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## VERTICAL TRANSMISSION OF CANINE EHRLICHIOSIS

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*María José Tintel Astigarraga*

Center for Veterinary Specialties (CEV)  
Department of Pathology. Asuncion,  
Paraguay

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**Abstract:** The tropical and subtropical climate is conducive to the massive dissemination of ectoparasites, which is why vector-borne diseases (VBDs) represent an emerging problem in veterinary medicine and their zoonotic potential is considered relevant to human health. Among them stands out Ehrlichiosis, caused by *Ehrlichia* spp. The contagion occurs through the bite of the infected tick, although the immunopathogenesis of rickettsiae are little known, transmissions by blood transfusions and vertically are also described. Diagnosis can be made by visualizing morulae in affected cells in peripheral blood as a direct method. The treatment of choice is doxycycline with good clinical response. Canine neonatal transmission with a favorable response to treatment in Asunción-Paraguay is mentioned.

## INTRODUCTION

Ehrlichiosis is a cosmopolitan zoonotic disease caused by bacteria of the Anaplasmataceae family, included in the Rickettsiales order, obligate intracellular and transmitted by ticks of the Ixodes<sup>8, 17</sup> genus. Depending on the affected cell line, they are classified as Monocytic Ehrlichia, Granulocytic Anaplasma and Anaplasma platys<sup>16</sup>

A history of exposure to ticks and immunocompromised patients increase the chances of infection. The non-specific clinical picture and the diagnostic limitations make the pathology a real challenge.

To date, there are few studies that mention vertical transmission in both veterinary and human medicine. The objective of the work is to identify *Ehrlichia* spp morulae in neonatal canines and the respective mother with non-specific clinical symptoms.

## BIBLIOGRAPHIC REVIEW

Ehrlichiosis can present an acute, subclinical or chronic form depending on the host's immune response<sup>22</sup>. The humoral immune response induces the production of antibodies that can persist for several years<sup>25</sup> and the antibodies can be passively transferred to neonatal canines or ingested with colostrum<sup>18</sup>. Related to the production of antibodies, it is mentioned that some dogs eliminate the infectious agent, induce an asymptomatic or chronically symptomatic carrier state, a common finding in Rickettsial infections. The production of IgA and IgM begins in 4 to 7 days of infection with *E. canis*<sup>25</sup> while IgG is detected in 15 days<sup>14</sup>

The infection occurs through the bite of the infected tick<sup>7</sup>. After an incubation period of 8 to 20 days, the agent multiplies in the organs of the Mononuclear-Phagocytic System (liver, spleen, lymph nodes). In the acute phase lymphoreticular inflammation<sup>11</sup> is produced with subsequent inflammation<sup>5, 7</sup>. Infected cells are transported by blood to various organs, especially the lungs, kidneys, and meninges. They adhere to the vascular endothelium, inducing vasculitis and tissue infection.<sup>1, 10</sup> Secondary to the vasculitis process, we have peripheral cell destruction or sequestration, leading to thrombocytopenia and leukopenia. After the acute phase, the sub-clinical phase<sup>4</sup> appears.

Diagnosis can be made directly by detecting morulae in peripheral blood smears<sup>9, 15</sup>. Indirectly by serology, IFI, ELISA, cell cultures or molecular methods by PCR<sup>3</sup>.

The immunopathogenesis of *Ehrlichia* spp infection is poorly understood<sup>24</sup>, as is vertical transmission.

## METHODOLOGY

### MATERIALS AND METHODS

The type of study was observational, descriptive and retrospective.

### ANIMALS AND SAMPLES

In June 2016, a patient of the canine species, 2 years old, English bulldog breed, with 50 days of gestation, was admitted to the clinic. Without manifest clinical symptoms. A routine peripheral blood sample (FSP) is taken.

6 newborn canines, English bulldog breed, 20 days old, 4 males and 2 females. With marked respiratory symptoms. Jugular venipuncture is performed for a complete blood count, FSP, and fecal matter sample.

### CYTOLOGICAL STUDY

All FSPs were fixed with methanol for 5 minutes and stained with a 10% Giemsa solution for 20 minutes and evaluated under the microscope with the immersion objective.

### EVALUATION OF THE RESULTS

The FSP with the presence of intracytoplasmic morulae in monocytes were considered positive for Canine Ehrlichiosis.

### RESULTS

Initially, the pregnant female enters the clinic for ultrasound control. The FSP revealed the presence of Ehrlichia spp morulae in monocytes (**Image 1**). As she was asymptomatic, no medication was given to the mother to avoid possible complications in the newborns.

A cesarean section was performed at 59 days of gestation, the mother presented generalized vasculitis before the surgical procedure and was stabilized with IV Hydrocortisone. During surgery, she presented symptoms of

hypotension of 30 mmHg that was reversed with the addition of Plasma EV.

Puppies at 20 days of age are admitted to the consultation for marked respiratory symptoms. Palliative medication based on Amoxicillin 25mg/kg IM is given, nebulization with saline solution, ambroxol hydrochloride, and dexamethasone 2 times a day.

After 24 hours they return for consultation because they did not present any improvement. The result of the complete blood count revealed evident anemia and leukocytosis (**Table 1**). The FSP expressed the presence of *Ehrlichia* spp Morulae (**Image 1**)

Doxycycline-based medication was established 5mg/kg every 12h IV for 2 applications, then PO for 4 weeks. 48h after the start of the medication, the puppies showed successful clinical improvement.

The fecal matter indicated Giardia cysts, once the patients were stabilized a therapy with Fenbendazole 25mg/kg was established for 3 days.

## DISCUSSION

Different studies have described the wide variety of clinical signs, related to various factors. Including the pathogenicity between strains, concomitant infections and the immune status of the dog<sup>25</sup>. In the acute phase, most cases resolve spontaneously and the subclinical phase begins, which can last from months to years, where some immunocompetent animals can eliminate the agent or the chronic phase begins<sup>2,3</sup>. This is related to the mother during pregnancy who did not manifest relevant clinical signs.

The peripheral blood smear is a diagnostic method where organisms can be visually identified in the form of morulae within the cytoplasm of infected cells, providing an early diagnosis, although the non-identification of the morula in the smears does not rule out the disease<sup>12</sup>. Once the intracytoplasmic

inclusions were visualized, the indicated treatment was established. It was not possible to complement the diagnostic means due to the lack of specific studies such as the culture of HL-60 leukemic promyelocytic cells, serology with immunofluorescence and molecular detection using polymerase chain reaction techniques.<sup>17, 19</sup>

The route of infection of the infant cannot be determined. The time of onset of the disease is compatible with the three possible routes of infection (Intrauterine, intrapartum or through lactation). As the route of infection in infants, discharges containing blood, minor skin abrasions, or during sucking cannot be excluded. Rickettsial agents have been identified in the milk leukocytes of cows<sup>20</sup>. Report of a case of perinatally transmitted granulocytic Ehrlichia in a child<sup>13</sup>. Strains that cause fetal death or abortion were determined in sheep and cows<sup>23, 6</sup>

Research has shown that *E. phagocytophila* can be transmitted through the placenta in cows<sup>21</sup>. Another ehrlichial species, *E. risticii* causes abortion and is transmitted transplacentally in horses<sup>18</sup>

More data are needed to determine the timing and choice of antibiotic for treatment of pregnant females and neonates to avoid adverse drug effects. It should be noted that in the reported case no reaction to the antibiotic was determined.

The diagnosis of this case was based on the clinical history, presence of intracytoplasmic morulae, and favorable response to therapy.

## CONCLUSIONS

The visualization of *Ehrlichia* morulae in neonates and their mothers are indicative of the possibility of transplacental transmission. More specific studies such as cell cultures and molecular reactions are needed to determine and compare the levels of antibodies and response to the agent between puppies.

It is important to take into account VTEs as differential diagnoses, regardless of the presence of ticks and the age of the pets.

Blood count	Result	Reference range
Hemoglobin	8.4 g/dl	12- 18 g/dl
Hematocrit	26 %	35- 55%
Red blood cells	3.613.000 mm <sup>3</sup>	(5-18) x10 <sup>6</sup> mm <sup>3</sup>
White blood cells	16.800 mm <sup>3</sup>	(5-15) x10 <sup>3</sup> mm <sup>3</sup>
Leukocyte formula		
Band neutrophils	03%	0-3%
Segmented neutrophils	60%	60- 77%
Lymphocytes	33%	15- 35%
Monocytes	00%	0-10%
Eosinophils	04%	0-10%
Basophils	00%	0-1%
Blood count indices		
MCV	72fl	64- 75 fl
MCH	23.3 pg	19.5- 24.5 pg
CHCM	32.3 g/dl	32- 36 g/dl
Erythrocyte sedimentation rate		
1° Time	3 mm	
2° Time	17 mm	
Platelets	320.000 mm <sup>3</sup>	120- 500 x 10 <sup>3</sup> /mm <sup>3</sup>
Total proteins	5.11 g/dl	5.30- 7.80 g/dl

Cuadro 1: Neonatal hematology.

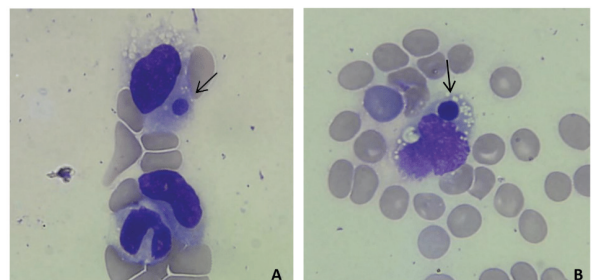


Imagen1: Microphotography Ehrlichia morulae in dog. Black arrows indicate intracytoplasmic morulae. A. Mother. B. Neonate. 100X. Giemsa stain.

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