 10,000 pigs tested in one study had positive titers for *M suis* by IHA.<sup>4</sup> However, titers declined rapidly and were negligible in chronically infected pigs. Detection of individual infected pigs should be facilitated by the recent development of a PCR-based diagnostic assay.<sup>63</sup>

The clinical signs of acute infection in sheep include pale or icteric mucous membranes caused by hemolytic anemia, and decreased exercise tolerance. The disease varies in severity, with acute herd outbreaks often lasting 14 to 28 days. Different strains of *M ovis* may vary in their ability to cause disease. In addition, anemia is often less severe if sheep have access to good-quality feed or pasture with trace element supplements in their diet and do not have a severe worm burden. The disease is more severe in young sheep and pregnant sheep on a low plane of nutrition.<sup>73</sup> Any activity that transmits infected RBCs from one sheep to another may spread the infection. Therefore, vaccination, ear tagging, shearing, or other stock management procedures that may cause bleeding can spread infection among flock members. Mosquitoes and midges may also transmit the disease.<sup>74</sup> Antibodies to *M ovis* have been detected in 4.5% of sheep on 47% of sheep farms sampled in Western Australia.<sup>74</sup> However, a serological survey in Victoria found that 90% of farms in northeastern Australia had *M ovis* infections, with 10% of weaned sheep and 51% of adult sheep testing positive for infection.<sup>75</sup> The prevalence of infection among sheep herds in the United States has not been reported.

'*Candidatus Mycoplasma haemolamae*' infection in camelids (llamas and alpacas) in the United States has been associated with mild to marked anemia. Rarely, death may occur in stressed, debilitated, and immunosuppressed animals. Anemia usually is not accompanied by icterus; however, animals may become hypoglycemic when large numbers of hemoplasma organisms are present in the blood.<sup>12</sup> Parasites also have been identified in low numbers on RBCs of apparently healthy animals. Clinical signs of acute disease, which include acute collapse, weight loss, depression, and lethargy, may be associated with shipping or other stresses.<sup>64</sup>

### Chronic disease

Chronic hemoplasma infections in animals, in which low or undetectable numbers of parasites are observed in peripheral blood smears, are well recognized. Before the availability of PCR, the best diagnostic test for latent *M suis* infection in a suspected carrier pig was

splenectomy. The diagnosis also was made by inoculation of a susceptible splenectomized pig with blood from the suspect animal. In one study, parasites were recovered from *M suis* antibody-positive pigs and even from some antibody-negative animals after inoculation of peripheral blood from the suspect animal into a splenectomized pig.<sup>8</sup> Chronic *M suis* infections have been associated with decreased reproductive efficiency in sows, including anestrus or delayed estrus, early embryonic deaths, and abortions. Among feeder pigs, increased incidence of respiratory and enteric infections and decreased weight gain have been associated with chronic *M suis* infection. Scrotal and hindlimb edema were reported in a Charolais bull that was chronically infected with *M wenyonii*.<sup>76</sup> In chronically infected dairy cows, swelling of the teats and the distal portions of the hind limbs, transient fever, prefemoral lymphadenopathy, rough haircoat, and dramatically decreased milk production have been reported, as well as subsequent weight loss and reproductive inefficiency.<sup>77</sup> Ill thrift, characterized by mild anemia, reduced weight gain, decreased wool production, and exercise intolerance, has been reported in sheep chronically infected with *M ovis*.<sup>78,79</sup> In addition to diseases associated with chronic infection, an acute and life-threatening exacerbation of hemolytic anemia in carrier animals may be activated by concurrent disease, stress, or immunosuppression.<sup>1,61,80,81</sup> Animals infected with hemoplasmas, even if treated with effective antibiotics, probably remain chronic carriers after clinical signs have resolved.<sup>4,61,82</sup>

### Properties and Pathogenicity of Hemoplasmas and Other Mycoplasmas

Most other members of the genus *Mycoplasma*, to which hemoplasmas belong, commonly colonize and infect humans; whereas, hemoplasmas infect primarily animals. *Mycoplasma* and *Ureaplasma* spp have been isolated from almost every domestic and laboratory animal and are of considerable importance in agriculture and biomedical research. Like hemoplasmas, other mycoplasmas cause both acute, life-threatening disease and chronic diseases associated with stunting of growth and variable clinical signs. Diseases associated with mycoplasma infection include arthritis, pneumonia, conjunctivitis, infertility, mastitis, and vulvovaginitis; the clinical signs of infection are dependent on the tissue tropism of the organism.<sup>83</sup> Hemoplasmas have unique features that allow them to parasitize RBCs, however, based on clinical evidence, infection of reproductive tissues also may be an important manifestation of chronic hemoplasma infections; this possibility has

not yet been investigated. New insights into the shared and divergent properties and pathogenicity of hemoplasmas and other mycoplasmas will enhance our understanding of diseases caused by both groups of organisms.

### The genomic basis of mycoplasma survival

Hemoplasmas, like other *Mycoplasma* organisms, are not found in nature as free-living organisms, but rather, depend on a host cell for essential compounds they cannot produce. The genomic sizes of *M suis* (745 kilobases [kb]) and *M haemofelis* (1245 kb) are comparable with those of other members of the genus *Mycoplasma*, which carry the smallest genomes recorded, ranging from 580 kb to ~2000 kb.<sup>84-86</sup> The cell wall and many biosynthetic systems of mycoplasmas have been lost during the process of reductive evolution from a branch of gram-positive walled bacteria.<sup>87</sup> These minimalist prokaryotes are believed to have retained only those genes that are essential for life. Their circular, double-stranded DNA molecule provides the genetic information for replication, transcription, and protein synthesis. The organisms also contain ribosomes upon which cell proteins are assembled and a single, limiting membrane that separates the cytoplasm of the organism from the external environment. A recent genome-sequencing survey of *M haemofelis* showed that 21% of genes identified were devoted to transport and metabolic functions.<sup>86</sup> Almost 50% of the genes appeared to encode for proteins that carry out required cellular functions, such as replication, cell division, transcription, and translation. *M haemofelis* also maintains some unique genes that may confer greater protection against oxidative stress and provide greater flexibility in amino acid biosynthesis.<sup>86</sup> To support the parasitic lifestyle, a considerable number of mycoplasma genes are devoted to adhesins and various genetic systems that provide a set of variable surface antigens for evading the host's immune system.<sup>88</sup> Several putative membrane lipoproteins and genes encoding adhesins of *M haemofelis* were identified in the genome-sequencing survey.<sup>87</sup> One gene encoded a protein having comparable homology at the amino acid level with the adhesin gene of *Mycoplasma genitalium*, MgPa.<sup>86</sup> These findings suggest that the family of mycoplasma cytoadhesins used for colonization is likely to be conserved among the hemoplasmas.

Unlike other mycoplasmas, the host-adapted survival of the hemoplasmas is achieved through surface parasitism of the RBC. They depend on the host cell for provision of amino acids, fatty acids, cholesterol, and vitamins. The failure of hemoplasmas and many other

mycoplasmas to grow in a defined medium is likely because of our inability to duplicate the complex nutritional support provided by the host.<sup>89</sup> With a diameter as small as 0.3  $\mu\text{m}$  and a genome as small as 745 kb, hemoplasmas are near the theoretical minimum size for a self-replicating organism.<sup>85</sup>

### Association with retroviral and immune-mediated diseases

Infections by pathogenic mycoplasmas rarely are fulminant in a healthy host; they more often follow a chronic course with wide-ranging clinical signs. Indeed, these organisms once were thought to live in relative harmony with their host, leading to the suggested concept of mycoplasmas as "ideal parasites."<sup>90,91</sup> However, disease associations with latent mycoplasma infections in both healthy and immunocompromised patients are now emerging. Possible associations of chronic mycoplasma infection in humans with rheumatic diseases, rheumatoid arthritis, and systemic lupus erythematosus (SLE) and as a cofactor in retroviral infections (eg, human immunodeficiency virus or acquired immune deficiency syndrome [AIDS]) recently were suggested.<sup>92,93</sup> Of particular interest are previously reported links between *Haemobartonella* infection and SLE in humans and *Haemobartonella*-like microorganisms in 6 anemic patients with AIDS.<sup>94,95</sup> Chronic mycoplasma infections also may play a role in malignant cell transformation and chromosomal alterations leading to neoplasia. They also have been linked to other unexplained illnesses, including the Gulf War syndrome and chronic fatigue syndrome.<sup>96,97</sup>

Although *M haemofelis* causes primary disease in cats, it also is commonly recognized as a pathogen in conjunction with retroviruses, including feline immunodeficiency virus and feline leukemia virus (FeLV), and with other debilitating diseases.<sup>98-101</sup> Cats experimentally coinfecting with FeLV and 'Candidatus Mycoplasma haemominutum' developed more severe anemia than did cats infected with the parasite alone.<sup>100</sup> Further, it was suggested that infection with 'Candidatus Mycoplasma haemominutum' could induce myeloproliferative disease in FeLV-infected cats. Chronic *M haemofelis* infection may promote neoplastic transformation of hematopoietic cells in FeLV-infected cats.<sup>81,102</sup>

### Mechanisms of pathogenicity

Several mechanisms have been proposed to explain the pathogenicity of mycoplasmas, including production of free radicals by the organism that induce oxidative

damage of host cell membranes,<sup>103,104</sup> secretion of mycoplasmal enzymes leading to localized tissue disruption,<sup>105,106</sup> and chromosomal aberrations.<sup>107</sup> Other pathogenic mechanisms include the depletion of nutrients or biosynthetic precursors by mycoplasmas leading to host cell damage,<sup>108</sup> and the development of autoantibodies that trigger an immune disorder.<sup>109</sup> The recent discovery of a new human mycoplasma, *M penetrans*, capable of active cellular penetration, provides another possible mechanism for cell damage.<sup>110</sup> The ability of mycoplasmas to establish chronic infections, thwarting both the host's immune responses and antibiotic therapies, may be the result of such intracellular infections. Some mycoplasmas also can produce superantigens, which bind directly to major histocompatibility complex molecules without being processed and which stimulate large numbers of lymphocytes. The production of inflammatory cytokines and potential suppression of host defenses by activated lymphocytes may have disastrous consequences, including the development of chronic, debilitating arthritis.<sup>111</sup> The mechanisms responsible for the pathogenicity of hemoplasmas have not yet been completely defined.

Strong evidence suggests that host immune reactions play an important role in the development of clinical signs associated with both acute and chronic mycoplasma infections. Postinfection sequelae of *M pneumoniae* in humans, affecting the central nervous system, blood, skin, joints, and other organs, may be attributed to autoantibodies that develop during the disease.<sup>112</sup> The best characterized of these are cold-reacting agglutinins directed at sialoglycoconjugates on the red cell membrane.<sup>113,114</sup> Cold-reacting red cell agglutinins and other autoantibodies also have been reported with hemoplasma infections in mice,<sup>115</sup> cats,<sup>116,117</sup> dogs,<sup>118,119</sup> and pigs.<sup>4</sup> On the basis of positive Coombs' test results, it has been postulated that the host's immune response to hemoplasma organisms may exacerbate the acute hemolytic episode. An Arthus-type reaction was the cause of scrotal and limb edema that developed in a bull chronically infected with a hemoplasma. It was speculated that a hypersensitivity reaction was triggered in the skin when IgG antibodies directed against the hemoplasma formed immune complexes locally.<sup>76</sup>

### Mechanisms of adherence to host cells

Mycoplasmas in humans and animals are primarily surface parasites, capable of colonizing epithelial cells that line the respiratory and urogenital tracts. With the inclusion of *Haemobartonella* and *Eperythrozoon* spp in

the genus *Mycoplasma*, the tissue specificity of mycoplasmal pathogens has been widened to include RBCs. Many mycoplasmal pathogens have a flask-shaped appendage containing a specialized tip organelle that mediates attachment to the target cell of the host.<sup>120</sup> This structure, composed of adhesins and accessory proteins, resembles a specialized cytoskeletal apparatus and is essential for adherence. To facilitate attachment, the accessory proteins help move and concentrate adhesins to the plasma membrane of the tip. Hemoplasma organisms lack distinct tip structures yet are fully capable of surface cytoadherence to RBCs. Hemoplasmas may use related genes or proteins or may have evolved an alternative mechanism of surface parasitism. Although sialylated sequences on erythrocyte membranes may serve as receptors for the adhesion molecules of many *Mycoplasma* spp,<sup>121</sup> colonization and growth on RBCs is unique to hemoplasmas. This suggests that strategies evolved for parasite survival by the hemoplasmas may be different than those used by other mycoplasmas. These intriguing possibilities should be further investigated.

Some mycoplasmas undergo a high-frequency cytoadherence phase and antigenic variation in vitro.<sup>122,123</sup> A novel characteristic displayed by *M haemofelis* suggests phenotype switching also may occur during natural infections in cats. *M haemofelis* rapidly and synchronously disappears from the host's red cells and cyclically reappears in vivo.<sup>61,82</sup> This phenomenon, involving rapid fluctuations in bacteremia from >90% of RBCs infected with multiple organisms to no detectable organisms, may occur in less than an hour in both splenectomized and intact animals. It is possible the organism undergoes phase variation, allowing it to detach from the RBC. There are plausible benefits to *M haemofelis* from a loss of capacity to adhere to the host's RBCs. The temporary loss of cytoadherence may contribute to survival of *M haemofelis* and subsequent development of a carrier state, for example, by changing or disguising certain immunodominant cytoadherence antigens or by enabling *M haemofelis* to establish infection of another cell type. The reappearance of cytoadherence may aid in initiation of a new cycle of infection, thereby facilitating the transmission of *M haemofelis* by a blood-sucking vector. Lappin et al<sup>124</sup> recently showed that fleas infected with *M haemofelis* can transmit the organism and produce disease in a susceptible cat; however, transmission of 'Candidatus *Mycoplasma haemominutum*' by fleas was not successful. It was suggested that the low number of fleas used in the study might be responsible for the negative findings. Experimental transmission of *M haemocanis* by the dog tick *Rhipicephalus sanguineus*<sup>125</sup> and transmis-

sion of *M haemomuris* by the rat louse *Polyplax spinulosa* and the flea *Xenosylla cheopis* have been reported.<sup>126</sup> Under experimental conditions, arthropod vectors, including lice, mosquitoes, and stable flies, can transmit *M suis* infection to pigs.<sup>4</sup>

### Summary and Future Directions

Phylogenetic evidence strongly supports the reclassification of *Haemobartonella* and *Eperythrozoon* spp as hemotrophic mycoplasma organisms. PCR assays now are available for the diagnosis of hemoplasma infection, and real-time quantitative PCR may provide additional information about the significance of a positive PCR result as well as help monitor treatment. Diseases caused by infection with hemoplasmas vary from overt life-threatening hemolytic anemia to subtle chronic anemia, ill-thrift, and infertility. Infection of reproductive tissues is a potentially important manifestation of chronic hemoplasma infections that warrants further investigation. Hemoplasma organisms also may act as cofactors in the progression of retroviral and other debilitating diseases. Intimate contact of hemoplasmas with RBCs leads to cell injury through immune-mediated and other mechanisms that still need to be completely defined. Despite an intense immune response and even with antibiotic treatment, infected animals probably remain chronic carriers after clinical signs have resolved. Strategies evolved for parasite survival by the hemoplasmas may be different than those used by other mycoplasmas and should be further investigated.

### References

1. Messick JB, Berent L, Ehrhart EJ, Wasmer CC. Light and electron microscopic features of *Eperythrozoon*-like parasites in a North American opossum (*Didelphis virginiana*). *J Zoo Wildl Med.* 2000;31:240-243.
2. Moulder JW. Order I. Rickettsiales. In: Buchanan RE, Gibbons NE, eds. *Bergey's Manual of Determinative Bacteriology*. 8th ed. Baltimore, MD: The Williams & Wilkins Co; 1974:882-890.
3. Henry SC. Clinical observations of eperythrozoonosis. *J Am Vet Med Assoc.* 1979;174:601-603.
4. Smith AR. Eperythrozoonosis. In: Straw B, Mengeling WL, D-Allaire SD, Taylor DJ. *Diseases of Swine*. 7th ed. Ames, IA: Iowa State University Press; 1992:470-474.
5. Uilenberg G, Zeeuwen AA, de Ruijter T. *Eperythrozoon parvum* (Rickettsiales) in swine in the Netherlands. *Tijdschr Diergeneeskd.* 1981;106:456.
6. Sheriff D, Clapp KH, Reid MA. *Eperythrozoon ovis* infection in South Australia. *Aust Vet J.* 1966;42:169-176.
7. Harbutt PR. The effect of *Eperythrozoon ovis* infection on body weight gain and haematology of lambs in Victoria. *Aust Vet J.* 1969;45:500-504.
8. Sutton RH, Charleston WA, Collins GH. *Eperythrozoon wenyoni*—a blood parasite of cattle. A first report in New Zealand. *N Z Vet J.* 1977;25:8-9.
9. Poole DB, Cutler RS, Kelly WR, Collins JD. *Eperythrozoon wenyoni* anaemia in cattle. *Vet Rec.* 1976;99:481.
10. Anziani OS, Tarabla HD, Ford CA. *Eperythrozoon teganodes* infection in splenectomized calves in the province of Santa Fe, Argentina. *Rev Argent Microbiol.* 1982;14:37-40.
11. Uilenberg G. *Eperythrozoon tuonii*, n.sp. (Rickettsiales), the 3rd species of *Eperythrozoon* of cattle in Madagascar. *Rev Elev Med Vet Pays Trop.* 1967;20:563-569.
12. McLaughlin BG, Evans CN, McLaughlin PS, et al. An *Eperythrozoon*-like parasite in llamas. *J Am Vet Med Assoc.* 1990;197:1170-1175.
13. Reagan WJ, Garry F, Thrall MA, et al. The clinicopathologic, light, and scanning electron microscopic features of eperythrozoonosis in four naturally infected llamas. *Vet Pathol.* 1990;27:426-431.
14. Messick JB, Walker PG, Raphael W, Berent L, Shi X. '*Candidatus* Mycoplasma haemodidelphis' sp. nov., '*Canidatus* Mycoplasma haemolamae' sp. nov. and *Mycoplasma haemocanis* comb. nov., haemotrophic parasites from a naturally infected opossum (*Didelphis virginiana*), alpaca (*Lama pacos*) and dog (*Canis familiaris*): phylogenetic and secondary structural relatedness of their 16S rRNA genes to other mycoplasmas. *Int J Syst Evol Microbiol.* 2002;52:689-693.
15. Bidwell DE, Voller A. The effect of *Eperythrozoon coccoides* on infections in mice [abstract]. *Parasitology.* 1967;57:22P.
16. Ewers WH. *Eperythrozoon mariboi* sp. nov., (Protozoa: order Rickettsiales) a parasite of red blood cells of the flying fox *Pteropus macrotis epularius* in New Guinea. *Parasitology.* 1971;63:261-269.
17. Berent LM, Messick JB, Cooper SK. Specific in situ hybridization of *Haemobartonella felis* with a DNA probe and tyramide signal amplification. *Vet Pathol.* 2000;37:47-53.
18. Foley JE, Harrus S, Poland A. Molecular, clinical and pathologic comparison of two distinct strains of *Haemobartonella felis* in domestic cats. *Am J Vet Res.* 1998;59:1581-1588.
19. Benjamin MM, Lumb WV. *Haemobartonella canis* infection in a dog. *J Am Vet Med Assoc.* 1959;135:388-390.
20. Donovan EF, Loeb WF. Hemobartonellosis in the dog. *Vet Med.* 1960;55:57-62.
21. Elko EE, Cantrell W. Phagocytosis and anemia in rats infected with *Haemobartonella muris*. *J Infect Dis.* 1968;118:324-332.
22. Frerichs WM, Holbrook AA. *Haemobartonella procyoni* sp. n. in the raccoon, *Procyon lotor*. *J Parasitol.* 1971;57:1309-1310.
23. Peters W, Molyneux DH, Howells RE. *Eperythrozoon* and *Haemobartonella* in monkeys. *Ann Trop Med Parasitol.* 1974;68:47-50.
24. Dillberger JE, Loudy DE, Adler RR, Gass JH. Hemobartonella-like parasites in cynomolgus monkeys. *Vet Pathol.* 1994;31:301-307.

25. Peters W, Howells RE, Molyneux DH. *Eperythrozoon* and *Haemobartonella* in primates. *Trans R Soc Trop Med Hyg.* 1973;67:21.
26. Ristic M, Kreier JP. Hemotropic bacteria. *N Engl J Med.* 1979; 301:937-939.
27. Archer GL, Coleman PH, Cole RM, Duma RJ, Johnston CL Jr. Hemotropic bacteria. *N Engl J Med.* 1980;302:1151-1152.
28. Uilenberg G, Lapeire C. The existence of infectious feline anemia (eperythrozoosis of cats) in Madagascar. *Rev Elev Med Vet Pays Trop.* 1967;20:355-357.
29. Harbutt PR. The incidence of *Eperythrozoon* (*Haemobartonella*) *felis* in Victoria. *Aust Vet J.* 1969;45:87-88.
30. Madsen M. Eperythrozoosis in swine—an overlooked disease? *Nord Vet Med.* 1986;38:57-67.
31. Barnett SF. *Eperythrozoon parvum* in pigs in Kenya. *Bull Epizoot Dis Afr.* 1963;11:85-95.
32. Sheriff D, Clapp KH, Reid MA. *Eperythrozoon ovis* infection in South Australia. *Aust Vet J.* 1966;42:160-176.
33. Schilling V. *Eperythrozoon coccoides*, eine neue durch splenektomie aktionierbare dauerinfektion der weissen. *Maus Klin Wchnschr.* 1928;1853-1855.
34. Alder S, Ellenbogen V. A note on two new blood parasites of cattle, *Eperythrozoon* and *Bartonella*. *J Comp Path Ther.* 1934;47: 219-221.
35. Kinsley AT. Protozoan-like body in the blood of swine. *Vet Med.* 1932;27:196.
36. Neitz WO. Eperythrozoosis in cattle. *Onderstepoort J Vet Sci Anim Industry.* 1940;14:9-28.
37. Lotze JC, Yiengst MJ. Eperythrozoosis in cattle in the United States. *North Am Vet.* 1941;22:345-346.
38. Jensen R. Eperythrozoosis in cattle and sheep of Louisiana. Preliminary report. *Louisiana Bull.* No. 366;8; 1943.
39. Flint JC, Moss LD. Infectious anemia in cats. *J Am Vet Med Assoc.* 1953;122:45-48.
40. Knutti RE, Hawkins WB. Bartonella incidence in splenectomized bile-fistula dogs. *J Exp Med.* 1935;61:115-119.
41. McNaught JB, Woods FM, Scott V. Bartonella bodies in the blood of a nonsplenectomized dog. *J Exp Med.* 1935;62:353-358.
42. Tanaka H, Hall WT, Sheffield JB, Moore DH. Fine structure of *Haemobartonella muris* as compared with *Eperythrozoon coccoides* and *Mycoplasma pulmonis*. *J Bacteriol.* 1965;90:1735-1749.
43. Small E, Ristic M. Morphologic features of *Haemobartonella felis*. *Am J Vet Res.* 1967;28:845-851.
44. Demaree RS Jr, Nessmith WB. Ultrastructure of *Haemobartonella felis* from a naturally infected cat. *Am J Vet Res.* 1972;33: 1303-1308.
45. Jain NC, Keeton KS. Scanning electron microscopic features of *Haemobartonella felis*. *Am J Vet Res.* 1973;34:697-700.
46. Simpson CF, Gaskin JM, Harvey JW. Ultrastructure of erythrocytes parasitized by *Haemobartonella felis*. *J Parasitol.* 1978;64:504-511.
47. Venable JH, Ewing SA. Fine-structure of *Haemobartonella canis* (Rickettsiales: Bartonellacea) and its relation to the host erythrocyte. *J Parasitol.* 1968;54:259-268.
48. McKee AE, Ziegler RF, Giles RC. Scanning and transmission electron microscopy of *Haemobartonella canis* and *Eperythrozoon ovis*. *Am J Vet Res.* 1973;34:1196-1201.
49. Keeton KS, Jain NC. *Eperythrozoon wenyoni*: a scanning electron microscope study. *J Parasitol.* 1973;59:867-873.
50. Pospischil A, Hoffmann R. *Eperythrozoon suis* in naturally infected pigs: a light and electron microscopic study. *Vet Pathol.* 1982;19:651-657.
51. Zachary JF, Basgall EJ. Erythrocyte membrane alterations associated with the attachment and replication of *Eperythrozoon suis*: a light and electron microscopic study. *Vet Pathol.* 1985;22:164-170.
52. Tully JG, Bove JM, Laigret F, Whiscomb RF. Revised taxonomy of the class *Mollicutes*: proposed elevation of a monophyletic cluster of arthropod-associated mollicutes to the ordinal rank (*Entomoplasmatales* ord. nov.), with provision for familial rank to separate species with nonhelical morphology (*Entoplasmataceae* farm. nov.) from helical species (*Spiroplasmataceae*), and emended descriptions of the order *Mycoplasmatales*, family *Mycoplasmataceae*. *Int J Syst Bacteriol.* 1993;43:378-385.
53. Weisburg WG, Tully JG, Rose DL, et al. A phylogenetic analysis of the mycoplasmas: basis for their classification. *J Bacteriol.* 1989;171:6455-6467.
54. Wilson KH, Blitchington RB, Greene RC. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *J Clin Microbiol.* 1990;28:1942-1946.
55. Rikihisa Y, Kawahara M, Wen B, et al. Western immunoblot analysis of *Haemobartonella muris* and comparison of 16S rRNA gene sequences of *H. muris*, *H. felis*, and *Eperythrozoon suis*. *J Clin Microbiol.* 1997;35:823-829.
56. Neimark H, Kocan KM. The cell wall-less rickettsia *Eperythrozoon wenyonii* is a *Mycoplasma*. *FEMS Microbiol Lett.* 1997;156:287-291.
57. Neimark H, Johansson KE, Rikihisa Y, Tully JG. Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of 'Candidatus *Mycoplasma haemofelis*', 'Candidatus *Mycoplasma haemomuris*', 'Candidatus *Mycoplasma haemosuis*' and 'Candidatus *Mycoplasma wenyonii*.' *Int J Syst Evol Microbiol.* 2001;51:891-899.
58. Neimark H, Johansson KE, Rikihisa Y, Tully JG. Revision of haemotrophic *Mycoplasma* species names. *Int J Syst Evol Microbiol.* 2002;52:683.
59. Foley JE, Pedersen NC. 'Candidatus *Mycoplasma haemominutum*', a low-virulence eperythrocytic parasite of cats. *Int J Syst Evol Microbiol.* 2001;51:815-817.
60. Messick JB, Berent LB, Cooper SK. Development and evaluation of a PCR-based assay for detection of *Haemobartonella felis* in cats and differentiation of *H. felis* from related bacteria by restriction fragment length polymorphism analysis. *J Clin Microbiol.* 1998;36:462-466.
61. Berent LM, Messick JB, Cooper SK. Detection of *Haemobartonella felis* in cats with experimentally induced acute and chronic infections, using a polymerase chain reaction assay. *Am J Vet Res.* 1998;59:1215-1220.
62. Jensen WA, Lappin MR, Kamkar S, Reagan WJ. Use of a polymerase chain reaction assay to detect and differentiate two strains of *Haemobartonella felis* in naturally infected cats. *Am J Vet Res.* 2001;62:604-608.

63. Messick JB, Cooper SK, Huntley M. Development and evaluation of a polymerase chain reaction assay using the 16S rRNA gene for detection of *Eperythrozoon suis* infection. *J Vet Diagn Invest.* 1999;11:229-236.
64. Tornquist SJ, Boeder LJ, Parker JE, Cebra CK, Messick JB. Use of a polymerase chain reaction assay to study the carrier state of infection with camelid *Mycoplasma haemolama*, formerly *Eperythrozoon* spp, infecting camelids [abstract]. *Vet Clin Pathol.* 2002;31:153-154.
65. Vandervoort JM, Bourne C, Carson RL, Heath AM, Boudreaux MK. Use of a polymerase chain reaction assay to detect infection with *Eperythrozoon wenyonii* in cattle. *J Am Vet Med Assoc.* 2001;219:1432-1434.
66. Tasker S, Binns SH, Day MJ, et al. Use of a PCR assay to assess the prevalence and risk factors for *Mycoplasma haemofelis* and 'Candidatus *Mycoplasma haemominutum*' in cats in the United Kingdom. *Vet Rec.* 2003;152:193-198.
67. Tasker S, Helps CR, Day MJ, Gruffydd-Jones TJ, Harbour DA. Use of real-time PCR to detect and quantify *Mycoplasma haemofelis* and 'Candidatus *Mycoplasma haemominutum*' DNA. *J Clin Microbiol.* 2003;41:439-441.
68. Cooper SK, Berent LB, Messick JB. Competitive, quantitative PCR analysis of *Haemobartonella felis* in the blood of clinically infected cats. *J Microbiol Methods.* 1999;34:235-243.
69. Westfall DS, Jensen WA, Reagan WJ, Radecki SV, Lappin MR. Inoculation of two genotypes of *Haemobartonella felis* (California and Ohio variants) to induce infection in cats and the response to treatment with azithromycin. *Am J Vet Res.* 2001;62:687-691.
70. Heinritzi K. The diagnosis of *Eperythrozoon suis* infection. *Tierarztl Prax.* 1990;18:477-481.
71. Bugnowski H, Horsch F, Muller D, Zepezauer V. Infection model for the reproduction of eperythrozoonosis in splenectomized SPF primary piglets. *Arch Exp Veterinarmed.* 1990;44:627-637.
72. Smith AR, Rahn T. An indirect hemagglutination test for the diagnosis of *Eperythrozoon suis* infection in swine. *Am J Vet Res.* 1975;36:1319-1321.
73. Gullard FM, Doxey DL, Scott GR. The effects of *Eperythrozoon ovis* in sheep. *Res Vet Sci.* 1987;43:88-91.
74. Kabay MJ, Richards RB, Ellis TE. A cross-sectional study to show *Eperythrozoon ovis* infection is prevalent in Western Australian sheep farms. *Aust Vet J.* 1991;68:170-173.
75. Nicholls TJ, Veale PI. The prevalence of *Eperythrozoon ovis* infection in weaner and adult sheep in north eastern Victoria. *Aust Vet J.* 1986;63:118-120.
76. Montes AJ, Wolfe DF, Welles EG, Tyler JW, Tepe E. Infertility associated with *Eperythrozoon wenyonii* infection in a bull. *J Am Vet Med Assoc.* 1994;204:261-263.
77. Smith JA, Thrall MA, Smith JL, et al. *Eperythrozoon wenyonii* infection in dairy cattle. *J Am Vet Med Assoc.* 1990;196:1244-1250.
78. Burroughs GW. The significance of *Eperythrozoon ovis* in ill-thrift in sheep in the eastern Cape coastal areas of South Africa. *J S Afr Vet Assoc.* 1988;59:195-199.
79. Daddow KN. *Eperythrozoon ovis*—a cause of anaemia, reduced production and decreased exercise tolerance in sheep. *Aust Vet J.* 1979;55:433-434.
80. Cotter S, Hardy W, Essex M. Association of feline leukemia virus with lymphosarcoma and other disorders in the cat. *J Am Vet Med Assoc.* 1975;166:449-454.
81. Bobade PA, Nash AS, Rogerson P. Feline haemobartonellosis: clinical, haematological and pathological studies in natural infections and relationship to infection with feline leukaemia virus. *Vet Rec.* 1988;122:32-36.
82. Harvey JW, Gaskin JM. Feline haemobartonellosis: attempts to induce relapses of clinical disease in chronically infected cats. *J Am Anim Hosp Assoc.* 1978;14:453-456.
83. Simecka, JW, Davis JK, Kavidson MK, et al. Mycoplasma diseases of animals. In: Maniloff J, McElhaney RN, Finch LR, Baseman JB, eds. *Mycoplasmas: Molecular Biology and Pathogenesis.* Washington, DC: American Society for Microbiology; 1992:391-415.
84. Razin S. Peculiar properties of mycoplasmas: the smallest self-replicating prokaryotes. *FEMS Microbiol Lett.* 1992;100:423-432.
85. Messick JB, Smith G, Berent L, Cooper S. Genome size of *Eperythrozoon suis* and hybridization with 16S rRNA gene. *Can J Microbiol.* 2000;46:1082-1086.
86. Berent LM, Messick JB. Physical map and genome sequencing survey of *Mycoplasma haemofelis* (*Haemobartonella felis*). *Infect Immun.* 2003;71:3657-3662.
87. Woese CR. Bacterial evolution. *Microbiol Rev.* 1987;51:221-271.
88. Yogeve D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev.* 1998;62:1084-1156.
89. Miles RJ. Cell nutrition and growth. In: Maniloff J, McElhaney RN, Finch LR, Baseman JB, eds. *Mycoplasmas: Molecular Biology and Pathogenesis.* Washington, DC: American Society for Microbiology; 1992:23-40.
90. Hutchison CA, Peterson SN, Gill SR, et al. Global transposon mutagenesis and a minimal *Mycoplasma* genome. *Science.* 1999;286:2165-2169.
91. Baseman JB, Tully JG. Mycoplasmas: sophisticated, reemerging, and burdened by their notoriety. *Emerg Infect Dis.* 1997;3:21-32.
92. Taylor-Robinson D. Mycoplasmas in rheumatoid arthritis and other human arthritides. *J Clin Pathol.* 1996;49:781-782.
93. Lo S-C. Mycoplasmas and AIDS. In: Maniloff J, McElhaney RN, Finch LR, Baseman JB, eds. *Mycoplasmas: Molecular Biology and Pathogenesis.* Washington, DC: American Society for Microbiology; 1992:525-545.
94. Kallick CA, Levin S, Reddi KT, Landau WL. Systemic lupus erythematosus associated with *Haemobartonella*-like organisms. *Nat New Biol.* 1972;236:145-146.
95. Duarte MIS, Oliveira MS, Shikanai-Yasuda MA, et al. *Haemobartonella*-like microorganism infection in AIDS patients: ultrastructural pathology. *J Infect Dis.* 1992;165:976-977.
96. Nicolson G, Nicolson NL. Gulf War illnesses: complex medical, scientific and political paradox. *Med Confl Surviv.* 1998;14:156-165.
97. Tsai S, Wear DJ, Shih JW, Lo SC. Mycoplasmas and oncogenesis: persistent infection and multistage malignant transformation. *Proc Natl Acad Sci U S A.* 1995;92:10197-10201.

98. Grindem CB, Corbett WT, Tomkins MT. Risk factors for *Haemobartonella felis* infection in cats. *J Am Vet Med Assoc.* 1990;196:96-99.
99. Lappin MR. Opportunistic infections associated with retroviral infections in cats. *Semin Vet Med Surg (Small Anim).* 1995;10:244-250.
100. George JW, Rideout BA, Griffey SM, Pedersen NC. Effect of preexisting FeLV infection or FeLV and feline immunodeficiency virus coinfection on pathogenicity of the small variant of *Haemobartonella felis* in cats. *Am J Vet Res.* 2002;63:1172-1178.
101. Harrus S, Klement E, Aroch I, et al. Retrospective study of 46 cases of feline haemobartonellosis in Israel and their relationships with FeLV and FIV infections. *Vet Rec.* 2002;151:82-85.
102. Shelton GH, Linenberger ML. Hematologic abnormalities associated with retroviral infections in the cat. *Semin Vet Med Surg (Small Anim).* 1995;10:220-233.
103. Almagor M, Kahane I, Gilon C, Yatziv S. Protective effects of the glutathione redox cycle and vitamin E on cultured fibroblasts infected by *Mycoplasma pneumoniae*. *Infect Immun.* 1986;52:240-244.
104. Somerson NL, Purcell RH, Taylor-Robinson D, Chanock RM. Hemolysin of *Mycoplasma pneumoniae*. *J Bacteriol.* 1965;89:813-818.
105. Rottem S, Hasin M, Razin S. Differences in susceptibility to phospholipase C of free and membrane-bound phospholipids of *Mycoplasma hominis*. *Biochim Biophys Acta.* 1973;323:520-531.
106. Bhandari S, Asnani PJ. Characterization of phospholipase A2 of mycoplasma species. *Folia Microbiol.* 1989;34:294-301.
107. Paton GR, Jacobs JP, Perkins FT. Chromosome changes in human diploid-cell cultures infected with mycoplasma. *Nature (London).* 1965;207:43-45.
108. Russel WC. Alterations in the nucleic acid metabolism of tissue culture cells infected by mycoplasmas. *Nature (London).* 1966;212:1537-1540.
109. Denman AM. Infectious arthritis in primary disorders of immunoglobulin synthesis. *Curr Opin Rheumatol.* 1991;3:634-638.
110. Lo S-C, Hayes MM, Tully JG, et al. *Mycoplasma penetrans* sp. nov., from the urogenital tract of patients with AIDS. *Int J Syst Bacteriol.* 1992;42:357-364.
111. Cole BC, Alkins CL. The *Mycoplasma arthritidis* T-cell mitogen, MAM: a model superantigen. *Immunol Today.* 1991;12:271-276.
112. Biberfeld G. Antibodies to brain and other tissues in cases of *Mycoplasma pneumoniae* infection. *Clin Exp Immunol.* 1971;8:319-333.
113. Smith GN, Weir WR. Cold agglutinins accompanying *Mycoplasma pneumoniae* infection. *Br Med J.* 1980;281:1391-1392.
114. Feizi T, Loveless RW. Carbohydrate recognition by *Mycoplasma pneumoniae* and pathologic consequences. *Am J Respir Crit Care Med.* 1996;154:S133-S136.
115. Cox HW, Calaf-Iturri G. Autoimmune factors associated with anaemia in acute *Haemobartonella* and *Eperythrozoon* infections of rodents. *Ann Trop Med Parasitol.* 1976;70:73-79.
116. Maede Y, Hata R. Studies of feline haemobartonellosis. II. The mechanism of anemia produced by infection with *Haemobartonella felis*. *Nippon Juigaku Zasshi.* 1975;37:49-54.
117. Zulty JC, Kociba GJ. Cold agglutinins in cats with haemobartonellosis. *J Am Vet Med Assoc.* 1990;196:907-910.
118. Bellamy JE, MacWilliams PS, Searcy GP. Cold-agglutinin hemolytic anemia and *Haemobartonella canis* infection in a dog. *J Am Vet Med Assoc.* 1978;173:397-401.
119. Bundza A, Lumsden JH, McSherry BJ, Valli VE, Jazen EA. Haemobartonellosis in a dog in association with Coombs' positive anemia. *Can Vet J.* 1976;17:267-270.
120. Kirchhoff H, Rosgarten R, Loz W, Fischer M, Lopatta D. Flask-shaped mycoplasmas: properties and pathogenicity for man and animals. *Isr J Med Sci.* 1984;20:848-853.
121. Baseman JB. The cytoadhesins of *Mycoplasma pneumoniae* and *M. genitalium*. In: Rottem S, Kahane I, eds. *Subcellular Biochemistry*. New York, NY: Plenum Press; 1993:243-259.
122. Su CJ, Chavoya A, Dallo SF, Baseman JB. Sequence divergence of the cytoadhesion gene of *Mycoplasma pneumoniae*. *Infect Immun.* 1990;58:2669-2672.
123. Peterson SN, Bailey CC, Jensen JS, et al. Characterization of repetitive DNA in the *Mycoplasma genitalium* genome: possible role in the generation of antigenic variation. *Proc Natl Acad Sci U S A.* 1995;92:11829-11833.
124. Lappin MR, Brunt J, Riley A, et al. *Mycoplasma haemofelis* and *Mycoplasma haemominutum* DNA in blood of cats and their fleas [abstract]. *Proceedings, 21st American College of Veterinary Internal Medicine Forum*, Charlotte, NC; 2003:929-930.
125. Seneviratna P, Weerasinghe, Ariyadasa S. Transmission of *Haemobartonella canis* by the dog tick, *Rhicephalus sanguineus*. *Res Vet Sci.* 1973;14:112-114.
126. Berkenkamp SD, Wescott RB. Arthropod transmission of *Eperythrozoon coccoides* in mice. *Lab Anim Sci.* 1988;4:398-401.