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Oxidative state markers and clinicopathological findings associated with bovine leukemia virus infection in cattle

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ABSTRACT

The aim of present study was to investigate hematological, biochemical and oxidative state parameters in cattle spontaneously infected with bovine leukemia virus (BLV). A total 500 cattle were examined for BLV infection by enzyme linked immunosorbent assay (ELISA). Eighty (16%) animals were positive for BLV infection. Biochemical and oxidative stress markers revealed significant increases in liver enzymes Alanine Transaminase, Aspartate Transaminase and Alkaline Phosphatase (ALT, AST and ALP) activities, creatinine level and superoxide dismutase (SOD) activity associated with a significant decrease in calcium level in seropositive cattle in comparison with seronegative cattle. Meanwhile, non-significant changes were reported in levels of malondialdehyde (MDA), Nitric oxide (NO), reduced glutathione (GSH) and hematological parameters in seropositive cattle in comparison with seronegative cattle.

1. Introduction

Enzootic bovine leukosis (EBL) is a lymphoproliferative infectious disease naturally occurring in cattle caused by the bovine leukemia virus (BLV) infection [1,2]. Once BLV infects a cell, it integrates as a DNA intermediate as a provirus both randomly and permanently, into the genome of lymphocytes. BLV preferentially infects B cells [3].

There are three different stages of BLV infection: first, BLV infection alone without any clinical expression; second, increase of the absolute number of peripheral blood lymphocyte (persistent lymphocytosis); third, lymphoma; the common form in adults [4,5]. Transmission of EBL between cattle occurs by exposure to infected lymphocytes in blood during parturition, rectal palpation, contaminated surgical instruments and blood sucking insects [6].

Enzootic bovine leukosis (EBL) has worldwide distribution. Whilst it has been eradicated in most of Western Europe and Scandinavia, it remains a problem in other regions, particularly Eastern Europe and South America [7]. Leukosis causes a huge economic damage both to the productive and breeding livestock through the culling of the diseased animals and elimination of virus carriers from breeding [8,9].

Virus infection may alter the oxidative status within a host cell [10]. Oxidative stress is a central issue in the process of aging and in the transformation or death of living cells [11]. BLV infection most often does not cause any clinical signs. Persistent lymphocytosis occurs in about one third of all BLV-infected cows, while the remaining cows have a normal cell count [3,12].

Therefore, the present study was aimed to investigate the changes in hemato-biochemical and oxidative status associated with BLV infection in dairy cattle.

2. Materials and methods

2.1. Animals and samples

The study was conducted according to principles of good practice and approved by the Ethical committee for animal experiments of Faculty of Veterinary Medicine, Benha University.

The sample size was estimated using Win episcope 2.0 with an expected prevalence of 10 and a 5% accepted error being 139 animals. We increased this sample size to 500 cattle, divided between 6 dairy herds located in different localities in Egypt during 2018 to study the seroprevalence of the disease among dairy cattle.

The area of study was divided into six sample districts that corresponded to six herds in three governorates in Egypt. Dairy farms from

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the interior of each district were chosen and animals were selected randomly. Cattle were used in this study were female Holstein-Friesian dairy cattle. The age of selected cattle was ranging between 2 and 7 years and 150 cattle were pregnant and rest of the animals were in lactating stage.

Regarding hemato-biochemical examination and oxidative state evaluation, whole blood samples were collected from seronegative and seropositive animals. The collected blood samples were divided into two portions. The first one was collected into a clean tube containing EDTA as anticoagulant for hemogram evaluation. The second portion was collected into plain tube to separate serum.

2.2. Serological examination using ELISA

All serum samples were examined serologically using IDEXX Leukosis Serum Screening Ab Test (IDEXX laboratories, Westbrook, Maine, USA) to detect antibodies against BLV according to manufacturer's instructions.

2.3. Hematological evaluation

Hematological parameters {Red blood cell (RBC) counts, hematocrit, White blood cell (WBC) counts, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte count} were estimated according to Feldman et al. [13]. Hemoglobin concentration was estimated according to Van Kampen and Zijlastra [14].

2.4. Biochemical parameters evaluation

Transaminases (ALT, AST) were assayed with commercial kits (Centronic, wartenberg, Germany). The activity of both enzymes was determined according to Reitman and Frankel [15]. Alkaline phosphatase (ALP) activity was assayed according to Stockham and Scott [16] with a kit of (Centronic, wartenberg, Germany).

Creatinine and Urea were determined according to Henry et al. [17], Patton and Crouch [18], respectively. Uric acid was determined by colorimetric method according to Morris and Macleod [19]. In addition, the blood concentrations of calcium was determined on an automated analyzer Erba Chem7 (Mannheim, Germany) with commercial kits (Arena Bioscien, Ismailia, Egypt).

2.5. Oxidative state evaluation

The measurement of reduced glutathione (GSH) and Malondialdehyde (MDA) were performed using commercial kits (Bio-Diagnostic, Giza, Egypt) according manufacturer. Similarly, commercially available colorimetric kits (Bio-Diagnostic, Giza, Egypt) were used for measurement of Nitric oxide (NO) and Superoxide dismutase (SOD).

2.6. Statistical analysis

Data were analyzed using software program (SPSS for Windows version 20, USA) [20]. The data obtained from BLV-infected cattle and control group were compared using independent *t*-test. Differences were considered statistically significant at $P < 0.05$.

3. Results

The seroprevalence of antibodies against BLV was detected in (80 out of 500) 16% of total examined cattle. The disease was significantly prevalent in older cattle more than young cattle (P = 0.02) and BLV infection is significant more appear in pregnant cattle than lactating cattle (P = 0.003) as in Table 1.

Hematological parameters evaluation revealed non-significant changes in erythrogram, leukogram and platelets count in seropositive cattle in comparison with seronegative cattle as in (Table 2).

Table 1
Seroprevalence of BLV in dairy cattle.

Category	Level	Number tested	Positive	% Seroprevalence	P value
Age group	2–4	200	20	10	0.02[a]
	4–7	300	60	20	
Pregnancy	Pregnant	150	35	23.3	0.003[a]
	Non-pregnant	350	45	12.8	

[a] The result was significantly different at $P < 0.05$.

Table 2
Hematological parameters (Mean ± SE) in seronegative and seropositive cattle.

parameters	Seronegative cattle	Seropositive cattle	P value
RBCS ($x10^6$/μl)	3.96 ± 0.16	4.02 ± 0.13	0.80
Hb (gm/dl)	13.85 ± 0.17	14.13 ± 0.18	0.32
PCV (%)	45.70 ± 0.56	46.64 ± 0.62	0.32
MCV (fl)	115.61 ± 3.31	116.69 ± 4.53	0.86
MCH (pg)	35.03 ± 1.00	35.36 ± 1.37	0.895
MCHC (%)	30.30 ± 0.01	30.30 ± 0.01*	0.01
TLC ($x10^3$/μl)	4.60 ± 0.24	4.82 ± 0.50	0.73
Neutrophils ($x10^3$/μl)	2.16 ± 0.20	2.53 ± 0.28	0.21
Lymphocytes ($x10^3$/μl)	2.07 ± 0.17	2.22 ± 0.29	0.55
Monocytes ($x10^3$/μl)	0.26 ± 0.05	0.17 ± 0.04	0.27
Eosinophils ($x10^3$/μl)	0.09 ± 0.01	0.07 ± 0.01	0.31
platelets count ($x10^3$/μl)	134.50 ± 5.56	125.00 ± 6.90	0.35

Concerning changes in liver enzymes (ALT, AST and ALP), there were significant increases in ALT ($p < 0.05$), AST ($p < 0.01$) and ALP ($p < 0.05$) activities in seropositive cattle compared with seronegative cattle. While changes in urea, creatinine, uric acid and Ca levels showed a significant increase in creatinine level ($p < 0.01$) associated with a significant decrease in Ca level ($p < 0.05$) in seropositive cattle in comparison with seronegative cattle. On the other hand, non-significant changes were reported in urea and uric acid levels (Table 3).

With respect to changes in antioxidant status, there was a significant decrease in SOD activity ($p < 0.001$). Meanwhile, non-significant changes were obtained in MDA, NO and GSH levels in seropositive cattle compared with seronegative cattle (Table 4).

4. Discussion

BLV is lifelong infection and most infections are asymptomatic can be recognized by serological testing [5] and by PCR techniques [21,22].

The result showed presence of antibodies against BLV in 16% of cattle using ELISA test. This sero-survey provide evidence that BLV infection is widespread around the world [23]. The seroprevalence rate of BLV in cattle in Egypt is nearly similar that recorded by Zaher and Ahmed [24] who reported rate of infection at 15.83%.

The obtained results revealed that the number of seropositive animals was increased significantly with the age and pregnancy. This evidence come in accordance with previous study which reported that the BLV infection increase with age and pregnancy [25].

The longer lifespan results in a longer exposure period of BLV, which is probable to lead to a greater incidence of BLV infection in dairy cattle [26,27].

With respect to the hematological parameters, the present study revealed non-significant changes in erythrogram, leukogram and platelets count in seropositive cattle in comparison with seronegative cattle. These results suggest that the animals under investigation were in the early stage of BLV infection.

These results is consistent with [28] who showed that infection with BLV may remain clinically silent in an aleukemic form. To clarify the

Table 3
Serum biochemical parameters (Mean ± SE) in seronegative and seropositive cattle.

Animal	ALT (U/l)	AST (U/l)	ALP (U/l)	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)	Ca (mg/dl)
Seronegative cattle	13.09 ± 1.81	40.15 ± 2.36	78.11 ± 9.89	21.81 ± 1.24	1.02 ± 0.09	0.99 ± 0.06	9.28 ± 0.95
Seropositive cattle	23.13 ± 2.78[a]	67.26 ± 5.73[b]	110.63 ± 7.00[a]	23.25 ± 2.14	1.57 ± 0.10[b]	1.24 ± 0.09	6.54 ± 0.42[a]
P value	0.01	0.004	0.05	0.57	0.01	0.08	0.02

[a] Value was significantly different at $P < 0.05$.
[b] Value was significantly different at $P < 0.01$.

Table 4
Serum SOD activity and NO, GSH and MDA levels (Mean ± SE) in seronegative and seropositive cattle.

Animal	SOD (U/ml)	NO (μmol/l)	GSH (nmol/dl)	MDA (nmol/ml)
Seronegative cattle	81.69 ± 6.20	4.87 ± 3.23	5.89 ± 2.50	16.47 ± 3.24
Seropositive cattle	44.62 ± 4.92[a]	5.03 ± 1.41	4.50 ± 1.40	22.82 ± 5.13
P value	0.007	0.51	0.43	0.10

[a] Value was significantly different at $P < 0.01$.

obtained results it is worth mention that EBL develops in three stages, about 60% of infected animals remain in the first stage (virus carriership), About 30% of animals are in the second (hematological) stage of the infection that characterized by leukocytosis and lymphocytosis and the third (neoplastic) stage that characterized by tumours formation in about 10% of infected animals [29].

Regarding the liver enzymes, there were significant increases in ALT, AST and ALP in seropositive cattle compared with seronegative cattle. The obtained results come in agreement with Krasnikova et al. [30] who reported significant increases in ALT, AST and ALP enzymes activities in cows infected with BLV.

Concerning renal function, the present study showed significant increase in creatinine level in BLV- infected cattle in comparison with seronegative cattle. The increase in creatinine level may be attributed to the impairment of filtrating ability of the kidneys as a consequence of nephrons damage in BLV-infected cows [30]. Taken together, liver and kidney function disorders that appear in the early stage of leukosis may be occurred as a result of leukemic lymphocytic cell infiltrations in hepatic and renal tissues [31].

In the current study, there was a significant decrease in Ca level in BLV-infected cattle compared with seronegative. Similar result was reported by Sandev et al. [31] who attributed hypocalcaemia to the alteration in permeability of the cell membranes that regulated by calcium. They added that hypocalcaemia is important in leukosis genesis. In another hand, Akalın et al. [32] reported non-significant changes in calcium level in BLV-infected cows. The variation may be due to different stages of leukosis.

In this study, there was a significant decrease in SOD activity associated with non-significant changes in MDA, NO and GSH levels in BLV-infected cattle compared with seronegative cattle. These findings is consistent, partially, with the previous results by Souza et al. [33] who study the oxidant status and the markers of oxidative stress in BLV-infected dairy cows and showed a significant decrease in SOD activity coincided with non-significant changes in MDA. To illustrate these findings, it is important to note that viral infection can alter the oxidative status either by increasing the formation of nitric oxide or by inhibiting the synthesis of enzymes involved in the oxidative defense within the host cell [10].

5. Conclusion

In conclusion, our results confirmed that infection with BLV could cause alterations in the internal homeostasis as well as some organs (liver and kidney) dysfunctions which should be considered during regimen of control.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2019.103662.

Conflicts of interest

The authors declare no conflict of interest.

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