

Bovine Leukemia Virus Infection in Dairy Cows in Egypt

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Abstract: Bovine leukemia virus (BLV) is a lymphotropic virus that affects cattle. In the current study 240 Holstein, Holstein Friesian and Red Sindhi dairy Cows in Kafr El Shekh, Alexandria and Monofia governorates of Egypt from five dairy farms and at small holder breeders were examined for the presence of BLV. The sera of tested dairy cows were subjected to commercial Agar Gel Immuno-Diffusion (AGID) and ELISA kits specific for BLV antibodies. Also the milk samples were subjected to another commercial ELISA kit for detection of BLV antibodies in milk. Meanwhile, PCR technique was conducted on serum samples for the specific identification of the viral genome. Clinical examination revealed lymph node enlargement which was a predominant sign in 6.25% of animals. In some cases protrusion of conjunctival membrane was found in 1.67% of animals. Lameness and respiratory manifestations, such as rale and dry cough and emaciation appeared in a few cases representing 0.42, 0.83 and 1.25%, respectively. Laboratory results showed that 15.83% of cows were serologically by both tests positive where 4.17% of animals which belonged to different cow's farms were infected with BLV, 11.67% of animals which belonged to small animal holders were infected by BLV. Concerning the disease risk factors, 7.08% of infected animals were 4-6 years and 5.42% were 2-4 years, while 3.33% were less than two years, pointing out that the occurrence of the disease increases with age. ELISA tests in blood showed the same or even upper limit of detection than the same test in milk samples, when the analytical sensitivities were checked reactivity, dilutions of 1:64 and 1:32. PCR confirmed the results of serology but considered more accurate for it detects the viral genome and is more sensitive than serological tests especially in high dilutions which is a serious problem in the used methods.

Key words: Dairy cows • BLV • ELISA • AGID • PCR • Egypt

INTRODUCTION

Bovine leukemia virus (BLV) is a lymphotropic single stranded RNA virus belongs to family *Retroviridae*. Following infection, the BLV can remain clinically dormant, cause a persistent lymphocytosis (PL) with increased B lymphocytes, or cause B-cell lymphomas in lymph nodes and other tissues. Only a few (<5%) BLV-infected animals develop tumors [1, 2]. The disease was first reported by Leisering in 1871, who described the presence of yellowish nodules in the enlarged spleen of a cow [3, 4].

There are three types of BLV: Enzootic Bovine Leukosis (EBL), where spleen disruption consecutive by a retrovirus called BLV (Bovine Leukemia Virus) and sporadic bovine leukosis which is not transmissible. Besides the lethal form of BLV-induced leukemia,

persistent lymphocytosis (PL) is characterized by a permanent and relatively stable increase in the number of B lymphocytes in the peripheral blood [5, 6].

Various indirect and direct methods have been used for BLV detection, e.g. AGID, ELISA and PCR [7-12]. In some cases the indirect methods fail in the detection of BLV infections [13- 15].

In Egypt, BLV was diagnosed by Madbouly *et al.* [16], through a serosurvey in Kafr El-Shekh that used lysozymes and hematology in virus diagnosis. In 1989 an outbreak occurred in Arab El-Aoumar, Assiut, Upper Egypt [17]. Serological screening by enzyme-linked immunosorbent assay was performed and diagnosis was confirmed by the detection of BLV proviral DNA using polymerase chain reaction with primers amplifying a fragment of the env gene.

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The goal of this work was to isolate the virus from suspected dairy farms and small holders and maintain a suitable accurate method for virus diagnosis. Another goal was to develop a PCR suitable for direct detection of BLV in blood samples in comparison with other tests to be applied for detection of the virus isolate from dairy herd in Egypt.

MATERIALS AND METHODS

This work was performed in the period of January to June 2012. The work was done in the NRC.

Sample Collection: Whole blood and milk samples were collected from 240 Holstein, Holstein Friesian and Red Sindhi dairy Cows in Kafr El Shekh, Alexandria and Monofia governorates of Egypt from five dairy farms and at small holder breeders. Sera and milk samples were collected and kept in -70°C till use.

Agar Gel Immuno-diffusion test (AGID): The test was done using Pourquier® AGID Leukosis Ab Test (IDEXX Laboratory P00410). The test was conducted according to manufacture's instruction.

ELISA Techniques for Detection of Antibodies Against BLV:

Detection of BLV Antibodies in Serum Samples: Pooling of serum samples was performed and ELISA was performed by IDEXX Leukosis Serum X2 antibodies Test (IDEXX Laboratories, Inc., USA. EBT1132T). The technique was performed step by step as manufacturer's instructions.

Detection of BLV Antibodies in Milk Samples: Pooling of milk samples was conducted and a commercial ELISA kit for detection of BLV antibodies in milk was used (IDEXX Leukosis Milk Screening Ab Test, P02210-10, IDEXX Laboratories, Inc., USA.). The technique was conducted as described by manufacturer's instructions.

DNA Extraction and PCR Amplification: For the preparation of DNA, DNA purification kit was used (QIAGEN, USA), while the PCR amplification was performed using RCR kit (QIAGEN, USA). Primers were constructed using Oligo Primer Analysis program 19 published by Tagawa *et al.* [18]. These primers were selected to amplify 521 base pair (bp) fragments within the gp51 env gene of BLV. They are Forward 5'GGG CCA TGG TCA CAT ATG ATT G-3'(genome position

5128-5149) and Reverse 5'CGT TGC CTT GAG AAA CAT TGA AC 3'(Genome position 5627-5649). The technique was performed as the manufacturer's instruction.

RESULTS AND DISCUSSION

Field Observations: The most common symptom among infected cows was lymph node enlargement which was a predominant sign in 6.25% of animals. In some cases, protrusion of conjunctival membrane was found in 1.67% of animals and this may be attributed to the enlargement of retro-ocular lymph nodes. Lameness and respiratory manifestations such as rale and dry cough and emaciation appeared in a few cases and represented 0.42, 0.83 and 1.25%, respectively (Table 1). These findings match with that obtained by Murphy *et al.* [1].

AGID Test Results: The commercial kit detects anti-gp51 antibodies and the internal protein p24 of the bovine leucosis virus by using the agar gel immunodiffusion method (AGID). Out of 240 animals only 38 cows (15.83%) were serologically positive while only 25 cows (10 %) were showing clinical signs (Table 2). Table 3 showed that 4.17% (n=10) of animals which belonged to different cows farms, were infected with BLV and 11.67% (n=28) of animals which belonged to small animal holders, were infected with the virus and this may be explained by the fact that farms imports animal from more trustful and may have some measures than in case of small animal holders. These findings match the reports of Ababneh *et al.* [19]; Erskine *et al.* [20] and Lee *et al.* [21].

The test results also revealed that 7.08% of infected animals were 4-6 years of age and 5.42% were 2-4 years, while 3.33% was less than two years, confirming the fact that the virus infection rate increases in elder ones (Table 4). This result comes in agreement with the findings of Erskine *et al.* and Erskine *et al.* [22, 23].

ELISA Technique Results in Sera and Milk: ELISA test was performed as OIE prescribes the ELISA as a test to be used for international trade OIE [24]. The commercial

Table 1: clinical signs of BLV in dairy cows

clinical signs	Number of animals	Percentage
Lymph nodes enlargement	15	6.25%
Protrusion of conjunctival membrane	4	1.67%
Lameness	1	0.42%
Respiratory manifestations	2	0.83%
Emaciation	3	1.25%
More than one sign	6	2.5%
χ^2 value	32%**	

**P<0.05

Table 2: Shows animals which were positive against BLV

Total numbers of animals	Clinically positive animals		Serologically positive animals	
	Number	Percent	Number	Percent
240	25	10.41%	38	15.83%

Table 3: number of infected animals and their distribution

place			Positive BLV animals	
Governorate	Owner	Total number	Number	Percent
Kafr El Shekh	Farm	65	3	1.25%
	Small holder	23	12	5%**
Alexandria	Farm	54	4	1.67
Monofia	Farm	48	3	1.25%
	Small holder	50	16	6.6%**
x2 value		33.4**		

**P<0.05

Table 4: Shows age risk of dairy cows to BLV infection

Age	Total number	Positive BLV infection	
		Number	Percent
= 2 years	40	8	3.33%**
2-4 years	100	13	5.42%**
4-6 Years	100	17	7.08%**
x2 value		33.9**	

**P<0.05

enzyme immunoassay kit was used for the detection of antibody to bovine leukemia virus (BLV) by detection of antibodies against viral proteins, including the highly immunogenic early-appearing glycoprotein gp51. ELISA tests in blood showed the same or even upper limit of detection than the same test in milk samples, when the analytical sensitivities were checked reactivity dilutions of 1:64 and 1:32, respectively (Fig.1). The results match the results of Sorge *et al*, [25].

PCR for detection of BLV: The PCR described in this study, utilizing primers for BLV-env, gene which coding for gp51 env protein, amplified a fragment of

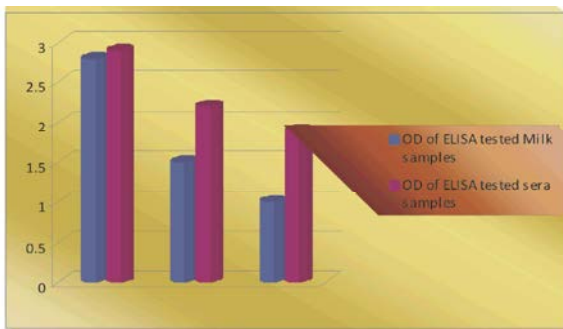


Fig 1: Shows comparison between ELISA on sera samples and milk sample.



Fig 2: PCR results: Positive white band at 521 bp. Lane 2: positive control, lane 5, 6, 7 contain positive samples; lane 4, 3 contain BLV DNA negative samples (reagents without sample); lanes 1 contain negative control; lane 1 contains molecular length markers.

approximately 521 bp. Fig. 2 shows PCR results obtained with DNA extracted from bovine blood samples where a white band was obtained at 521bp in positive samples. This result matches with the results reported by Adabadi *et al*. [26] and Tagawa *et al*. [18].

All three techniques detected almost the same numbers of infected animals these results were also confirmed by Ballagi-Pordany *et al.* [27], Kuzmak *et al.* [28] and Dube *et al.* [29]. Abd El Hafeiz *et al.*, [30] reported in their study the dilution of AGID antigen 80 times and using it for coating the ELISA plates. Also the tested sera in the same study was diluted 1/160. By this reported method, ELISA proved to be more sensitive than AGID as it was able to detect lower titer of antibodies than AGID. This fact is also mentioned in the manufacturer's leaflet guide.

The current study recommends the use of PCR in BLV diagnosis as it detects the viral genome and due to the high sensitivity of this technique even in high dilution of the tested samples, Also it is considered as the most economic method compared with commercial ELISA and AGID tests. The study also recommends importation of dairy cows from countries that are free from BLV with certificates that confirm this fact. Furthermore, the imported animals should be tested in the quarantine before introduction to farms where all recommended tests should be applied on them. Moreover, milk also should be periodically checked as a routine work by ELISA on blood followed by PCR to ensure that the colostrum of the dams are free from the disease to avoid transmitting the disease to young calves.

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