

Epidemiology/Diseases

DIAGNOSTIC OF THE CAPRINE ARTHRITIS ENCEPHALITIS VIRUS IN UTERINE FLUID AND EMBRYOS OF GOATS BY VIRUS ISOLATION IN CELL CULTURE AND PCR NESTED

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Transmission of caprine arthritis encephalitis virus (CAEV) through embryo transfer procedures has always been hypothesized and has great importance in the control programs of the caprine arthritis encephalitis (CAE) having direct influence on embryo market. The aim of this study was to evaluate the presence of CAEV in embryo, uterine fluid and embryo washing solution, by virus isolation and nested polymerase chain reaction (nPCR). Embryos and fluids were collected from 10 Saanen goats, 4 to 6 years old, seropositive for CAEV and fertilized with bucks also seropositive. Fifty embryos with intact zona pellucidae were washed (25) or not (25), according to the IETS guidelines. The washed embryos and no-washed ones, uterine fluid and the embryo washing solution were then inoculated 24 hours after the recovery, in the goat primary synovial semi-confluent monolayer culture. The cultures were maintained during 63 days in an incubator at 37°C in 5% CO₂ atmosphere, with passage of cells every 21 days. The monolayers were evaluated daily for characteristic cytopathic effects (CPE). After the last cells passage, half sample of the monolayer was stained with violet crystal (0.1%) for better visualization of CPE characteristic, and the other part was trypsinized to nPCR. After extraction of DNA with hypertonic buffer and proteinase K (0.1mg/mL), the samples were submitted to nPCR with two pairs of oligonucleotide primers to amplify a gag sequence of CAEV (185pb). The DNA was observed in 1% agarose gel and submitted to enzymatic restriction with Bal I enzyme. CAEV was not detected by virus isolation as well as nPCR in the samples of washed embryo, no-washed embryos and in the embryo washing solution. However, 37.5% of uterine fluid was positive by virus isolation and 70% was positive for nPCR, with sensibility among 10^{4.5} a 10^{-4.5} TCID₅₀/50μL. The specificity was confirmed by enzymatic restriction. The presence of CAEV in the uterine fluid showed the risk of the fetal-maternal transmission, alerting for the presence of the virus in the embryo media. However, as the CAEV was not detected in the embryo and in the embryo washing solution, the use of the embryo transfer procedure, following the sanitary IETS guidelines, could be a safer alternative in the use of the genetic material from females infected by CAEV.

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