Hemotrophic mycoplasmas, occurrence and detection methods in animals of veterinary importance

Micoplasmas hemotrópicos, presencia y métodos de detección en animales con importancia veterinaria

Rosa Estela Quiroz-Castañeda, Itzel Amaro-Estrada, Sergio Rodríguez-Camarillo, José Hugo Aguilar-Díaz

INTRODUCTION

The interest in hemotrophic mycoplasmas (also called hemoplasmas) is growing worldwide, mainly due to the detection of hemoplasmas by molecular methods. Either alone or in co-infection with other microorganisms, hemoplasmas are associated with clinical signs in domestic and wild animals (1,2). In addition, hemoplasmas can act as opportunistic agents that silently infect animals and humans (3).

Hemoplasmas are small, Gram-negative and cell wall-less bacteria, considered to be obligate erythrocyte bacteria, which up to now have been uncultivable, in contrast to mucosal mycoplasmas (3). Hemoplasmas are pleomorphic (cocci, rods, rings), 0.3 to 1 µm in diameter, with small genomes (0.5-1.0 Mb). They are usually attached to the outer surface of the red blood cells forming slits (2).

ABSTRACT: Hemotrophic mycoplasmas (hemoplasmas) pose a threat to animal health. During the last years, the presence of hemoplasmas around the world has significantly increased thanks to recent molecular detection methods that have been developed. Hemoplasmas cause severe alterations in the health of the host animal, either alone or in co-infection. These include cats, dogs, mice, pigs, and cattle. They cause severe anemia to chronic infection, clinical signs for which the animal may eventually die. Current interest in hemoplasmas is based on their pathogenic role at the molecular level in the host animal. This review presents information on hemoplasmas diversity and the potential for them to be considered a zoonotic risk. It also highlights the development of several molecular techniques for the diagnosis of hemoplasmas, which allow a quick and accurate detection to make decisions in case of an infection event.

Key words: hemoplasmas diversity, phylogenetic reconstruction, diagnosis, co-infections.

RESUMEN: Los micoplasmas hemotróficos (hemoplasmas) representan una amenaza para la salud animal. Durante los últimos años, la presencia de hemoplasmas alrededor del mundo se ha incrementado significativamente gracias a los recientes métodos de detección moleculares que se han desarrollado. Los hemoplasmas causan alteraciones severas en la salud del animal hospedador, ya sea solos o en coinfección. Entre estos se incluyen los gatos, perros, ratones, cerdos y el ganado bovino. Causan anemia severa hasta una infección crónica, signos clínicos por los que eventualmente el animal puede morir. El interés actual en los hemoplasmas se basa en su papel patogénico a nivel molecular en el animal hospedador. En esta reseña se presenta la información sobre la diversidad de los hemoplasmas y el potencial para ser considerados como riesgo zoonótico. Se resalta también el desarrollo de diversas técnicas moleculares para el diagnóstico de los hemoplasmas, que permiten una detección rápida y precisa para tomar decisiones ante un evento de infección.

Palabras clave: diversidad de hemoplasmas, reconstrucción filogenética, diagnóstico, coinfección.
Hemoplasmosis can cause hematological disorders in several mammalian species, ranging from severe anemia to chronic infection without clinical signs. Those animals with acute infections may present hemolysis, anorexia, dehydration, fever, loss of weight, lethargy, and even sudden death (3).

The transmission of hemoplasmosis mediated by different vectors depending on the pathogen. Fleas, ticks, lice, and flies are responsible for the transmission of hemoplasmas in cats, dogs, mice, pigs, and cattle (4,5). Although hemoplasmosis is not strictly considered a tick-borne disease, they may play a role in the epidemiology of these bacteria as some hemoplasmas are occasionally detected in cattle ticks (6).

HEMOPLASMAS CLASSIFICATION AND PHYLOGENETIC ANALYSES

Based on the morphological characteristics, response to antibiotics, Gram-negative staining, erythrocyte tropism, and putative arthropod transmission, hemoplasmas were initially classified in the order Rickettsiales, family Anaplasmataceae and genera Eperythrozoon and Haemobartonella. Currently, based on their 16S rRNA gene sequence, they have been reclassified from the genus Rickettsia to the genus Mycoplasma since phylogenetic reconstruction shows a robust relationship with members of the Mycoplasmataceae family (2,7,8). The genus Mycoplasma groups microorganisms that can establish commensal or virulent or both relations with the host.

The phylogenetic reconstruction of hemoplasmas is based on the 16S rRNA gene and the RNA subunit of the RNAase P (rnpB) gene (9-12). These phylogenies are important tools to classify new hemoplasma species and to denote the group of hemoplasmas from the rest of Mycoplasma groups (9). Hemoplasmas are present in domestic and wild animals, including cats, dogs, bovines, buffaloes, mice, sheep, goats, feral cats, among others. Both genome sequencing of some hemoplasmas (so far, existing 11 hemoplasma genomes) and 16S rRNA sequences allowed the identification of hemoplasmas of veterinary health importance, many of them named as Candidatus (Ca.) since they are uncultivable (13-15).

HEMOPLASMAS IN COMPANION ANIMALS

Hemoplasmas of felines are widely reported, including Mycoplasma haemofelis, Candidatus Mycoplasma haemominutum, Candidatus Mycoplasma haematoparvum, and Candidatus Mycoplasma turicensis (4,16-18). These four species produce hemolytic anemia in cats; however, M. haemofelis is the most pathogenic. Ca. M. haemominutum and Ca. M. turicensis may induce anemia when the host is immunosuppressed or when a concurrent disease is present, for instance, those caused by the feline leukemia virus (FeLV) (18,19).

Clinical signs caused by feline hemoplasmas include anemia, pallor, lethargy, anorexia, weight loss, pyrexia, and dehydration. Tetracyclines or fluoroquinolones are an effective treatment, although the infection may persist (18).

The most common hemoplasma species in dogs are Mycoplasma haemocanis and Candidatus Mycoplasma haematoparvum (20); other dog hemoplasmas are Candidatus M. haemominutum and Candidatus M. turicensis (21,22).

HEMOPLASMAS IN PRODUCTION ANIMALS

Usually, cattle infected with hemoplasmas look healthy. In Mexico, Candidatus Mycoplasma haemobos and Mycoplasma wenyonii were recently identified in cattle and their genomes were reported (23,24). Brazilian studies on the detection and occurrence of Ca. M. haemobos in cattle revealed that infected animals might represent chronically asymptomatic carriers with the risk of transmission to those healthy animals (12). In some cases of females and calves infection, symptoms such as transient fever, anorexia, lymphadenopathy, decreased milk yield, and weight loss, are observed (25).

The detection of Ca. M. haemobos and M. wenyonii in sick animals during a fatal anaplasmosis outbreak in Switzerland suggested that co-infection of both hemoplasmas could increase their pathogenicity in cattle (26). In
cattle, the prevalence of these hemoplasmas is higher in adults and rare in calves, perhaps due to the immune protection of the mother.

Swine hemoplasmas *Mycoplasma suis* and *Mycoplasma parvum* parasitize the surface of red blood cells causing membrane deformations and damage, which lead to anemia and icterus in pigs. The adverse effects of hemoplasmas in pigs include decreased reproductive efficiency in sows, delayed estrus, early embryonic death, and late-term abortion; newborn and weaned piglets shows severe anemia and pyrexia (27).

*M. parvum* is a non-pathogenic bacterium of pigs. It is often accumulated on the red blood cells infecting only a few cells. Frequently, this pathogen is unnoticeable in the absence of clinical signs even in splenectomized pigs (28). The comparative genomic analyses have shown the different pathogenicity levels between *M. suis* and *M. parvum* (29), and the similarities in the number of coding DNA sequences (CDS) related to metabolic functions, transporters, and putative virulence factors.

The molecular epidemiology of sheep and goat hemoplasmas is poorly studied. *Mycoplasma ovis* and *Candidatus Mycoplasma haemovis* are the two species identified in these animals (30). *M. ovis* infecting reindeers (*Rangifer tarandus*) causes weight loss and moderate anemia, among other symptoms (31).

### HEMOPLASMAS CO-INFECTIONS

Infectious agents are continually threatening animal health, and often they establish relationships with other pathogens that might severely impact the infection process. This interaction between pathogens may be useful as mobility support, enhanced contagiousness, and accelerated virulence. (32). In hemoplasmas, the role of each pathogen during a co-infection and their molecular interactions are still unknown. Table 1 shows several co-infections reported in animals.

### DETECTION OF HEMOPLASMAS

In the last ten years, the number of reports related to hemoplasmosis in different hosts increased significantly due to the use of molecular detection methods.

The detection of hemoplasmas includes the detection of specific antibodies, microscopic visualization of the organisms, and more recently, molecular-based methods. Giemsa staining and acridine orange are the most widely used methods for visualizing hemoplasmas, providing information on the presence of the pathogen but not its identity (33).

The electron microscopy is a specific technique used for the observation of *M. suis* in infected tissues (34). Besides the scanning electron microscopy allows observing the replication and attachment of hemoplasmas to erythrocytes as well as the damage they cause to the endothelial cells and other host tissues.

*In situ* hybridizations of fixed tissue sections allowed locating the attachment site of *M. haemofelis* to the liver, kidney cells and red blood cells (35). In this case, besides visualizing, the speciation of the causal agent was also attained.

Pathogen detection is the first step to characterize the agent or agents of a co-infected host. Especially, PCR-based tests of the 16S

<table>
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<tr>
<th>Co-infection</th>
<th>Hemoplasmas</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Dogs</td>
<td><em>M. haemocanis</em> and <em>Ca. M. haematoparvum</em></td>
<td>(40)</td>
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<tr>
<td>Dogs</td>
<td><em>M. haemocanis</em> and <em>Anaplasma platys</em></td>
<td>(40)</td>
</tr>
<tr>
<td>Dogs</td>
<td><em>Ca. M. haematoparvum</em> and <em>A. platys</em></td>
<td>(40)</td>
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<tr>
<td>Dogs</td>
<td><em>M. haemocanis</em> and <em>Babesia vogeli</em></td>
<td>(40)</td>
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<tr>
<td>Dogs</td>
<td><em>M. haemocanis</em> and <em>Ehrlichia canis</em></td>
<td>(1)</td>
</tr>
<tr>
<td>Cats</td>
<td><em>Babesia conradae, Ca. M. haematoparvum, M. haemocanis</em></td>
<td>(41)</td>
</tr>
<tr>
<td>Dairy cattle and Water Buffalo</td>
<td><em>Mycoplasma wenyonii</em> and <em>Ca. M. haemobos</em></td>
<td>(43, 44)</td>
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rRNA gene followed by sequencing is the most used molecular method to detect and identify hemoplasma species. New hemoplasma genomes allow finding sequences to design specific primers for proper identification, besides 16S rRNA PCR followed by restriction fragment length polymorphism (RFLP), which has been successfully used for the diagnosis of *M. haemofelis* and *M. haemominutum* in cats (36).

Real-Time TaqMan or SYBR green PCR assays have some advantages over more traditional detection methods as they allow quantification, have minimal risk of amplicon carryover and are highly specific. However, due to their specificity, these assays are unlikely to detect novel hemoplasma species (37).

Diagnostic methods that permit fast differentiation are necessary when a limited number of hemoplasmas are suspected. Thus, the analysis of the melting curve of SYBR green-based RT-PCR products is an excellent tool to differentiate between hemoplasma species. For instance, hemoplasma prevalence in the Miyagi Prefecture of 109 bovine cattle was as follows: 67 animals (61.5 %) infected with *M. wenyonii*, 25 animals (22.9 %) infected with *Ca. M. haemobos*, and 14 (12.8 %) infected with both (38). A similar method was used for feline hemoplasmosis with encouraging results as the authors discerned among seven different mycoplasmas from the blood of suspect cats and other felines (39).

Undoubtedly, the combination of several detection methods enhances the possibility to confirm the presence of hemoplasmas. For instance, the propidium iodide staining of blood smears and the end-point PCR for the detection of *Ca. M. haemobos* and *M. wenyonii* confirm the presence of both pathogens in Mexican cattle (Fig. 1). Diagnostic methods allowing a fast differentiation are necessary when a limited number of hemoplasmas are suspected. Table 2 shows a summary of primers and techniques used for hemoplasmas identification.

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**Table 2**

<table>
<thead>
<tr>
<th>Primers and Techniques</th>
<th>Hemoplasmas Identified</th>
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<tr>
<td></td>
<td><em>M. haemofelis</em></td>
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<tr>
<td></td>
<td><em>M. haemominutum</em></td>
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<tr>
<td></td>
<td><em>M. wenyonii</em></td>
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**Figure 1.** Micrography of staining of (A) *Ca. M. haemobos* and (B) *M. wenyonii* with propidium iodide correlates with the end-point PCR detection of both pathogens in Mexican cattle. Micrography of *Ca. M. haemobos* taken from (52). Micrografía de la tinción de *Ca. M. haemobos* and *M. wenyonii* con yoduro de propidio y detección de ambos patógenos por PCR punto final en muestras de sangre de ganado mexicano. Micrografía de *Ca. M. haemobos* tomada de (52).
CONCLUSION

The distribution of hemoplasmas comprises different hosts. Over time, hemoplasmas found either alone or in co-infections become a risk to companion and production animals and even to human health. The absence or presence of clinical signs in infected animals could be related to the interactions established by the pathogens in the host. Hence, the importance to develop more precise molecular tools for early diagnoses.

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REFERENCES


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