Detection of IgG antibody to Bovine Leukaemia Virus in urine and serum by two enzyme immunoassays

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2024/98: received and accepted 29 March 1999

K.T. CARLI, A. SEN, H. BATMAZ AND E. KENNERMAN. 1999. Four hundred blood sera from a cattle production unit were tested for BLV-(Bovine Leukaemia Virus) antibody with IP (Institut Porquier) and SB (Svanova Biotech) ELISA kits. Seventy-seven cattle with BLV-antibody (19·25%) and 77 without the antibody were used. No significant difference was found between O.D. of sera of PL + (Persistent Lymphocytosis Positive) and PL– (Negative) cattle. The mean O.D. of urine samples of 77 seropositive cattle was significantly higher than that of 77 seronegative cattle ($P<0·01$). There were also differences between urine O.D.s of seropositive (PL +) and seropositive (PL–) groups of cattle with IP ($P<0·05$) and SB ($P<0·01$) kits. All the results revealed the presence of BLV-antibody in the urine of the cattle without any urinary dysfunction.

INTRODUCTION

Studies using enzyme immunoassay and some other assays for the detection of specific antibodies to some retroviruses, especially to Human T cell-lymphotropic virus (HTLV) types I, II and III, and Bovine Leukaemia Virus (BLV), in different body fluids, including serum, have been carried out by several authors (Mammerickx *et al*. 1985; Hoff-Jorgensen 1989; Connel *et al*. 1990). Reagan *et al*. (1990) used commercial ELISA kits for anti-HIV antibody and found that all O.D. values of urine samples from seropositive (SP) individuals were above the calculated threshold level, but that those from seronegative (SN) individuals were below the threshold level. Carli *et al*. (1993) reported on the presence of anti-BLV antibody in 15 urine, milk and serum samples from the same cattle naturally infected with BLV using a commercially available ELISA, and observed that all samples from SPs had higher O.D. values than SNs. In the diagnosis of BLV infection with IgG capture ELISAs, milk, but not urine, has been used widely as reliably as serum (Hoff-Jorgensen 1989; Mammerickx *et al*. 1985), although both milk and urine contain sufficient detectable IgG type antibody (Butler 1973).

The aim of this study was to determine the presence of anti-BLV antibody in urine samples of a group of cattle having the antibody in their sera by the two most sensitive commercial ELISA for serum (Kramps 1994).

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MATERIALS AND METHODS

Sample collection

Serum samples from 400 cattle over 6 months of age in a cattle production unit were used to determine SP and SN.

Diagnosing of PL stage

Two blood samples were taken from SP cattle at 1 monthly intervals. Leucocyte formulae of these samples were made and the SP cattle population was divided into two groups, PL+ and PL– (Schalm *et al*. 1975).

Urine analysis

Urine samples taken from SP and SN cattle used in ELISAs were routinely analysed with Multstix 10 SG Bayer Diagnostic kits.

Enzyme immunoassays

ELISAs were performed according to the directions of the manufacturers of the kits (IP: Institute Pouquier, Montpellier, France; and SB: Svanova Biotech, Uppsala, Sweden).

Urine samples were taken in screw-capped tubes, placed in a plastic box containing ice, and used within 1 h without dilution and without delay. The cut-off values for serum were calculated as described in the test procedures while those for the urine samples were calculated using the formula: threshold positivity for urine = 2·5 × average score of O.D. scores of negative controls in the plate.

Statistics

Statistical analysis was performed using the student's *t*-test and Minitab 7.1 standard version statistical software.

RESULTS

The results showed that 77 cattle sera had anti-BLV IgG-type antibody (Table 1). Of these, 31 and 46 were identified as PL + and PL–, respectively. Serum O.D. were not significantly different between PL + and PL– groups of cattle in both tests. Significant differences ($P < 0{\cdot}001$) were found between the mean O.D. scores of urine samples of the SP and SN groups of cattle, showing that BLV-infected cattle had anti-BLV antibody in their urine (Table 2). It was observed that the urine samples of PL + cattle had a higher mean O.D. than that of urine samples of PL– cattle in both tests (Table 3) and the two different EIAs could detect anti-BLV antibody in the urine, with low sensitivity and without any false positivity (Table 4).

There were no abnormal parameters in urine analysis with respect to renal dysfunction of the cattle.

Table 1 Distribution of IP- and SB-O.D. scores of serum samples from SP and SN cattle

O.D.* scores	SP†–IP‡ cattle no.	SP-SB§ cattle no.	SN¶–IP cattle no.	SN–SB cattle no.
<0·500	—	—	77	77
0·501–1·000	—	3	—	—
1·001–1·500	—	7	—	—
1·501–2·000	1	36	—	—
2·001–2·500	33	29	—	—
2·500–3·000	43	2	—	—

* Optical density; † Seropositive; ‡ Institut Pourquier kit; § Svanova Biotech kit; ¶ Seronegative.

Table 3 The mean O.D. scores of urine samples from SP (PL+) and SP (PL−) cattle in both tests

Group	(X ± Sx)§
SP (PL+)‡-OD-IP	1·383 ± 0·169*
SP (PL+)-OD-SB	0·313 ± 0·060†
SP (PL−)‡-OD-IP	0·807 ± 0·107*
SP (PL−)-OD-SB	0·165 ± 0·010†

* Significance was found at $P < 0{\cdot}05$; † significance was found at $P < 0{\cdot}01$; ‡ persistent lymphocytosis positive; ‡ persistent lymphocytosis negative; § mean O.D. and standard.

DISCUSSION

BLV infection is present in the Marmara region in Turkey (Batmaz *et al*. 1995). In this study, the infection prevalence was determined as 19·25% in a cattle production unit in the same region.

The two commercially available BLV-Enzyme Immunoassay kits, which had been produced for detecting the antibody in serum, and previously found to be the most sensitive (Kramps 1994), were used in the present study. Both kits detected the same serum samples as positive for BLV-anti-

Table 2 Distribution of IP- and SB-O.D. scores of urine samples from SP and SN cattle

O.D. scores	SP–IP cattle no. (1·377)* (0·551 ± 0·060)†	SP–SB cattle no. (0·162) (0·064 ± 0·006)	SN–IP cattle no. (0·687) (0·275 ± 0·045)	SN–SB cattle no. (0·152) (0·061 ± 0·021)	SP–IP cattle no. (1·030) (0·412 ± 0·110)	SP–SB cattle no. (0·150) (0·060 ± 0·012)	SN–IP cattle no. (0·757) (0·303 ± 0·140)	SN–SB cattle no. (0·122) (0·049 ± 0·020)
<0·500	13	35	39	39	13	32	38	38
0·501	8	—	—	—	9	—	—	—
10··1–1·500	5	3	—	—	3	—	—	—
1·501–2·000	5	—	—	—	4	—	—	—
2·001–2·500	1	—	—	—	1	—	—	—
2·501–3·000	6	—	—	—	2	—	—	—

* Calculated threshold score for each plate (2·5 × Mean O.D., X); † X ± Sx.

Table 4 Detection of anti-BLV antibody in the urine by two different EIAs

Test	N* (SP)	SP sample number (%)		N (SN)	SN sample number (%)	
		RP†	FN‡		RN§	FP¶
SB	70	25 (35·7)	45 (64·3)	77	77 (100)	0
IP	70	23 (32·9)	47 (67·1)	77	77 (100)	0

* Number of sample; † real positivity; ‡ false negativity; § real negativity; ¶ false positivity.

body. Almost similiar results were also obtained from urine samples with the use of both test kits.

The presence of anti-BLV antibody in the urine samples has confirmed our previous report (Carli *et al.* 1993). In this work, O.D. scores of urine samples from SN individuals were below the calculated threshold positivity, but 23 samples (29·9%) of SP cattle with IP and 25 (32·5%) with SB were above, indicating that there was no false positivity and sufficient sensitivity to detect all BLV-infected cattle. Connell *et al.* (1990) and Reagan *et al.* (1990) also faced similar problems with anti-HIV antibody detection kits for serum when they used them for urine, and the problems were solved by producing specific kits for urine samples (Hashida *et al.* 1993). In addition, concentrating urine samples before use was also advised as an alternative procedure to increase the sensitivity of the test (Connell *et al.* 1990; Reagan *et al.* 1990).

In general, immunoglobulins found in urine are derived from two sources. The first is the local production of immunoglobulins in response to local infections of the urinary tract (Kantele *et al.* 1994). The second source is transfusion from blood serum (Butler 1973). In this study, anti-BLV antibody in the urine samples was considered as a transfusion product derived from blood serum, because no urinary dysfunction could be detected in the cattle tested. There are also other studies suggesting possible transfussion of anti-BLV antibody in bovine urine and of anti-HIV antibodies in human urine (Connell *et al.* 1990; Carli *et al.* 1993).

Results of the present study suggest that anti-BLV antibody detection in bovine urine could be possible with low sensitivity and without any false positivity by the EIA kits for serum.

ACKNOWLEDGEMENTS

This work was supported by Veterinary Medicine and Animal Husbandry Research Grant VHAG-1037 from the Scientific and Technical Research Council of Turkey (TUBITAK).

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