Diagnosis of Clinical Leukosis in Cattle and its Correlation with Bovine Leukemia Virus Antigens Using the Agar Gel Immunodiffusion (AGID) Test

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Summary

During an 18-month period a total of 317 bovine sera were tested for antibody to bovine leukemia virus (BLV) using the agar gel immunodiffusion (AGID) test. Of these field submissions, 130 (41%) were positive for antibody to BLV and 34 (26%) had a clinical diagnosis of bovine leukosis. A second (AGID) precipitin line was found in tests with sera from five of these cattle. Four cows had grossly enlarged palpable lymph nodes or subcutaneous masses. The two antigens identified in the sera of the 5 cattle were the glycoprotein gp envelope antigen, typically found in cattle exposed to BLV, and the p24 antigen which is part of the virus core. We found that in these 5 cattle with antibodies to both antigens the concentration of antibody to the gp antigen was greater than that of the p24 antigen. The presence of antibody to the p24 viral antigen in cattle with clinical disease and its implication in the confirmation of a diagnosis of bovine leukosis is discussed.

Introduction

The etiologic agent for enzootic bovine leukosis (EBL) in cattle is a C-type, RNA retrovirus (1, 2). Lymphosarcoma, the prevalent adult form of malignancy, is the tumorgenic form which occurs in cattle infected with bovine leukemia virus (BLV). Other sporadic forms of bovine lymphocytic leukosis (calf, thymic and cutaneous) also occur, for which the etiology is unknown (1, 2, 3). Cattle infected with BLV produce antibodies to the virus and the detection of these antibodies is a measurement of infected animals (3, 4).

The diagnosis of BLV-positive cattle has been by the measurement of persistent lymphocytosis, a benign lymphoproliferative response (3) and by the detection of antibodies produced in BLV-infected cattle by using several immunological tests; agar-gel immunodiffusion (AGID) (4, 5, 6), radioimmunoprecipitationassay (RIA) (4, 7), complement-fixation (2), and syncytia-inhibition tests (8). The hematologic keys which measure persistent lymphocytosis in BLV-infected cattle do not detect every infected animal (3), however, the AGID, RIA, and syncytia-inhibition tests have been accurate indicators of BLV-infected cattle. Various immunologic tests for antibodies to BLV have been compared and the RIA test appears to be the most sensitive (4).

The AGID test measures antibodies to a glycosylated (gp) envelope antigen with a molecular weight of 70,000 daltons of the virus. (1, 2). Antibodies to a non-glycosylated polypeptide (p24), a major core viral antigen with a molecular weight of 24,000 daltons, may also be present in cattle sera and can be detected by the AGID test. A reaction with the p24 antigen by most BLV-positive cattle sera is not usually seen due to lower concentrations of antibodies to p24 antigen (4, 5, 7) and the relationship of this antigen p24 to malignancy is not known.

The purpose of this report is to present data on the number of sero-positive cattle in Oklahoma found by our laboratory and to describe our observations of the presence of additional precipitin bands (p24) in the AGID test in 5 cattle with a definitive clinical diagnosis of lymphosarcoma.

Materials and Methods

Sera: Between January 1980 and May 1981, a total of 317 bovine serum samples were tested for antibody to BLV by the AGID test.

History of Animals Producing 2 Precipitin Bands:

Case History No. 1: A 10-year old, 1500 pound Holstein cow was in a herd of 78 cows. When the cow was examined, three pelvic lymph nodes of softball size were palpated. The left front quarter of the udder was also enlarged. The heart, eyes, spine, uterus were normal and the abomasum palpated normal. A hematologic cell count was normal. No other clinical signs were observed. An offspring, a 3-year old female, within the herd was sero-positive for BLV.

Case History No. 2: A 900 pound Holstein cow was first noticed sick in April, 1981. The animal was recumbent, anemic and had enlarged lymph nodes. This animal susequently died.

Case History No. 3: A 1000 pound, 3-year old Santa Gertrudis cow in a herd of 35 animals, was observed in November of 1980 to have multiple subcutaneous masses ranging from egg to softball size. The largest mass was located perirectal while other masses were located on the shoulders and ribcage. The prescapular and prefemoral lymph nodes were also enlarged. A rectal examination revealed no internal abnormalities. The red cell packed cell volume was 30% and the body temperature was slightly elevated (102°F). The lung and heart auscultated normally.

Case History No. 4: A Holstein cow in a herd of 50 was found to have multiple masses and enlarged inguinal and prefemoral lymph nodes when examined in April of 1980. No additional history was available.

Case History No. 5: A serum sample from a cow was submitted during November of 1980 for detection of antibodies to *Anaplasma marginale* and bovine leukemia virus. The cow was jaundiced and had been treated unsuccessfully for Anaplasmosis. A negative serum titer by CF to *Anaplasma marginale* was found. The cow subsequently died.

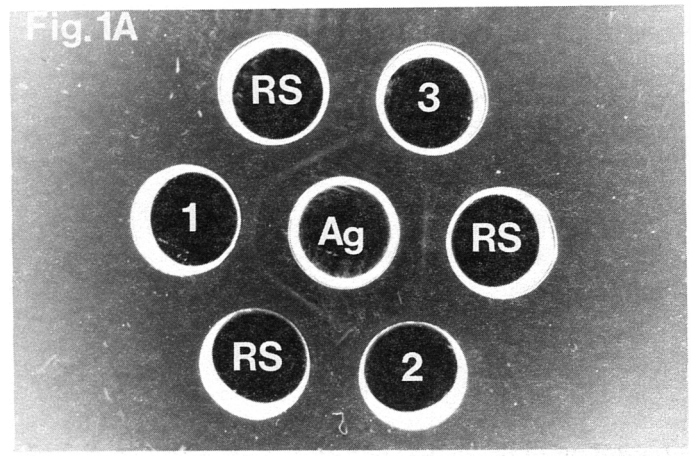
Test Procedure:

The AGID test for the measurement of antibodies to BLV was done as described by Miller and Van der Maaten (6, 9) using commercial reagents (a) (Fig. 1A).

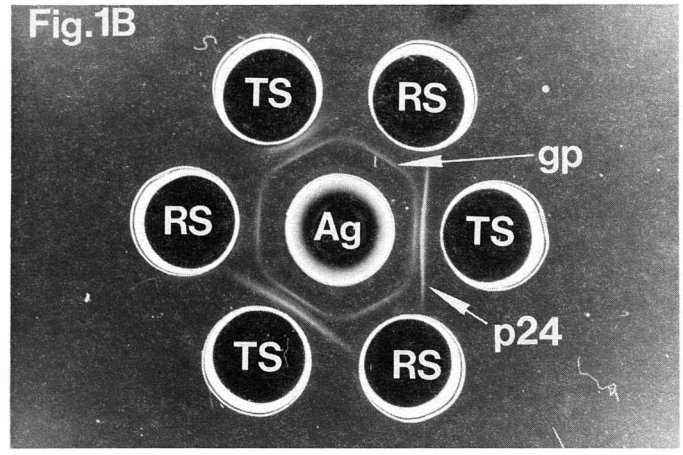
Results

Of the 317 cattle sera submitted for antibodies to BLV, 130 (41%) were positive for antibody to the gp antigen and 187 were negative. Of the 130 cattle with antibody to the gp antigen, 34 or 26% had the attending veterinarian diagnose leukosis. Within the BLV-positive cattle sera, we found 5 sera in which a second precipitin line was present (Figure 1B). The additional precipitin band which was seen outside the gp precipitin line was identified as the viral polypeptide (p24) antigen by serum specific for the viral p24 core antigen (b). The contiguous precipitin band (line of identity) for the reaction between gp antigen and antibody to the test serum was located closest to the antigen (Ag) well. (Fig. 1B, C). The faster migrating precipitin band was identified as a reaction

Figure 1: Immunodiffusion of Bovine Sera Using BLV Antigens



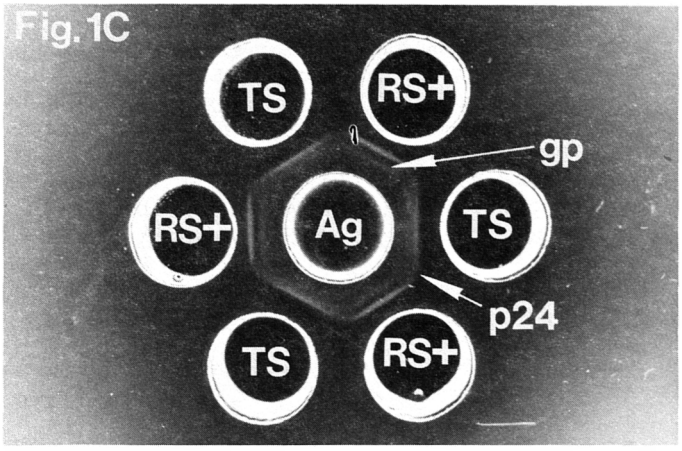
(A) AGID test with cattle sera, well no. 1 is a strong BLV-positive, well no. 2 a weak positive and well no. 3 a negative.



(B) Effect of dilution of p24 seropositive sera in AGID test. Dilutions tested were 1:2, 1:4, 1:6 located in wells 1, 2, 3 respectively. Note the intensity of the p24 precipitin band which decreases with dilution.

(a) Pitman-Moore, Washington Crossing, New Jersey 08560.

(b) Sera specific for p24 antigen (BLV) was kindly provided by Dr. Janice Miller, Science and Education, Administration, NADC, Ames, Iowa.



(C) Identification of antibodies to p24 antigen, inner band is antibody reaction to gp antigen, outer band is p24 reaction line.

RS=BLV-positive reference serum, RS+ = reference serum to p24 antigen TS=test serum, AG= BLV antigen.

between viral p24 antigen with specific antibody (Fig. 1C). When the bovine sera with antibody to both gp and p24 antigens were serially diluted, sharp precipitin lines were obtained for gp antigen (Fig. 1B), however, the precipitin line to the p24 antigen disappeared. This result, following dilution of each cattle serum tested, indicated that antibody to p24 antigen was present in less concentration in cattle sera than antibody to gp antigen.

Of the 5 cattle whose sera produced 2 precipitin bands in the AGID test, four cows had confirmed clinical signs of lymphosarcoma as determined by enlargement of various lymph nodes, loss of weight, drop in milk yields and subsequent death. The breeds represented by the 5 cattle were 3 Holsteins, 1 Santa Gertrudis and 1 in which the breed was not reported. Of the number (187) of sero-negative cattle, none had a diagnostic or clinical history of enzootic bovine leukosis or lymphosarcoma.

Discussion

Diagnosis of enzootic bovine leukosis (EBL) has been enhanced by the development of various diagnostic procedures, such as, hematologic keys, agar-gel immunodiffusion (AGID), syncytia-inhibition assay, and radioimmunoprecipitinassay (RIA) (1, 2). Of these, the simplest immunologic test is the AGID test due to its reproductibility, ease of performance and commercial availability of reagents to diagnostic laboratories. Within the AGID test lies a complex system of antigens and antibody reactions because of the number of antigens associated with BLV (3, 4). Two precipitating antigens have been described for BLV (3, 6); they are a viral core protein (p24) and the viral envelope glycosylated protein (gp). The presence of precipitin lines against these viral components by antibody present in cattle sera is the current diagnostic criterion used in the identification of BLV-infected animals.

By the present diagnostic immunologic procedures, a clinical diagnosis of lymphosarcoma cannot be predicted in cattle sero-positive for BLV. Thus, when cattle test as positive for antibodies to BLV they are considered exposed to BLV and potential carriers of BLV, however, many BLV-infected animals do not develop malignancy (3).

Persistent lymphocytosis is a benign lymphoproliferative response in BLV-infected cattle and can be determined by the Bendixen hematologic key (3). The AGID test identifies cattle with antibody to BLV, however, neither the AGID test nor the hematologic keys can be used to predict if a positive animal will eventually develop clinical lymphosarcoma. Although the RIA test is more sensitive than AGID (1, 11) it also cannot be used to predict which animals will develop lymphosarcoma. However the AGID and RIA tests are comparable since both measure antibody to the viral gp and p24 antigens and both tests detect a greater concentration of antibody in cattle sera to viral gp when compared to viral p24 antigen (1, 11).

Our case sampling of 317 field specimens for antibodies to BLV has indicated that certain cattle (four) with a definitive clinical field diagnosis of lymphosarcoma also have measureable amounts of antibody to the p24 antigen of BLV. This preceding result suggests that the presence of measureable concentrations of antibody to p24 antigen of BLV may be indicative of clinical disease or lymphosarcoma developing in these animals.

Of 130 sero-positive cattle for antibodies to BLV, 48 were dairy cattle, 68 were beef cattle and 14 were of unknown breed. This probably reflects a greater number of submissions of beef breeds due to their greater numbers within the state of Oklahoma. Of the 34 cattle with a diagnosis of clinical leukosis 22 or 64% were dairy cattle and 12 or 36% were beef cattle. It was of interest that the diary cattle (Holstein) (three) were more prevalent in our positive sampling for the p24 antigen of BLV as opposed to beef cattle. This result is similar to that obtained by other investigators (10) who found a greater percent of reactors to BLV (10%) in diary cattle than in beef cattle (1%). The presence of antibody to the p24 antigen of BLV was not found in 29 cattle with a clinical diagnosis of leukosis. This may be the reflection of the stage of disease in each individual animal. However, the increased number of clinically positive cattle for lymphosarcoma which contained antibody to p24 antigen suggests a relationship of malignancy with elevated antibody against p24 antigen.

Although our sampling was small, if the observation of a positive serologic result to p24 antigen is consistently linked to malignancy, it would enhance the confirmation of a diagnosis of clinical lymphosarcoma in cases where no overt signs of disease are observed.

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