Competitive Inhibition Enzyme-Linked Immunosorbent Assay for Antibody in Sheep and Other Ruminants to a Conserved Epitope of Malignant Catarrhal Fever Virus

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Malignant catarrhal fever (MCF) is a severe, usually fatal, acute systemic disease syndrome of certain domestic and wild ruminants caused by members of the family Gammaherpesvirinae. Two distinct but closely related viruses cause clinically indistinguishable syndromes: one that is indigenous to the wildebeest and the other that apparently is indigenous to domestic sheep. Neither the pathogenesis nor the epidemiology of sheep-associated MCF (SA-MCF) is understood, primarily because of a lack of adequate detection methods for the etiologic agent or antibody against it. No acceptably documented isolates of SA-MCF virus have been reported, and existing antibody assays suffer from significant cross-reactivity with other viruses. As a basis for a specific serologic assay, an attempt was made to identify an epitope conserved among all isolates of MCF viruses, by using a monoclonal antibody (MAb) produced against a previously reported U.S. isolate of MCF virus. A MAb (15-A) which bound a conserved epitope present on all four isolates of MCF virus examined was found. MAb 15-A did not react with eight common sheep and goat viruses or five common bovine viruses. Immunoprecipitation revealed that the 15-A epitope was located on the viral glycoprotein complex, with molecular masses of 115, 110, 105, 78, and 45 kDa. Sera from experimentally and naturally infected animals which yielded a similar glycoprotein complex immunoprecipitation pattern competed with MAb 15-A for its epitope. A competitive inhibition enzyme-linked immunosorbent assay (ELISA) based on MAb 15-A was therefore developed. The assay detected antibody in infected infected sheep and in cattle, deer, and bison with clinical MCF. Of the 149 serum samples from sheep associated with MCF outbreaks, 88 (55%) were seropositive by competitive inhibition ELISA.

Malignant catarrhal fever (MCF) is a frequently fatal lymphoproliferative and inflammatory disease syndrome of certain domestic and wild ruminants caused by closely related members of the family Gammaherpesvirinae (3). Two major epidemiologic forms of MCF, defined by the reservoir ruminant species from which the causative virus arises, are recognized. One, known as the African form, is referred to as wildebeest-associated MCF (4, 32, 33). The etiology is a relatively well-characterized lymphotropic gammaherpesvirus, named alcelaphine herpesvirus 1 (AHV-1) (37), which is indigenous to the wildebeest (subfamily Alcelaphinae) to which it is well adapted and nonpathogenic.

The other major, worldwide source of an MCF agent is thought to be domestic sheep. Though never successfully propagated in vitro, there is substantial epidemiologic and serologic evidence that a virus closely related to AHV-1 exists in sheep and is transmitted, often at lambing, to susceptible, poorly adapted ruminants such as domestic cattle, deer, and bison (29, 31, 35). Sheep-associated malignant catarrhal fever (SA-MCF) is one of the few known infectious diseases for which an etiologic agent has never been isolated. Moreover, experimental transmission of MCF to cattle by inoculation of material from suspect sheep has been almost uniformly unsuccessful (8, 15). However, recent demonstration of SA-MCF virus (SA-MCFV) DNA sequences with homology to Epstein-Barr virus and herpesvirus saimiri in leukocytes from normal sheep and SA-MCF-affected cattle (2) strengthens the notion that sheep are indeed carriers of a gammaherpesvirus. So far, productive replication in vitro from infected sheep leukocytes has not been achieved, which suggests that sheep cells may not be highly permissive. Restricted gene expression in lymphocytes is a common feature of the gammaherpesviruses, e.g., Epstein-Barr virus (18), herpesvirus saimiri (9), and Marek's disease virus (40).

MCF usually is sporadic but occasionally occurs epizootically, causing significant losses (5, 28, 30, 36). The lack of available confirmed sheep-associated isolates has hindered development of efficient reagents for diagnosis and control of SA-MCF. The identification of MCFV-infected sheep currently is based on the detection of antibodies to AHV-1, the wildebeest isolate (14, 26, 41). Only low titers of neutralizing antibody to AHV-1 are found in infected sheep (11, 38), limiting the utility of the neutralization test. Indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA), using AHV-1-infected cells as antigens, are the tests currently in most common use (14, 38, 41). Their accuracy is also limited, however, by the fact that MCFVs share antigens with other bovine herpesviruses (13, 23). Thus, insensitivity and nonspecificity seriously limit the usefulness of the currently available tests for studying the epidemiology, pathogenesis, and control of SA-MCF (11, 12, 22). We report here a test for MCFV antibody, based on a single epitope shared by all MCFV isolates but no other ruminant viruses that we have examined to date, and describe its use in the detection of MCFV antibody in sheep and other ruminant species.

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MATERIALS AND METHODS

Viruses and viral antigens. The Minnesota isolate of MCFV, originally derived from a cow involved in a sheep-associated MCF outbreak in Minnesota (10) (herein designated MN-MCFV), WC-11 (cell culture passaged strain of AHV-1), and fetal mouflon sheep kidney (FMSK) cells were kindly provided by W. Heuschele, Center for Reproduction of Endangered Species, Zoological Society of San Diego, San Diego, Calif. Bovine herpesvirus 1 (BHV-1) (LA strain, ATCC VR-188), BHV-4 (DN-599, ATCC VR-631), and bovine turbinate cells (ATCC CRL-1390) were obtained from the American Type Culture Collection (Rockville, Md.). Bovine viral diarrhea virus, parainfluenza virus 3, ovine syncytial virus, bovine respiratory syncytial virus, ovine adenoviruses 5 and 6, ovine herpesvirus 1, and caprine herpesvirus 1 were kindly supplied by J. Evermann, Washington Animal Disease Diagnostic Laboratory, Pullman. The MN-MCFV was propagated on FMSK cells at the 16th to 22nd passage level in high glucose Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum. All other viruses were produced in bovine turbinate cells grown in DMEM with 10% fetal bovine serum. MCFV antigens were prepared by infection of FMSK cell monolayers in 900-cm² roller bottles at a multiplicity of infection of 0.1. When 80 to 90% of the cells exhibited cytopathic effect, the supernatant was harvested and clarified by centrifugation at 4,300 × g for 30 min, and virus was pelleted at 125,000 × g for 90 min through a 2-cm 35% (wt/wt) sucrose column. The pellets were resuspended in phosphate-buffered saline (PBS), sonicated, and stored at -20°C.

Sera. During 1992 and 1993, serum samples were obtained from sheep, cattle, and deer in seven states that had been associated with outbreaks of MCF (see Table 2), diagnoses having been established by clinical signs and pathologic lesions. MCFV antibody-negative sheep sera were obtained from 5- to 6-month-old lambs in a local farm flock in Pullman, Wash. Antisera to MN-MCFV were raised in rabbits by immunization with MN-MCFV antigen prepared as described above. Rabbit antiserum against the WC-11 strain of AHV-1 was purchased from a commercial source (Cytimmune, San Diego, Calif.). Sera from naturally infected wildebeest and other exotic ruminants were supplied by W. Heuschele. Sera from sheep experimentally inoculated with a U.S. isolate of AHV-1 (4, 41) were kindly provided by A. Castro, Pennsylvania State University. Bovine antisera to MCFV 732, a putative SA-MCFV isolate from Austria (39), and to BHV-1, BHV-2, BHV-4, and bovine viral diarrhea virus were kindly provided by J. Pearson, National Veterinary Service Laboratory, USDA Animal and Plant Health Inspection Service, Ames, Iowa. Antisera to CAEV and OPPV were obtained from the Washington Animal Disease Diagnostic Laboratory, Pullman. Antisera to ovine herpesvirus 1 and caprine herpesvirus were raised in mice by inoculation of virus propagated on sheep kidney cells and purified by sucrose gradient centrifugation as described above.

Radioimmunoprecipitation. Radioimmunoprecipitation of labeled viral proteins was performed as previously described (20). Briefly, monolayers of FMSK cells were infected with MN-MCFV at a multiplicity of infection of 3.0. [35S]Methionine or [3H]glucosamine (40 µCi/ml; New England Nuclear, Boston, Mass.) was added to the medium, and the cultures were harvested at 90% cytopathic effect. For radioimmunoprecipitation, labeled cell lysates were mixed with polyclonal antisera or monoclonal antibodies (MAbs), and the antigen-antibody complexes were allowed to bind to recombinant protein G-coated beads (GENEX Co., Gaithersburg, Md.). The beads were then precipitated and washed, bound complexes were solubilized, and the solubilates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

IIF assay. FMSK cells infected at a multiplicity of infection of 0.5 with MN-MCFV were harvested at 24 h postinfection. Bovine turbinate cells infected with other viruses were harvested at 50% cytopathic effect. Harvested cells were washed with PBS, dried onto glass slides, and fixed with 100% acetone at 25°C for 5 min. Thirty microliters of twofold dilutions of antisera or MAbs was layered onto the cells and incubated at 37°C for 1 h. After the cells were washed with PBS, 30 µl of a predetermined optimal dilution of commercial recombinant protein G conjugated with fluorescein isothiocyanate (Zymed Lab, Inc., San Francisco, Calif.) was added to each well and incubated at 37°C for 1 h. Uninfected control cells were stained in the same manner.

CI-ELISA. A panel of MAbs to MN-MCFV was produced and characterized as described elsewhere (21). MAbs with high activity in ELISA were evaluated for their ability to compete for their epitopes with antibodies from sheep seropositive for MCFV as defined by IIF and radioimmunoprecipitation. Competitive inhibition ELISA (CI-ELISA) was performed by using a modification of a previously described procedure (1, 19). Ninety-six-well polystyrene plates (Immulon 4; Dynatech Lab., Inc., Chantilly, Va.) were coated at 4°C for 18 to 20 h with 0.25 µg of the above-described MCFV antigen in 50 mM carbonate-bicarbonate buffer (pH 9.6) per well. Control wells were coated with the proteins from uninfected FMSK cultures subjected to the same purification procedure. After the wells were blocked with 20% nonfat milk in PBS at 25°C for 2 h and washed with PBS with 0.1% Tween 20, 250 µl of dilutions of test serum and 50 µl of MAb (0.2 µg) were added and incubated at 25°C for 1 h. The wells were washed three times with PBS-Tween 20 and incubated with alkaline phosphatase anti-mouse immunoglobulin G (Sigma, St. Louis, Mo.) at 25°C for 1 h. After a final wash, 50 µl of substrate buffer containing 1 mg of p-nitrophenyl phosphate (Sigma) per ml was added, the mixture was incubated, and the optical density at 414 (OD414) was determined. Serum samples were run in triplicate. Negative control sera were defined by an absence of MCFV antibody detectable by IIF and immunoprecipitation. When the mean of the three replicate OD readings of a serum was more than 3 standard deviations below the mean of a panel of six negative control serum samples, run in triplicate, the serum was considered positive for MCFV antibody.

RESULTS

Identification of an epitope conserved among isolates of MCFV. Sixty-four MAbs with high reactivity in ELISA were selected and tested against sheep sera by CI-ELISA. MAb 15-A was inhibited by sera from sheep that tested positive for MCFV antibody in IIF and immunoprecipitation. MAB 15-A of isotype immunoglobulin G2b, when used in immunoprecipitation, bound an epitope located on a glycoprotein complex which, on reducing gels, resolved into five bands with apparent molecular masses of 115, 110, 105, 78, and 45 kDa (Fig. 1A). MAb 15-A identified only a 45-kDa protein in immunoblots (Fig. 1B).

Figure 2 shows viral proteins of MN-MCFV which reacted in immunoprecipitation with sera from wildebeest, sheep, cattle, and deer experimentally or naturally infected with a variety of isolates of MCFV. The predominant proteins recognized by these sera were a single 130-kDa protein; a complex of proteins with molecular masses of 115, 110, 105, 78, and 45 kDa; and a single 110-kDa protein which comigrated with the 110-kDa moiety of the complex. To identify a conserved epitope, sera
from the animals listed above that were positive for MCFV by immunoprecipitation were examined for their ability to block the binding of MAb 15-A in CI-ELISA. MAb 15-A was efficiently inhibited by antisera against a variety of MCFV isolates of both wildebeest and sheep origin but was not significantly inhibited by the preinoculation sera from the same animals or from other un inocculated control animals of the same species (Fig. 3).

**Specificity of MAb 15-A for MCFV.** To provide evidence of the specificity of MAb 15-A, rabbits were immunized by intramuscular injection with purified MN-MCFV with Titer-Max adjuvant (Vaxcel, Inc., Norcross, Ga.) and tested for antibody by CI-ELISA. Sera from these rabbits competed effectively with MAb 15-A in CI-ELISA, in contrast to sera from the rabbit immunized with uninfected FMSK cell antigen (Fig. 4). Moreover, sera from rabbits immunized with the

FIG. 1. (A) Immunoprecipitation of [35S]methionine-labeled proteins of MN-MCFV-infected (lanes V) or uninfected (lanes C) cell lysate with MAb 15-A (lanes labeled 1) and isotype MAb control (lanes labeled 2). (B) Immunoblotting of purified MN-MCFV (lanes V) and FMSK cell (lanes C) antigens with MAb 15-A (lanes labeled 3) and isotype MAb control (lanes labeled 4). Molecular mass markers (in thousands) are indicated on the left.

FIG. 2. Immunoprecipitation of [35S]methionine-labeled proteins of MN-MCFV-infected (lanes V) or uninfected (lanes C) cell lysate with antisera (lanes labeled +) and preinoculated (lanes labeled −) or negative control sera (−/−) from the following animals inoculated or infected with different MCFV isolates: a rabbit immunized with AHV-1 (strain WC-11) (lanes 1), a rabbit immunized with MN-MCFV (lanes 2), a calf inoculated with a putative SA-MCFV isolate from Austria (MCFV 732) (lanes 3), sheep experimentally inoculated with a U.S. isolate of AHV-1 (lanes 4), wildebeest naturally infected with MCFV (lanes 5), deer with clinical SA-MCF (lanes 6), and sheep naturally infected with MCFV (lanes 7). Molecular mass markers (in thousands) are indicated on the left.

FIG. 3. The conservation of 15-A epitope among MCFV isolates. Expressed as the percentage of inhibition in CI-ELISA by sera from a rabbit immunized with AHV-1 (strain WC-11) (Ra), sheep experimentally inoculated with a U.S. isolate of AHV-1 (Sh), a calf inoculated with a putative SA-MCFV isolate from Austria (MCFV 732) (Bo), deer with clinical SA-MCF (Dr), and wildebeest naturally infected with MCFV (Wb). See Materials and Methods for serum sources.

WC-11 strain of AHV-1, from sheep inoculated with a U.S. isolate of AHV-1 (4, 43), and a calf infected with a putative SA-MCFV isolate (MCFV 732) from Austria, which has recently been reported (39), strongly competed with the MAb 15-A in CI-ELISA (Fig. 3). When tested by IIF and CI-ELISA, MAb 15-A did not react with any of eight common sheep and goat viruses or five common bovine viruses (Table 1).

**Detection of MCFV antibody in sheep and other ruminants.** Sera from 12 sheep that were seronegative by IIF and immunoprecipitation yielded an OD value of 0.8 ± 0.05 (mean ± standard deviation) by CI-ELISA, representing approximately 20% inhibition of the OD of MAb 15-A alone. The mean OD of these 12 negative serum samples, as well as the MAb isotype control of irrelevant specificity, yielded an OD value of ≤0.1. Comparison of both CI-ELISA ODs and immunoprecipitation patterns of seronegative lambs 5 to 6 months of age and 36 seronegative adult sheep revealed no significant differences between the two age groups. Of 149 serum samples collected from sheep associated with MCF outbreaks in seven states, 88
TABLE 1. Reactivity of MAb 15-A with ovine, caprine, and bovine viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>IIF reactivity to:</th>
<th>CI-ELISA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immune serum 15-A</td>
<td>MAb</td>
</tr>
<tr>
<td>Ovine herpesvirus 1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Caprine herpesvirus 1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>OPPV</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CAEV</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ovine adenovirus 5</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Ovine adenovirus 6</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Ovine respiratory syncytial</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>virus 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovine parainfluenza virus 2</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>BHV-1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>BHV-2</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td>BHV-4</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Bovine viral diarrhea virus</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Bovine respiratory syncytial</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN-MCFV</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MCFV 732</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AHV-1 strain WC-11</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Sera negative for MCFV had less than 35% inhibition of MAb 15-A.
* The IIF titer of all antisera exceeded 1:500.
* MAb 15-A negative at 40 μg/ml; MAb 15-A positive at 0.002 μg/ml.
* ND, not done.
* Putative SA-MCFV from Austria.

were positive by CI-ELISA, inhibiting the test by >3 standard deviations more than the mean inhibition value for the negative controls. All sera positive by CI-ELISA were also positive by IIF. The converse, however, was not true: some serum samples that were positive by IIF were negative by CI-ELISA (Table 2). Of 65 lambs tested that were less than 1 year old, only 2 were seropositive by CI-ELISA, whereas a high percentage of sheep over 1 year old were seropositive (Table 3). The test detected antibody in six of eight cows, two of three deer, and two bison with clinical MCF.

The sensitivity of CI-ELISA was comparable to that of IIF. Antibody titers of sera from sheep experimentally infected with a U.S. isolate of AHV-1 (4, 41) as measured by the two assays were not significantly different (Fig. 5). Moreover, when samples were compared at only a single dilution, the percentage inhibition in CI-ELISA was generally proportional to the immunofluorescent staining intensity (data not shown). Careful optimization of the coating antigen concentration significantly improved the sensitivity of the assay (data not shown). The concentration of the MAb, however, was less critical, suggesting that it might have a low binding affinity (16).

TABLE 2. Seroprevalence of MCFV antibody in sheep associated with MCF outbreaks

<table>
<thead>
<tr>
<th>Species clinically affected</th>
<th>Location of outbreak</th>
<th>No. of sheep tested</th>
<th>No. positive by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CI-ELISA</td>
</tr>
<tr>
<td>Cattle</td>
<td>California</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Cattle</td>
<td>Georgia</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Cattle</td>
<td>North Dakota</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Cattle</td>
<td>Wyoming</td>
<td>48</td>
<td>19</td>
</tr>
<tr>
<td>Deer</td>
<td>North Dakota</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Deer</td>
<td>Tennessee</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Deer</td>
<td>Washington</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td>Bison</td>
<td>Kentucky</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>149</td>
<td>88</td>
</tr>
</tbody>
</table>

TABLE 3. The relationship between age of sheep and presence of MCFV antibody by CI-ELISA

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>65</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1–3</td>
<td>57</td>
<td>46</td>
<td>81</td>
</tr>
<tr>
<td>&gt;3</td>
<td>49</td>
<td>45</td>
<td>92</td>
</tr>
</tbody>
</table>

**DISCUSSION**

MCF is a very complex syndrome that, in spite of many studies over more than 50 years, is only superficially understood. The sheep-associated form, in particular, has eluded efforts to define its etiology, epidemiology, and pathogenesis. Though considerable evidence has accumulated implicating domestic sheep as the reservoir, no confirmed isolate yet exists and Koch's postulates have not been fulfilled. Its usually sporadic nature, obscure transmission patterns, and lack of specific diagnostic tests for inapparent carriers have hindered development of control measures for the disease. The design of this study was to identify an epitope specific for MCFV and conserved among all of its known isolates, of both sheep and wildebeest origin, and develop a seroassay based on the specificity of a MAb for that epitope.

The data of this report characterize a murine immunoglobulin G2b (MAb 15-A) that defines a conserved carbohydrate-dependent epitope on MCFV. This epitope was present in all isolates of MCFV tested but was not present in 13 common sheep and cattle viruses.

Parallel testing of 149 serum samples from sheep that had been in contact with cattle, deer, and bison during MCF outbreaks revealed 83% concordance between CI-ELISA and IIF. The number of seropositive sheep detected by IIF was always equal to or greater than the number detected by CI-ELISA. Sharing of antigens between MCFV and other ruminant herpesviruses, such as BHV-1, BHV-2, and BHV-4 and goat herpesvirus 1, is well-known (13, 23). Cross-reactivity between MCFV and viruses such as these apparently is responsible for the greater number of sheep scored seropositive by IIF than by CI-ELISA. Another source of nonspecificity potentially affecting an immunofluorescence assay is binding of antibodies to Fc receptors on herpesvirus-infected cells, as has

FIG. 5. Comparison of sensitivities of CI-ELISA and IIF (inset) in detection of specific antibody in sera from sheep experimentally inoculated with a U.S. isolate of AHV-1 (A and B) and control (C).
been shown with herpes simplex virus (42, 43) and human cytomegalovirus (17, 34).

Two hundred forty-seven of 555 (44.55%) sheep serum samples collected from seven different states were positive by CI-ELISA. One hundred forty-nine of the 555 samples were from sheep known to have been associated with MCF outbreaks in cattle, deer, or bison. Eighty-eight of these 149 serum samples (55%) were positive. This relatively high percentage of seropositivity in sheep is consistent with earlier studies based on IIF and indirect ELISA, in which infection rates ranging from 22 to 97% were reported (22, 23, 38, 41). The wide range may reflect differences in the ages of sheep examined, as well as the relatively low specificities of these assays. In the present study, a very low rate of seropositivity was present for lambs under 1 year of age. It was much higher for sheep 2 years of age and older. A better definition of the relationship between seropositivity and age requires examination of a larger set of sequentially collected samples, which is underway.

The pattern suggested by the data herein, perhaps surprisingly, contrasts with that which is usually seen in wildebeest calves infected with AHV-1, apparently at birth (25, 31). The infected calves shed virus into the environment, serving as an important source of disease transmission (27). Neutralizing antibody to AHV-1 is usually detectable in wildebeest calves by 3 months of age (24), after which shedding ceases. The seroconversion pattern in sheep, as suggested by this study, is reminiscent, however, of that of natural infections of squirrel monkeys with herpesvirus saimiri, another gammaherpesvirus, wherein the young are infected not at birth but sometime after 12 months of age. By 2 years of age, virtually all young squirrel monkeys born of persistently infected mothers have seroconverted (6, 7). Whether the pattern in sheep reflects delayed infection or a delayed immune response to an earlier infection remains to be determined.

Only limited numbers of serum samples were examined from other ruminant species, both domestic and wild. A seroprevalence rate generally resembling that found in domestic sheep was found in domestic goats and bighorn sheep. In contrast, other ruminant species, such as llamas and mountain goats, were found not to exhibit significant rates of seropositivity (data not shown). Preliminary studies have found significant concordance between CI-ELISA and detection of the agent by PCR, using primers derived from a recently reported sequence of an SA-MCFV-specific DNA fragment (2). Studies to better define the epidemiologic patterns of this agent in various wild and domestic ruminant species are currently ongoing.

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