RESUMO SIMPLES - PROTOZOÁRIOS E AGENTES TRANSMITIDOS POR VETORES DE IMPORTÂNCIA EM SAÚDE

DETECTION OF TOXOPLASMA GONDII IN A FREE-LIVING COUGAR (PUMA CONCOLOR) RESCUED IN RESENDE, RJ

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Toxoplasma gondii is a widely distributed protozoan capable of infecting various species of mammals, including wild cats, which act as definitive hosts. Serological detection is essential for assessing previous exposure and epidemiological risks. The Modified Agglutination Test (MAT) is widely used because it has good sensitivity, low cost, and no species restrictions, making it particularly useful for studies in wildlife. The objective of this study was to evaluate the presence of anti-T. gondii IgG antibodies in a specimen of Puma concolor rescued in the municipality of Resende, RJ, and received by the Volta Redonda Municipal Zoo. The blood sample was obtained by puncturing the cephalic vein into tubes without anticoagulant and with EDTA. To obtain the serum, the tube without anticoagulant was centrifuged, and the serum was kept

refrigerated at -20°C until analysis. MAT was performed using tachyzoite antigens from the RH strain of T. gondii prepared in a solution containing borate buffer, Evans blue dye, and 2-β-mercaptoethanol. In 96-well U-shaped microplates, 25 µL of the antigen suspension was added, followed by the addition, in a 1:1 ratio, of serum samples previously diluted in PBS at an initial dilution of 1:25. The plates were incubated at 37 °C for 12 hours. Positive control serum and negative control serum were included in each assay. After incubation, samples were considered negative when a blue button formed at the bottom of the well, and positive when the bottom was clear, indicating agglutination. To determine the final titer, positive samples were titrated by serial dilutions ranging from 1:25 to 1:3200, following the same incubation and reading protocol. To complement the diagnosis, EDTA blood samples were used for molecular analysis, and DNA extraction was performed using the Wizard Genomic DNA Purification kit (Promega®), according to the manufacturer's instructions. After extraction, the viability of the samples for biomolecular testing was assessed by amplifying a 400-base pair (bp) fragment of the endogenous GAPDH gene. Conventional PCR was used to detect T. gondii DNA by amplifying a 194-bp fragment of the B1 gene. The serology result revealed a titer of 1:3200, which is considered positive and indicative of previous exposure to the parasite. Molecular analysis did not identify amplification of T. gondii DNA, indicating the absence of parasitemia or active infection at the time of collection. Most epidemiological studies in cats are based on determining seroprevalence in the analyzed population through the detection of anti-Toxoplasma IgG antibodies. This allows the identification of infected individuals, but does not determine whether they are in the acute phase (intestinal, with release of oocysts in the feces) or in the chronic phase (with tissue cysts). The serodiagnostic tests most commonly used in clinical and epidemiological studies for screening T. gondii infection in humans and animals include the enzyme-linked immunosorbent assay (ELISA) and the MAT. both tests have demonstrated good sensitivity and specificity for the detection of chronic T. gondii infection (IgG) in humans and animals, but in veterinary studies, MAT is often preferred because it is simple, economical, and has no species restrictions, unlike ELISA. The association between a high-titer positive MAT and a negative PCR suggests that the animal had previous contact with the agent and is in the chronic phase of infection, with no evidence of systemic parasitic activity. It is concluded that MAT is an efficient tool for screening toxoplasmosis in wild cats and useful in health surveillance programs in conservation institutions.

Palavras-chave: toxoplasma gondii; puma concolor ; modified agglutination test.