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BOVINE LEUKEMIA VIRUS INFECTION IN TAIWAN: EVALUATION OF THE ENZYME-LINKED IMMUNOSORBENT ASSAY AND AGAR GEL IMMUNODIFFUSION TEST

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Key Words: bovine leukemia virus, enzyme-linked immunosorbent assay, Agar gel immunodiffusion test.

SUMMARY

I evaluated an enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID) test simultaneously for the detection of bovine leukemia virus (BLV) antibodies. Total 1,293 serum samples were tested for ELISA and AGID test and the results were compared. The results of ELISA and AGID agreed by 1,156 out of 1,293 (89.4%). All of AGID-positive 356 sera were positive by ELISA. However, of 451 ELISA-positive sera, 95 sera were either negative or equivocal by AGID test. Eleven animals which showed ELISA-positive but AGID-negative or equivocal became AGID-positive in a year.

It may be inferred that ELISA detects infected cattle earlier and with greater sensitivity than AGID.

INTRODUCTION

Bovine leukemia virus (BLV) is an oncogenic RNA virus which causes enzootic bovine leukosis (5). Cattle with positive BLV antibody have been considered a source of BLV infection since BLV-infected animals carry the virus persistently in their peripheral lymphocytes for life. In fact, the effective eradication of BLV was achieved by segregation or elimination of BLV antibody-positive cattle (1, 15). Consequently, serological surveys provide the basis for an effective eradication program. However, the success of such a program depends mainly on the sensitivity and reliability of the test used to identify the infected animals. Several serological methods have been described for the detection of serum BLV antibodies. Among them, AGID test is the

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most common and simplest procedure, but it is not sensitive enough to detect antibodies in the early stages of the infection or in milk samples (6, 10).

Recently, ELISA has been used and recognized to be a sensitive and reliable procedure for testing serum, milk, or tissue fluids in identifying infected animals in Europe (2, 9, 12). In this paper, we compared sensitivity and diagnostic value of ELISA and AGID, and evaluated their combination to screen sera from BLV-infected herds for effective control of BLV infection.

MATERIALS AND METHODS

ELISA : The culture fluid collected weekly from fetal lamb kidney cells persistently infected with BLV was used as the source of BLV. BLV was purified by sucrose gradient centrifugation as described previously (11). Purified BLV was treated with 0.1% Triton X–100 for 1 hour and used as ELISA antigen. This antigen had glycoprotein (gp) antigenicities of 16 units and protein (p) antigenicities of 500–1,000 units in the AGID test.

The ELISA procedure was performed as described by Takahashi et al (11). The serum samples to be tested were diluted 1 : 100 in phosphate-buffered saline containing 10% horse serum and 0.15% Tween 20 (PBST) and added to positive and control antigen coated wells. After incubation for 1 hour at 37°C, the plates were washed 3 times with PBST and rabbit anti-bovine IgG peroxidase conjugate (H and L chains-specific, Jackson Immunoresearch Laboratories, Inc. West Grove, Pennsylvania) diluted 1 : 2,400 with diluent solution added to each well and the plates were incubated for 1 hour at 4°C. After washing, the enzyme substrate (100ml of phosphate citrate buffer added with 34mg of O-phenylendiamine dihydrochloride and 50 μl of 30% H_2O_2) was added, and the plates were kept in the dark for 30 minutes. The reaction was stopped by adding 3N H_2SO_4. Absorbance at 490 nm was determined with an Auto Reader MR 700 (Dynateck Instruments, Inc., Sussex, England). The result of the ELISA for each serum was expressed in terms of the difference in optimal density (OD) values recorded in the viral and control antigen wells.

Antigen and Procedure for AGID : The antigen preparation and AGID procedure were performed according to the methods described previously (7, 13).

Sera : The following 2 groups of sera were used in these experiments : 1) Sera collected from 100 cattle, aged 1–8 years in a BLV-free herd which had been known to be BLV negative during a 2-year investigation by the AGID test ; 2) Sera collected from 1,293 cattle, aged 1–16 years in 6 BLV-positive dairy farms in the northern and southern areas of Taiwan. Two reference sera, one known to be BLV negative (AGID test negative) collected from a BLV-free dairy farm and one standard anti-BLV positive serum, were also used.

RESULTS

Optimum antigen dilution for ELISA: Fig. 1 summarizes the effect of serial dilution of BLV antigen (VA) on ELISA values. The optimum dilution was found to be between 1 : 160 and 1 : 640 for reaction with the 1 : 100 dilution of positive sera. No reaction occurred in the control antigen (CA). Therefore, we selected 1 : 640 dilution as the ELISA antigen.

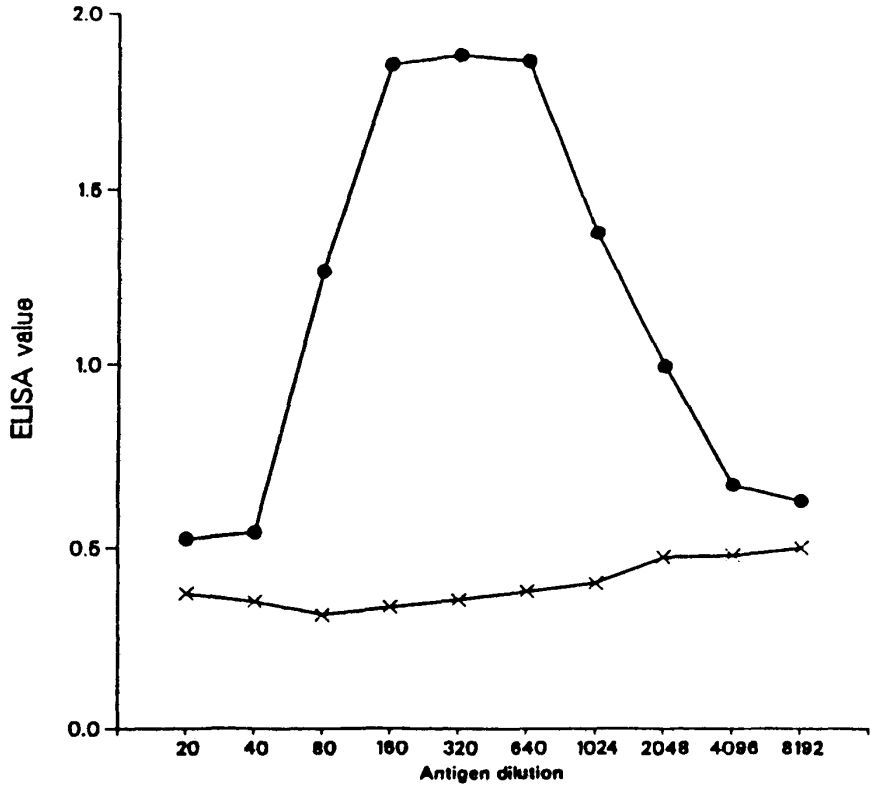

Fig 1. Titration curves of the ELISA antigens
● —— ● virus antigen (VA)
x —— x control antigen (CA)

Determination of negative, equivocal and positive reactors by OD values in ELISA : Normal range of ELISA values was determined by testing sera collected from BLV-free herds. The ELISA values of the sera is skewed to the right and showed a gamma distribution as shown in Fig. 2. Therefore, assuming a 1.2% probability of misclassification (i. e. $\bar{X}+3SD$), the upper limit of an ELISA value for the negative sera was determined as 0.1524. Cattle with values greater than 8 SD from the mean, 0.3141, were regarded as positive reactors with a 0.0008% probability of misclassifica-

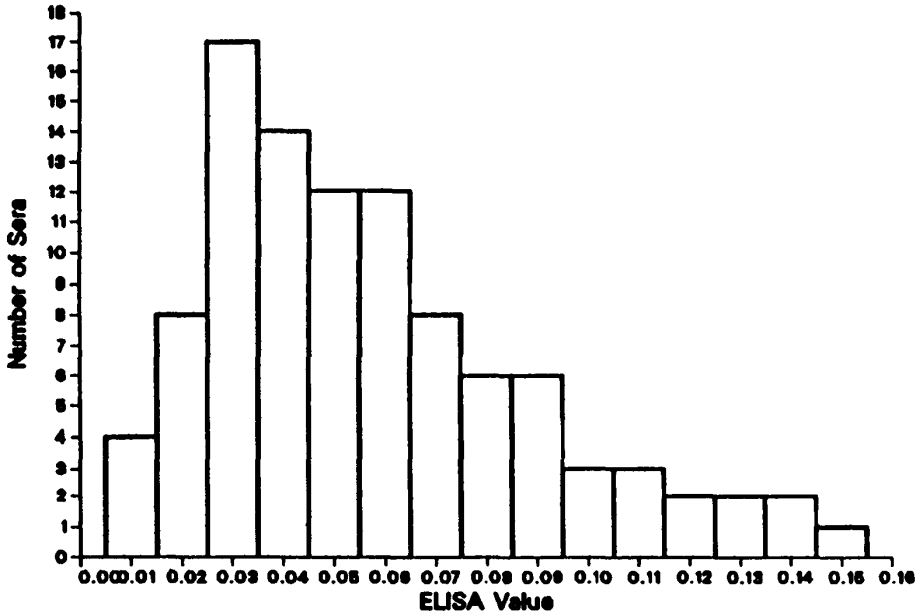

Fig 2. Distribution of ELISA values from a BLV-free herd

tion, and those with values between 0.1525 and 0.3140 as equivocal.

Comparison of ELISA and AGID test: A total 1,293 sera from 6 farms were tested with ELISA and AGID procedures. There was a total of 89.4% (1,156/1,293) agreement between ELISA and AGID tests, 451 or 34.9% reacted positively by ELISA, and 356 or 27.5% positively by AGID (Table 1). All 356 AGID positive sera were also positive by ELISA, but among the AGID-equivocal 62 reacted positively and 32 reacted equivocally by ELISA. Among the AGID-negative sera 33 showed positive and 31 equivocal with ELISA tests.

Table 1. Comparison of ELISA and the AGID-test for Detection of Antibodies to BLV in 1293 Cattle in BLV-postive herds

ELISA	AGID			Total (%)
	+	±	−	
+	356	62	33	451(34.9)
±	0	32	31	63
−	0	11	768	779
Total	356	105	832	1293
(%)	(27.5)			

Fig. 3 depicts the distribution of the ELISA values of 1,074 sera. Among the 378 ELISA-positive sera, 92.6% (350) had values greater than OD 0.500, which consisted of 18 (5.1%) AGID-negatives, 42 (12%) AGID-equivocal, and 290 (82.9%) AGID-positives. On the contrary, among the 28 ELISA-positive sera with ELISA values between 0.3141 and 0.500, 10 (35.7%) were AGID-negative, 14 (50%) AGID-equivocal, and 4 (14.3%) AGID-positive ; and among 61 ELISA-equivocal sera, 31 (50.8%) showed AGID negative, 30 (49.2%) AGID equivocal.

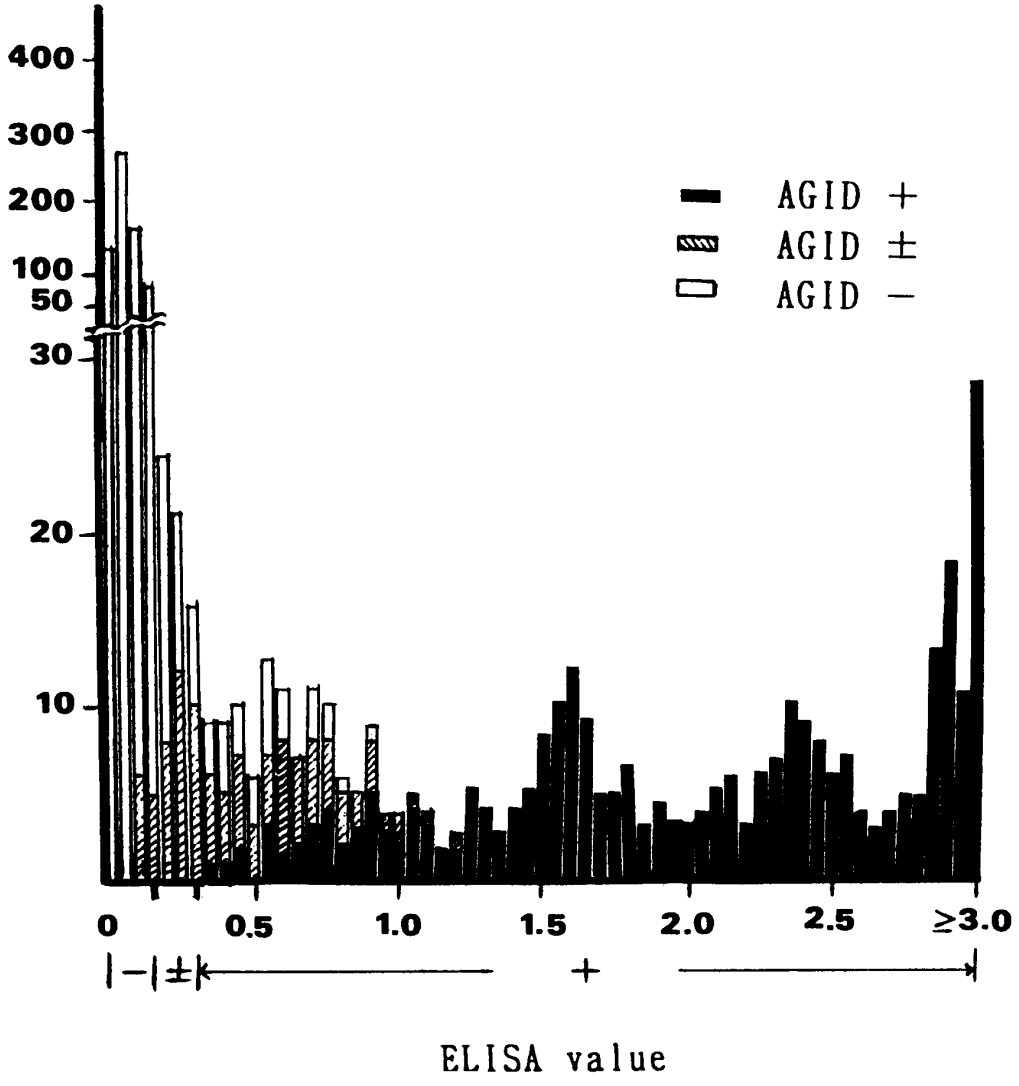

Fig 3. Distribution of AGID − , AGID ± , and AGID + sera in relation to ELISA value, ELISA values are : ELISA − , ≦ 0.1524 ; ELISA ± , 0.1525–0.3140 ; and ELISA + , ≧ 0.3141.

Table 2 summarizes the results of successive sampling of the 16 ELISA-positive or-equivocal cattle over a 15-month period. Six ELISA^{+}/AGID$^{\pm}$ cattle became ELISA^{+}/AGID^{+} within 5–12 months (100%), five of nine (55.6%) ELISA^{+}/AGID^{-} became ELISA^{+}/AGID^{+} and of the remaining 4, 2 (No. A5 and No. 283) remained unchanged, and 2 (No. A2 and No. 1340) became ELISA^{+}/AGID$^{\pm}$. One ELISA$^{\pm}$/AGID$^{\pm}$ (No. 383) became ELISA^{+}/AGID^{+}.

Table 2. Comparison of ELISA and AGID-test on Successive Sampling of the Sixteen Cattle Which Showed Equivocal Results in ELISA and AGID test

Cattle No.	Age	Date of Sampling				
		July 1988	Dec. 1988	Apr. 1989	July 1989	Oct. 1989
3211	6	+/±*	+/±	+/+	Eliminated	
3074	6	+/±	+/+	Eliminated		
3184	6	+/±	+/±	+/±	+/+	+/+
3160	6	+/±	+/±	+/+	+/+	+/+
4272	6	+/±	+/+	Eliminated		
2396	5	+/±	+/±	+/+	Eliminated	
383	4	±/±	NT	+/±	+/+	+/+
2922	5	+/−	+/+	+/+	Eliminated	
2086	5	+/−	+/−	+/+	Eliminated	
290	2	+/−	+/−	+/+	Eliminated	
292	2	+/−	NT	+/±	+/+	+/+
283	2	+/−	NT	+/−	+/−	+/−
352	4	+/−	+/−	+/−	+/+	+/+
1340	4	NT	NT	+/−#	+/−##	+/±
A2	1	+/−**	+/−	+/−	+/−	+/±
A5	1	+/−**	+/−	+/−	+/−	+/−

* ELISA/AGID # late pregnant NT : not tested
** young cattle ## parturition

Discussion

I used both ELISA and AGID tests to examine the serum samples from naturally infected herds and evaluated their diagnostic values. The preliminary results indicate ELISA is more sensitive and reliable than AGID test. Furthermore ELISA detects BLV infection in its early stages where AGID test could not. Early detection of BLV infection by ELISA made it possible to segregate infected animals earlier than the measure based on the ADID-test result.

I compared the efficacy of ELISA and AGID procedures of detecting infected cattle under field conditions in 6 naturally infected farms. The results showed 89.4% agreement between ELISA and AGID tests, 34.9% reacted positively by ELISA, and 27.5% positively by AGID. The results agree with those reported previously in that ELISA detects a greater number of positive BLV cases than AGID test (1,4,8). Furthermore, some BLV-negative or equivocal cases by the AGID test were also ELISA positive, in agreement with the report of Takahashi et al (11). More importantly, 6 AGID-equivocals, and 5 AGID-negatives became $ELISA^{+}/AGID^{+}$ on subsequent tests (Table 2) indicating that ELISA detects BLV infection earlier and with greater sensitivity than AGID test. Both the calf No. A2 and the pregnant cattle No. 1340 tested earlier as $ELISA^{+}/AGID^{-}$ and later become $ELISA^{+}/AGID^{\pm}$ on subsequent tests.

This is probably due to the fact that AGID has limitations, particularly with reference to the difficulty of demonstrating antibody in young cattle in the early stages of infection because of low antibody production. Pregnancy and parturition might be responsible also for low serum antibody level in virus carriers (1). The disagreements between ELISA and AGID test observed by us have been also reported in experimentally infected cattle or sheep (11, 14), and cattle in dairy herds under field conditions (2, 3).

As we have demonstrated in this study, ELISA showed a greater sensitivity and efficacy than AGID. Although the AGID test has been useful and simple to use, it failed to identify a fair share of infected animals. We recommend all AGID-negatives or equivocal cattle in naturally infected farms should be retested with ELISA, or by using a combined method as we have demonstrated here.

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