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Evaluation of Agar Gel Immunodiffusion Serology Using Caprine and Ovine Lentiviral Antigens for Detection of Antibody to Caprine Arthritis-Encephalitis Virus

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The sensitivity of the agar gel immunodiffusion (AGID) test for the detection of antibody to caprine arthritis-encephalitis virus (CAEV) was investigated with CAEV or ovine progressive pneumonia virus (OPPV) as the source of antigen. A total of 218 goat serum specimens were tested for anti-CAEV antibody by AGID and immunoprecipitation of [^{35}S]methionine-labeled CAEV. In comparison with that of immunoprecipitation, the sensitivity of the CAEV AGID test was 0.91, and that of the OPPV AGID test was 0.56. The AGID test with either antigen was 100% specific. The lower sensitivity of the OPPV AGID test in detecting caprine antibody to CAEV indicates that OPPV antigen is of limited value for use in CAEV diagnosis and control programs.

Caprine arthritis-encephalitis virus (CAEV) is a lentivirus which causes multisystemic disease (4, 7). The most consistent clinical sign, periarticular carpal swelling, appears in 12 to 40% of infected goats (4, 6, 9). The remaining infected goats may never show clinical signs; however, since CAEV causes a persistent infection, infected goats are a continuous source of CAEV for transmission. Therefore, accurate serologic detection of persistently infected, clinically normal goats is a necessity for the success of currently recommended eradication programs (2, 13, 15).

The most widely used serologic test for detecting caprine anti-CAEV antibody is the agar gel immunodiffusion (AGID) test (3). The sensitivity of the AGID test for detecting anti-CAEV antibody is dependent upon the antigen used (1). It was demonstrated that an AGID assay with CAEV gp135 surface glycoprotein afforded greater sensitivity than an AGID assay with CAEV p28 core protein (1). A commercially available AGID test, the caprine arthritis-encephalitis–ovine progressive pneumonia antibody test kit (Veterinary Diagnostic Technology, Wheat Ridge, Colo.), for the detection of anti-CAEV and anti-ovine progressive pneumonia virus (OPPV) antibodies uses OPPV as the antigen. OPPV, also a lentivirus, is closely related to CAEV (10).

In an immunoprecipitation assay, the major structural proteins of CAEV and OPPV share antigenic determinants (10); however, the degree of antigenic cross-reactivity has not been defined. A comparison of the deduced amino acid sequences of the CAEV and OPPV *gag* and *env* gene products showed divergences of 25% in core proteins and 40% in envelope proteins (16). The significance of this divergence of CAEV and OPPV proteins in terms of their use in the AGID assay for the detection of heterologous antiviral antibody is not known. Since current CAEV eradication programs (2, 13, 15) depend in part on the accuracy of the AGID test, the sensitivity and specificity of the AGID test with CAEV and OPPV antigens were determined.

The serologic reactivity of 218 goat serum specimens to CAEV was tested. These serum specimens originated from Atlantic Antibodies, Windham, Maine (121 serum specimens), submissions to the Washington Animal Disease Diagnostic Laboratory (39 serum specimens), and the Washington State University College of Veterinary Medicine CAEV-free Saanen goat herd (58 serum specimens). Two additional goat serum specimens (17 and 05) were positive and negative controls previously described (12). Reference sera for the OPPV AGID test were provided with the commercial test kit.

The AGID test with CAEV was performed as previously described (3). AGID CAEV test antigen CAEV-63 was propagated in goat synovial membrane cells (5), and OPPV antigen was obtained as part of the commercial test kit. The OPPV AGID test was performed and interpreted as directed in the product insert. For both AGID assays, inward deviation of the reference line or fusion of a line between the sample and antigen wells with the reference line was considered a positive result (1).

Immunoprecipitation of [^{35}S]methionine-labeled CAEV was performed as previously described (5, 10, 12). It has been shown that goats infected with CAEV have 10^2- to 10^3-fold higher titer against the gp135 surface protein than against the p28 core protein (11, 12). Therefore, test serum was designated positive in immunoprecipitation when a protein migrating at a molecular mass of approximately 135 kDa was visually present, in comparison with negative and positive control sera (Fig. 1).

The OPPV AGID test identified 60 of the 218 serum specimens as positive, 37 less than were identified as positive by the CAEV AGID test. Immunoprecipitation identified 107 serum specimens as positive. All sera found positive by the CAEV AGID test or the OPPV AGID test were also found positive by immunoprecipitation. Totals of 121, 158,

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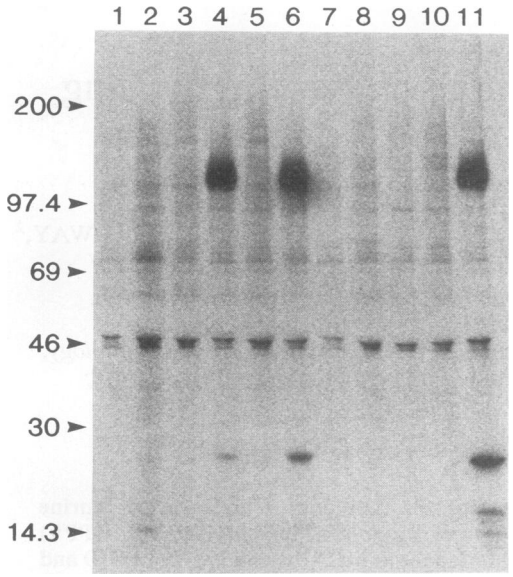

FIG. 1. Immunoprecipitation of ^{35}S-labeled CAEV with goat test sera. Shown are serum from negative control goat 85G05 (lane 1), sera from test goats (lanes 2 to 10), and serum from positive control goat 85G17 (lane 11). Sera from test goats in lanes 4 and 6 are positive. Lanes 2, 3, 5, and 7 to 10 represent negative test sera. The migration of ^{14}C-labeled marker proteins is indicated (in kilodaltons).

and 111 serum specimens were identified as negative by the CAEV AGID test, the OPPV AGID test, and immunoprecipitation, respectively.

The collective data were analyzed with a microcomputer spreadsheet program (Borland International Inc., Scotts Valley, Calif.). Sensitivity and specificity were computed by use of 2 × 2 tables as described previously (14). Kappa analysis for the degree of concordance beyond chance was performed as described previously (14). In the comparison of tests, a kappa value of at least 0.4 to 0.5 indicates a moderate level of agreement (14). Table 1 shows the test performance data obtained by comparing the CAEV AGID test and the OPPV AGID test with immunoprecipitation for the detection of anti-CAEV antibody. Although the CAEV AGID test and the OPPV AGID test were less sensitive than immunoprecipitation for the detection of anti-CAEV antibody, both tests were specific. No sera identified as positive by either AGID test yielded a negative immunoprecipitation result. Therefore, the difference in test performance values between the CAEV AGID test and the OPPV AGID test in comparison with immunoprecipitation is due to a higher frequency of false-negatives yielded by the OPPV AGID test.

The most likely reason for the difference in sensitivity between the CAEV AGID test and the OPPV AGID test is the divergence of the *gag* and *env* gene products of CAEV and OPPV (16). Although the immunoprecipitation reaction (10) requires only the binding of a single epitope by antibody to obtain a positive result, precipitation in an agar gel requires multiple epitope-antibody interactions (17). It was shown by immunoprecipitation that CAEV and OPPV share antigens (10); however, the extent of antigenic homology has not been determined.

The data in this report clearly show that in comparison with immunoprecipitation, the CAEV AGID test is considerably more sensitive than the OPPV AGID test for the detection of anti-CAEV antibody. The lower sensitivity of the OPPV AGID test indicates that OPPV antigen is of limited value for use in CAEV diagnosis and control programs. Furthermore, the marked variability in the reported prevalence of CAEV (3, 6, 8, 9) may be due in part to the use of the OPPV AGID test in some surveys (8, 9).

TABLE 1. Comparison of AGID (CAEV and OPPV) test performance with that of immunoprecipitation[a]

Type of AGID	Concordance	Sensitivity	Specificity	Kappa value
OPPV	0.78	0.56	1.0	0.536
CAEV	0.95	0.91	1.0	0.908

[a] Statistical analysis was performed as described in the text.

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