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Detection of Antibodies to Caprine Arthritis-Encephalitis Virus by Protein G Enzyme-Linked Immunosorbent Assay and Immunoblotting

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Sera from goats suffering from caprine arthritis-encephalitis contained antibodies to virus proteins of 15, 17, 28, 40, and 130 kilodaltons in immunoblots of maedi-visna virus. We propose to use immunoblotting as a validation test for enzyme-linked immunosorbent assay and demonstrate that the specificity of indirect enzyme-linked immunosorbent assay can be improved by replacing second antibody by a protein G-avidin-biotin conjugate.

Various techniques are used for the serologic diagnosis of infection with caprine and ovine lentiviruses. These include agar gel precipitation (1, 5, 8), indirect enzyme-linked immunosorbent assay (ELISA) (4, 6), and ELISA based on a double-antibody sandwich blocking procedure (7). The main problems with all assays are validation and standardization. These must be done by independent methods such as radioimmunoassay (3). In this work we chose to use immunoblotting because this method has been successfully used in the validation of ELISA for human lentivirus infection (10, 12–14). Maedi-visna virus (strain ZZV 1050; donated by D. Houwers, Lelystad, The Netherlands) was grown on sheep plexus chorioideus cells and purified by precipitation with ammonium sulfate and density gradient centrifugation as described elsewhere (16). The pellet was suspended in phosphate-buffered saline after traces of sucrose were removed and was used as virus antigen. For preparation of control antigen, mock-infected cells and supernatant were frozen and thawed twice and sonified in a B-12 sonifier (Branson Sonic Power Co., Danbury, Conn.) before precipitation and centrifugation as previously described for virus antigen (16). All subsequent steps were carried out in parallel for virus and control antigen. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described elsewhere (9), with 12% polyacrylamide gels and Protean II minigel equipment (Bio-Rad Laboratories, South Richmond, Calif.). Proteins of maedi-visna virus were either visualized directly by the silver staining method of Morrissey (11) or were transferred to nitrocellulose essentially as described previously (15), with a Trans-Blot electrophoretic transfer cell (Bio-Rad) at 100 V for 1 h in a buffer composed of 25 mM Tris, 192 mM glycine, 0.01% sodium dodecyl sulfate, and 20% ethanol (pH 8.5). The nitrocellulose was then cut into strips 0.2 cm wide, saturated in washing buffer (10 mM Tris hydrochloride, 500 mM NaCl, 0.5% Tween 20, 0.01% Merthiolate, 1% [wt/vol] skim milk [pH 8.0]) for 1 h at room temperature, washed, and subsequently incubated for 3 h with goat serum diluted 1:50 in washing buffer. After being washed, the strips were incubated for 90 min with an alkaline phosphatase-conjugated swine anti-goat immunoglobulin G antibody (heavy and light chain specific; Tago; Medac, Hamburg, Federal Republic of Germany), washed, and stained with fast red TR-salt and naphthol AS-MX-phosphate (0.3 and 0.05% [wt/vol], respectively; Sigma Chemical Co.) in 200 mM Tris hydrochloride (pH 8.2).

The pattern of maedi-visna virus proteins recognized by goat antibody was established by using sera obtained from goats suffering from carpitis. Infection with caprine arthritis-encephalitis virus in these goats was verified by the demonstration of antibodies cross-reactive with maedi-visna virus antigen in ELISA (4). The partially purified virus preparation shown in Fig. 1, lane 1, contains a multitude of proteins in a pattern similar to that of the control obtained from uninfected cells (lane 2). It can also be estimated from lanes 1 and 2 that similar amounts of protein are present in the two preparations. Only a minority of these proteins are of viral origin (lanes 3 and 4). In particular, immune serum contained antibodies that bound to proteins of 15, 28, 40, and 130

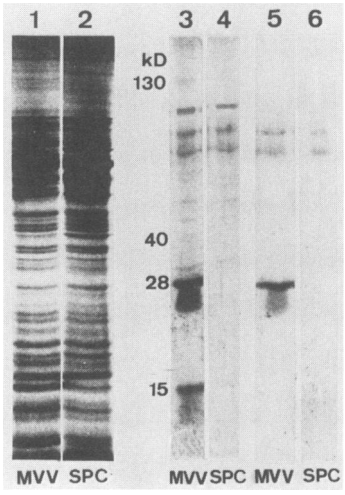

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of maedi-visna virus antigen and immunoblots of sera from caprine arthritis-encephalitis virus-infected goats. For technical details see the text. Lanes: 1 and 3, proteins of maedi-visna virus (MVV) antigen preparation visualized with the silver staining method and reacted with serum from a goat infected with caprine arthritis-encephalitis virus, respectively; 2 and 4, control antigen prepared from uninfected sheep plexus chorioideus (SPC) cells treated as for lanes 1 and 3; 5, immunoblot of virus antigen treated with Triton X-114; 6, control antigen treated with Triton X-114.

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TABLE 1. Seroconversions of seven colostrum-deprived goats from negative to equivocal or positive in ELISA and in immunoblotting

Goat no.	Age (mo)	Result by[a]:	
		ELISA	Immunoblot
1	6	−	+
	11	±	+
2	8	−	−
	13	−	+
	22	±	ND
3	8	−	−
	13	+	+
4	9	−	+
	15	+	+
	22	+	+
5	36	±	+
	41	±	+
6	36	−	−
	42	−	−
	49	±	+
	54	+	+
7	?[b]	±	+
	+6[c]	+	+

[a] Reactions: −, negative; ± equivocal; +, positive; ND, not done.
[b] Age of goat 7 was unknown at first testing.
[c] Six months after first testing.

kilodaltons (kDa). When ELISA-positive sera from sheep (infrequently also goat) were used, one additional protein of 17 kDa could be demonstrated (not shown). Treatment of partially purified virus with Triton X-114 resulted in the loss of the 15-kDa protein in immunoblots. This indicates that this protein is hydrophobic and suggests that it may be a matrix protein (compare lanes 3 and 4 with 5 and 6, respectively).

The pattern of proteins recognized showed some variance between individual serum specimens. Of 10 ELISA-positive specimens obtained from clinically affected goats, 7 were found to react with the 15-, 28-, 40-, and 130-kDa proteins. Two serum specimens showed no reaction with the 15-kDa protein, and one serum failed to bind to the 40-kDa protein. To assess the sensitivity of immunoblotting, we chose 10 ELISA-negative serum specimens from goats with carpitis and sera of 7 colostrum-deprived goats which seroconverted in ELISA in a time range of 7 to 49 months postpartum. Of the 10 ELISA-negative specimens from goats with carpitis, 5 were recorded as positive in immunoblotting. Two of these contained antibodies to the 28-, 40-, and 130-kDa proteins, two were positive for the 28- and 130-kDa proteins, and one had antibodies reacting with the 15-kDa protein only. Of the seven animals whose sera converted from negative to equivocal or positive, six could be diagnosed as antibody positive by immunoblotting at a time when they still showed negative or equivocal results in ELISA (Table 1). Again, the reactions to individual viral proteins were not uniform, one serum reacting with the 28-, 40-, and 130-kDa proteins, two reacting with the 28-kDa protein only, and one reacting with the 15-kDa protein only (immunoblots not shown). None of these sera reacted with the virus-free control coat. However, we occasionally observed that certain other sera bound to a protein of 40 kDa present on the virus-free control coat, indicating that a sole reaction with a protein of 40 kDa on the virus antigen may not be taken as evidence of infection.

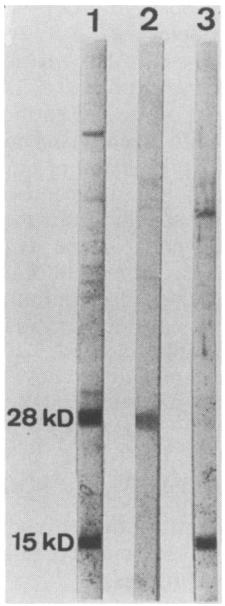

FIG. 2. Immunoblots of goat sera with maedi-visna virus antigen showing representative reaction patterns to virus proteins. For details, see the text.

The previous experiments had established that the immunoblot method was more sensitive than ELISA and that it allowed the recognition of nonspecific reactions, thus minimizing false-positive results. This allowed us to test the possibility of using immunoblotting as a validation test for the ELISA used in routine diagnostic work. This ELISA is essentially based on the protocol proposed by Houwers et al. (6). We selected 100 serum specimens, 20 of which were either negative or positive and 60 of which were equivocal in ELISA. Of the 100 specimens, 54 were recorded as positive, mainly because of clear binding to the 28- and 15-kDa proteins. Of these 54 specimens, 28 reacted with both proteins, 23 reacted with the 28-kDa protein only, and 3 reacted with the 15-kDa protein only (see Fig. 2 for three representative patterns). All ELISA-positive sera were also positive by immunoblotting, and concordant results were obtained also with all ELISA-negative sera. An important point, however, is that the large group of sera that showed equivocal results in ELISA was clearly resolved in immunoblotting: 34 serum specimens were found to be positive and 26 were found to be negative.

Preliminary experiments had indicated that the performance of the ELISA could be improved by using a protein G-biotin-avidin–biotin-peroxidase conjugate instead of a rabbit anti-goat immunoglobulin G-peroxidase conjugate in the detection of goat antibody bound to the ELISA plates. Protein G has been shown to bind to ruminant immunoglobulin G with high avidity (2). Of the 54 blot-positive serum specimens, 20 were positive in ELISA when a second antibody was used as the detection system compared with 33 when protein G was used. Of the 46 blot-negative specimens, 20 were negative in ELISA when a second antibody was used compared with 38 when protein G was used. An important point is that the number of serum specimens that gave equivocal results could be reduced from 60 to 28. Four

specimens of the blot-positive group were ranged negative with the protein G system.

Taken together, our results demonstrate that immunoblotting is useful in resolving equivocal ELISA results and that using protein G instead of second antibody in the detection of goat antibody bound to maedi-visna antigen on the ELISA plates may reduce the proportion of equivocal results. The double-antibody sandwich assay proposed by Houwers et al. (7) is potentially more specific than the protein G ELISA described here but depends on the availability of suitable monoclonal antibodies. Immunodiffusion and indirect ELISA protocols may therefore continue to be useful in the serologic diagnosis of caprine arthritis-encephalitis when combined with immunoblotting.

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ADDENDUM IN PROOF

An immunoblot analysis of the antibody response of sheep to maedi-visna virus was recently completed (D. J. Houwers et al., Vet. Microbiol., in press).

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