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Evaluation of a Voluntary Control Program for the Detection of Bovine Leukemia Virus Antibodies Based on Agar Gel Immunodiffusion Test in Dairy Farms in Costa Rica

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Abstract

Cattle from 20 dairy farms were serologically tested over a five-year period using agar gel immunodiffusion test (AGIDT) as part of a voluntary Bovine Leukemia Virus (BLV) control program. After five years of removing infected animals from the herds based on BLV-AGIDT serological status, blood samples from 332 cattle in these farms were collected and analyzed side by side by AGIDT and enzyme-linked immunosorbent assay (ELISA) to detect antibodies against BLV. AGIDT detected 29.2% (97) and 16.0% (53) of the animals as positive and weak positive respectively, whereas ELISA detected 58.2% (193) cattle as positive. The prevalence of BLV-antibodies determined with AGIDT in the dairy farms oscillated between 0% and 86%, whereas prevalence determined by ELISA ranged between 28% and 100% in the same farms. Although both techniques showed similarly results in farms with high BLV-prevalence, ELISA detected a larger proportion of BLV-positive, especially in farms with low or no BLV-prevalence based on AGIDT, leading to wrong assumptions in terms of farm level control efforts. Our results strongly suggest that AGIDT alone is inadequate to implement BLV control programs and ELISA is a more adequate test for BLV surveillance and control programs.

Keywords

BLV, Serological Testing, Control Programs

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1. Introduction

Bovine leukemia virus (BLV), an exogenous retrovirus, is the infectious agent responsible for causing enzootic bovine leucosis (EBL), the most frequent neoplasm of lymphatic tissue in cattle [1]. This agent can induce lymphocyte transformation and eventually, development of lymphosarcomatous tumors after long incubation periods. However, BLV replicates in B-lymphocytes and cattle become infected by exposure to virus infected lymphocytes. Cattle with antibodies against BLV are persistently infected and represent a source of infection for other animals [2].

Control and eradication programs are mainly based on detection of seropositive animals followed by elimination, segregation or implementation of corrective management [3]. The agar gel immunodiffusion test (AGIDT) has been the test of choice for routine diagnosis and is still prescribed for international trade [4] [5]. Although AGIDT is a simple and reliable procedure, it has a relatively low sensitivity, which might favor the occurrence of low-titer BLV infections in clinically normal herds [5]-[7]. This is of remarkable importance in herds which had been tested repeatedly for some time with AGIDT [8] [9]. Previous results showed that control based solely on low sensitivity precipitation assays might complicate the epidemiology of BLV by selecting animals which, although infected, do not produce detectable antibodies in AGIDT. For its high sensitivity, the enzyme-linked immunosorbent assay (ELISA) is considered to be the most adequate test for future survey and control programs [9]-[13]. Several immunoenzymatic assays have been developed, even molecular assays, and compared to AGIDT [7] [10] [14]-[16]. This study was designed to compare and evaluate AGIDT and ELISA for the detection of antibodies to BLV in twenty dairy farms in Costa Rica that were tested repeatedly during five years with AGIDT as part of a voluntary EBL control program.

2. Material and Methods

2.1. Bovine Sera

332 bovine sera from dairy cattle were collected from 20 farms located in the outskirts of the Poás volcano in the Vara Blanca region in Heredia, Costa Rica.

2.2. Agar Gel Inmunodiffusion Test (AGIDT)

Leukassay B^R (Pittman Moore Inc., Mundelein, IL, USA) was performed as recommended by the manufacturers. A layer, 3.5 mm thick, of 0.8% type IV agarose, dissolved in 0.05 M Tris-HCl, 8.5% NaCl, pH 7.2, was poured into bacteriological polystyrene plates. The pattern of six peripheral wells filled with positive control sera and bovine sera, and one central well filled with antigen was used.

2.3. Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA was performed using the leukosis indirect ELISA kit kindly donated by the Joint FAO/IAEA Programme. This ELISA was conducted according to the test protocol submitted with some modifications. BLV-antigen was diluted 1:1000 with 0.05 M carbonate buffer, pH 9.6. Microtiterplates (Polysorp, Fa. Nunc) were coated with BLV-antigen (100 µl per well) and incubated 16 h at 4˚C. The plates were washed three times with washing buffer (0.02 M PBS, 0.05% Tween-20). Bovine control and test sera diluted 1:50 in PBS (containing 1 M NaCI, 0.05% Tween-20) were added to the wells (100 µl per well) and incubated 1 h at 37˚C. Positive, weak positive and negative control sera were added in quadruplicate, and test sera in duplicate to the plates. After washing the plates, the peroxidase conjugate, a mouse monoclonal antibody against bovine IgG_1 (M23) was diluted 1:20.000 in PBS, 0.05% Tween-20, and added to each well, and the plate was incubated for 1 h at 37˚C. After washing, 100 µl peroxidase substrate (0.05 M phosphate citrate buffer, pH 5.0, containing 0.416 mM TMB, 1.75 mM H_2O_2) was added to each well. After 15 minutes substrate chromogen incubation 100 µl of stopping solution (2 M H_2SO_4) was added to each well. The optical density (OD) was measured at 450 nm in a Titertek Multiscan^R Plus MKII (Flow Laboratories). The optical densities were expressed as positive percentage (PP) with respect to the OD of the mean of the positive controls of each plate (mean OD of the positive control sera = 100% PP). All tested sera that showed PP values of 25% and higher with respect of the positive control of the plate were considered positive in this investigation.

2.4. Statistical Analysis

Sensitivity, specificity, predictive positive and predictive negative values for the AGIDT, compared to the ELISA, were calculated with Epinfo Software.

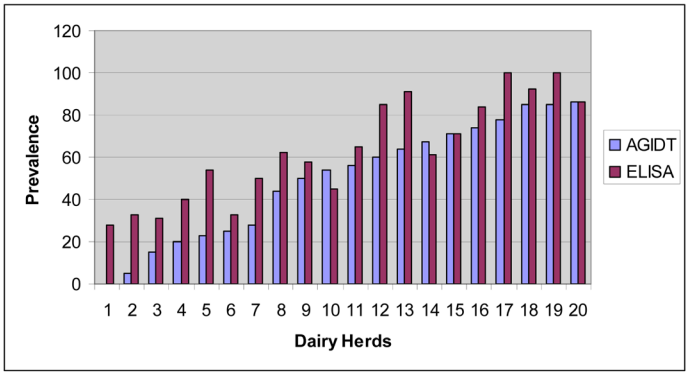
3. Results

From 332 sera analyzed, 97 (29.2%) showed positive, 53 (16.0%) showed weak positive reactions and 182 (54.8%) reacted negative in AGIDT. In contrast, 193 (58.2%) reacted positive and 139 (41.9%) negative in ELISA (**Table 1**). The relative sensitivity and specificity of the AGIDT with respect to the ELISA for the 332 sera were 73.0% and 96.0%, respectively; predictive positive and negative values were 74.6% and 95.6%, respectively. From 97 sera with positive reactions in AGIDT, 96 (98.9%) were confirmed positive and one (1.1%) reacted negative in ELISA. From 53 sera with weak positive reactions in AGIDT, 48 (90.5%) were confirmed positive and five (9.5%) reacted negative in ELISA. From 182 sera that reacted negative in AGIDT, 49 (26.9%) reacted positive and 133 (73.1%) reacted negative in ELISA (**Figure 1**).

The prevalence of BLV-antibodies determined with AGIDT in 20 dairy farms oscillated between 0% and 86%, however, the ELISA determined prevalence ranged from 28% - 100% (**Figure 1**). Although both techniques showed similar results in farms with high BLV-prevalence, ELISA detected a larger proportion of BLV-positive cows in farms with low prevalence of BLV-antibodies in AGIDT (**Figure 1**).

4. Discussion

The results of AGIDT with 332 sera of dairy cattle collected in the Poás region of Costa Rica (45.2% positivity) were in accordance with those from other authors [9] [17]-[19]. However, the proportion of positive animals increased to 58.1% when ELISA was used. The determined relative sensitivity and specificity of AGIDT was also in accordance with other reports that conferred AGIDT a lower sensitivity compared to ELISA [4] [7] [9]-[13].



Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AGIDT	0	5	15	20	23	25	28	44	50	54	56	60	64	67	71	74	78	85	85	86
ELISA	28	33	31	40	54	33	50	62	58	45	65	85	91	61	71	84	100	92	100	86

Figure 1. Prevalence of BLV-infection in twenty dairy herds of Costa Rica detected by AGIDT and ELISA.

Table 1. Detection of antibodies against BLV in 322 bovine sera, comparison AGIDT and ELISA.

		ELISA		
		+	−	Σ
AGIDT	+	96 (28.9%)	1 (0.3%)	97 (29.2%)
	±	48 (14.5%)	5 (1.5%)	53 (16%)
	−	49 (14.8%)	133 (40.0%)	182 (54.8%)
	Σ	193 (58.2%)	139 (41.8%)	332 (100%)

+ = positive reactions; ± = weak positive reactions; − = negative reactions; Σ = total.

The use of the AGIDT failed generally in dairy farms with low BLV-prevalence. A possible explanation is that the antibody content in the sera of the animals tested was under the detection limit of the assay. The same behavior was described by Manz and Bauer [8] and Dolz and Moreno [9]. They showed a failure of the AGIDT compared to ELISA in herds controlled during a long time with AGIDT due to the fact that many AGIDT negative animals were low titer reactors in ELISA. Although non-specific reactions are relatively common in ELISA, and difficult to distinguish from specific ones, the ELISA used in this study was determined previously as high specific (100%), using Western Blotting as confirmatory test [9].

In countries with high prevalence of BLV-infection such as Costa Rica, where the management of dairy herds and probably the presence of bloodsucking vectors promote the spreading of the virus, tests with relatively low sensitivity are not adequate for control programs, since they may favor the maintenance of BLV in clinically normal cattle [4] [7] [9]-[13]. Our results with sera from the 20 dairy farms strongly suggest that control based solely on low sensitivity precipitation assay might complicate the epidemiology by selecting animals, which although infected, do not produce detectable antibodies in AGIDT. Based on the fact the ELISA is more sensitive, allows expedited testing of large number of samples and the test outcome is an objective numerical reading, we consider ELISA as the most adequate test for survey and control programs in Costa Rica.

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