Comparatively, Immuno-prevalence of Bovine Leukemia Virus in Cattle Blood and Milk Samples by an IDEXX ELISA Technique

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Abstract

Bovine leukemia virus is an onchogenic pathogenic agent that affecting on bovine B-cell lineage and resulting in a long latency period-disease. In this study, a totally of (92) cattle were undergone for blood samples collection, which involved 44 lactating cows subjected, additionally, for milk samples collection, and then, both samples were submitted for serological examination by IDEXX ELISA test that applied for first time in Iraq. The overall seroprevalence results revealed that 14.13 % of blood samples and 6.82 % of milk samples were sero-positives. In relation to sex factor, the ser-positive infection rates were 7.41 % and 16.92 % in males and females, respectively, whilst in relation to age factor, the sero-positive infection results of (≥ 1-3), (3-5) and (> 5) years age's groups were 3.23%, 10.81% and 33.33%, respectively. Statistically, the significant differences were reported between the sero-positive results for both tested samples, blood and milk, as well as within both sex groups and age groups at ($P \geq 0.05$) level.

Keywords: Bovine leukemia virus, Blood serum, Milk, Cattle, ELISA

بالمقارنة ، الانتشار المناعي لفيروس سرطان الدم البقري في عينات دم وحليب الابقار باستعمال تقنية الايديكس اليزا

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الخلاصة

فيروس سرطان الدم البقري هو مسبب مرضي سرطاني يصيب سلالة الخلايا اللمفية البقرية B–cell ويؤدي الى مرض كامن لفترة طويلة . في هذه الدراسة ، خضعت بالاجمال (٩٢) بقرة الى جمع عينات الدم ، التي من ضمنها (٤٤) بقرة مدرة للحليب خضعت ، اضافيا ، الى جمع عينات الحليب ، وبعد ذلك خضعت كلا العينتين لاختبار الايديكس اليزا المصلي الذي يستعمل لاول مرة في العراق . كشفت نتائج الانتشار المصلي الكلية بان ١٤,١٣ % من عينات الدم و ٦,٨٢ % من عينات الحليب كانت موجبة مصليا . فيما يتعلق بعامل الجنس ، كان معدل الاصابة الموجبة مصليا هو ٧,٤١ % و ١٦,٩٢ % للذكور والاناث ، على التوالي ، اما فيما يتعلق بعامل العمر ، كانت نتائج الاصابة الموجبة مصليا للمجاميع العمرية (≤١– ٣) ، (٣ – ٥) و (< ٥) هي ٣,٢٣ % ، ١٠,٨١ % و ٣٣,٣٣ % ، على التوالي . احصائيا ، سجلت الاختلافات المعنوية فيما بين النتائج الموجبة مصليا لكلا العينتين المختبرتين (الدم والحليب) ، اضافة الى ذلك ، داخل مجاميع الجنس والمجاميع العمرية عند مستوى ($P \leq 0.05$).

الكلمات المفتاحية : فيروس سرطان الدم البقري ، مصل الدم ، الحليب ، ابقار ، اليزا

Introduction

Bovine leukemia virus (BLV) is an oncogenic single-stranded RNA virus belongs to *Deltaretrovirus* genus of *Retroviridae* family, which first identified by Miller et al., (1969) and confirmed its transmissibility in 1972 **(1).** The virus is undergoing a transcription in a DNA of infected cell, inserted into host genomes and remains dormant **(2).** Although, the major target cell of BLV is usually in B lymphocytes, it can be persist in T lymphocytes, monocytes, macrophages, granulocytes and in other tissues **(3).** BLV is distinguish to be the etiological pathogen of enzootic

bovine leukosis (EBL), a disease that results in large, directly and indirectly, losses such as mortality, weight loss, growth retardation, reproductive damage with abortion and reduced milk production **(4).** The virus is worldwide distribution and had been reported in most countries. Although, EBL infections in almost does not related, clinically, with any clinical signs, the virus can be result in a persistent lymphocytosis in about 30% of infections, and by lymphoid tumors (lymphosarcoma) in 5% of BLV cattle **(5).** Once infected, cattle remain carrier for virus for life and start to show a serological reaction within a few weeks after infection **(6).** Thus, the early diagnosis of infected carriers is very important in cattle industries to estimate the prevalence of virus and to application an intensive eradication programs **(7).** Several direct and indirect methods have been applied for detection of BLV in infected carriers as serological and molecular techniques that have been with variable degrees of sensitivity and specificity **(8).** Although, the agar-gel immunodiffusion (AGID) test was used widely for long period and considered as the test of choice for detection of BLV, but the sensitivity of this serological test is limited and instances have been reported were infected animals failed to produce a detectable antibody response **(9).** Recently, the IDEXX ELISA test was applied in several studies and reported a high efficacy because of its simplicity, rapidity, high sensitivity and specificity **(10, 11).** The main goals of present study were to seroprevalence of IgG antibodies against BLV in blood serum and milk samples by application of IDEXX ELISA test, for first time in Iraq, and to compare between sero-positive results of both samples to evaluate their validity in detection of virus; as well as to determine the relationship between infection with sex and age factors.

Materials and Methods

- **Samples collection**: From rural areas in Wasit province, and during the period of (April-August) / 2016, an overall (92) cattle from both sexes more than 1 year old age, were submitted to this study. These cattle were divided into two groups according to their sex, included 27 males and 65 females; and to three groups according to age, included 31 (≥1-3 years), 37 (3-5 years) and 24 (>5) years. About 10 ml of whole blood sample, without anticoagulant, were collected from all tested cattle, allowed for clotting and then centrifuged at 1200 ×g for 15 minutes. Serum sample for each one was inserted into a special micro-tube (1ml), and kept until be analysed at -20°C. Also, samples of milk were gathered

from 44 adult cows, labeled in correspondence with blood samples, defatted and centrifuged at 2500g for 15 minutes. The skim milk below the fat layer was transferred into labeled tubes and kept at - 40°C until be analysed **(12, 13).**

- **Serological technique**: in September / 2016, the serum samples have been analysed by using an IDEXX ELISA test (IDEXX Laboratories, USA), which based on demonstration of existence of the specific antibodies against BLV in examined cattle. According to manufacturer's recommendations, the samples were measured at (450 nm) and the Optical densities values for samples and controls were recorded. The test's results were obtained by application the following equations found in (Table 1).

Table (1): Calculations of Test's Results

1	**Controls**	Positive control (PC)	**PC $\bar{x}$ = PC1 + PC2 / 2**
		Negative control (NC)	**NC $\bar{x}$ = NC1 + NC2 / 2**
2	**Validity Criteria**		**PC $\bar{x} \geq 0.350$**
			PC $\bar{x}$: NC ≥ 3
3	**Samples**	**S/P % = Sample – NC / PC $\bar{x}$ – NC**	
4	**Interpretation**	Negative	**S/P % ≤ 50**
		Positive	**S/P % > 50**

Statistical Data Analysis: all the diagnostic data were tabled as positive or negative and stored as a database. The confidence intervals and statistical tests for clustered data were analysed by using the Microsoft Office Excel (v.12) and IBM/SPSS (v.20) computer programs. The statistical differences have been, significant, at a level of ($P \leq 0.05$) **(14).**

Results

- In (Table 2), in a totally (92) cattle submitted for blood serums testing by IDEXX ELISA, 13 (14.13%) of cattle were sero-positives, and 79 ones (85.87%) were sero-negatives.

Table (2): Results of Blood Serum Testing in All Study's Cattle

Total No.	Positives		Negatives	
	No.	%	No.	%
92	13	14.13 %	79	85.87 %

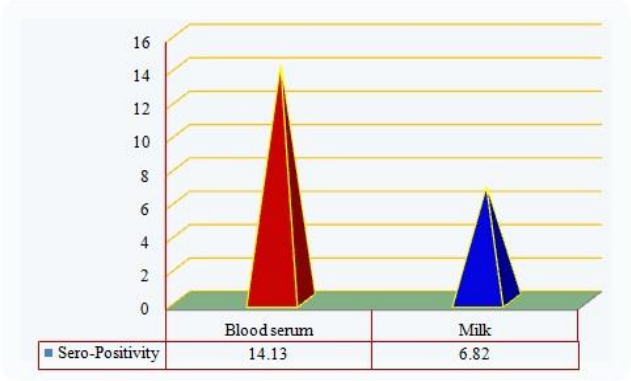
- In (Table 3), the milk samples of 44 lactating cows were tested by IDEXX ELISA test that detected, only, 3 (6.82%) sero-positive cows. In this study, the sero-positive milk samples cows were reported previously as, also, sero-positives with blood samples examination.

Table (3): Results of Milk Samples Testing in Lactating Cows

Total No.	Positives		Negatives	
	No.	%	No.	%
44	3	6.82%	41	93.18%

- In (Figure 1), the obtained results of both tested samples, blood serum and milk, were compared and revealed that 13/99 (14.13%) and 3/44 (6.82%) were ser-positives, respectively.

Figure (1): Comparison Sero-positive Results of Blood Serums



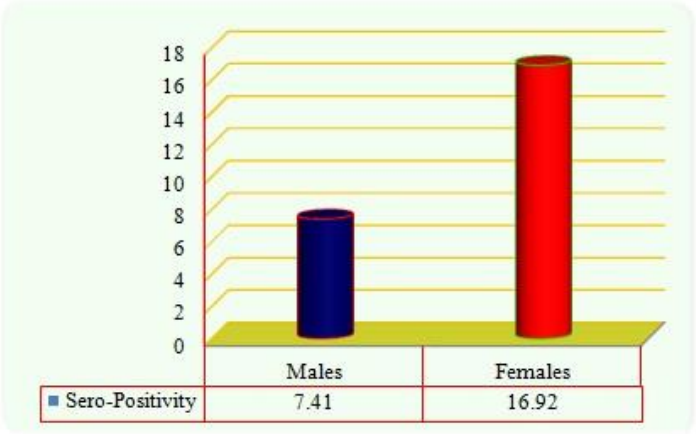
- In (Table 4 and Figure 2), the study's cattle were divided into to two sex groups included (27 males) and (65 females), and the sero-positive infection rates were 2 (7.41 %) and 11 (16.92 %), respectively.

Table (4): According to Sex, Positive Blood Sampling Cattle

Sex	Total No.	Positives	Negatives
Male	27	2 (7.41 %) [b]	25 (92.59 %)
Female	65	11 (16.92%) [a]	54 (83.08 %)

Difference in small letters, referred to a significant difference at ($P \leq 0.05$)

Figure (2): Results of Sero-positive Cattle According to Sex



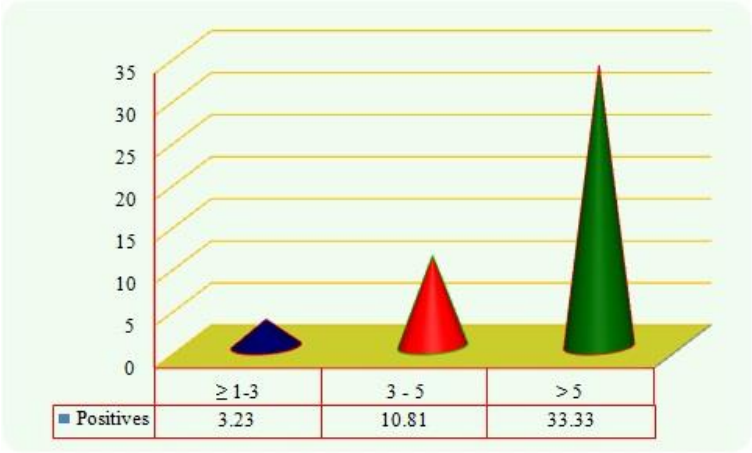
- According to results of (Table 5 and Figure 3), dealt with an association between sero-positivity and age of tested cattle that divided into three age groups, which revealed the sero-positivity results for three age's groups (≥ 1-3, 3-5 and ≥ 1-3) as 1 (3.23 %), 4 (10.81 %) and 8 (33.33 %), respectively.

Table (5): Results of Positive Cattle According to Age

Age	Total No.	Positives	Negatives
≥ 1-3	31	1 (3.23 %) [c]	30 (96.77 %)
3-5	37	4 (10.81 %) [b]	33 (89.19 %)
> 5	24	8 (33.33 %) [a]	16 (66.67

Difference in small letters, referred to a significant difference at (*P≤0.05*)

Figure (3): Results of Sero-positive Cattle According to Age



Discussion

Infectious bovine leukemia virus (BLV) is a significant viral pathogen of cattle that found worldwide. In present study, the results of testing blood and milk serums were 14.13% and 6.82%, respectively, which, both have higher outcome than those reported in previous serological studies have been done in Iraq by **(15, 16).** Globally, the prevalence of BLV was varies within and between countries, appreciably, between countries and has been found to be as low as 5% in Taiwan and Cambodia, 17% in turkey, 22.3 in Iran, 25.7% in Canada and as high as 83.9% in United States **(17, 18).** Several factors might be describe, practically, the variation in sero-positives of BLV infections such as geographical locations, cattle population, herd density, breeds, age, sex, origin and managements **(19).** Also, a significant difference between blood and milk sero-positive results were reported in this study, which might be related to the low level of milk's BLV-antibodies, technique or apparatus-performance, milk degeneration, udder complications or infections. Although, **Jaworski et al., (2016)** had the ability to detect BLV-specific antibodies in 94% of plasma samples and 90% of milk samples, he found that the mean of these antibodies titer in plasma was 10-fold than in milk and the DNA was detected in 82% and 59% of blood and milk samples, respectively **(20).** In veterinary medicine, the diagnostic tests have many important applications including research, epidemiological surveillance with certification for areas free of

disease and prevalence estimate studies **(21).** Serological tests have been used more extensively to identify the infected cattle with virus due to their rapidity, cost-effectiveness and easy for interpretation **(3).** Although, agar gel immunodiffusion test is the prescribed diagnostic technique for international trade and the most common test used for detection of BLV-specific antibodies, it influenced by cattle's type and age, serum antibody level as well with lactation phase **(9). Kobayashi et al., (2014)** confirmed that the accuracy and efficiency of a commercially ELISA test were, reasonably, higher than other serological tests, and the level of performance was very acceptable **(22). Jaworski et al., (2016)** detected that the sensitivity and specificity of ELISA were 97.2 and 97.5, respectively. In relation to sex, the study's results showed that the females have an infection rate more than males. Worldwide, the studies and information which correlated between BLV and the sex, as risk factor, were actually absent or not available. Though, this result can be attributed to low number of tested males, most tested males were young in age, ecological and reproductive causes or hormonal and genetic variations **(20).** However, cows appear to be more ability for transmitting the virus by blood during gestation or blood transfusion as well as milk during suckling **(23).** In this study, the results revealed that the BLV infections were more pronounced in older age (> 5years) and increased, progressively, with advancing age. Although, **Tiwari et al., (2005)** has found that the relationships of BLV with age of dairy cattle aren't statistically significant **(24). Erskine et al., (2012)** showed that there was a negative relation of prevalence of BLV with cow's age, which evaluated as the rate of cattle group in 3rd or high lactation **(25).** However, many studies determined that all cull rates increased significantly as BLV-serologically positive cow's age and this may be especially important among BLV-infected that transform to lymphocytosis **(26). Mousavi et al., (2014)** demonstrated that the morbidity rate of BLV was tending to be elevated among cattle with older ages **(18).**

In conclusion, the study has shown that the virus is widespread and seroprevalence of BLV is continued to be increased in an accelerated steps due to management problems such as poor condition of sanitation and high density population. As no therapy or vaccines are existing; commercially, the control schemes depended on detection and removal of infected cows can be realized in main by identification of antiviral antibody. In Iraq, an eradication plan based on sero-grouping and segregation of sero-positive from sero-negative animals had facing several practical

difficulties as well as high economic losses. Also, further investigations are required to explain the actual causes for increasing of virus spread and elucidating the keys of risk factors in an attempt to control new infections.

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