Detection of Antibody to Alcelaphine Herpesvirus-1 by ELISA in Cattle with Sheep-Associated Malignant Catarrhal Fever

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Summary

An enzyme-linked immunosorbent assay (ELISA) and a serum-virus neutralization (SVN) test were used to measure antibody specific for the alcelaphine herpesvirus-1 of malignant catarrhal fever (MCF) in sera from healthy cattle and cattle with clinical signs of sheep-associated MCF. Of 128 cattle associated with outbreaks of MCF in California and Colorado, 30 were seropositive for MCF by ELISA but seronegative by the SVN test. In cattle sera from 47 experimental and clinical cases of MCF in Colorado, 11 were seropositive for MCF by ELISA and 4 had low levels of virus neutralizing (VN) antibody. Seroconversion was demonstrated in 3 paired sera from cattle experimentally infected with inocula from clinical cases of sheep-associated MCF. By ELISA, 11 of 35 cattle sera from Indiana which had elevated levels of antibody to bovine herpesvirus-1 (infectious bovine rhinotracheitis virus) were seropositive for MCF. Of 88 tested sera from cattle in Oklahoma which had antibody to IBR virus, all were seronegative by ELISA. The significance of the finding of antibodies to alcelaphine herpesvirus-1 by the ELISA and SVN test in sera of domestic cattle with sheep-associated MCF is discussed.

Introduction

The sheep-associated form of MCF is similar clinically to the wildebeest-associated or African form of MCF (5, 13, 17). The etiological agent for wildebeest-associated MCF has been characterized as an alcelaphine herpesvirus (1, 2, 10, 17). However, the etiological agent of sheep-associated MCF has yet to be identified. Outbreaks of sheep-associated MCF in the United States, which has led to major losses in domestic cattle, have been reported in California and Arizona (11), Colorado (12) and Minnesota (3). The diagnosis of sheep-associated MCF in cattle or red deer is usually based on a history of contact with sheep, the clinical signs in the affected animals, and the histopathologic lesions (3, 11, 12, 16). The characteristic lesions in the tissues of cattle infected with alcelaphine herpesvirus-1 have been described as a vasculitis with lymphoproliferation associated with a monocytic infiltrate (5, 31) and these described lesions are identical to those reported in cattle with sheep-associated MCF (8, 9, 10, 11, 12).

In a series of investigations (20-22) on sera from different flocks of sheep associated with outbreaks of MCF, specific antibodies to alcelaphine herpesvirus were detected using an indirect immunofluorescence (IIF) procedure. Furthermore, antibodies to alcelaphine herpesvirus were identified by the IIF test in cattle with sheep-associated MCF (20), and also in cattle infected with alcelaphine herpesvirus (21). However, VN antibody to alcelaphine herpesvirus was not detected in sera from either sheep (22) or cattle with sheep-associated MCF (20). In contrast, VN antibodies of low titer were detected in sera of cattle infected with alcelaphine herpesvirus (21).

Previously, we reported on the development of an enzyme-linked immunosorbent assay (ELISA) for the detection of specific antibody to alcelaphine herpesvirus-1 in various zoological species of ruminants (29). The purpose of this report is to present the data of a serologic study for antibody to alcelaphine herpesvirus-1 as determined by the ELISA and the SVN test on domestic cattle sera obtained from various geographical areas of the United States. The sera assayed included specimens from documented clinical cases in cattle of sheep-associated MCF and cattle sera from non-MCF cases.

Materials and Methods

Sera

The first group of specimens comprised 128 sera obtained from cattle with a diagnosis of MCF as determined by

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clinical histories and histopathologic lesions. These sera were provided by Dr. J. Storz of the Department of Veterinary Microbiology and Parasitology, Louisiana State University and represented cattle involved in MCF outbreaks in California and Colorado and also cattle experimentally infected with inocula from clinical cases in cattle of sheep-associated MCF.

In the second group, 69 sera from 47 cattle in Colorado were provided by Dr. J. C. DeMartini of the Department of Pathology, Colorado State University. These cattle sera included 24 preinoculation and 21 post-inoculation specimens which were collected from cattle experimentally inoculated with clinical materials from suspected cases of sheep-associated MCF (7) and 24 sera from clinical cases of cattle with MCF. In addition, 3 specimens from synovial fluids of hock joints and 3 cerebrospinal fluids (CSF) from the clinical cases of MCF were also examined.

In the third group, 35 sera with moderate to high titers of VN antibody to bovine herpespvirus-1 were supplied by Dr. C. Kanitz of the Veterinary Diagnostic Laboratory, Purdue University.

In the fourth group, 88 cattle sera randomly selected from cattle sera submitted to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for a serologic profile of bovine respiratory disease were examined.

Enzyme-Linked Immunosorbent Assay (ELISA)

The method used for preparation of the viral antigens for the ELISA has been previously described (29). Briefly, trypsinized bovine fetal kidney cells infected with the greater kudu strain of alcelaphine herpesvirus-1 (1) were allowed to swell in a hypotonic buffer (0.01M NaCl, 0.01M Tris HCl, 0.001M $MgCl_2$, pH 7.4) for 5 to 10 minutes and then ruptured by homogenization using a Dounce homogenizer. The intact nuclei were pelleted by centrifugation[a] at 500 x g for 2 minutes. The nuclei, which were essentially free of cytoplasmic membranes as determined by staining (29), were then sonicated in a sonicator[b] for 1.5 to 2 minutes at maximal setting. The sonicated nuclear homogenates were then diluted in the coating buffer (carbonate buffer, pH 9.6) and added to microtiter plates[c] which then were incubated for 3 hours at 37 C. The concentrations for the viral antigens (positive) and the host cell antigens (negative) to be used in the ELISA were determined by the titration of each antigen on each batch of coated plates against a 1:20 dilution of a positive and negative serum for MCF. All test sera were non-heat inactivated and were assayed at a 1:20 dilution in the ELISA. The procedures for the ELISA for the detection of antibodies to alcelaphine herpesvirus-1 were similar to those described for pseudorabies virus (27).

To standardize the absorbance values obtained for each test serum in the microtiter plate at a wavelength of 405/450[d], the formula below was developed:

$$\text{relative absorbance} = \frac{\bar{X} \text{ of net absorbance of test serum}}{\bar{X} \text{ of net absorbance of positive serum}}$$

The mean (X) for each serum assayed was composed of the absorbance readings of 4 wells. A positive threshold for relative absorbance of a serum positive for antibodies to alcelaphine herpesvirus was previously established as 0.150 absorbance units (29). Therefore, a test serum with an absorbance value greater than 0.150 was considered seropositive for alcelaphine herpesvirus-1.

Serum-Virus Neutralization (SVN) Test

The SVN test on the cattle sera were performed by a microtiter procedure (4). The procedure was as follows: serial 2-fold (25 ul) dilutions of heat-inactivated (56 C for 30 minutes) serum were made in a 96-well microtiter plate using growth medium containing 10% fetal bovine serum (FBS) and 200 ug/ml of gentamicin. To each serum dilution was added 25 ul containing 100 to 300 $TCID_{50}$ of a cell-free preparation of the WC-11 strain (14) of alcelaphine herpesvirus-1. Serum-virus mixtures were allowed to react for one hour a 37C in a CO_2 incubator. Following incubation of the serum-virus mixtures, a 10^6 cells/ml suspension of bovine turbinate (BT) cells in growth medium was prepared and 150 ul were added to each well on the plate. The inoculated plates were then placed at 33 C in an incubator with 5% CO_2. The titer of each serum was determined by the inhibition of viral cytopathology at the 10th day of incubation and serum titers were calculated by the method of Karber (6). A serum titer of 4 or greater was considered to be seropositive for alcelaphine herpesvirus-1.

Results

Of 128 serum samples in group 1 from cattle associated with MCF outbreaks in California and Colorado and assayed by ELISA, 30 sera (23%) were positive for antibody to alcelaphine herpesvirus-1 (Table 1). Sixty-nine of the 128 sera which were received at a 1:4 in phosphate buffer were tested at a 1:8 dilution for VN antibody to alcelaphine herpesvirus (W-11 strain). Thirty-six cattle were negative for VN antibody and 33 were toxic to the BT cells. Clinical histories were not available for any of the 30 seropositive cattle.

In the cattle from group 2 which were experimentally

[a] *IEC HN-SII Centrifuge, DAMON/IEC Division, Needham Heights, Mass. 02194.*

[b] *Biosonik II.*

[c] *Immunlon I microtiter plates, Dynatech Lab., Inc., Alexandria, VA 22314.*

[d] *MR580, MicroELISA Auto Reader, Dynatech Lab., Inc., Alexandria, VA 22314.*

TABLE 1. Antibody to alcelaphine herpesvirus-1 in sera from domestic cattle with sheep-associated MCF and other respiratory diseases.

Group No.	Origin of cattle	Clinical History of cattle	ELISA No. of Positive Sera No. Tested	ELISA % Positive	SVN Test No. of Positive Sera No. Tested	SVN Test % Positive
1	California and Colorado	Clinical Outbreaks of MCF	30/128	23	0/69	0
2	Colorado	Clinical and Experimental MCF Cases**				
		Preinoculation	1/24	4	0/10	0
		Post-inoculation	4/21	19	1/14	7
		Clinical MCF	6/24	35	3/15	20
3	Indiana	Respiratory Disease	11/35	31	N.D.	
4	Oklahoma	Respiratory Disease	0/88	0	N.D.	

* Sera were tested at a starting dilution of 1:8 and 33 sera were toxic to cell cultures.
** Total 69 sera were collected from 47 cattle naturally or experimentally infected with sheep-associated MCF.
N.D.: Not Done.

infected with MCF, 11 of 69 tested cattle sera were seropositive for MCF by ELISA. Seroconversion was detected by ELISA in 3 of 16 paired sera but in only one paired serum by the SVN test. Of sera collected from cattle when clinical signs of MCF appeared, only 1 of 6 sera was seropositive. One of the preinoculation serum from the experimentally inoculated cattle was detected as positive by ELISA, but the post-inoculation serum from this animal was seronegative; however, all other preinoculation sera were negative by ELISA. Of 5 post-inoculation cattle sera which were unpaired, only 1 was seropositive by ELISA.

Sera from 18 cattle which developed clinical signs of MCF after receiving an inoculum of clinical materials from cattle with sheep-associated MCF or had been naturally-infected were tested by ELISA and 5 were seropositive. Of these 5 positive sera, only 1 had a VN titer of 4, all the others were negative by SVN test. However, from these 18 cattle sera, 2 which were negative by ELISA had a VN titer of 4 and 8 respectively.

In sera from group 3 which were from cattle in Indiana and contained VN titers to IBR virus of 128 to 256, 11 of 35 sera were positive for antibody to alcelaphine herpesvirus-1 by ELISA. Clinical histories available on these cattle did not indicate a prior infection with alcelaphine herpesvirus-1 but did document a respiratory problem. Due to insufficient volume, sera were not assayed by the SVN test for antibodies to alcelaphine herpesvirus-1.

In group 4, cattle sera selected at random and representing 88 different cattle which had been submitted to OADDL for a virus respiratory profile were all negative for antibody to alcelaphine herpesvirus-1 by ELISA. Most of these sera contained VN titers to IBR virus which ranged in titer from 4 to 32. Furthermorem, 23 of these sera contained antibody to parainfluenza virus type 3 and 50 of the sera contained antibody to bovine viral diarrhea virus.

Discussion

In this serologic study, antibodies to alcelaphine herpesvirus-1 were detected by ELISA in a portion of cattle sera with clinical MCF. The low percent of seropositive sera for MCF as detected by the ELISA and SVN test in our serologic survey was in agreement with previous assays on MCF in naturally and experimentally infected cattle as measured by the IIF and SVN tests (18, 19, 20, 21). Previous serologic surveys on MCF have shown that VN antibody was detectable only in cattle which had recovered following clinical MCF (14) and also in cattle that were hyperimmunized with either inactivated or attenuated alcelaphine herpesvirus-1 (15).

The absence of demonstrable humoral antibody to alcelaphine herpesvirus-1 may be related to the tropism of alcelaphine herpesvirus-1 for the cells of the immune system. Recent investigation of rabbits infected with alcelaphine herpesvirus-1 have shown that the target cells with MCF virus were thymus-derived "T" lymphoblasts (24). Therefore, a dysfunction of either B or T-lymphocytes in MCF-affected ruminants has been cited as possible reason for the absence of antibody production (25). Additionally, immunosuppressive factors have been found in acute sera from rabbits infected with alcelaphine herpesvirus (30). This immunosuppressive factor in acute rabbit sera was measured by the inhibition of blastogenic transformation in lymphocytes following mitogen stimulation (30). In contrast in another study, lymphocytes from cattle and rabbits infected with alcelaphine herpesvirus were found to have an enhanced activity to mitogens in the presence of acute-phase rabbit sera to alcelaphine herpesvirus (26). This finding provided no evidence to indicate a dysfunction of B or T-lymphocytes in MCF-affected cattle or rabbits. Furthermore, a 4-fold or greater increase in the

concentration of immunoglobulins G and M have been demonstrated in sera of rabbits following their infection with alcelaphine herpesvirus, but VN antibodies were not detected in either whole or fractionated rabbit sera (23). This finding suggests that certain antibodies produced by the host following an infection with alcelaphine herpesvirus may not be measurable by SVN test.

The ELISA detected more seropositive sera than did the SVN test which suggested that the ELISA was more sensitive in the detection of antibodies to alcelaphine herpesvirus-1. However, the SVN test was done on only a portion of the sera because of insufficient amounts of sera and thus, no correlation between assays could be done. Nevertheless, we found that 2 seropositive sera by the SVN test from group 2 were ELISA negative. One explanation for this conundrum may be the use of an affinity purified peroxidase-labeled antibovine IgG (H+L) conjugate in the ELISA since early antibody belongs to the IgM class (28). Thus, the affinity purified antibody to bovine IgG used in the ELISA might not recognize this early IgM and might account for a negative ELISA.

In group 2, although 1 serum labeled preinoculation was positive by ELISA, post-inoculation serum from the same animal was negative. This may be a result of mislabeling since the sera has been collected, coded and stored for several years prior to their use in the ELISA. Nevertheless, in certain paired sera from cattle receiving an inoculum of sheep-associated materials, the preinoculation sera were negative by ELISA and the post-inoculation sera were seropositive by ELISA. This finding implies that the etiological agent of sheep-associated MCF is serologically related to alcelaphine herpesvirus-1.

Because the complete histories for the California and Colorado serum samples in group 1 and 2 were not available or the time of collection of sera or the duration of the clinical disease, this serologic findings will require further conformation. However, a bovine cytomegalovirus and a bovine leukemia virus has been isolated from some of these cattle in group 1[e] and the possibility that these viruses have epitopes similar to alcelaphine herpesvirus-1 which are detected by the ELISA has not been investigated.

Detection of seropositive cattle for MCF by ELISA in the sera from group 3 was unexpected. Based on previous findings (Wan, unpublished results), and on 88 sera from group 4, we found no evidence of a cross-reaction of the alcelaphine herpesvirus antigens with cattle sera containing high, moderate or low levels of antibody to IBR virus. As further evidence of the absence of cross-reaction of bovine herpesvirus-1 positive sera with alcelaphine herpesvirus-1, all the ELISA negative sera from the Indiana cattle also had VN titers of 128 to 256 to IBR virus.

Nevertheless, our present findings do not eliminate the possibility that an antibody response to a previous infection to other bovine herpesviruses (e.g. cytomegaloviruses and a variety of orphan bovine herpesviruses) may have cross-reacting antibodies for antigens of alcelaphine herpesvirus-1. Another explanation for these MCF-seropositive cattle may have been a previous exposure to sheep-associated MCF by association with lambing ewes but such information was not available.

Our serologic findings supported the hypothesis (17) and findings (20-22) that the putative virus of sheep-associated MCF is serologically related to alcelaphine herpesvirus-1. Therefore, the ELISA provides a rapid supplemental diagnostic assay for the screening and study of sheep-associated MCF in domestic cattle. Until the virus of sheep-associated MCF is isolated and the pathogenesis of MCF is fully investigated, the interpretation of the significance of seropositive healthy cattle or cattle clinically ill with sheep-associated MCF remains tenuous.

[e] *Dr. J. Storz, personal communication, 1985.*

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Abstracts

Use of ELISA to assess lungworm infection in calves

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The enzyme-linked immunosorbent assay (ELISA) technique was applied to the detection of lungworm infections in calves. In experimentally infected animals different responses to larval and adult worm antigens were observed. The response to adult worm antigens was delayed in vaccinated animals when infection occurred by the gradual uptake of infective larvae from contaminated pasture. A serological survey in The Netherlands demonstrated a high incidence of lungworm infection in both vaccinated and unvaccinated herds. There was a good correlation between anti-adult worm and anti-larval ELISA-titres. ELISA appeared to be a useful technique for assessing the level of lungworm infection in a herd.

The vaginal mucus agglutination test in the diagnosis of bovine brucellosis

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Of 1140 vaginal mucus agglutination tests (VMAT) on specimens obtained in 1971–72 from 663 dairy cows in seven herds infected with brucellosis, 97 were positive. When the VMAT was positive one or more serological tests were also positive. Of the 97 corresponding serum agglutination tests 80 sera had titres of more than 533 international units. Only 69·8 per cent of VMAT from serologically positive cows were positive. No evidence was found of non-specific agglutinins in vaginal mucus and positive VMAT reactions appeared to be specific for field infection. Three cows showed evidence of local agglutinins in the vagina. Hence herd testing by VMAT has no advantage over tests of blood serum but the test could be an aid in establishing whether individual cattle are infected.