

Lumpy Skin Disease in Cattle: Hematological, Biochemical and Oxidative Changes

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ABSTRACT:

This study was conducted to determine the clinical, haematologic, biochemical findings, and oxidative stress parameters in cattle naturally infected with lumpy skin disease virus (LSDV) in the Kafrelshikh governorate, Egypt. Twenty animals were divided into 2 groups. Ten apparent healthy mixed Holstein female cows with age ranged between 1.5-3 years did not show any symptoms of LSD and considered the control group. Other 10 mixed Holstein pregnant females with an age range between 2-3 years considered diseased group by clinical examination and laboratory confirmation by polymerase chain reaction and analysis of the blood samples. The diseased cows suffered from fever (above 40°C), anorexia, emaciation, skin nodules distributed in various body parts, lacrimation, nasal discharge, oedema, enlarged prescapular, and prefemoral lymph nodes. Haematological examination of the blood collected from infected animals showed a significant reduction in the number of erythrocytes, various leukocyte types, and thrombocytes as well as macrocytic hypochromic anemia.

Keywords: cattle, hematology, antioxidant, biochemistry, lumpy skin disease.

INTRODUCTION:

Lumpy skin disease (LSD) is reasonably an important contagious viral disease of cattle. Lumpy skin disease virus (LSDV) is a member of the genus Capripoxvirus in the Poxviridae family that also including sheep pox and goat poxviruses. LSD virus is transmitted among cattle by biting insects such as (mosquitoes, flies, and ticks) (Şevik *et al.*, 2016). It causes significant economic losses due to reduced milk production, enduring hide damage, increased abortion rates, and decreased weight gain. It causes a chronic weakness in infected animals with high morbidity rates, but with lower mortality that never exceeds 1-3% (Coetzer and Tuppurainen, 2004).

LSD is endemic in Africa and first observed in Egypt in the Suez Governorate in May 1988. Then, several outbreaks were observed and spread to many countries in the Middle East. Now LSD becoming endemic in some countries in this region (Davies, 1991). The disease is manifested by fever, then followed by the development of skin nodular lesions that may cover the whole body, necrotic plaques in the mucosal membranes, especially in the upper respiratory tract; generalized lymphadenitis, and commonly limbs oedema. Throughout the disease's first stages, the infected cattle show loss of appetite, nasal discharge, and lacrimation (Coetzer and Tuppurainen, 2004).

LSD is a systemic disease, with obvious lymphadenopathy and cell-associated viremia. It means that monocytes in blood play an important role in virus distribution. Similar to most members of the subfamily Capripoxvirus display a distinctive tropism for keratinocytes (Maclachlan and Dubovi, 2016). Proliferative nodular lesions can take place within forestomachs and lungs, and less frequently in the kidneys, tongue, and liver. The viral proliferation inside the cells destroys them directly or indirectly by cellular dysfunction as a result of the host immune response to the existence of virus proteins. considerable changes can be seen in serum biochemicals when cellular damage occurs (Şevik et al., 2016). During the immune response for infection, large amounts of reactive nitrogen species (RNS) and reactive oxygen species can be produced, which causes oxidative damage to tissues (Bozukluhan *et al.*, 2013).

There is incomplete data in the literature about the serum oxidative changes and biochemical findings in cattle naturally infected with LSDV. Serum biochemical markers might be a valuable tool for evaluating animal wellbeing and aid better handling of the infection. Therefore, the reason for this investigation is to find out the changes in some serum biochemical markers as well as assessing the extent of oxidative stress in cattle naturally infected with LSDV.

MATERIA AND METHODS:

Animals:

This study was carried out on 20 animals. They divided into 2 groups. The first group is the control group which including 10 mixed Holstein female cows with age ranged between 1.5-3.0 years. Those animals were belonged to a private farm in Kafrelshikh governorate. The second group is the diseased group, which included 10 mixed Holstein pregnant females with age range between 2-3 years. The animals were presented to a private animal clinic in a village in Kafrelshikh governorate, Egypt. The samples were collected during disease outbreak between August and October 2017. All animals were exposed to complete clinical examination including rectal temperature, respiratory rate and pulse rate and ruminal contraction.

The animals were held in strict adherence to accepted standards for the humane treatment of animals and according to the guidelines of the Committee of Animal Care and Welfare,

Faculty of Veterinary Medicine, University of Sadat City.

Samples:

Blood samples with anticoagulant (EDTA), were collected from the jugular vein of each animal for haematological examination. In addition, blood samples without anticoagulant were collected and then centrifuged at 3000rpm for 10min for serum separation. The serum samples were stored at -20°C until testing for further laboratory examination.

PCR analysis:

Skin nodules were aseptically collected from infected cows and kept at -20 °C until extraction of DNA. DNA was extracted using Introbio DNA/RNA kit (Gentaur, France).

PCR was performed to confirm the diagnosis of LSD using primers specific to the gene encoding for the viral attachment protein of *Capripoxviruses* (Ireland and Binopal, 1998).

The forward primer (5'TTTCCTGATTTTCTTACTAT3'), and the reverse primer (5'AAATTATATACGTAAATAAC3') were used. These primers amplify a 192 bp fragment.

An ultimate volume of 25 µl reaction mixture containing 2.5 µl of 10× PCR buffer for KOD – Plus- Neo (Toyobo), 1.5 µl of MgSo4 (25 mM), 2.5 µl of dNTPs (2 mM), 0.75 µl of the forward primer, 0.75 µl of the reverse primer, 1 µl of DNA template, 0.25 µl (0.25 U) of KOD –Plus- Neo DNA polymerase (Toyobo), and 15.75 µl of nuclease-free water.

The thermal profile is as follows: an initial cycle at 98 °C for 2 min, and then 35 cycles of 98 °C for 10 s, 50 °C for 30 s and 68 °C for 10 s and a last extension step of 68 °C for 5 min.

Haematological examination:

Following parameters were estimated: red blood cell count (RBCs), haemoglobin concentration (Hb), packed cell volume (PCV), total leukocytic count (TLC) and differential leukocytic counts (DLC) as described by (Feldman *et al.*, 2000).

Serum biochemical analyses:

Aspartate aminotransferase (AST), total protein (TP), albumin and glucose were colorimetrically assayed using commercial kits of Spectrum (Germany) and according to the methods of (Reitman and Frankel, 1957), (Henry *et al.*, 1974), (Doumas *et al.*, 1971; Werner *et al.*, 1970), respectively. Serum globulin was estimated by subtraction of serum albumin from serum total protein and then A/G ratio was estimated by dividing albumin on

globulin. Serum urea and creatinine levels were by the method of (Putton and Crouch, 1977), (Husdan and Rapoport, 1968), respectively. Sodium (Na) was detected by colorimetric method using the commercial kits according to (Tietz, 1986). Calcium (Ca), inorganic phosphorus (iP) and magnesium (Mg) levels were assayed by using colorimeter and commercial kits of Spectrum (Germany) according to methods described by (Barnett, 1965). Some oxidative stress parameters such as catalase (CAT) which was determined by colorimetric method using commercial kits provided by (Bio-Diagnostic, Giza, Egypt) according to (Aebi, 1984) and the instruction of the enclosed pamphlet. Reduced Glutathione (GSH), hydrogen peroxides (H₂O₂), nitrogen oxide (NO), and malondialdehyde (MDA) were estimated according to (Beutler *et al.*, 1963), (Koracevic *et al.*, 2001), (Menaka *et al.*, 2009) and (Satoh, 1978), respectively.

Statistical Analysis:

The results were typed as mean \pm standard error (SE). Two-sample *t*-test was used for comparison between the LSDV-infected group and healthy group. Probability (*P*) < 0.05 was considered statistically significant. All statistical analysis was performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA) according to (Snedecor and Cochran, 1996).

RESULTS:

Clinical signs:

The diseased cows suffered from fever (above 40 °C), anorexia, emaciation, decrease in milk production and skin nodules distributed in various body parts involving the neck, chest, abdomen, udder, limbs, perineal area, muzzle, and in some cases, all over the body (Fig.1, A).

Table (1). Hematological parameters in lumpy skin diseased group compared to control group.

Parameters	Groups (N=10)	
	Control	Diseased
RBCs (x 10 ⁶ /ul)	7.39 \pm 0.06	6.34 \pm 0.09*
Hb (g/dl)	9.14 \pm 0.11	6.59 \pm 0.11*
PCV (%)	29.78 \pm 0.22	27.03 \pm 0.23*
MCV (fl)	40.31 \pm 0.34	42.66 \pm 0.56*
MCH (pg)	12.38 \pm 0.19	10.38 \pm 0.09*
MCHC (%)	30.73 \pm 0.52	24.36 \pm 0.34*
TLC (x 10 ³ /ul)	10.48 \pm 0.16	6.87 \pm 0.09*
Neutrophils (x 10 ³ /ul)	2.66 \pm 0.05	2.76 \pm 0.04
Lymphocytes (x 10 ³ /ul)	7.53 \pm 0.097	3.81 \pm 0.03*
Monocytes (x 10 ³ /ul)	0.17 \pm 0.01	0.18 \pm 0.01
Esinophils (x 10 ³ /ul)	0.16 \pm 0.02	0.09 \pm 0.01*
Platelets (x 10 ³ /ul)	420.00 \pm 0.60	260.56 \pm 0.32*

Values are means \pm SE. Significant differences in the values between control and diseased group were indicated by (*) at *P*<0.05.

Lacrimation, nasal discharge, oedema and enlarged prescapular and prefemoral lymph nodes were observed in most infected animals (Fig. 1, B & C).

PCR confirmation:

Capripoxvirus infection was confirmed by the PCR analysis of skin nodules extracted DNA using viral attachment protein encoding gene primers. The expected amplicon size (192 bp) was found in all examined samples (Fig. 2).

Hematologic and biochemical assessments:

Erythrogram analysis showed significant decrease (*P*<0.05) in erythrocyte count (RBCs), haemoglobin (Hb), packed cell volume (PCV %), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). While, the mean corpuscular volume (MCV) revealed significant increase (*P*<0.05) when compared to healthy animals (Table 1). Regarding to the leukogram results, the present data showed significant decrease (*P*<0.05) in the total leucocytic count, lymphocytes and eosinophils count. Furthermore, significant decreases (*P*<0.05) in total thrombocytes count in diseased group than in healthy one (Table 1). Concerning to serum biochemical analysis, the current study showed a significant increase in serum activity of AST enzyme, urea and creatinine levels compared to the control group (*P*<0.05) (Table 2). Meanwhile, total protein, albumin, globulin, Na, Ca, iP and Mg were significantly reduced in LSD infected cattle compared to healthy group (*P*<0.05). The oxidative biomarkers showed a significant reduction (*P*<0.05) in catalase and reduced glutathione while there was a significant elevation in MDA, H₂O₂ and NO in diseased cattle (*P*<0.05) in comparison to control group (Table 2).

Table (2). Serum biochemical parameters in lumpy skin diseased group compared to control group.

Parameters	Groups (N=10)	
	Control	Diseased
AST (U/L)	29.38±0.49	54.88±1.09*
TP (g/dl)	8.18±0.08	5.76±0.14*
Alb (g/dl)	3.21±0.08	2.47±0.12*
Glob (g/dl)	4.96±0.01	3.29±0.09*
A/G ratio	0.64±0.01	0.76±0.04*
Glucose (mg/dl)	97.00±0.57	70.83±0.91*
Na (mmol/L)	122.62±0.37	93.85±1.49*
Urea (mg/dl)	42.25±0.37	49.38±0.33*
Creatinine (mg/dl)	1.48±0.03	1.80±0.03*
Ca (mg/dl)	11.24±0.13	8.46±0.22*
iP (mg/dl)	5.61±0.13	4.01±0.02*
Mg(mg/dl)	3.39±0.04	2.16±0.08*
CAT (U/L)	340.13±0.12	149±0.16*
GSH (mmol/ml)	22.25±0.41	10.68±0.20*
MDA (mmol/ml)	2.56±0.04	11.04±0.16*
H2O2 (ng/ml)	284.25±0.13	534.5 ± 0.76*
NO (ng/ul)	26.00±1.30	55.26±0.44*

Values are means ± SE. Significant differences in the values between control and diseased group were indicated by (*) at $P < 0.05$.



Figure (1): (A) Cutaneous skin nodules in a diseased cow with LSD. (B), Oedema in the dewlap and fore legs of a diseased cow with LSD. (C), Enlargement of prescapular lymph node in a diseased cow with LSD.

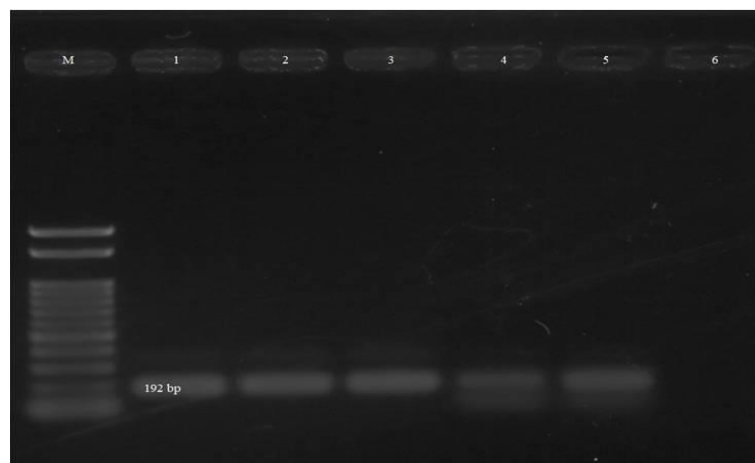


Figure (2). Electrophoretic pattern for LSDV-PCR assay, Lane M is 100 bp DNA ladder, Lanes 1–5 are positive (amplicon size 192 bp), Lanes 6 is negative.

DISCUSSION:

Lumpy skin disease is an important endemic disease in Africa causing considerable economic loss mainly due to permanent hide damage, decrease of milk production and emaciation. The disease is described by proliferative nodules appear on the skin and other internal organs that causes cellular damage and hematological and biochemical changes.

The diseased cows suffered from fever, anorexia, emaciation, drop in milk production and skin nodules, lacrimation, nasal discharge, edema and enlarged pre-scapular and pre-femoral lymph nodes. The previously mentioned clinical signs were also described by (Hunter and Wallace, 2001; Sharawi and Abd El-Rahim, 2011).

The frequent occurrence of LSD in Egypt in summer and fall months, can be attributed to the exaggerated insect population throughout summer and fall, reduction of vector population due to unfavorable climatic conditions leads to disease quietness, LSD outbreaks are usually linked to reduction of the host immunity (Hunter and Wallace, 2001).

In the present investigation, PCR was highly sensitive (100%) in detecting the LSDV-RNA in cutaneous nodular samples. Similar finding were observed (Sharawi and Abd El-Rahim, 2011), and this may be attributed to LSDV tropism to skin tissues.

Erythrogram results of the blood samples obtained from cattle naturally infected with LSD virus revealed a significant decrease in RBCs, HB, PCV, and MCHC with a significant increase in MCV when compared by healthy animals. These results indicated presence of macrocytic hypochromic anemia, which , also

observed by Weiss et al. (Weiss and Wardrop, 2011), who documented that haemolytic anemia could be occurred with viral infection. In addition, the increase in MCV and decrease in MCHC suggested haemolytic or haemorrhagic anemia. LSD can cause destruction of RBCs as it increases production of reactive oxygen species (ROS) which have adverse effects on membrane and macromolecules of erythrocytes as they induce osmotic fragility, decrease cellular deformability and damage to cell membrane (Nashwa *et al.*, 2017).

Regarding to the leukogram results, the current work denoted a significant decrease in WBCs count, lymphocytes, and eosinophils in LSD animals compared with control healthy animals. Leukopenia was observed in ruminants, especially during acute period of infection and could be attributed to increase tissue demand and neutrophilic margination. Furthermore, lymphopenia is a common finding in viral infections than in bacterial ones. Widespread circulation of antigens in systemic infectious diseases results in lymphopenia, due to re-distribution of recirculating lymphocytes; they remain transiently sequestered in the lymphoid tissues rather than entering efferent lymph and blood (Chapman, 2013). These results be consistent with Ismail and Yousseff (2006) who attributed the incidence of lymphopenia, due to release of large quantities of endogenous corticosteroid.

In the LSD-infected cattle platelet count was significantly lower than in the healthy controls, which was in agreement with findings of (Abutarbush, 2015). Thrombocytopenia may be the result of decreased platelet production in

the bone marrow or sequestration of platelets due to splenomegaly (Morris, 2002).

In present investigation infected cattle showed significantly higher concentrations of serum AST had which it is a sensitive indicator of hepatocellular damage, even if the damage is of a subclinical nature (Stockham and Scott, 2013). LSD lesions can occur in the muscle fascia as well as in the muscle (Davies, 1991). Regarding to the increased level of urea concentration in serum, this could be attributed to the effect of LSD virus on renal tissues resulted in renal damage or reduction of glomerular filtration rate and increase protein catabolism (Neamat-Allah, 2015). Furthermore, a significant high concentration in serum creatinine was recorded in LSD animals, this data confirms the direct effect of LSDV on the renal tissue (Kaneko *et al.*, 1997). The total serum protein concentrations, albumin and globulin in infected cattle were significantly decreased than those of healthy cattle. This may be attributed to anorexia and decrease food intake during the period of illness. This can be also explained the decreasing of serum glucose level in infected cattle others attributed these results to decreased synthesis and higher protein catabolism as well as damaged of hepatocellular tissues.

Calcium, magnesium, and phosphate are necessary for several biologic and cellular functions. The etiology of lowered levels of serum calcium is not well known but hypocalcemia could be associated with hypoalbuminemia due to about one-half of all calcium is bound to serum albumin (Latimer, 2012). The kidneys play a central role in the homeostasis of these ions (Blaine *et al.*, 2015). In this study, decreased levels of Ca, P, Mg and Na may indicate kidney dysfunction. At the same time, hyponatremia could be referred to reduction in food intake or decreased absorption.

Reactive-Oxygen-Species (ROS) and nitric oxide (NO) are the early responses of host innate immunity to infectious disease. They are highly toxic to pathogens and are capable of degrading cellular protein, nucleic acid and membrane lipids causing lipid peroxidation and cellular injury (Halliwell *et al.*, 1992). Lipid peroxidation is a sign of oxidative stress in the tissues and the most abundant lipid peroxide byproduct is Malondialdehyde (MDA) (Heidarpour *et al.*, 2013). The significant

elevation in MDA, H₂O₂ and NO was accompanied with the significant reduction in catalase and reduced glutathione in diseased cattle, indicate the presence of oxidative stress condition. Oxidative stress happens when the levels of oxygen radical formation such as (H₂O₂) and (NO) exceed out the antioxidants, such as catalase and reduced glutathione. This coincided with Zalba *et al.* (2006) and Circu and Aw (2010), who reported that antioxidants depleted with increasing of ROS.

In conclusion, LSD in cattle was associated with pancytopenia (an overall reduction in the number of erythrocytes, various leukocyte types and thrombocytes), Serum biochemical alteration especially in AST activities, total protein, albumin, globulin, glucose, magnesium, calcium, and phosphate concentrations. Furthermore, oxidative stress plays critical role in the pathogenesis of LSD. This study gives additional insight on pathogenesis and consequently improves strategies for proper treatment.

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CONFLICT OF INTEREST:

The authors declare that they have no conflicts of interest.

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