Investigation of *Toxoplasma gondii* and *Neospora caninum* in the amniotic fluid and placenta of pregnant women seropositive for toxoplasmosis

SOUSA, J.C.[1]; PASCHOINI, M.C.[1]; MICHELETTI, A.M.R.[1]; OLIVEIRA, K.R.[2]; CANDIDO, P.A.[1]; CABRINE-SANTOS, M.[1]; OLIVEIRA-SILVA, M.B.[1]

[1] Universidade Federal do Triângulo Mineiro, Uberaba, MG
[2] Universidade Federal de Uberlândia, Ituiutaba, MG

Toxoplasma gondii is an Apicomplexa protozoan, a parasite that infects humans, and causes toxoplasmosis, responsible for abortions in pregnant women and neonatal death, while *Neospora caninum* causes a similar disease, mainly in cattle. Some studies have demonstrated seropositivity for *N. caninum* in humans, but no reports of neosporosis are yet available. The transmission of these protozoa occurs through the ingestion of water, food or meat from contaminated intermediate hosts. Congenital transmission in toxoplasmosis may occur during primoinfection during gestation, when *T. gondii* tachyzoites can be transmitted to the fetus via the transplacental route. Infection with *T. gondii* or *N. caninum* can be diagnosed by serological methods or by PCR. Taking into account the need for the standardization of the PCR technique for the diagnosis of congenital toxoplasmosis at the Clinic Hospital at the Triângulo Mineiro Federal University (HC-UFTM) and the shortage of studies involving neosporosis in humans, the present study aimed to detect *T. gondii* and/or *N. caninum* DNA by the PCR technique in the amniotic fluid and placentas of pregnant women (fixed in formaldehyde) diagnosed with toxoplasmosis by the serological method, as well as to evaluate PCR as a diagnostic method for congenital toxoplasmosis. Twenty-three pregnant women followed by the HC-UFTM Gynecology and Obstetrics Course presenting a positive serological diagnosis (ELISA: IgM and IgG) for toxoplasmosis and indicative of amniocentesis participated in this study. Twenty-four amniotic fluid samples (22 samples from different patients and 2 samples from the same patient at different times) and 16 placental samples, collected from July 2014 to March 2017, were evaluated in the study. DNA extraction from the amniotic fluid and placenta was performed using the MagaZorb® DNA Mini-Prep Kit, following the manufacturer's protocol for fluid and tissue extraction. The presence of *T. gondii* DNA was determined by a nested-PCR amplifying a fragment of approximately 97bp from the B1 gene. Two specific primers (Np21plus and Np6plus) that amplify a fragment of approximately 337bp from the Nc-5 genomic region were used to search for *N. caninum* DNA. Three of the 24 amniotic fluid samples and only one of the 16 placenta samples were positive by nested-PCR for *T. gondii*, indicating possible transplacental transmission and allowing for early treatment and, consequently, less consequences to the fetus. This demonstrates that nested-PCR applied for tissues is less sensitive than DNA detection in amniotic fluid, which may be explained by the greater difficulty in extracting DNA from tissues, especially when fixed in formaldehyde. No amniotic fluid or placenta samples showed the presence of *N. caninum* DNA, indicating that no infection or vertical transmission of this parasite in the studied population.