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Evaluation of a Caprine Arthritis-Encephalitis Virus/Maedi-Visna Virus Indirect Enzyme-Linked Immunosorbent Assay in the Serological Diagnosis of Ovine Progressive Pneumonia Virus in U.S. Sheep[▽]

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A caprine arthritis-encephalitis virus (CAEV)/maedi-visna virus (MVV) indirect enzyme-linked immunosorbent assay (iELISA) was validated with samples from U.S. sheep and by the use of radioimmunoprecipitation as the standard for comparison. The sensitivity and the specificity were 86.0% (±5.8%) and 95.9% (±2.9%), respectively. The iELISA format and phylogenetic differences based on the MVV *gag* sequence contribute to the reduced sensitivity.

The diagnosis of small ruminant lentivirus (SRLV) infections in sheep and goats is most commonly determined by the detection of anti-SRLV antibodies in serum by an enzyme-linked immunosorbent assay (ELISA) that is typically created by the use of maedi-visna virus (MVV) or caprine arthritis-encephalitis virus (CAEV) isolates from sheep or goats of a given region or country (1). ELISA formats are typically validated against reference standard tests, including the agar gel immunodiffusion (AGID) assay, the radioimmunoprecipitation (IP) assay, or Western blot analysis. Although most seropositive sheep and goats do not show clinical signs of SRLV disease, they are persistent and potential reservoirs for transmission. Therefore, highly specific and sensitive serological diagnostic assays are essential for the early detection of SRLV.

Three hundred ten of 332 serum samples from U.S. sheep from a previous CAEV competitive ELISA (cELISA) validation study (4) were tested in duplicate by using a Chekit CAEV/MVV antibody test kit (IDEXX Laboratories, The Netherlands), according to the manufacturer's instructions. The CAEV/MVV indirect ELISA (iELISA) results were compared with those of the ovine progressive pneumonia virus (OPPV) WLC1 radio-IP assay, which has been described previously (4). The CAEV/MVV iELISA utilizes whole virus from Swiss MVV strain OLV as the antigen (15, 16). With a value of ≥60% being defined as a CAEV/MVV iELISA-positive serum sample, the sensitivity and the specificity of the CAEV/MVV iELISA were 74.0% (±7.6%) (95% confidence interval) and 98.3% (±2.0%), respectively, compared to the results of the radio-IP assay. Since the sensitivity was less than adequate, we reassessed the cutoff by calculating the mean value (in percent) ± 2 standard deviations for the radio-IP assay-negative serum samples. The results of that analysis placed the cutoff mean value at 33.1%. By using the new cutoff value, the sensitivity of the iELISA improved to 86.0% (±5.8%) and the specificity decreased slightly to 95.9% (±2.9%) compared to the results of the radio-IP assay. However, compared to the CAEV cELISA, which has a sensitivity of 98.6% and a specificity of 96.9% when the results of the radio-IP assay are used as the reference standard, the iELISA had a reduced sensitivity.

Since the sera were taken from a number of different U.S. sheep kept under different husbandry and management conditions, we also wanted to test the performance of the CAEV/MVV iELISA with sera from one flock in which the sheep are exposed to the same husbandry and management conditions. Sera from an Idaho sheep flock (n = 405) consisting of sheep of the Rambouillet, Polypay, and Columbia breeds ages 3, 4, 5, and 6 years were tested by the iELISA. The results were compared to those of the CAEV cELISA by using the new iELISA cutoff value of 33.1%, and the discrepant samples were analyzed by Western blotting with OPPV WLC1 and by previously published methods (2). The positive and negative concordances of the CAEV cELISA and the CAEV/MVV iELISA were 92.5% (±3.1%) and 99.3% (±1.4%), respectively. Eighteen of 20 CAEV/MVV iELISA-negative and CAEV cELISA-positive serum samples tested positive by Western blot analysis, and the 2 remaining discrepant serum samples tested negative by Western blot analysis. One CAEV/MVV iELISA-

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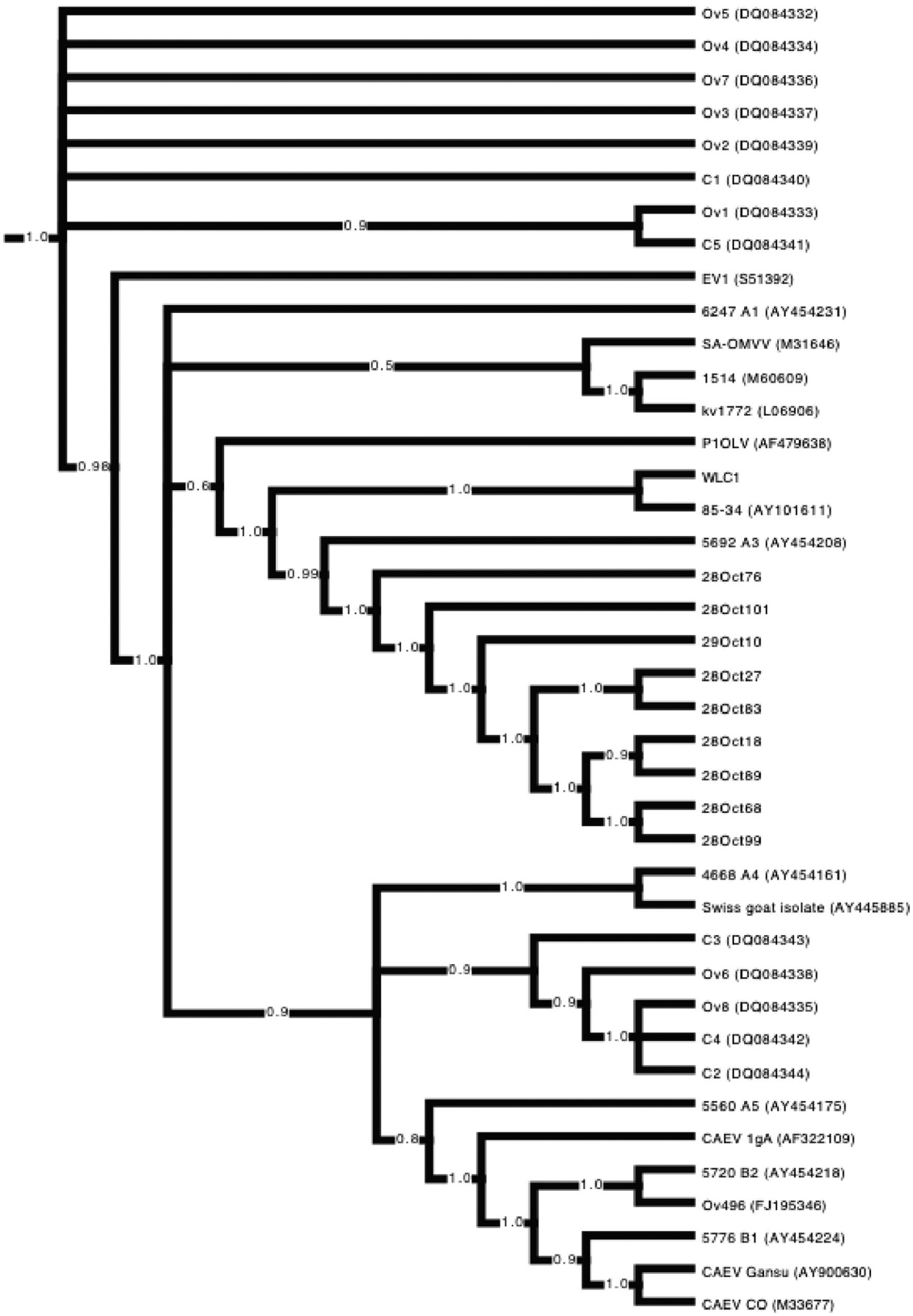

FIG. 1. A 50%-majority-rule consensus tree of SRLV *gag* nucleotide sequences obtained by Bayesian phylogenetic analyses with the general time-reversible model with invariant sites and a measure of the rate of heterogeneity. Consensus *gag* nucleotide sequences from Idaho sheep (sheep 28Oct76, 29Oct10, 28Oct101, 28Oct27, 28Oct83, 28Oct18, 28Oct89, 28Oct68, and 28Oct99) and a consensus *gag* nucleotide sequence for WLC1 were utilized and compared to the *gag* nucleotide sequences from other U.S. OPPV strains (85-34), MVV strains, and CAEV strains. The GenBank accession numbers are given in parentheses next to the name or identification number given in previous studies. The numbers on the branches represent the Bayesian posterior probabilities.

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positive and CAEV cELISA-negative sample tested negative by Western blot analysis. The 95% confidence interval for the positive and the negative concordances of the results of the CAEV/MVV iELISA relative to those of the CAEV cELISA for Idaho sheep and U.S. sheep overlapped (data not shown).

A difference in the limit of detection between the CAEV/MVV iELISA and the CAEV cELISA may be a major reason for the reduced sensitivity of the CAEV/MVV iELISA (86%) compared to that of the CAEV cELISA (98.6%) with sera from U.S. sheep. Sera require dilution 1:10 for testing by the CAEV/MVV iELISA, whereas undiluted sera are used for the CAEV cELISA. To test whether the limit of detection is greater for the CAEV cELISA than the CAEV/MVV iELISA, 15 Western blot analysis-positive, CAEV cELISA-positive, and CAEV/MVV iELISA-negative serum samples from the Idaho flock were diluted 1:10 and 1:50 with 1× phosphate-buffered saline, pH 7.5, and retested by the CAEV cELISA. Twelve of these 15 serum samples tested positive by the CAEV cELISA at a 1:10 dilution, and 7 of 15 tested positive by the CAEV cELISA at a 1:50 dilution. This indicates that the CAEV cELISA has a higher dilution limit for the detection of anti-SRLV antibodies than the CAEV/MVV iELISA with sera from U.S. sheep. This high dilution limit of detection is likely due to the format of the CAEV cELISA, in which this assay is reliant upon anti-OPPV serum antibodies to inhibit the binding of a peroxidase-labeled monoclonal antibody (monoclonal antibody 74A) to a single, specific epitope on the CAEV-63 surface envelope glycoprotein.

A previous report of 95.5% sensitivity and 97.2% specificity has been reported for the iELISA with sera from Swiss sheep (15). Large SRLV strain differences between Swiss MVV and U.S. OPPV strains may account for the lower sensitivity of the iELISA with sera from U.S. sheep. Therefore, *gag*, which encodes the capsid protein, was evaluated because it is a B-cell-immunodominant viral antigen in sheep naturally infected with MVV and OPPV and is more conserved than other viral genes (2, 7, 17). Unfortunately, no *gag* sequence is available for the Swiss MVV OLV (the strain used in the iELISA); however, several representative *gag* sequences from SRLV strains from Swiss goats and sheep that were previously reported for SRLV clades A1, A3, A4, A5, B1, and B2 were utilized (13). Genomic DNA was isolated from peripheral blood leukocytes from nine cELISA- and iELISA-positive Idaho sheep by previously described methods (6). The *gag* gene encoding the capsid was amplified by PCR with the following primers: primer *GAG*PSf (5′-TGG-CGA-CGC-AAG-GCT-CAA-A-3′) and primer *GAG*PSr (5′-GCG-GAC-GGC-ACC-ACA-CG-3′) (Integrated DNA Technology, Coralville, IA). The PCR mixture consisted of 100 ng of genomic DNA from sheep peripheral blood leukocytes or goat synovial membrane cells infected with WLC1, 2.5 mM_f (final concentration) $MgCl_2$, 0.2 mM_f deoxynucleoside triphosphates, and 1 U of *Taq* polymerase (Fisher Scientific, Pittsburg, PA). Primers *GAG*PSf and *GAG*PSr bind to nucleotides 734 to 752 and 2038 to 2054, respectively, of the sequence with GenBank accession number AY101611. The amplification conditions for the *gag* PCR were as follows: 95°C for 4 min, followed by 25 cycles of 95°C for 30 s, 58.1°C for 30 s, and 72°C for 2 min, followed by 72°C for 7 min and 4°C indefinitely. The *gag* PCR products were analyzed, cloned, and sequenced by previously described methods (5). The *gag* sequences were further refined by hand by using the Se-Al (version 2.0) program (http://tree.bio.edu.ac.uk/software/seal/), and a consensus *gag* sequence was generated from *gag* sequences from four sheep and OPPV WLC1.

For phylogenetic analysis, the appropriate model of nucleotide substitution was selected by using hierarchical likelihood ratio testing with the MrModelTest2 command block (11) executed in the PAUP program (14). A general time-reversible model (also called the GTR or REV model) (12) with invariant sites and a measure of the rate of heterogeneity determined by use of the gamma distribution was chosen for full Bayesian analysis by using the program MrBayes (8). A 50%-majority-rule consensus tree based on the *gag* sequences was constructed by using the Mesquite software package (10). The resulting Bayesian phylogenetic tree showed that the sequences from the Idaho sheep formed one clade with OPPV WLC1, OPPV 85/34, and a single Swiss goat SRLV strain (strain 5692 A3) with high posterior probabilities (0.99 to 1.0) (Fig. 1). The finding that three *gag* sequences from Swiss sheep are found in other clades aside from the U.S. sheep OPPV clade (6247 A1, 5720 B2, and 5776 B1) suggests that Swiss sheep SRLV strains are quite different from U.S. OPPV strains, and these differences contribute to the reduced sensitivity of the iELISA with sera from U.S. sheep compared to that achieved with sera from Swiss sheep.

This combination of information suggests that U.S. sheep maintain strains of small ruminant lentiviruses phylogenetically different (determined on the basis of the *gag* sequences) from those from most other parts of the world. This suggests that the creation and validation of one serological assay that is based on one viral strain and that is accepted worldwide might not be successful due to the diversity of SRLV strains worldwide (3, 9).

Nucleotide sequence accession numbers. Fifty-two partial *gag* nucleotide sequences from nine Idaho OPPV-infected sheep were assigned GenBank accession numbers GQ255381 to GQ255432. Four partial *gag* nucleotide sequences from goat synovial membrane cells infected *in vitro* with OPPV WLC1 were assigned GenBank accession numbers GQ255433 to GQ255436.

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