Serological Diagnosis of Infection with Bovine Leukemia Virus in Dairy Cattle, Camel and Ovine

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ABSTRACT

Bovine leukemia Virus (BLV) is world wide pathogen infecting white cells and causing wide variety of multiple persistent diseased syndromes in dairy cattle. A total of 926 serum samples (480 bovine, 214 camels and 232 ovine) were randomly collected and used to determine the seroprevalence of infection with BLV. The 480 bovine samples representing 18 unvaccinated dairy herds of more than 20 month age by scheme of 15 to 44 (10% of herd capacity) while the ovine and camel's sera were collected individually and randomly during slaughtering. All the serum samples were tested for antibodies to BLV using indirect ELISA. The individual sample positive ratios for antibody by ELISA were ranged from 0.518 to 1.650by means of 0.532 to 1.089. The positive dairy herds were 14 herd of 18 by at least one positive sample have. A total of 82 bovine sera of 480 were positive by a percentage of17%. The BLV seropositivity and percentages in camel and ovine sera were 15/214 (7%) and 18/232 (7.8%) respectively. Significantly high percentages of BLV seropositive were reported among the oldest cows, camels or ovine samples that had persistent lymphocytosis and enlarged lymph nodes, as compared to apparently healthy ones. The results provide the first prevalence of natural BLV infections in the dairy, camels and ovine herds that already carried the virus as immunotolerants.

Key Words: BLV, Dairy cattle, Camels, Ovine, Seroprevalence, ELISA.

INTRODUCTION

BLV is an insidious worldwide, transmissible virus, associated with sub clinical enzootic bovine leukosis (EBL) infection in cattle, camels and ovine; in Canada *(1)*, in Ireland, *(2)*; USA *(3)*. It is classified within *Genus Delta viruses*, of the *Family Retroviridae*. It is transmitt to animals, horizontally by direct contact, consumption of colostrums and/or milk; transplacentally by unknown mechanism; iatrogenically, via multiple examinations, vaccinations or samplings using the same equipments; Biting flies during the summer may contribute. Although the BLV do not spread via embryo transfer or AI; infected females after natural matings with infected bulls have been reported **(4)**. BLV rarely found as free virus as it infects B-lymphocytes and integrates as proviral DNA into the host chromosomes, so any duplication of the host cell automatically duplicates the virus DNA resulting in established, prolonged life long asymptomatic period of 4-8 years despite of the active virus specific humoral and cellular immune responses that are incapable of eliminating or clearing the viral DNA form *(5)* It associated with 5% multicentric fatal lymphoid neoplasia by aggregation of B lympocytes, leukemic infiltration and enlargement of organs and tissues distributed in thymus, spleen, heart, abomasums, lymph nodes, uterus, kidneys, eye, spinal meninges and brain. About 30% of the infected cows develop persistent B-lymphocytosis, immunosuppression and deaths ***(6, 7, 8, 9).***

BLV has economic impacts on cattle industry, lower on-farm productivity from reduced milk production, increased morbidity, mortality rates of 10 to 50%, culling, reduced weight gain, abortion and reduce import or export competitiveness among cattle trade. Further, BLV infection is associated with secondary infections and stresses of parturition *(10, 11)*. Ovine is susceptible to experimental infection with the BLV, with immunocmpetence and development of

lymphosarcoma *(12)*. Previous reports failed to detect the BLV antibodies of natural infection in Camelus dromedaries *(13)*.. Diagnosis based on clinical signs alone is often difficult because of the wide range of symptoms. Up to 90% of infected host become seropositive 3-12 weak post infection with or without clinical signs that remain a potential reservoirs and recognized only by serology (**9**). This study was to estimate the seroprevalence of BLV infection in unvaccinated dairy cattle, camels, and ovine using indirect antibody ELISA.

MATERIALS and METHODS

Animals and collection of blood samples: Holstein dairy herds older than 20 month of age represented by 10% of the herd capacity, in addition ovine and camel's sera that were collected individually and randomly. There was neither contact between the herds nor previous history of BLV infection nor vaccination against the virus. Most of animals showing frequent viremia, enlarged cutaneous and lymphoid nodules. A total of 926 serum samples (480 bovine, 214 camels and 232 ovine of sheep and goat) were randomly collected. The blood samples were allowed to clot then centrifuged at 3000 rpm / 10 minutes. Sera were separated inactivated at 56^0C for 30 minutes and then stored at -20^0C til tested by ELISA.

Measurement of BLV specific antibody using BLV ELISA:

A- Screening Assay: Antibody of BLV in the cattle, ovine and camel sera in comparison to positive and negative controls were assessed using BLV ELISA (IDEXX Lab., Westbrook, USA) of sensitivity 98.5% and specificity 99.9%; *(14,15)*. The sera were screened by a dilution of 1:25 that was conducted and performed according to the manufacturer's instructions. The formed antigen-antibody complex was detected by the subsequent addition of BLV anti-bovine HRP conjugate, chromogen TMB substrate and stop solutions. The optical density (OD) / absorbance of the plates were measured using ELISA reader blanked with air (Dynatech, MR5000, Ireland) at the wave length of 650 nm.

B-Verification assay: The reactive samples by the screening test were confirmed using a verification assay depended upon the same samples above dilution and the use of strips coated with BLV and normal host cell antigens in duplicates and measured as above.

Test validity: The validity of the assay was considered as the negative control mean (NCx) for BLV and the normal host cell (NHC) wells had to have an OD at 650 nm (OD_{650}) of $\leq$ 0.200, with a difference of $\geq$ 0.075 with the positive control mean (PCx - NCx).

Interpretation of results: The means of OD for both positive and negative controls were calculated and then the Corrected Optical Density (cOD) was calculated. The positivity or negativity for BLV antibody in the sera was calculated from Sample/Positive ratio (S/P) as:

:

$$S/P = \frac{\text{Sample OD Absorbance - Mean of Negative OD Absorbance}}{\text{Corrected Optical Density (COD)}}$$

As:

Negative	Positive
S/P < 0.500	S/P ≥ 0.500

RESULTS

Measurement of BLV specific antibody using BLV ELISA:

The intensity of color was directly proportional to the level of BLV antibody in the sera. The individual OD values of the samples were varied from 0.381 to 1.853 in comparison to the averages of the reference negative and positive sera of 0.165 and 0.586 respectively. The most herds showed at least one sample was seropositive for BLV. The sample positive ratios among the individual sera for antibody by ELISA were 0.518 to 1.650 by means of 0.532 to 1.089 at herd levels. The number of seropositive herds were 14/18 (78%) by percentages ranged from 0 to 28%. The number of positive cows' sera for BLV was 82 samples of 480 by a percentage of 17%. Herds 2, 3, 4, 5, 10, 11, 12, 14, and 17 showed higher seropositivity by percentages of25%, 23%, 27%, 21.4%, 28%, 19%, 20%, 28%, and 20% respectively (table1, figure1). The seropositivity for BLV in camel and ovine sera was 15/214 and 18/232 by percentages of 7% and 7.8% respectively (table2 & figure 2). Significantly high BLV positive sera were reported among the oldest cows, camels or ovine that had persistent lymphocytosis and enlarged lymph nodes, as compared to apparently healthy ones.

Table (1): Scheme of blood sampling and prevalence of BLV antibodies in dairy cattle using ELISA:

No. of Herd	No. of Samples	Mean of Ab S/P	No. & % of Positive
1	24	0.740	3/24 (13%)
2	28	0.532	7/28 (25 %)
3	40	0.640	9/40 (23 %)
4	44	1.089	12/44 (27%)
5	28	0.640	6/28 (21 %)
6	28	0.533	4/28 (14%)
7	16	0.560	2/16 (13%)
8	20	0	0/20 (0%)
9	34	0.640	4/34 (12 %)
10	29	0.533	8/29 (28 %)
11	42	0.650	8/42 (19 %)
12	30	0.754	6/30 (20 %)
13	18	0	0/18 (0 %)
14	25	1.089	7/25 (28 %)
15	18	0.533	1/18 (4 %)
16	16	0	0/16 (0 %)
17	25	0.685	5/25 (20 %)
18	15	0	0/15 (0 %)
Total = 18	480	Mean = 0.532 to 1.089	82/480 (17%)
Number of positive herds = 14 / 18 (78 %)			

S/P: Sample to Positive ratio. **Ab: Antibody**

Table (2): Scheme of blood sampling and seroprevalence of BLV antibodies in Camel and Ovine sera using ELISA:

Camel			Ovine		
# of Samples	No of Positive	% of Positive	No of Samples	No of Positive	% of Positive
214	15	7%	232	18	7.8%

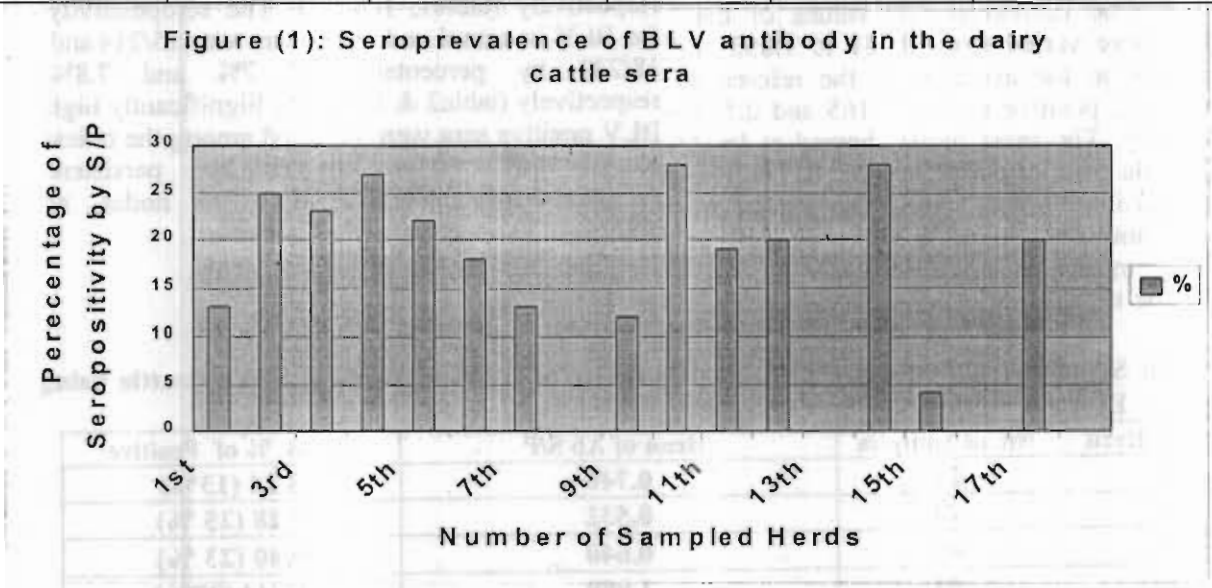

Figure (1): Seroprevalence of BLV antibodies in dairy cattle using ELISA.

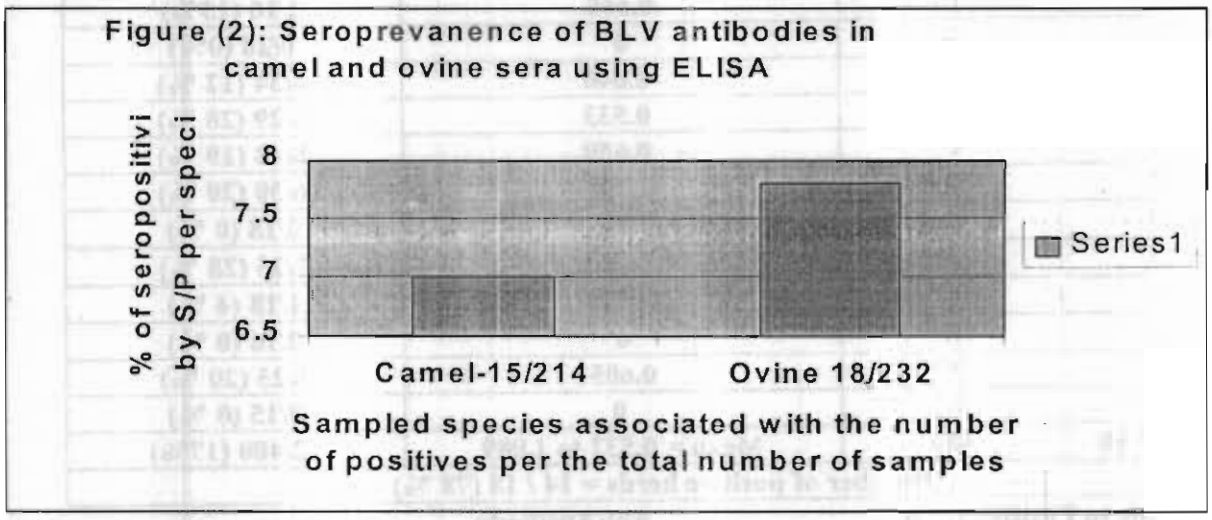

Figure (2): Prevalence of BLV antibodies in camel and ovine using ELISA.

DISCUSSION

The prevalence and distribution of enzootic bovine leukosis (EBL) caused by BLV was tremendously increased. Therefore, serologic diagnosis by ELISA provided sufficient sensitivity, specificity and quantitative for determining the infection status of the virus in the susceptible animals. The presence of antibodies to BLV indicates that the animals have been exposed to and may be chronically infected. The sample positive ratios by ELISA among the individual sampled animals were 0.518 to 1.650 by means of 0.532 to 1.089 at herd levels. The number of positive cows was 82 samples of 480 by a percentage of 17%. Although the prevalence rate was not so high (17%) as compared to previous studies in other areas, it provides information on the wide spread and the potential co-infection with BLV and in accordance with ***(16,17,18,19)*** who reported seroprevalecne of BLV in dairy herds but by percentages around 52%. Moreover, most of the cows included in this study in addition to their nursing calves had been previously tested positive for the BVDV antigens using Buffy coat cells ***(20)***. A percentage of BLV seronegative cattle were BVDV seropositive, thus, such cattle infected with BVDV may have diminished antibody responses to BLV infection with false negative results when tested for BLV and in accordance with ***(21)***. A correlation between BLV seropositivity and the presence of lymphoid abnormalities was reported in a few sampled animals that had significantly higher antibody titers than apparently healthy ones. Therefore, the presence of lymphocyctosis and enlarged nodules may have been coincidental, related to a more advanced stage of the infection. These findings indicated the wide spread of BLV persistent infection and in a close similarity and concordance with the previous findings reported by ***(22,23,24,25,26)*** whose reported the infected cattle become sero-converted by 3 weeks post infection.

A few studies elsewhere had been reported on experimental infection with BLV in sheep with a mild lymphosarcoma and slow immunocompetence by 1-3 months post infection ***(27)***. In other studies, seroprevalence for the natural infection with the virus failed to detect specific anti-BLV antibodies in either sheep ***(28, 29)*** or in dromedary mares (*Camelus dromedaries*) ***(13)***. In this study, seropositive rates for BLV in ovine was 18/232 by percentage of (7.8%) while for camels was 15 / 214 by percentage of (7%). The percentages of seropositivity are not too high but relatively close to the previous reports. The lowest results for ovine and camel's samples might be due to the different natural host susceptibility, short life span of the ovine as the virus is slow viral developing disease, different breeds as well as different used assay which is not ELISA that was used in the current study. However, the prevalence of virus infection was variable, not uniform, increases or decreases with the age, management and densities of animals that would facilitate the virus transmission within herds. Because the cattle tested were all less than 3 years of age, this may also account, in part, for the low prevalence that in agreement with ***(30,31)***.

ELISA used was based on the use of ultra purified virus lysate, with both screening and verification assays demonstrated the presence of all types of antibodies against the BLV (envelope proteins, capsid proteins gP51, etc) and enables to take into account possible non-specific interactions ***(21)***. In this study, the sampled animals were neither vaccinated nor previously infected with BLV. Therefore, the seropositivity must be due to natural exposure and is consider the first report documenting the BLV infections in the sampled dairy cattle, camel and ovine herds that already carried the virus as immunotolerants. Future studies should be deigned to isolate the virus, investigate its pathogenesis, prepare and/ or

import a vaccine for control and prophylaxis against such virus infection.

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التشخيص السيرولوجى للعدوى بفيروس لوكيميا/ سرطان كريات الدم البيضاء في الأبقار والجمال/ الإبل والأغنام

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-إن الغرض من هذه الدراسة تشخيص مدى تواجد العدوى بفيروس سرطان كريات الدم البيضاء في بعض قطعان الأبقار والجمال/ الإبل والأغنام.
-يتميز هذا الفيروس بالإنتشار واسع النطاق مسبباً مجموعة من الأعراض المرضية المزمنة.
تم تجميع **926** عينة مصل موزعة كالأتي: ٤٨٠ من الأبقار الحلابة و٢١٤ من الجمال/ الابل و٢٣٢ من الأغنام والماعز الغير محصنة.
-تم قياس الأجسام المناعية المضادة الخاصة بالفيروس فى جميع عينات المصل باستخدام اختبار الإليزا.
-معدل إيجابية وقراءة العينات الفردية تراوحت من ٠,٥١٨ إلى ١,٦٥٠ وبمتوسطات بين المجاميع/ القطعان من ٠,٥٣٢ إلى ١,٠٨٩.
-بالنسبة للعدد الكلي لعينات الأبقار فإن ٨٢ عينه من ٤٨٠ بنسبة ١٧% أظهرت إيجابية للأجسام المضادة للفيروس وكان عدد قطعان الأبقار الإيجابية ١٤ من إجمالي ١٨ وبنسبة كلية ٧٨%.
.إن ١٥ عينه من عينات الجمال الكلية الـ ٢١٤ بنسبة ٧% بينما ١٨ من الأغنام والماعز الـ ٢٣٢ وبنسبة ٧,٨% قد أعطت إيجابية للأجسام المضادة بقراءت متماثلة مع عينات الأبقار.
-بوجه عام فإن عينات المصل الإيجابية كانت من الحيوانات ذات الأعمار الكبيرة و التى تزيد عن ٣ سنوات.
-أثبت هذا البحث من خلال استخدام اختيار الإليزا ذات الحساسية العالية فى التشخيص والكشف عن العدوى بالفيروس فى قياس مدى الإنتشار الواسع للفيروس بين القطعان المنتجة من الأبقار والجمال/الإبل والأغنام والماعز والذى يؤثر بالسلب على الإنتاج العائد مما يتطلب عزل ودراسة خصائص الفيروس وبالتالى تحضير أو استيراد اللقاح اللازم والمناسب للوقاية والتحكم فى العدوى بذلك الفيروس.