Short Communications

Use of a Recombinant Maedi-Visna Virus Protein ELISA for the Serologic Diagnosis of Lentivirus Infections in Small Ruminants

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ABSTRACT

Highly purified recombinant *gag* and *env* proteins derived from Icelandic strain 1514 of maedi-visna virus were used in an indirect enzyme immunoassay (ELISA) to detect antibodies to small ruminant lentiviruses in sheep and goat sera. The recombinant protein-based ELISA performed very well relative to whole maedi-visna virus and whole caprine arthritis-encephalitis-virus-based ELISAs in its ability to detect anti-maedi visna virus and anti-caprine arthritis-encephalitis virus antibodies, despite the antigenic and genomic variablity that is known to exist within and between these two small ruminant lentiviruses. The data suggest that these recombinant maedi-visna virus proteins can be reliably used in an ELISA for the routine serodiagnosis of lentiviral infections in sheep and goats.

RÉSUMÉ

Les protéines recombinantes purifiées *gag* et *env* dérivées de la souche islandaise 1514 du virus maedi-visna ont été utilisées dans une épreuve immuno-enzymatique indirecte (ELISA) afin de détecter, chez des moutons et des chèvres, des anticorps sériques dirigés contre les lentivirus des petits ruminants. Les résultats de l'épreuve ELISA effectuée à l'aide des protéines recombinantes se comparaient favorablement à ceux obtenus avec les virus du maedi-visna ou de l'arthrite-encéphalite caprine complets pour ce qui est de la détection d'anticorps dirigés contre le virus du maedi-visna et le virus de l'arthrite-encéphalite caprine, et ce malgré les variations antigéniques et génomiques connues existant à l'intérieur et entre de ces deux groupes de lentivirus. Les résultats laissent entrevoir la possibilité que ces protéines recombinantes du virus du maedi-visna pourraient être utilisées de façon fiable dans une épreuve ELISA pour le sérodiagnostic routinier des infections à lentivirus chez la chèvre et le mouton.

(Traduit par docteur Serge Messier)

Maedi-visna (MV), also known as ovine progressive pneumonia (OPP), and caprine arthritis-encephalitis (CAE) are lentiviral diseases of sheep and goats. MV occurs in most sheep producing countries with the possible exception of New Zealand and Australia. The first Canadian reports of MV came out of Quebec in 1974 (1), and Nova Scotia in 1978 (2). A Canadian serological survey conducted between March, 1988 and February, 1989, involving 14 047 sheep over one y of age from 286 randomly selected flocks, estimated the national seroprevalence at 19% and the mean flock prevalence at 12% (3). Infection of goats with CAEV also has a worldwide distribution. A number of serologic surveys for CAEV have been conducted within the United States. The most comprehensive of these involved 3790 goats from 28 states and revealed a seroprevalence to CAEV of 31% (4). Results from an unpublished serologic survey conducted in the early 1990s involving 4701 goats from 216 herds from across Canada estimated the Canadian seroprevalence at greater than 50%.

The cosmopolitan nature of these two pathogens is reflected in the many isolates of each that have been characterized. Examples are: the two North American isolates of CAEV, CO (5) and strain 63 (6), the Icelandic visna virus strain 1514 (7), the British MVV isolate EV1 (8), the South African ovine MVV SA-OMVV (9), and the New York ovine lentivirus isolate CU1 (10).

The CO strain of CAEV and Icelandic 1514 strain of MVV are 74.8%, 77.5%, and 60% homologous at the amino acid level in their *gag, pol*, and *env* proteins, respectively (5). In contrast, the 1514 strain of MVV and the South African strain SA-OMVV are 81.5%, 87%, and 80% homologous at the amino acid level in their *gag, pol*, and *env* proteins (5), while CAEV strains CO and 63 share 85% homology in their *env* proteins (11). The high degree of phylogenetic relatedness between MVV and CAEV demonstrated at the nucleic acid level is strengthened by immunologic data showing that CAEV and MVV have at least one epitope in common in each of their structural proteins (12,13).

Despite evidence supporting the view that these two viruses are closely related, significant viral antigenic variation can also occur within the same animal over time in CAEV and MVV infections (14,15). Consequently, concerns have been raised regarding antigenic and genomic variability and the effects these may have

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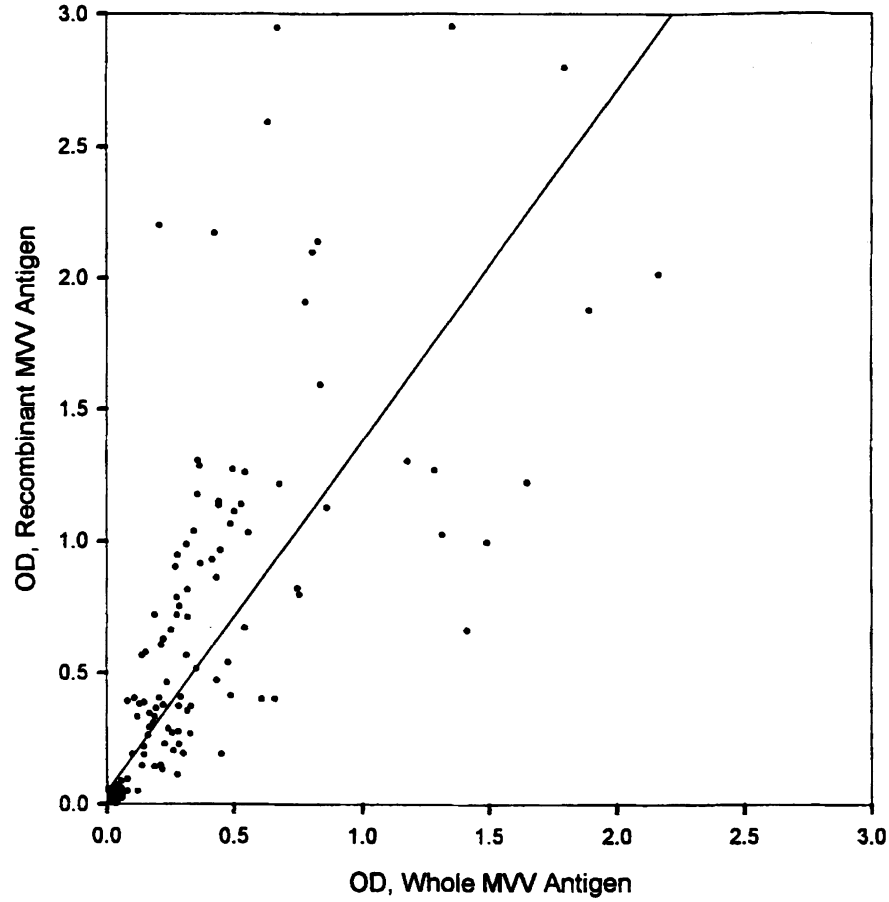

Figure 1. Scatter plot of ODs for 331 sheep sera tested by whole MVV antigen and recombinant MVV antigen ELISAs.

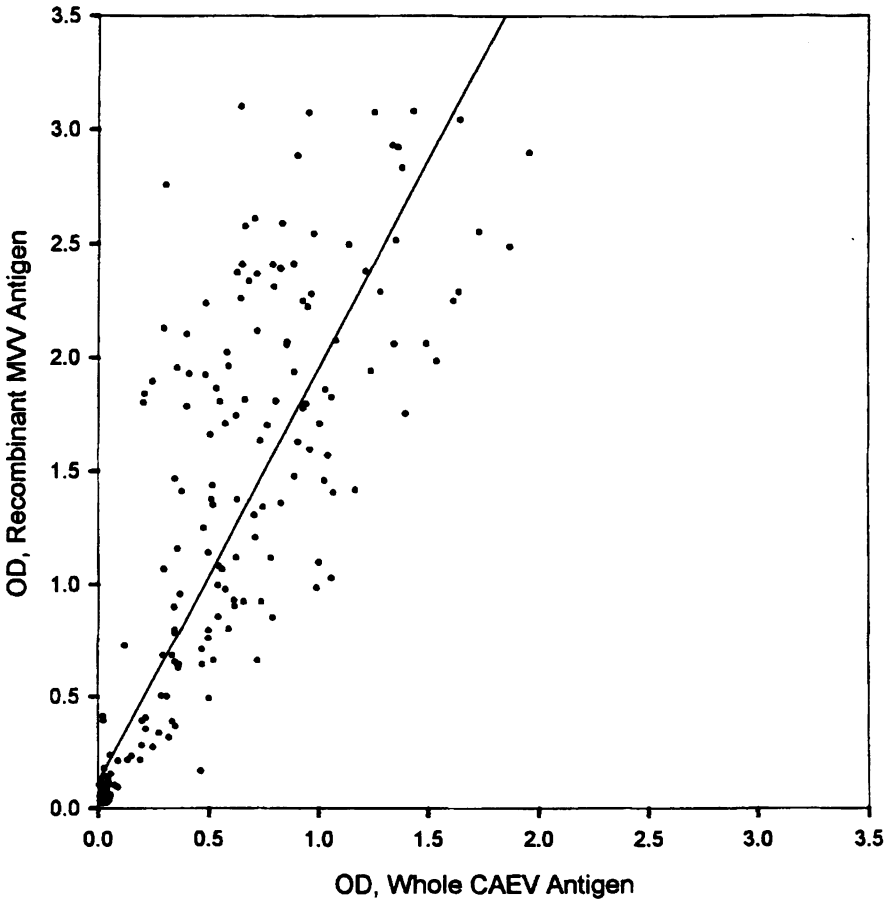

Figure 2. Scatter plot of ODs for 300 goat sera tested by whole CAEV antigen and recombinant MVV antigen ELISAs.

on the ability to diagnose small ruminant lentiviral infections. This report addresses the significance MVV and CAEV homology and antigenic variability may have on the ability to detect these infections by serological means.

An indirect enzyme-linked immunosorbent assay (ELISA), using a cocktail of recombinant MVV *gag* and *env* proteins (16), was used to test sheep and goat sera for antibodies against MVV and CAEV, respectively. These results were compared with those obtained using whole MVV antigen-based (17) and whole CAEV antigen-based (18) ELISAs.

The recombinant MVV antigens used in this study were in the form of glutathione S-transferase fusion proteins, purified by polyacrylamide gel electrophoresis under reducing and denaturing conditions, as described previously (16). They included a 159-amino acid fragment from the amino terminal portion of the transmembrane domain of *env*, and a 330-amino acid fragment of *gag* consisting of the carboxy terminal, 79% of the matrix protein p16, and all but the last three carboxy terminal amino acids of the capsid protein p25. The above viral sequences were PCR-amplified from LV1-KS1, an infectious molecular clone of Icelandic MVV strain 1514. The whole MVV and CAEV antigens were prepared as previously described (19) and employed a Kemptville, Nova Scotia isolate of MVV, grown on primary fetal lamb lung cells, and a Moncton, New Brunswick isolate of CAEV, grown on primary fetal lamb corneal cells. The primary cell cultures used for virus antigen production were derived from specific-pathogen-free (MVV, pestivirus, parainfluenza virus 3, *Coxiella burnetti*, and *Leptospira* spp.) sheep.

The optical density (OD) cutoffs for the recombinant MVV-antigen-based ELISA was established using 200 MV-negative sheep sera, tested 3 times. Based on this, samples were declared postive if they produced an OD > 0.116 in addition to having a

postive/negative OD ratio (PN ratio) > 2.82, suspect if they produced an OD > 0.083 but < 0.116 in addition to having a PN ratio > 2.82, and negative if they produced an OD < 0.083. The same panel was used to establish the OD cutoffs for the whole MVV antigen-based ELISA. The corresponding parameters in this case were: positive = OD > 0.072 and PN ratio > 1.95; suspect = OD > 0 .062 but < 0.072 and PN ratio > 1.95; negative = OD < 0.062. A 200-member, CAE-negative, goat serum panel tested 3 times was used in establishing the OD cutoffs of the whole CAEV-antigen-based ELISA. The corresponding parameters were: positive = OD > 0 .093 and PN ratio > 2.34; suspect = OD > 0.069 but < 0.093 and PN ratio > 2.34; negative = OD < 0.069. The negative goat panel was not, however, used to establish the OD cutoffs for goat sera on recombinant MVV-antigen-coated plates. In this case, the parameters that were established using the MV-negative panel on recombinant-antigen-coated plates were also used for determining the status of goat sera tested on recombinant-antigen-coated plates. Lastly, strong positive, weak positive, and negative control sera, in addition to a diluent blank, were run on each plate. Results from plates were accepted only if controls stayed within experimentally determined upper and lower control limits.

Sheep and goat sera submitted to the Retrovirology Centre of Expertise were tested by whole MVV antigen-based and recombinant MVV antigen-based indirect ELISAs and whole CAE-antigen-based and recombinant MVV-antigen-based indirect ELISAs respectively. The relative diagnostic sensitivities and specificities as well as the level of agreement between test pairs are summarized in Tables I and II. A kappa value of 0.97, indicating excellent agreement beyond chance between the recombinant and whole MVV-based ELISAs, was not surprising, since significant homology is known to exist among various MVV isolates (5,8,11,20). However, a kappa value of 0.86, indicating very good agreement between the recombinant MVV and whole CAEV-based ELISAs, was not anticipated, since greater amino acid sequence divergence exists between MVV and CAEV. Most of the disagreement occurred with sera that were classified as reactors when tested on recombinant MVV-antigen-coated plates but non-reactors when tested on whole CAEV-antigen-coated-plates. The majority of these were suspects or weak positives having ODs that ranged from 0.082 to a high of 0.394. Figure 1 is a scatter plot for the ODs of sheep sera tested on whole MVV-antigen-coated plates versus recombinant MVV-antigen-coated plates with a regression line drawn through the data. The correlation coefficient is 0.796, $P < 0.001$, N = 331. The scatter plot for the ODs of goat sera tested on whole CAEV-antigen-coated plates versus recombinant MVV-antigen-coated plates is shown in Figure 2. The corresponding correlation coefficient is 0.869, $P < 0.001$, N = 300. Both figures show that there is a fairly strong relationship between ODs of sera tested by whole virus and recombinant ELISAs. Further improvements in the level of agreement between whole CAEV and recombinant MVV ELISAs for serodiagnosing CAE in goats may have been possible if a CAE-negative goat serum panel had been used in establishing the negative OD cutoff on recombinant MVV-antigen-coated plates. Alternatively, receiver-operating-characterisitic (ROC) methods can be employed to determine the cutoff that will optimize the agreement between the two tests.

The most widely used serologic test for detecting caprine antibodies to CAEV is the agar gel immunodiffusion (AGID) test. An AGID test that is commercially available for serodiagnosing CAEV-infected goats (Veterinary Diagnostic Technology, Wheat Ridge, Colorado) uses ovine progressive pneumonia virus as the antigen. However it has been reported that a CAEV antigen based AGID test had a significantly higher sensitivity (0.91) and kappa value (0.908) relative to an immunoprecipitation assay than did an OPPV-antigen-based AGID test (sensitivity = 0.56 and kappa value = 0.536) for detecting anti-CAEV antibodies in goat sera (21). The conclusion reached from that study was that OPPV antigen is of limited value for use in CAEV serodiagnosis and that the most likely reason for the difference in sensitivity between the CAEV and OPPV AGID tests is the divergence of the *gag* and *env* gene products of the two viruses. The reason MVV antigens gave better results in this study is likely due to the fact that the indirect ELISA is a primary binding assay and is not dependent on the occurrence of complex secondary phenomena like precipitation, as is the case with the AGID test. Furthermore, it is probable that antibody binding to antigen in the case of recombinant MVV proteins likely involves linear rather than conformational epitopes since purification of these proteins takes place under reducing and denaturing conditions. Hence, it may be that linear epitopes within *gag* and the transmembrane portion of the *env* protein are highly conserved between CAEV and MVV explaining the observed cross-reactivity in the present study.

A number of ELISAs for the serodiagnosis of CAEV-infected goats have been reported in the literature. These assays have been based on antigen derived from CAEV-infected cell cultures (18,22,23), recombinant MVV gag antigen (20), recombinant CAEV *gag* and *env* antigens (24), and recombinant MVV major core protein p25 in combination with recombinant transmembrane protein gp40 (25). Although it has been established for some time that CAEV and MVV are

TABLE I. Performance of recombinant MVV antigen ELISA relative to whole MV virus antigen ELISA for the serodiagnosis of MVV infection in sheep

Recombinant MVV Antigen ELISA Result	Whole MV Virus ELISA Result		
	Reactor	Non-Reactor	Totals
Reactor	98	3	101
Non-Reactor	2	284	286
Totals	100	287	387

Relative Diagnostic Sensitivity = 98%
Relative Diagnostic Specificity = 98.8%
Kappa = 0.97
Data are from 387 sera from 22 flocks

TABLE II. Performance of recombinant MVV antigen ELISA relative to whole CEA virus antigen ELISA for the serodiagnosis of CAEV infection in goats

Recombinant Result	Whole CEA Virus ELISA		
MVV Antigen ELISA Result	Reactor	Non-Reactor	Totals
Reactor	231	25	256
Non-Reactor	5	187	192
Totals	236	212	448

Relative Diagnostic Sensitivity = 97.9%
Relative Diagnostic Specificity = 88.2%
Kappa = 0.86

antigenically related (12,13), the exact extent to which they are related has not been fully determined. The present study suggests that the degree of relatedness is sufficient to be successfully exploited in primary binding serologic assays like the indirect ELISA and confirms the work published by others (20,25,26).

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