Detection of Caprine Arthritis-Encephalitis Virus by Polymerase Chain Reaction

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Detection of caprine arthritis-encephalitis virus (CAEV) infection in goats is currently limited to serologic testing or cell culture. We developed a polymerase chain reaction (PCR) assay to detect CAEV sequences in peripheral blood mononuclear cells (PBMC), synovial fluid cells (SFC), and milk cells (MC) obtained from infected goats. Results were positive for 18 of 20 PBMC, 8 of 8 MC, and 5 of 5 SFC samples from seropositive goats, whereas 3 of 33 PBMC samples and none of 8 MC or 5 SFC samples from seronegative goats were positive. Two of the PCR-positive and seronegative goats seroconverted upon follow-up testing 2 months later. This PCR assay provides a useful method for detecting CAEV infection in goats.

Caprine arthritis-encephalitis virus (CAEV) belongs to the lentivirus subfamily of retroviruses (1, 10). CAEV integrates into the host cell DNA, causing a persistent infection with tropism mainly for the cells of the monocyte-macrophage lineage (6). The virus causes chronic progressive arthritis, pneumonia, and mastitis in adult goats and leukoencephalomyelitis in young kids (2, 3, 5). In one survey, the prevalence of infection was found to be as high as 81% among dairy goats in the United States (4). Transmission occurs mainly through ingestion of colostrum and milk, although intrauterine and contact transmissions have not been ruled out (9, 11). The success in controlling the spread of CAEV infection depends largely on early detection and removal of infected animals from their herds. Screening for CAEV has been limited to virus isolation by cell culture, which is time-consuming and costly, or to serologic testing, which is based routinely on an agar-gel immunodiffusion (AGID) test that uses as antigens CAEV and the ovine progressive pneumonia virus. Besides the potential specificity problem because of the presence of ovine progressive pneumonia virus, the usefulness of the AGID test may be limited by other factors such as the lack of information about its sensitivity and the interference from passively transferred maternal antibodies in young kids. In this report, we describe a polymerase chain reaction (PCR) assay that is sensitive and specific for detecting proviral sequences of CAEV.

Of 53 goats at Tuskegee University used in the study, 8 had evidence of arthritis and five had evidence of mastitis, three kids had typical neurologic signs of CAEV infection and pneumonia, and the remaining goats appeared to be healthy. Serum samples were screened for antibodies to CAEV by using the AGID test kit (Veterinary Diagnostic Technology, Denver, Colo.). Peripheral blood mononuclear cells (PBMC) were isolated from blood samples by density gradient centrifugation. Synovial fluid (0.5 to 1 ml) collected from goats was diluted in 10 ml of phosphate-buffered saline (PBS) and then centrifuged at 800 × g for 10 min to collect the synovial fluid cells (SFC). About 50 ml of milk was collected from lactating goats, and 5 to 10 ml of fluid was collected from the mammary glands of nonlactating multiparous goats. The tubes were centrifuged to pellet the milk cells (MC). Contaminating erythrocytes in PBMC, SFC, and MC preparations were removed by treating the samples with 0.85% ammonium chloride solution and washing with PBS.

The PBMC, MC, and SFC were adjusted to a concentration of 6 × 10^6 cells per ml and then lysed in PCR lysis buffer supplemented with 60 μg of proteinase K per ml for 1 h at 56°C (7). Primers and probes were designed on the basis of the published sequence of the CAEV Co strain (Table 1) (12). Lysate aliquots of 25 μl were cycled 35 times at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and the products were Southern blot hybridized to a 32P-labeled internal probe under conditions described previously (7). Measures to avoid PCR contamination as previously suggested (8) were strictly followed. Positive controls included CAEV Co- and CAEV 63-infected fetal goat synovial membrane (GSM) cells, while negative controls included uninfected GSM cells. CAEV Co and CAEV 63 (ATCC VR-905) were propagated in primary GSM cells as described previously (5). Initial experiments using the positive and negative controls indicated that the GAG primer pair (CA1-CA3) successfully detected both CAEV 63 and CAEV Co strains, while the uninfected GSM cells were negative (Fig. 1).

All samples from seropositive and seronegative goats were tested by PCR blindly, using CA1-CA3 (Table 2). Of the 20 PBMC samples from seropositive goats, 18 were positive. MC and SFC samples collected from five of these PCR-positive goats and MC samples from three other seropositive animals were also PCR positive by CA1-CA3. Our inability to detect CAEV from the PBMC of two seropositive goats may have been due to a low viral load in these apparently

<table>
<thead>
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<th>Oligonucleotide</th>
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<tr>
<td>CA1</td>
<td>CCAGGGCGATCCCAACTGTTACAAGGAC</td>
<td>1355-1379</td>
</tr>
<tr>
<td>CA3</td>
<td>CTTGCGCTTATGCTGCTGTCATAACA</td>
<td>1617-1593</td>
</tr>
<tr>
<td>CA2 (probe)</td>
<td>GTGAAAGCAGTTAGATGCAAGAC</td>
<td>1420-1442</td>
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<tr>
<td>CP1</td>
<td>AATTGGAAAAGGGATTACGCGGGCTC</td>
<td>2189-2212</td>
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<tr>
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<td>TTTCTTTCTTTGTCCTGCCTCGCAAGATG</td>
<td>2463-2493</td>
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<tr>
<td>CP2 (probe)</td>
<td>AAGAAAATCAAGGGAAATGGAGAATG</td>
<td>2350-2374</td>
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* Corresponding author.
healthy animals. No attempts to culture any virus from these two animals have been made. This relatively high sensitivity of CA1-CA3 may be due in part to a lack of intrasolate sequence variation in this region.

Although the number of milk samples tested was relatively low, our data indicate that screening milk samples for CAEV by PCR is sensitive and has the advantages of ease and convenience of sample collection from dairy goats. The ability to detect CAEV consistently by PCR in MC and SFC confirms the previous findings for the distribution of the virus in these tissues (9, 13). Of the samples from 33 seronegative goats, 3 PBMC samples were found to be positive by CA1-CA3 (Table 2) and (Fig. 1). The eight MC and five SFC samples collected from different seronegative goats were all found to be negative by CA1-CA3 (Table 2). Of the three PCR-positive but seronegative goats, two were kids about 2 months of age and the third was an adult that was removed from the herd and could not be followed up. Upon further serologic testing, the two kids were found to have seroconverted at 4 months of age. Our data indicate that these PCR assays are specific and may be more sensitive than AGID in detecting early infection. The use of Southern blot hybridization in these tests may have contributed to the high specificity and sensitivity as seen in other studies (7).

With respect to AGID and within its limitations, the overall sensitivity of the PBMC PCR assay was 91% (20 of 22) and the specificity was 97% (30 of 31). To further confirm our results, we subjected a subset of samples from both seronegative and seropositive goats to another amplification reaction with a polymerase primer pair (CP1-CP3) with subsequent probing with CP2 (Table 1). Results from these amplifications correlated fully with those from the amplifications with CA1-CA3 (data not shown). These assays provide a new molecular tool for the detection of CAEV in infected animals and cell cultures and may allow studies of tissue distribution of the virus in animals and the genetic variation of CAEV.

**Nucleotide sequence accession number.** The sequences in Table 1 have been obtained from GenBank (accession number M3367).

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**REFERENCES**


