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Molecular assessment of the transplacental transmission of *Toxoplasma gondii*, *Neospora caninum*, *Brucella canis* and *Ehrlichia canis* in dogs

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ABSTRACT

Given the fact that numerous microbial species can be detected in pregnant female dogs, the objective of this study was to assess the transplacental transmission of *Brucella canis, Ehrlichia canis, Neospora caninum* and *Toxoplasma gondii* in stillborn puppies. This study involved 41 stillborn puppies, 78.6% of which were positive for *T. gondii*, 52.4% for *N. caninum* and 59.5% for *B. canis*. *E. canis* was not detected in any of the analyzed puppies. Pregnancy is an important physiological condition for the transmission of infectious agents to puppies and transplacental transmission may be epidemiologically relevant in the spread of these opportunistic agents.

1. Introduction

Toxoplasma gondii and *Neospora caninum* are parasitic infectious agents that cause a variety of clinical signs in dogs because they spread to several organs and tissues [1,2]. Most commonly, these parasitic protozoa cause neurological disorders in dogs. Moreover, *T. gondii* and *N. caninum* are also widely recognized as organisms that cause disorders in the reproductive system of domestic animals. Both of these agents have been associated with transplacental transmission and canine miscarriage [3–8]. Among the causal agents of reproductive problems in dogs, *Brucella canis* is a major causative agent of prostatitis and epididymitis in males, while in females it is associated with cases of abortion due to embryonic or fetal death and the presence of stillbirth or sick neonates [9,10].

There is a very large amount of data on *T. gondii* and *N. caninum* infection in dogs, both in Brazil and worldwide [11,12]. The presence of anti-*T. gondii* and anti-*N. caninum* antibodies in canine populations varies according to their location and is frequently associated with poor local sanitation conditions, contact with susceptible animals, and food handling [13]. In parallel, *B. canis* appears to be widely distributed and is considered an occupational zoonosis transmitted by close contact with infected dogs [14].

Ehrlichia canis, a species of the genus *Ehrlichia*, is the cause of canine monocytic ehrlichiosis (CME). The main vector of *E. canis* is the brown dog tick, *Rhipicephalus sanguineus* s.l. This infectious disease occurs worldwide and is one of the diseases most commonly reported in Brazil [15]. Although the vertical transmission of *E. canis* has not been reported elsewhere, Aguiar et al. [16] suggest that there are other forms of transmission besides the tick bite, given the high percentage of seropositive or chronically infected

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Table 1
Possible hypothesis for the detection of *Toxoplasma gondii*, *Neospora caninum* and *Brucella canis* in bitches and their puppies by Indirect Immunofluorescence Assay (IFA) and Polymerase Chain Reaction (PCR).

Situations	Bitches	Puppies	Hypothesis
Toxoplasma gondii			
IFAT + /PCR+	2	PCR + = 1 PCR – = 1	transplacental transmission in active parasitemia
IFAT + /PCR –	4	PCR + = 6 PCR – = 2	transplacental transmission occurring previously to the blood collection/reactivated parasitemia
IFAT –/PCR +	4	PCR + = 7 PCR – = 0	transplacental transmission in active parasitemia
IFAT –/PCR –	13	PCR + = 19 PCR – = 6	transplacental transmission occurring previously to the blood collection/transient parasitemia
–			
Neospora caninum			
IFAT + /PCR +	5	PCR + = 5 PCR – = 4	transplacental transmission in active parasitemia
IFAT + /PCR –	5	PCR + = 5 PCR – = 5	transplacental transmission occurring previously to the blood collection; reactivated parasitemia
IFAT –/PCR +	5	PCR + = 7 PCR – = 4	transplacental transmission in active parasitemia
IFAT –/PCR –	8	PCR + = 5 PCR – = 6	transplacental transmission occurring previously to the blood collection; transient parasitemia
–			
Brucella canis			
PCR +	12	PCR + = 18 PCR – = 4	transplacental transmission in active bacteremia
PCR –	11	PCR + = 7 PCR – = 13	transplacental transmission occurring previously to the blood collection; transient bacteremia

dogs and the low frequency of infection in *R. sanguineus* ticks. CME is manifested by a variety of clinical signs that may include fever, bleeding disorders, bone marrow failure, and death in irreversible cases [17].

The objective of this study was to evaluate the transplacental transmission of *T. gondii*, *N. caninum*, *B. canis* and *E. canis* in pregnant bitches undergoing obstetric procedures and in their stillborn puppies.

2. Material and methods

2.1. Sampling of pregnant bitches and stillborn puppies

This study was conducted from June 2011 to November 2012 at the Veterinary Hospital of the Federal University of Mato Grosso (HOVET-UFMT), in Cuiabá (15° 35′ 45″S, 56° 5′ 49″W), Brazil. A total of 23 pregnant bitches of different ages and breeds were involved in this study. All the bitches underwent a cesarean section because they were diagnosed with dystocia. After obtaining the owners' consent, 41 stillborn puppies were included in this study. Sample collection was conducted according to the Ethical Principles for Animal Research under the institutional watch of the UFMT Committee for Ethics in Animal Research (23108.022391/11-8).

2.2. Material collection

After dystocia was diagnosed in the pregnant bitches, blood samples were drawn by jugular venipuncture into sterile vacuum tubes with and without an anticoagulant. Fragments of the stillborn dogs' spleen, thymus and heart blood clots were analyzed. All the samples were kept frozen at −20 °C until the examinations were concluded.

2.3. Indirect-immunofluorescence assay

An indirect immunofluorescence assay (IFA) was used to detect IgG anti-*T. gondii* [18], anti-*N. caninum* [19] and anti-*E. canis* [16] antibodies in the pregnant bitches.

2.4. DNA extraction and PCR

The collected spleen, thymus and heart blood clot samples obtained from the puppies and the total blood collected from the bitches were subjected to DNA extraction as described by Sangioni et al. [20]. For *T. gondii*, the primers TOXO 1 (GGAACTGCATCCGTTCATGAG) and TOXO 2 (TCTTTAAAGCGTTCGTGGTC) were used according to Burg et al. [21], which amplify a 194 bp fragment of *B1*gene. For *N. caninum*, the nested-PCR method was carried out to detect the *ITS-1* region, using the primers NN1 (TCAACCTTTGAATCCCAA) and NN2 (CGAGCCAAGACATCCATT) in the first reaction and NP1 (TACTACTCCCTGTGAGTT) and NP2 (TCTCTTCCCTCAAACGCT) in the second reaction, which amplify a 213 bp fragment [22]. Research into *B. canis* genetic material was performed according to the protocol described by KIM et al. [23] using the primers B2N-1 (GTCGCGGATTCTACCTCACCT) and B2N-2 (TAAGCAGGTAAGAGGCAATTT), which amplify a 280 bp of *virB2* gene. For the detection of *E. canis* DNA, real time PCR (q-PCR) was employed with the primers 321 (TTGCAAAATGATGTCTGAAGATATGAAACA) and 671 (GCTGCTACACCAGTAAATGTATCCCCTA) for the *dsb* gene and a specific probe was used for *E. canis* (AGCTAGTGCTGCTTGGGCAACTTTGAGTGAA; 5′ FAM/BHQ-1 3′) [24].

2.5. Statistical analysis

Puppies born from both infected and non-infected bitches were evaluated by the chi-square test or by Fisher's exact test when appropriate, and the value of $P < 0.05$ was considered significant.