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

IDENTIFICATION OF NEOSPORA CANINUM IN EAR-NOTCH TISSUE FROM AN ABORTED BOVINE FETUS

Daniela Cristina Rocha De Freitas (danielaa.freitas@hotmail.com)

Samuel Rodrigues Bonamichi Do Couto (samuel_piquete@yahoo.com.br)

Myleide Aparecida Da Silva Costa (myleide.silva@yahoo.com.br)

Fabíola De Souza Lemes Dos Santos (fabiolasouzabio@gmail.com)

Marcia Mayumi Fusuma (mmayumi06@gmail.com)

Juliana Ferreira Rocha Mello (jurocha.me@gmail.com)

Marilene De Farias Brito Queiroz (mfariasbrito@uol.com.br)

Vera Lúcia Teixeira De Jesus (jesus@ufrrj.br)

Marco Roberto Bourg De Mello (mmrural@gmail.com)

Liria Hiromi Okuda (liria.okuda@sp.gov.br)

Neospora caninum, a parasite from the Apicomplexa phylum, is one of the major etiological agents of abortion in cattle. The preferred samples for protozoan diagnosis are placental and fetal tissues, mainly liver, skeletal muscle, and brain. This study reports the detection of N. caninum in the ear tip of an aborted bovine fetus. A dairy farm located in the countryside of Rio de Janeiro, with a history of abortions caused by neosporosis, sent a fetus of approximately six months gestation to the Universidade Federal Rural do Rio de

Janeiro for necropsy. The collected samples were frozen and sent to Instituto Biológico for differential diagnosis of abortion. The tested samples included: umbilical artery, heart, intestines, Longissimus dorsi muscle, pancreas, skin, several central nervous system (CNS) regions, thymus, an organ pool (kidney, spleen, and lung), and the ear-notch. All samples were macerated in PBS buffer (1:5) for genomic DNA extraction using a magnetic beads commercial kit (Magmax Core, ThermoFisherScientific®) in an automated extraction system (KingFisher, ThermoFisher®), following the manufacturer's instructions. The final DNA volume was 90 µL, and samples were stored at -20 ºC until nested-PCR completion. For the first amplification, JB primers (JB1: 5'-AGGAAGGAGAAGTCGTAACAAGG-3' and JB2: 5'-GAGCCAAGACATCCATTGC-3') were used at 0.5 µM concentration in a PCR Master mix (Promega®) with 5 µL of DNA, amplifying a 500 bp fragment (ITS1 ribosomal region). For nested-PCR, a second set of primers (SF1: 5'-CCTGTGAGTTGTATCGCCTTC-3' and SF2: 5'-TCTCTTCCCTCAAACGCTATCC-3') were used at 0.5 µM concentration in a PCR Master mix (Promega®) with 2 µL of the product to amplify a 250 bp fragment (NC5 region of N. caninum). The first amplification protocol was: 1 cycle at 95 ºC for 5 minutes, 25 cycles at 94 ºC for 1 minute, 60 ºC for 1 minute, 72 ºC for 1 minute, and 1 cycle at 72 ºC for 10 minutes, followed by maintenance at 4 ºC. The second amplification was the same, using 5 µL of the first amplification product. Amplification products were applied to 2% agarose gel prepared in TBE 1X buffer (Promega®) and subjected to electrophoresis at a constant voltage of 100 V for 1 hour. Amplicons were visualized under ultraviolet light after GelRed staining. DNA fragment sizes were compared with a standard molecular weight marker (100 bp DNA ladder – ThermoFisherScientific®). Neospora caninum DNA was detected in the heart, Longissimus dorsi muscle, CNS, thymus, and ear-notch. The ear-notch is commonly used for BVDV diagnosis, and this case suggests it is also a feasible tissue for neosporosis diagnosis, as it does not interfere with the humoral response. This case demonstrates the possibility of using the ear-notch for the diagnosis of N. caninum.

Palavras-chave: neosporosis; molecular diagnosis; cattle; matrix; abortion.