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Equine Hemoplasmosis (Brief Review)

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Abstract

Hemoplasmosis is a worldwide spread disease with significant clinical and epidemiological importance due to the related to its health crises it causes, which negatively impacts infected animals. In horses, this disease requires careful attention because of the systemic reactions, hemolytic anemia, and weakness it can trigger. Additionally, postmortem features may display general emaciation, paleness of internal organs, obvious splenomegaly, and enlarged (liver, kidneys, and heart), which showed white patches. Furthermore, histopathological changes indicate degeneration and necrosis in cardiac muscles, besides atrophic glomeruli, accompanied by the dilatation of renal tubules in the kidneys, as well as a marked reduction in the proportion of white pulp of splenic tissue and degenerative changes in the cytoplasm of hepatocytes, accompanied by nuclear alterations characterized by loss of chromatin content in the hepatic tissue. As the disease is life-threatening, it often results in the death of the affected animal; therefore, essential preventive measures should be applied to limit the disease and its spread by implementing advanced methods that protect animals from infection and death.

Keyword: Hemoplasma, Equine, Review

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Introduction

Parasites may pose a major threat to animal health. Horses are susceptible to over 60 different types of parasites and can harbor multiple species of worms simultaneously. However, it has been shown that the effects of parasites are more pronounced in young than in adult horses (1). Generally, parasitic diseases are the most common illnesses reported in horses (2). Additionally, blood parasitic infections in horses have a significant impact (3). The most common blood parasitic infectious agents in horses are *Thileria equi*, *Babesia caballi,* and Hemomycoplasma infection. These parasites always invade red blood cells and are known as piroplasm due to their specific shape, such as maltos-cros, pear-shaped, and dot-like shapes seen inside erythrocytes or invading the cell wall (1).

Causative agent

Hemoplasmosis, also known as Hemotropic mycoplasma, or Hemoplasma is found as a Gram-negative, small, cell-wall-less, uncultivable bacterium, pleomorphic, appearing in different forms such as cocci, ring, and rode shapes of 0.3 to 1 µm in their diameter, Nonetheless, they typically attach to the outer surface of red blood cells and can be found individually or in chains (4,5). The organism is one of the smallest microorganisms, divided into two groups: Hemotropic and non-Hemotropic mycoplasmas. The first one was therefore included in the genus Mycoplasma, designated as “hemoplasmas” to characterize and differentiate this microorganism group with Hemotropic abilities (6).

Examination of hemoplasmas using optical and electron microscopy has been valuable for the morphological characterization of these microorganisms. The latter technique has demonstrated its epicellular location and revealed a high level of pleomorphism, with the microorganisms appearing as rods, donuts, disks, cocci, or cones. They are found in depressions along the surface of erythrocytes, with a space of 15–25 nm between them and the erythrocyte membrane, in which fibrils connecting to the membrane can be observed (7). Infected erythrocytes undergo morphological changes, losing their typical biconcave shape (8). Areas where the microorganisms have attached show depressions and well-defined erosion lesions. These organisms are enclosed by a single membrane, and although no cytoplasmic organelles have been identified, several granules of varying sizes and densities have been observed. The images also suggest that two types of cell division may occur: binary fission and budding (9).

Epidemiology

It has been known that comparing the incidence and prevalence of Hemoplasmosis in animals among different countries could be affected by topographic variations; however, different diagnostic techniques played a good role in addition to climatic changes with geographic location (3). Locations with higher temperatures are associated with higher prevalence, and this is also verified in areas where several risk factors coalesce. (10).

In horses, Hemomycoplasmosis is mainly caused by *Hemomycoplasma haemofelis* (*Mycoplasma haemofelis*). Organisms classified within the genus Mycoplasma are identified based on the 16S rRNA gene and the RNase P RNA gene. According to Constable et al. (2017) (3) and Messick

(2004) (5), a pleomorphic organism, which often appears as coccoid, rod, and ring shapes, invades the outer surface of red blood cells and is transmitted mechanically by arthropod parasites (9,11). In horses, the organism was first identified in 1978 during a large and noticeable epidemic in Nigerian horses, and its presence was confirmed by molecular diagnosis in 2020, resulting in substantial economic losses (9,12,13,14).

It was indicated that the season will also play a certain role in determining the infection rate of the disease, where summer and autumn are considered the target seasons for high prevalence of Hemomycoplasmosis in animals, as the insect population is too active in transmitting the disease. However, bad management, along with stress factors, might increase the spread of infection. On the other hand, there may also be genetic differences in host susceptibility (15)

Transmitters such as *Dermacentor andersoni*, Haematopinus lice, flies, and fleas also serve as vectors for transmission. Moreover, Blood transfusions, the common use of contaminated needles and surgical instruments, transplacental transmission, and Transmission via contaminated food were also taken into consideration, but this route is still not confirmed (16).

Pathogenesis

While the organism attaches to the red cell membrane, this mechanism might create cell wall changes in the erythrocyte cell wall, reflecting cell injuries after the detachment of the microorganism (3), Therefore it was shown that electrolyte imbalance with subsequent osmotic fragility, which could end with haemolytic destruction of the red blood cells, depends on the size and depth of the erosion, Nevertheless, This osmotic fragility has been detected in several studies (17).

In addition to these mechanical and osmotic changes caused by Hemomycoplasma attachment (leading to both intravascular and extravascular hemolysis), it has also been suggested that competition for nutrients and glucose may cause early removal from circulation. This exploitation results in decreased energy production, leading to oxidative stress and a shorter lifespan in circulation; these mechanisms may also trigger programmed self-destruction of red blood cells, as shown with *Mycoplasma suis*, contributing to intravascular hemolysis (18,19).

Different works have previously documented the presence of Hemotropic mycoplasma in equine blood. These studies also confirm that the species can infect more than one type of animal, as horses have been recorded as infected with *Mycoplasma ovis* (20), and some studies confirm that horses have been infected with *Mycoplasma haemofelis* (12,21).

Clinical signs

Infected horses showed various clinical signs, including increased body temperature, loss of appetite, enlargement of different lymph nodes, and pale mucous membranes, especially those of 3ed eyelids and conjunctivae. At that time, diagnosis was based on detecting the organism in blood smears, but a definitive classification of hemoplasmas was not possible. In 2010, a hemoplasma infection in horses was confirmed, and initial characterization of the agent was performed using nucleic acid sequencing. The report involved two horses presented to a veterinary clinic with rough hair coats, poor condition, weight loss, and unthriftiness. Hematologic tests revealed that both horses had mild anemia (1,3).

Concerning various clinical documents related to equine Hemoplasmosis, Diseased horses exhibited different manifestations, including continuous fever, complete anorexia, weakness, increased respiration, pale and/or icteric mucous membranes, excessive sweating, and petechial haemorrhages detected on the sclera. In addition, diseased horses might show nose bleeding after strenuous exercise, along with stumbling, and enlargement of lymph nodes could also be detected. Moreover, Severe signs were seen in horses that were already stressed. (16).

Anemia, which is the characteristic feature of the disease, arises from destruction and /or phagocytosis of the erythrocytes, resulting in low haematocrit, including hypo hemoglobinemia expressed by anemic hypoxia, reflected by reduced oxygen transport, which is clinically exhibited by rapid breathing, exhaustion, exercise intolerance, paleness of mucous membranes, weakness, and easy fatigue. Moreover, diseased horses also show signs of rapid heart rate, murmurs on auscultation, and haemoglobinuria (3,14). On the other hand, Haemolytic anemia often results in elevated liver enzymes and increased total bilirubin (19). Further, pathological hemolysis increases haemoglobin breakdown and bilirubin production, which exceeds the liver's capacity to uptake, conjugate, and excrete bilirubin into bile, resulting in bilirubinaemia. (12,20, 21).

Post-mortem changes:

Post-mortem changes of Hemotropic mycoplasma infections involve examining tissues, particularly the heart, spleen, kidney, and liver, for macroscopic and microscopic changes. Histopathology, including staining with hematoxylin and eosin, is crucial for identifying characteristic lesions. It has been documented that enlargement of the spleen (splenomegaly), congestion, and a soft consistency are common findings. The white pulp may be prominent. On the other hand, enlargement and a distinctive rust-brown discoloration due to hemosiderin deposition are characteristic. Further, the liver may show moderate periacinar necrosis (30). Moreover, Battisti et al (31) added that gelatinous epicardial fat on the heart, moderate non-suppurative pneumonia, distended gallbladder (cholecystitis), and evidence of vasculitis (edema and exudates) may also be observed

Diagnosis

Cytologic diagnosis:

Cytologic diagnosis of Hemotropic mycoplasma involves examining stained blood smears for detecting hemoplasma on the surface of red blood cells (22). While this method can indicate infection, it is known to have low sensitivity and specificity, which could be due to cross-reactions, meaning it may miss infections or misdiagnose other conditions (23). Blood smears are examined for the visual presence of the causative organism. It should be noted that an experienced examiner can often distinguish between bacteria parasitizing the cell walls of red blood cells and other elements, such as Rickettsia, *Anaplasma marginale*, and the Howell–Jolly bodies (22).

Fluorescence diagnosis of Hemotropic mycoplasma involves using fluorescent dyes to detect these microorganisms in red blood cells. Specifically, fluorescent dyes like Hoechst (this dye stains DNA and is commonly used to detect mycoplasma contamination in cell cultures, according to a resource) or DRAQ5 (this dye specifically targets nucleic acids and is used for detecting hemoplasmas in red blood cells), as well as the acridine orange dye, are utilized for visualization

and quantification. Samples are observed under a fluorescence microscope to visualize the fluorescently labeled bacteria. (24,25)

ELISA (Enzyme-Linked Immunosorbent Assay):

It is a biochemical technique used to detect the presence of antibodies or antigens in a sample. It involves using antibodies that bind to a specific antigen, then employing a detection system to measure the reaction. It can be used to diagnose Hemotropic mycoplasma infections, but it is not always the most reliable method. While ELISA helps screen rodent colonies for *Mycoplasma pulmonis*, it has limitations such as cross-reactivity with other mycoplasmas and variability in results (26).

Molecular diagnosis (The PCR assay):

There are two PCR techniques available: conventional PCR (cPCR), where reaction products are analyzed using electrophoresis and interpreted from agarose gel bands, and quantitative PCR (qPCR), which detects reaction products through emitted fluorescence. The Polymerase Chain Reaction (PCR) shows higher sensitivity and specificity than serological and cytological tests for detecting Hemotropic mycoplasma infections (27). PCR is a molecular diagnostic technique that amplifies specific DNA sequences of the mycoplasma, providing high sensitivity and specificity. PCR can also identify the exact species of Hemotropic mycoplasma (28). It is a highly effective method for diagnosing Hemotropic mycoplasma infections, especially when microscopic examination of blood smears is inconclusive or challenging. PCR assays enable rapid and accurate detection and identification of various hemoplasma species, such as *Mycoplasma haemofelis* (29).

The real-time PCR assay described provides a reliable method for diagnosing infections and quantifying equine hemoplasma DNA in blood samples. It offers advantages such as faster processing, higher throughput, and a lower risk of false-positive results compared to conventional PCR. It is hoped that quantification will assist veterinarians in determining the significance of a positive PCR result, which can be problematic with infections like *Mycoplasma haemofelis*, as these always cause disease in the host. The assay could also be used to monitor response to treatment. Moreover, Ajaj et al (28) added that real-time PCR (qPCR) is based on the methodology of PCR but with the capability to monitor amplification during the reaction. Denaturation of target DNA, annealing of DNA with oligonucleotide primers, extension of the DNA strand, and detection of amplification all occur within the same tube. DNA amplification is efficient during the exponential phase, allowing for the measurement of the product. This means that the amount of target DNA in a sample can be determined based on when the fluorescent signal reaches a threshold level (29).

Economic impact:

The economic impact of these diseases is significant, mainly caused by their harmful effects on animal health. Financial losses come from slowed growth and weight gain, reduced productive output, and delayed development. In the end, these impacts are worsened by the immediate losses from severe clinical disease and case mortality (32)

Treatment

Neither antibiotic treatment consistently eliminates hemoplasmas from circulation, nor do other factors that may contribute to the development of resistance. An example of this is the discovery that *Mycoplasma suis* can penetrate erythrocytes and evade antibiotics. The administration route may also play a crucial role. Initial intravenous administration may help maximize the antibiotic concentration in circulation, improving its efficiency and ability to eliminate the bacteria. (2,3). Although some cases may be refractory to the antibiotics used, a positive clinical response is usually observed. Some studies are testing the efficacy of protocols that use different antibiotics sequentially (e.g., Doxycycline and Marbofloxacin), with variable results (21).

Imidocarb dipropionate (brand name Imizol) is a drug used to treat infections caused by protozoa. For example, it is used to treat Infection with Babesia spp. Sometimes, imidocarb dipropionate is used for the treatment of Ehrlichiosis or other blood parasitic infections in animals like Anaplasma spp.Hemomycoplasma spp. Imidocarb is an antiprotozoal drug with a dual mechanism of action that targets susceptible parasites by interfering with nucleic acid metabolism and by producing anticholinergic effects (3-5).

Control of Hemotropic mycoplasmas (Hemoplasmas)

Control of Hemotropic mycoplasmas requires a comprehensive approach focusing on prevention, management, and treatment. The following key strategies are essential:

Starting with the prevention of transmission through vector control, it is essential to use rigorous flea and tick management with topical insecticides, collars, or oral medications, since these vectors are the main sources of transmission. Furthermore, Injury management, through preventing animal fights and injuries, reduces bite-related transmission. Additionally, screening blood donors: all blood donors should be tested for hemoplasma via PCR to prevent iatrogenic transmission (33). On the other hand, managing carrier animals is important, as asymptomatic carriers are common and can remain PCR-positive for extended periods. While treatment may not eliminate the infection, it reduces the risk of transmission and clinical disease. Nonetheless, stress reduction is vital, as immunosuppression can trigger recurrence of clinical signs (21). Furthermore, environmental and housing measures include regular disinfection of living areas and grooming tools, as well as isolating infected animals during treatment, since casual contact is not a significant transmission route (34). Moreover, monitoring and diagnosis using blood smears and PCR testing is the gold standard for detection and species identification (35).

For scientific knowledge, old and new studies, as well as all scientific developments related to this disease, are important and necessary and must be continuously reviewed to minimize the economic losses it causes. Therefore, this review was conducted to demonstrate and understand the importance of the disease in animals, especially horses.

Conclusion

It was shown that Hemomycoplasmosis is now considered an important infectious disease affecting most domestic animals, especially horses. Therefore, taking strict preventive measures, including controlling disease-carrying insects, especially arthropods, along with using laboratory methods for early diagnosis and treatment of infected horses, was all advised.

Conflicts of interest

The authors declare that there is no conflict of interest.

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داء الهيموبلازما الدموية الخيلية

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الخلاصة

داء الهيموبلازما الدموية، مرض منتشر عالميًا، وله أهمية سريرية ووبائية بالغة، نظرًا لما يسببه من أزمات صحية تؤثر سلبًا على الحيوانات المصابة. يتطلب هذا المرض لدى الخيول عناية فائقة نظرًا لما قد يسببه من تفاعلات جهازية، وفقر دم انحلالي، وضعف عام. بالإضافة إلى ذلك، أظهرت الجثث الميتة هزالاً عامًا، وشحوبًا في الأعضاء الداخلية، وتضخمًا واضحًا في الطحال، وتضخمًا في الكبد والكلى والقلب، مع ظهور بقع بيضاء. علاوة على ذلك، تشير التغيرات النسجية المرضية إلى تنكس ونخر في عضلات القلب، بالإضافة إلى ضمور الكبيبات، مصحوبًا بتوسع في الأنابيب الكلوية في الكلى، بالإضافة إلى انخفاض ملحوظ في نسبة اللب الأبيض في نسيج الطحال، وتغيرات تنكسية في سيتوبلازم الخلايا الكبدية، مصحوبة بتغيرات نووية تتميز بفقدان محتوى الكروماتين في نسيج الكبد وغالبًا ما يؤدي إلى نفوق الحيوان المصاب؛ لذلك، ينبغي اتباع الإجراءات الوقائية الأساسية للحد من المرض وانتشاره، من خلال تطبيق أساليب متطورة تحمي الحيوانات من العدوى والنفوق.

الكلمات المفتاحيه: هيموبلازما,الخيول,مراجعه بحثية.