

## RESEARCH ARTICLE

### Detection of Lumpy Skin Disease during an Outbreak in Summer 2019 in Menoufia Governorate, Egypt using Clinical, Biochemical and Molecular Diagnosis

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*Article History: Received: 22/08/2020 Received in revised form: 25/09/2020 Accepted: 26/10/2020*

#### Abstract

Lumpy skin disease is an infectious disease affecting the bovines and caused by Lumpy skin disease virus (LSD virus; LSDV). It is considered an endemic disease in African continent and was recorded in Egypt since 1988. This study aimed to monitor the cattle health condition along the course of the disease through the estimation of biochemical parameters. Besides, investigations on the incriminated strains of LSD virus circulating during outbreak in summer 2019 were performed. One hundred and eighteen cattle of different ages and sex were examined for LSD in some villages of Menoufia Governorate, Egypt. The clinical signs were recorded, and blood chemical analyses were applied on the infected animals. Conventional PCR was used to identify the LSDV using two conservative genes, fusion and P32 coding genes. The infected animals showed typical symptoms of the LSDV infection. Morbidity rates in cattle were high in males (43%) and in 6–12 months of age (44.4%). The hemogram showed significant ( $P < 0.05$ ) decrease in the count of red blood cells (RBCs) ( $6.13 \pm 0.51 \times 10^6/\mu\text{l}$ ), hemoglobin (Hb) ( $9.07 \pm 0.15 \text{ g\%}$ ), hematocrit (HCT) ( $17.47 \pm 0.48\%$ ), mean corpuscular volume (MCV) ( $41.77 \pm 0.79\text{fL}$ ), mean corpuscular hemoglobin (MCH) ( $13.53 \pm 0.29\text{Pg}$ ), and mean corpuscular hemoglobin concentration (MCHC) ( $30.43 \pm 0.53\text{g/dL}$ ) in infected animals compared with control. The leukocytes showed significant ( $P < 0.05$ ) increase in the count of white blood cells (WBCs) ( $15.13 \pm 0.03 \times 10^3/\mu\text{l}$ ), lymphocyte (LYM) ( $7.17 \pm 0.12 \times 10^3/\mu\text{l}$ ), monocytes and some eosinophil (MID) ( $2.93 \pm 0.08 \times 10^3/\mu\text{l}$ ), and granulocytes (GRAN) ( $5.03 \pm 0.09 \times 10^3/\mu\text{l}$ ) in infected animals. Serum biochemistry of the infected animals showed significant ( $P < 0.05$ ) increase in all biochemical parameters except serum albumin ( $2.61 \pm 0.35\text{g/dL}$ ). Lumpy skin disease virus was detected by Polymerase chain reaction (PCR) for both genes and the sequences of the fusion and P32 proteins were deposited in the GenBank by the accession numbers MN699855 and MN699856, respectively. Thus, attention should be directed to the circulating LSDV strain in the field and comparing it with the previously isolated strains. Field practitioners should depend on the blood parameters of the infected animals to select the appropriate remedies.

**Keywords:** Blood chemical analyses, Cattle, Egypt, Lumpy Skin Disease virus, Phylogenies.

#### Introduction

Lumpy skin disease is a viral infection of cattle and triggered by Lumpy skin disease virus (LSDV). The virus together with the poxviruses of the sheep and goat belong to the genus *Capripoxvirus* (CaPV), subfamily *Chordopoxvirinae*, of the family *Poxviridae*

[1]. LSD is characterized by skin nodules, which cover all body parts with generalized lymphadenopathy [2]. LSDV is considered as an endemic disease in most of the African countries, and the mechanical transmission by arthropod vectors is considered the main method for viral transmission [3]. LSD is

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endemic in Egypt since 1988 [4], and the incidence of the disease markedly increase where the disease prevalence in 2008 reached 25.47% [5]. In addition, the disease was recorded during summer 2016 even in vaccinated animals with the Romanian sheeppox virus (SPPV) vaccine [6]. LSD results in great economic losses as a result of skin damage, mastitis, decrease milk production and various genital problems such as infertility, abortion, and occasionally death due to secondary bacterial infections [2].

The clinical signs of LSD are the primary methods for disease diagnosis. However, there are many methods for definite diagnosis of the disease as virus isolation, electron microscopy [7], and polymerase chain reactions (PCR) [8, 9].

Recently, PCR is an accurate and rapid method for LSDV detection in suitable specimens [10], where it is more sensitive and specific in comparison with immunoassays [11]. The PCR tools can speedily identify the caprine poxvirus (CaPV) from infected animals, within a short period from the collection of samples, presenting a suitable molecular platform that can be integrated into field diagnostics. The appropriateness of this assay to detect CaPV genomic material in the clinical samples was assessed and attested [9]. Diagnosis of LSD is often based on distinguishing clinical signs. Yet, mild and subclinical cases necessitate fast and consistent laboratory analysis to authorize the diagnosis [10]. Laboratory identification of LSD comprised either revealing of the virus using: electron microscopy, egg inoculation, isolation on tissue culture, fluorescent antibody technique; or detection of its related antibody using serological investigations [10]. Several PCR assays have been established in recent times for more precise and prompter detection of LSDV in appropriate specimens. The PCR has been evidenced further sensitive and specific if matched with immunoassays [11].

Mechanical transmission of LSDV occurs by numerous blood-sucking insects such as mosquitoes and midges [3]. The virus can be transferred through blood, nasal discharge, lacrimal secretions, semen, and saliva. It can also be transmitted through infected milk to

suckling calves [7]. Lumpy skin disease virus can persist viable in infected tissue for additional 120 days, and quarantine restrictions are of limited use [12].

Vaccination with an attenuated virus offered the most promising method of control. Even with annual bulk vaccination with sheep pox vaccine (Veterinary Serum and Vaccine Research Institute, Egypt) by the Egyptian authorities, LSDV still mingles nearly each summer. For instance, during the summer of 2006, a widespread LSD epidemic had hit Egypt, affecting 16 provinces [12], and it come back in 2011, 2014 and 2016 [6].

From the economic view, LSD has a very important economic impact on cattle farms because of the long period of the disease course, which leading to decrease of milk production, infertility, abortion, and sometimes death occurs as a result of secondary bacterial infections. Animal death has a negative impact on meat production and consequently affect animal protein source leading to public health importance [2]. The veterinary practitioners play a crucial role in the control and eradication of the animal diseases, development and supervision of food hygiene practices, and increase sources of animal proteins. Commercial damages might be high as result of the condemnation of carcass arising from fever as well as the cost of examining the meat. Furthermore, severe and perpetual damage can occur to leathers and consequently lessening their marketable value. Therefore, attention should be given to the control of animal diseases through vaccination, diagnoses, and treatment due to their economic effects [2].

The objective of this study was to monitor the cattle health condition during LSD through the biochemical investigations, which will help in the treatment approach. Besides, the study investigated the strains of the *LSD virus* which were circulating during the lumpy skin disease outbreak in summer 2019.

## Materials and Methods

### *Temporal and spatial data*

One hundred and eighteen cattle of different ages (6-12 months) and sex were examined for suspicion of LSD during the period of August to October 2019 at Shebin el kom villages (Elkom Elakhdr, Met Mousa,

Kafr Tanbdi, Met Khakan, Almay, and Shanawan) Menoufia Governorate, Egypt (Figure 1 and Table 1).

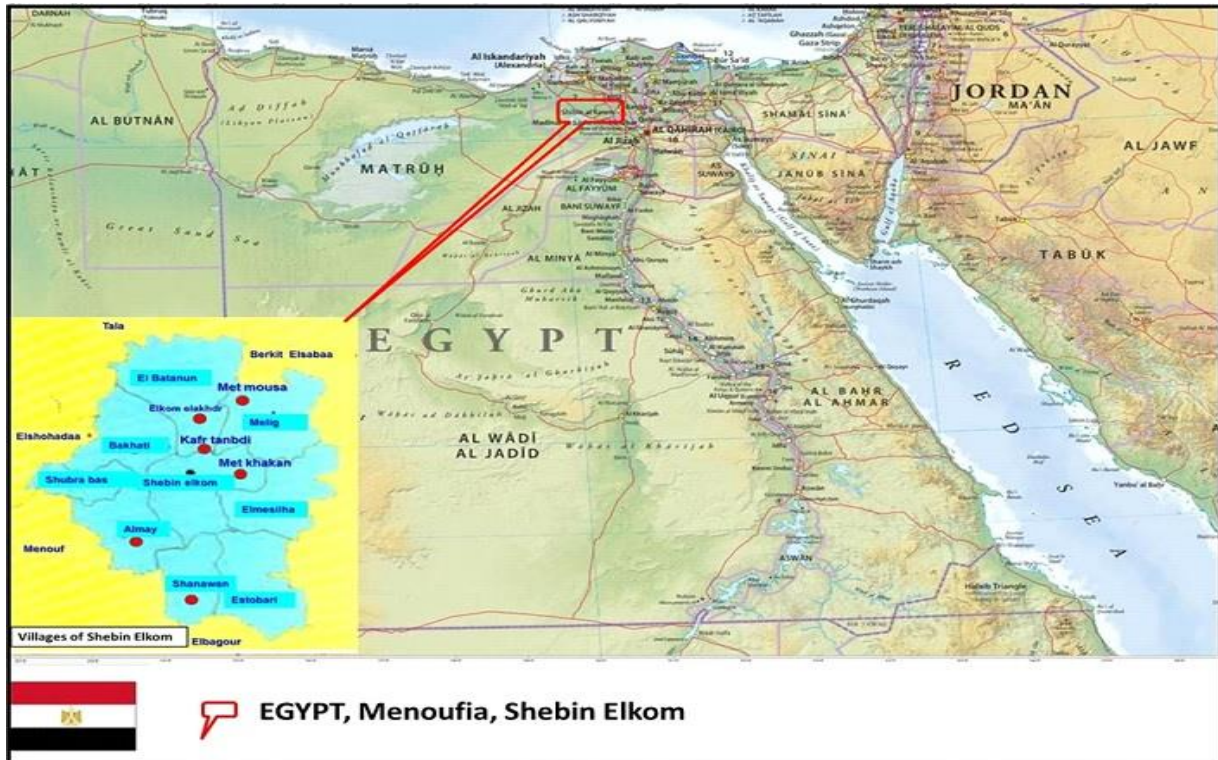


Figure 1. The map of Egypt showing the geographical area of study.

Table 1. Numbers of examined and infected cattle according to locality, sex and age

Localities	Total Numbers of examined animals					Total Numbers of infected animals									
	Sex		Age (months)			Sex		Age (months)							
	M	F	0-6	6-12	> 12	M	%	F	%	0-6	%	6-12	%	> 12	%
<b>Shanwan</b>	10	16	10	8	8	2	20	4	25	3	30	2	25	1	12.5
<b>Almay</b>	14	8	8	9	5	6	43	2	25	2	25	4	44.4	2	40
<b>Met Khakan</b>	10	8	7	6	5	3	30	2	25	2	28.6	1	16.7	1	20
<b>Elkom Elakhdr</b>	6	10	8	5	3	1	16.7	2	20	1	12.5	1	20	1	33.3
<b>Kafr Tanbdi</b>	8	8	9	4	3	2	25	2	25	2	22	1	25	1	33.3
<b>Met Mousa</b>	12	8	8	7	5	3	25	1	12.5	1	12.5	2	28.6	2	40
<b>Total</b>	60	58	50	39	29	17	28	13	22.4	11	22	11	28	8	27.5

M, Male  
F, Female

### **Clinical examination**

It was done as described previously [13], with the history of no vaccination for the past 2 years.

### **Sample collection**

Thirty whole blood samples were collected from the jugular vein of the female cattle at 6-12 months of age. Part of the blood was collected on ethylenediaminetetraacetic acid (EDTA) as anticoagulant for hematological examination and the other part without anticoagulant for serum separation (at  $1500 \times g$  /15 min), which stored at  $-20^{\circ}\text{C}$  until analysis.

### **Biochemical analyses**

The samples were used for measuring of aspartate transaminase (AST) and alanine transaminase (ALT) activities, total protein, albumin, urea, and creatinine in the serum by using commercial kits (Bio-Diagnostic Company, Egypt).

### **DNA Extraction**

Extraction was carried out as previously described [11]. Nodules from some clinical cases were ground using sterile mortar and pestle, suspended in sterile phosphate buffer saline (PBS) containing 10% antibiotic solution. Each tissue homogenate was centrifuged at  $1500 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The clear supernatant was frozen at  $-70^{\circ}\text{C}$  until used in PCR. DNA was extracted from freeze supernatants of the skin lesions (nodules) by GF-1 Tissue DNA Extraction Kit (Vivantis TM, Malaysia). The DNA was stored at  $-20^{\circ}\text{C}$  until use.

### **Primers design**

The PCR oligonucleotide primers were selected from distinctive LSDV sequences within the gene for viral fusion protein [11]. The amplicon size of the PCR product is 472 bp.

Forward primer: 5'-ATG GAC AGA GC TTT A TCA -3', Reverse primer: 5'-TCA TAG T GTT GTA CT TCG-3'.

Also, primer pairs for the of viral P32 protein Gene that is definite to the *Capripoxvirus* genus [14] were used and the amplicon size of the PCR product is 587 bp.

Forward primer: 5'- CGC GAA ATT TCA GA TGT AGT TCC A -3', Reverse primer: 5'- TGA GCC ATC CAT TTT CC AAC TC -3'

### **Polymerase chain reaction**

PCR reaction was applied by T100™ Thermal Cycler (Bio-Rad, Laboratories, Inc. USA) in a total volume of 20  $\mu\text{L}$  using Master Mix, specific primers concentration, and template as recommended by PCR Master Mix manufacturer (i-Taq™ iNtRON Biotechnology, USA). PCR

condition was run as follows, one cycle of  $94^{\circ}\text{C}$  for 5 min; 34 cycles of  $94^{\circ}\text{C}$  for 1min,  $50^{\circ}\text{C}$  for 30 sec, and  $72^{\circ}\text{C}$  for 5 min; followed by one final cycle of  $72^{\circ}\text{C}$  for 5 min.

PCR products were separated by agarose gel electrophoresis using a 100-bp DNA ladder (100bp DNA Ladder h3 RTU, GeneDirex, USA) as a molecular marker on 1.5% agarose (Agarose superior grade type II, Sisco Research Laboratories Pvt. Ltd. India), containing 1  $\mu\text{g}/\text{mL}$  Safe Red dye® and was visualized by using ultraviolet light transilluminator (BIOTEC-FISCHER, USA).

The PCR amplicons of proper predicted sizes were gel purified using DNA gel purification kit (MEGAquik-spin™ plus, USA) and forwarded to sequencing and phylogenetic analysis.

### **Multiple sequence alignment and phylogenetic analysis**

PCR amplicons subjected to DNA sequencing were selected randomly as all samples were collected from the same Governorate. PCR products of P32 proteins coding genes of LSDV were sequenced commercially by Sanger sequenced using primer walking at MacroGen Inc. (Seoul, South Korea). Results were collected, modified, and analyzed using BioEdit version 5.0.6 [15].

Initial analysis was made by alignment by means of web-based BLAST <http://www.ncbi.nlm.nih.gov/BLAST>. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1 (Arizona State University, USA) [16]. Phylogenetic trees based on the fusion and P32 proteins coding genes sequences were explained with the neighbor-joining (NJ) tree [16].

### **Statistical analysis**

The obtained numerical data were statistically analyzed using SPSS version 16 (USA) using independent t test.

## **Results**

### **Clinical signs**

By clinical examination of suspected animals, 30 out of 118 (25.4 %) were suffering from fever ( $41-42^{\circ}\text{C}$ ) for more than 10 days accompanied by cutaneous lumps ranged from localized (mammary gland and testis) to generalized nodules scattered all over the body of the animal (Figure 2 and Tables 1, 2). Lymphadenitis and edema of limbs developed in 10 out of 30 clinically diseased animals. Anorexia, depression, emaciation, recumbences, and salivation were also present (Table 2).





Figure 2. Skin nodules at different stages of the disease, edema, recumbence, and skin nodules on mammary gland and testis of affected cattle (summer, 2019)

Table 2. Number of animals showing different symptoms of the lumpy skin disease (N= 30)

Clinical signs	Number of animals showing the symptoms n (%)
Generalized nodules	18 (60)
Localized nodules	12 (40)
Limb edema	10 (33.3)
Brisket edema	6 (20)
Udder nodules	13 (43.3)
Scrotal nodules	12 (40)
Oral, nasal and vulvar nodules	8 (26.6)

The results of the examined animals in different localities showed that the morbidity rate of LSD in cattle at Almay village was the highest in males (43%), and in age group of 6–12 months (44.4%), while the morbidity rate at Met Khakan was the lowest in cattle of age group 6–12 months.

#### **Hemogram and serum biochemistry**

Regarding the results of the hemogram, there was significant ( $P < 0.05$ ) decrease in the count of RBCs ( $6.13 \pm 0.51 \times 10^6 / \mu\text{l}$ ), Hb ( $9.07 \pm 0.15\text{g}\%$ ), HCT ( $17.47 \pm 0.48\%$ ), MCV ( $41.77 \pm 0.79\text{fL}$ ), MCH ( $13.53 \pm 0.29\text{Pg}$ ), and MCHC ( $30.43 \pm 0.53\text{g/dL}$ ) in the LSD group

when compared to the control group. Results of leukocytes showed a significant ( $P < 0.05$ ) increase in the count of WBCs ( $15.13 \pm 0.03 \times 10^3 / \mu\text{l}$ ), LYM ( $7.17 \pm 0.12 \times 10^3 / \mu\text{l}$ ), MID ( $2.93 \pm 0.08 \times 10^3 / \mu\text{l}$ ), and GRAN ( $5.03 \pm 0.09 \times 10^3 / \mu\text{l}$ ) in the LSD group in comparison with the control group (Table 3). Results of the serum biochemistry of the LSD group showed a significant ( $P < 0.05$ ) increase in all measured serum biochemical parameters except serum albumin ( $2.61 \pm 0.35\text{g/dL}$ ) that was nearly equal in comparison with the control group (Table 4).

**Table 3. Hematogram of control and cattle infected by lumpy skin disease.**

Parameter	Control group	LSD group
RBCs count ( $\times 10^6/\mu\text{l}$ )	7.58 $\pm$ 0.22 *	6.13 $\pm$ 0.51
Hb (g%)	11.14 $\pm$ 0.20 *	9.07 $\pm$ 0.15
HCT (%)	27.80 $\pm$ 0.78 *	17.47 $\pm$ 0.48
MCV (fL)	46.27 $\pm$ 0.29 *	41.77 $\pm$ 0.79
MCH (Pg)	15.20 $\pm$ 0.29 *	13.53 $\pm$ 0.29
MCHC (g/dL)	34.53 $\pm$ 2.31 *	30.43 $\pm$ 0.53
WBCs count ( $\times 10^3/\mu\text{L}$ )	9.20 $\pm$ 0.25	15.13 $\pm$ 0.03 *
LYM ( $\times 10^3/\mu\text{L}$ )	5.50 $\pm$ 0.26	7.17 $\pm$ 0.12 *
MID ( $\times 10^3/\mu\text{L}$ )	1.43 $\pm$ 0.09	2.93 $\pm$ 0.08 *
GRAN ( $\times 10^3/\mu\text{L}$ )	2.27 $\pm$ 0.12	5.03 $\pm$ 0.09 *

Values are expressed as mean  $\pm$  standard errors. Means in the same row with (\*) significantly differ at ( $P \leq 0.05$ ). LSD = Lumpy skin disease group, RBCs = red blood cells, Hb = hemoglobin, HCT = hematocrit, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, WBCs = white blood cells, LYM = lymphocyte, GRAN = Neutrophil, eosinophil and basophil, MID = Monocytes and some eosinophil

**Table 4. Different serum biochemistry parameters of the control and diseased cattle**

Parameter	Control group	LSD group
Total protein g/dL	6.43 $\pm$ 0.29	7.10 $\pm$ 0.97 *
Albumin g/dL	2.60 $\pm$ 0.31	2.61 $\pm$ 0.35
Globulin g/dL	3.83 $\pm$ 0.17	4.49 $\pm$ 0.63 *
Creatinine mg/dL	0.67 $\pm$ 0.12	1.33 $\pm$ 0.06 *
Urea mg/dL	21.33 $\pm$ 1.45	41.67 $\pm$ 3.48 *
AST U/L	36.67 $\pm$ 2.03	67.33 $\pm$ 4.98 *
ALT U/L	20.33 $\pm$ 1.45	40.67 $\pm$ 1.20 *
ALK U/L	41.00 $\pm$ 3.21	143.33 $\pm$ 2.03 *

Values are expressed as mean  $\pm$  standard errors. Means in the same row with (\*) significantly differ at ( $P \leq 0.05$ ). LSD = Lumpy skin disease group. Aspartate transaminase (AST) and Alanine transaminase (ALT). Alkaline phosphatase (ALK).

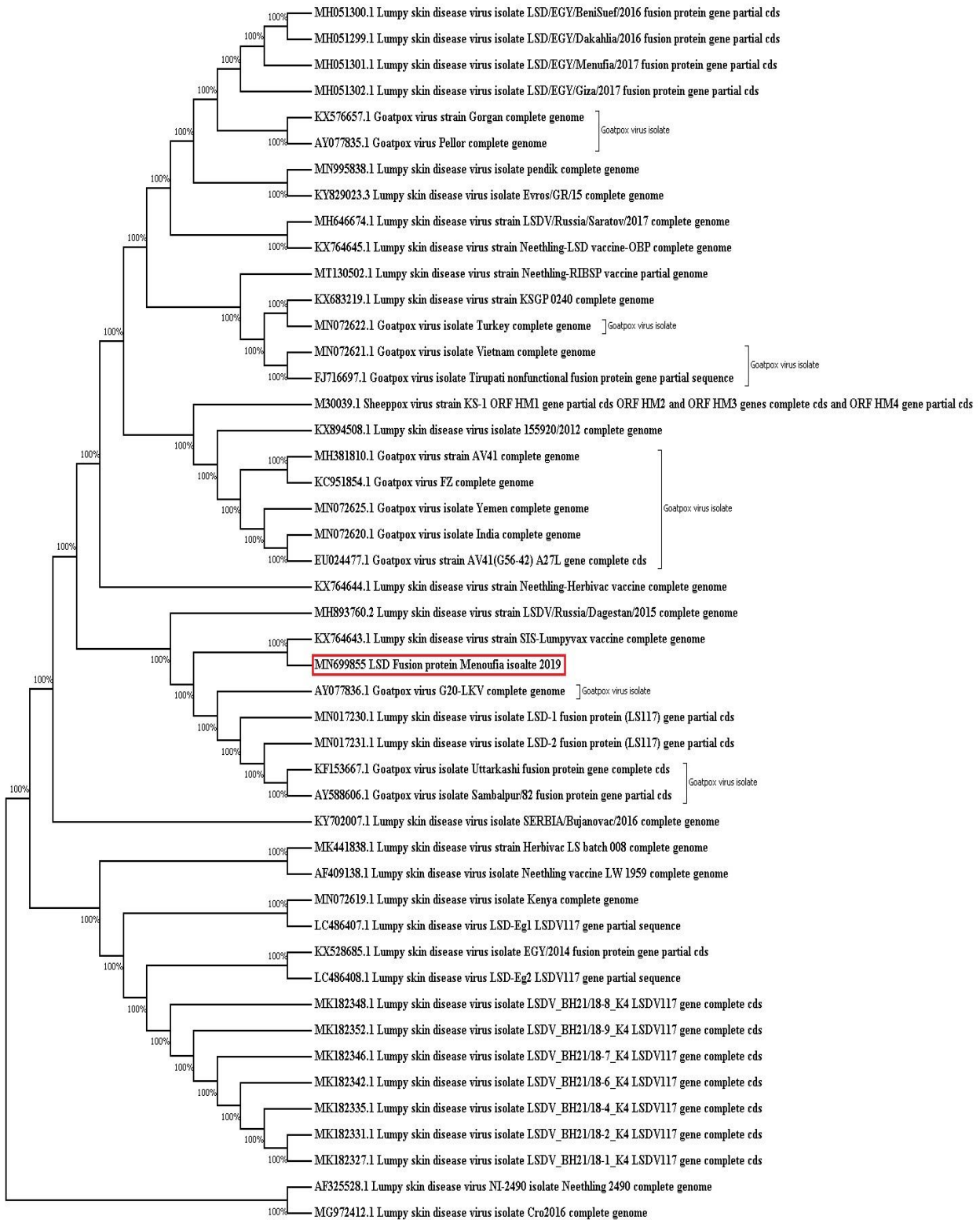
### PCR results

PCR products showed the approximate size for each gene Fusion and P32 of 472 and 587 bp, respectively. All skin biopsy (100 %) was positive for the PCR assay. Negative controls produced no amplicon.

### Multiple sequence alignment and phylogenetic analysis

The sequences of nucleotides of selected genes were aligned with others registered in GenBank and revealed close by sequence identity (98%). Sequences of the detected genome sequences were presented to the BLAST search versus the GenBank database.

The nearby sequences to the native LSDV isolates were those to sheep poxvirus (SPV), goat poxvirus (GPV), and LSDV as well. The created phylogenetic trees (Figures 3 and 4) approved the results which were achieved from both sequence alignments and BLAST search as demonstrated. These results showed that the isolates were consistent to LSDV and strictly associated with SPV and GPV. The sequences of the fusion and P32 proteins were registered in the GenBank with the accession numbers MN699855 and MN699856, correspondingly.



**Figure (3).** Phylogenetic tree of LSDV fusion gene nucleotides sequences of Lumpy skin disease virus detected in cattle (summer, 2019); red square, after multiple sequence alignment by using the maximum likelihood method.



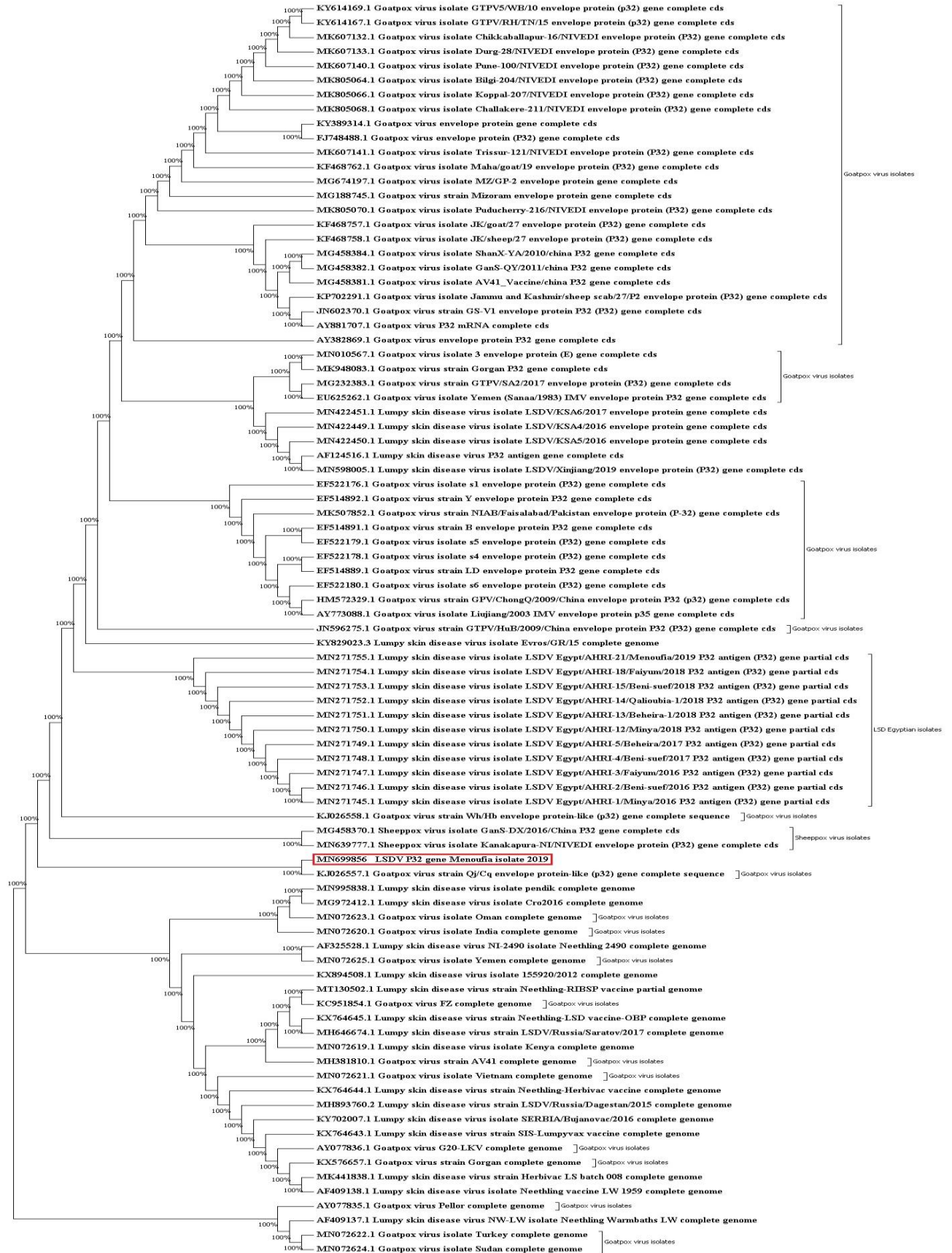


Figure (4). Phylogenetic tree of LSDV P32 gene nucleotides sequences of Lumpy skin disease virus detected in cattle (summer, 2019) red square, after multiple sequence alignment by using the maximum likelihood method.



## Discussion

Lumpy skin disease is a significant economic sickness of cattle, which resulted in chronic debility in infected cattle with a high mortality rate [17] and a devastating disease characterized by characteristic skin nodules. The clinical signs such as generalized nodules, limp and udder edema and scrotal nodules come in harmony with those described previously [6]. On the other hand, the other clinical signs as fever and off food were previously documented [5].

The effect of age on the infection rate of LSD was obvious, where the age group of 6–12 months was the highest in the infection rate. This may be due to the absence of protective antibodies in calves, which may be attributed to their absence in mothers or their natural decrease a few months after their passive acquisition from the colostrum. These results are compatible with Shalaby and coauthors [18]. Meanwhile, the variance in the prevalence of LSD in the studied localities would be attributed to immunological status of the animals whether infected or vaccinated.

The pathogenesis and prognosis of the disease can be elucidating by the measurement of haemato-biochemical parameters, which significantly affected especially in case of infection [19]. The evaluation of hematological and biochemical parameters in each animal is an important method of assessment of health [20, 21]. Also, a previous study [22] reported that changes in the values of blood chemistry may be an aid during the treatment of diseased animals. In the present investigation, there was haemato-biochemical alteration as a result of LSDV infection that was supported previously [23]. In detail, the erythrogram (RBCs count, Hb, HCT, MCV, MCH, and MCHC) were decreased significantly, which attributed to LSDV infection. In normal conditions, the free radical's reactive oxygen species (ROS) and nitric oxide (NO) are produced during metabolism. These free radicals increase in some diseases, stress, and inflammatory conditions [24]. The ROS can induce lipid peroxidation resulting in cellular damage [25]. On the other hand, Weiss and Wardrop [26]

reported that viral infection could result in hemolytic anemia. Regarding leucogram white blood cells (WBCs) count, lymphocytes (LYM), monocytes and some eosinophil (MID), and granulocytes (GRAN) in infected animals, there was a leukocytosis, which may be attributed to acute secondary bacterial infection. These results supported by a previous study [27] but disagree with the finding of another one [28]. This difference may be attributed to the stage of the disease where after infection, a large amount of corticosteroids were secreted, which appear in terms of leukopenia [29]. From the above-mentioned results, LSD infection needs supportive treatment to overcome anemia, immunostimulant drugs, and antibiotics to overcome the secondary bacterial infection.

Regarding serum biochemical profile, the serum activities of AST, ALT, and ALK and the concentration of the whole protein and globulin were increased significantly in ill animals. These results were confirmed by Şevik and coauthors [30], which may be attributed to the inflammatory reactions of viral diseases [31]. These elevations in hepatic serum enzymes indicate hepatic injury [32]. Also, the result of serum protein was normal. Serum biochemical profile results may be attributed to the extended half-life of albumin (about 18 days) in cattle. Also, normal serum protein with a significant increase in serum globulin indicates liver disease [15]. These results indicated that serum samples were taken at the early stage of the disease. Also, elevated serum urea and creatinine use as an indicator of renal diseases [23]. So, liver and renal supportive drugs needed for these animals to avoid the liver and renal complications.

On the other hand, PCR proved to be the best choice prompt detection of LSDV outbreaks especially other methods are believed to be time-consuming [10]. The PCR assay detected LSDV in all biopsies collected from infected cattle, which in agreement with several studies [10, 11] in which the virus in skin lesions was reported with the level of success 100%. The World Organization for Animal Health (OIE) classifies LSD as

notifiable disease where the molecular diagnostic investigations take part in keep an eye on the spread of these agents in susceptible livestock. A range of conventional agarose-gel-based polymerase chain reaction (PCR) assays [7, 11], or real-time PCR assays [9] are used in diagnostic laboratories. Yet, inadequately equipped laboratories often face obscurities applying these molecular techniques (mainly real-time PCR) that are subject to expensive and relatively fragile equipment. In particular, the ability to perform nucleic acid-based tests such as PCR in field settings has proven to be a tough goal in principal due to the dependence upon pre-processing of samples (nucleic acid extraction), the deficient of stable reagents that are suitable for use in environments where it is not possible to maintain a cold chain [9].

Viral sequence assessments and phylogenetic analyses of the current LSDVs on annual basis are compulsory for recognizing new circulating strains, noting the viruses spread, and selecting appropriate vaccines as a prophylactic measure against the disease [33]. Both genes have been used as a diagnostic tool for the virus of this disease where it is considered highly conserved among the whole family [10] and for both SPP and GTP viruses [33]. The sequencing results confirmed the complete identity to isolates of *LSDV*. The sequences showed 100% likeness at the nucleotide level to each other and to isolates submitted from Egypt (MH051302.1, MH051301.1, MH051300.1, MH051299.1, LC486408.1, LC486407.1, KX528685.1) and more than 98% to African isolates (MK182352.1, MN072619.1, MK182356.1, KX683219.1) as well.

### Conclusion

Animal gatherings without vaccination are considered the real threat for flaring up of new outbreaks of the LSD. Vaccination of cattle in an endemic area is necessary. Attention should be directed to isolate the circulating LSDV strain and produce a vaccine from this local strain, which will help in diminishing the spread of the infection and containing the virus every year.

### Conflict of interest

There is no conflict of interest.

### Acknowledgement

The authors acknowledge the support of all staff members in Faculty of Veterinary Medicine, Matrouh University, Matrouh, Egypt, and in National Research Centre, Egypt, as well.

### References

- [1] Buller, R.M.; Arif, B.M.; Black, D.N.; Dumbell, K.R.; Esposito, J.J.; Lefkowitz, E.J.; McFadden, G.; Moss, B.; Mercer, A.A.; Moyer, R.W. and Skinner, M.A., (2005): Poxviridae. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. editors. *Virus Taxonomy: Eight Report of the International Committee on the Taxonomy of Viruses*. Elsevier/Academic Press, Oxford, pp.117-133.
- [2] Ahmed, A.M. and Dessouki, A.A. (2013): Abattoir-based survey and histopathological findings of lumpy skin disease in cattle at Ismailia abattoir. *Int J Biosci Biochem Bioinforma.* 3(4), 372-375.
- [3] Tuppurainen, E.S. and Oura, C.A., (2012): lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. *Transbound Emerg Dis.* 59(1), 40-48.
- [4] Ali, A.A.; Esmat, M.; Attia, H.; Selim, A. and Abdel-Hamid, Y.M. (1990): Clinical and pathological studies on lumpy skin disease in Egypt. *Vet Rec.* 127(22), 549-550.
- [5] Ahmed, W.M. and Zaher, K.S. (2008): Observations on lumpy skin disease in local Egyptian cows with emphasis on its impact on ovarian function. *Afr J Microbiol Res.* 2(10), 252-257.
- [6] Abdallah, F.M.; El Damaty, H.M. and Kotb, G.F. (2018): Sporadic cases of lumpy skin disease among cattle in Sharkia province, Egypt: Genetic characterization of lumpy skin disease virus isolates and pathological findings. *Vet World.* 11(8), 1150-1158.
- [7] Tuppurainen, E.S.; Venter, E.H. and Coetzer, J.A. (2005): The detection of lumpy skin disease virus in samples of

- experimentally infected cattle using different diagnostic techniques. Onderstepoort J Vet Res. 72(2), 153-164.
- [8] Menasherow, S.; Rubinstein-Giuni, M.; Kovtunencko, A.; Eyngor, Y.; Fridgut, O.; Rotenberg, D.; Khinich, Y. and Stram, Y. (2014): Development of an assay to differentiate between virulent and vaccine strains of lumpy skin disease virus (LSDV). J Virol Methods, 199, 95-101.
- [9] Armon, B.; Fowler, V.L.; Tuppurainen, E.S.; Howson, E.L.; Madi, M.; Sallu, R.; Kasanga, C.J.; Pearson, C.; Wood, J.; Martin, P. and Mioulet, V. (2017): Detection of capripoxvirus DNA using a field-ready nucleic acid extraction and Real-time PCR platform. Transbound Emerg Dis, 64(3):994-997.
- [10] El-Kholy, A.A.; Soliman, H.M. and Abdelrahman, K.A. (2008): Polymerase chain reaction for rapid diagnosis of a recent lumpy skin disease virus incursion to Egypt. Arab J Biotechnol. 11(2): 293-302.
- [11] Ireland, D.C. and Binopal, Y.S. (1998): Improved detection of capripoxvirus in biopsy samples by PCR. J Virol Methods, 74(1): 1-7.
- [12] Awadin, W.; Hussein, H.; Elseady, Y.; Babiuk, S. and Furuoka, H. (2011): Detection of lumpy skin disease virus antigen and genomic DNA in formalin-fixed paraffin-embedded tissues from an Egyptian outbreak in 2006. Transbound Emerg Dis, 58(5): 451-457.
- [13] Constable, P.D.; Kenneth, W.H.; Stanley, H.D. and Walter, G. (2017): Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs, and Goats, eleventh edition.
- [14] Mansour, K.A. and HachimNaser, H. (2015): Molecular and Clinical investigation of lumpy skin disease in cattle in AL. Qadissiyya province. Kufa J. Vet. Sci., 6(1):8-14.
- [15] Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic acids symposium series (Vol. 41, No. 41, pp. 95-98). [London]: Information Retrieval Ltd., c1979-c2000.
- [16] Kumar, S.; Tamura, K.; Jakobsen, I.B. and Nei, M. (2001): MEGA2: molecular evolutionary genetics analysis software. Bioinformatics. 17(12), 1244-1245.
- [17] Davies, F.G. (1991): Lumpy skin disease of cattle: a growing problem in Africa and the Near East. World Anim Rev, 68(3): 37-42.
- [18] Shalaby, M.A.; El-Deeb, A.; El-Tholoth, M.; Hoffmann, D.; Czerny, C.P.; Hufert, F.T.; Weidmann, M. and El Wahed, A.A. (2016): Recombinase polymerase amplification assay for rapid detection of lumpy skin disease virus. BMC Vet Res. 12(1), 244.
- [19] Akalın, P.P.; Ataseven, V.S.; Fırat, D.; Ergün, Y.; Başpınar, N. and Özcan, O. (2015). Selected biochemical and oxidative stress parameters and ceruloplasmin as acute phase protein associated with bovine leukaemia virus infection in dairy cows. Bull Vet Inst Pulawy, 59(3): 327-230.
- [20] Milner, J.M.; Stien, A.; Irvine, R.J.; Albon, S.D.; Langvatn, R. and Ropstad, E. (2003): Body condition in Svalbard reindeer and the use of blood parameters as indicators of condition and fitness. Can J Zool, 81(9): 1566-1578.
- [21] Girling, S.J.; Campbell-Palmer, R.; Pizzi, R.; Fraser, M.A.; Cracknell, J.; Arnemo, J. and Rosell, F. (2008): Haematology and serum biochemistry parameters and variations in the eurasian beaver (*Castor fiber*). PLoS One. 10(6) e0128775.
- [22] Jenkins, J.R. (2008): Rodent diagnostic testing. J Exotic Pet Med, 17(1): 16-25.
- [23] Neamat-Allah, A.N. (2015): Immunological, hematological, biochemical, and histopathological studies on cows naturally infected with lumpy skin disease. Vet World. 8(9), 1131-1136.
- [24] Abou-Zeina, H.A.A., Hassan, S.G., Sabra, H.A. and Hamam, A.M., (2009): Trials for elevating adverse effect of heat stress

- in buffaloes with emphasis on metabolic status and fertility. *Global Veterinaria*, 3, 51–62.
- [25] Gaschler, M.M. and Stockwell, B.R., 2017. Lipid peroxidation in cell death. *Biochem Biophys Res Commun.*, 482(3): 419–425.
- [26] Weiss, D.J. and Wardrop, K.J. EDS., (2011): *Schalm's veterinary hematology*. John Wiley & Sons.
- [27] Kumar, V. and Cotran, R.S. (2007): *Robbins' basic pathology*. Arch Pathol Lab Med, 118(2): 203-203.
- [28] El-Mandrawy, S.A. and Alam, R.T. (2018): Hematological, biochemical and oxidative stress studies of lumpy skin disease virus infection in cattle. *J Appl Anim Res*, 46(1): 1073-1077.
- [29] Ismail, S.M. and Youssef, F.M. (2006). Clinical, hematological, biochemical and immunological studies on lumpy skin disease in Ismailia Governorate. *SCVMJ*, 10(1): 393–400.
- [30] Şevik, M.; Avcı, O.; Doğan, M. and İnce, Ö.B. (2016): Serum biochemistry of lumpy skin disease virus-infected cattle. *Biomed Res Int.*, 2016:6257984.
- [31] Jalali, S.M.; Rasooli, A.; Seifi, Abad Shapuri M. and Daneshi, M. (2017): Clinical, hematologic, and biochemical findings in cattle infected with lumpy skin disease during an outbreak in southwest Iran. *Arch Razi Inst*, 72(4): 255-265.
- [32] Kaneko, J. J.; Harvey, J. W. and Bruss, M. L. (Eds.). (2008): *Clinical biochemistry of domestic animals*. Academic press.
- [33] Varshovi, H.R.; Keyvanfar, H.; Aghaiypour, K.; Pourbakhsh, S.A.; Shoostari, A.H. and Aghaebrahimian, M. (2009): Capripoxvirus identification by PCR based on P32 gene. *Archives of Razi Institute*, 64(1): 19-25.

### الملخص العربي

الكشف عن مرض الجلد العقدي أثناء وباء صيف 2019 في محافظة المنوفية , مصر بواسطة إستخدام البحث السريري

والكيميائي الحيوي والجزئي

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مرض الجلد العقدي (LSD) هو عدوى فيروسية تصيب الماشية وينتج عن فيروس الجلد العقدي. يعتبر مرضا متوطنا في القارة الأفريقية وتم تسجيله في مصر منذ عام 1988. هدفت هذه الدراسة إلى مراقبة الحالة الصحية للماشية أثناء مسار المرض من خلال فحوصات الاختبارات البيوكيميائية. إلى جانب ذلك ، تم التحقق من سلالات فيروس LSD التي كانت تنتشر خلال تفشي المرض في صيف 2019 بمحافظة المنوفية. تم فحص مائة وثمانية عشر رأسًا من الأبقار من مختلف الأعمار والجنس في بعض قرى محافظة المنوفية ، مصر. تم تسجيل الأعراض السريرية وإجراء التحاليل الكيميائية للدم على الحيوانات المصابة. تم استخدام تفاعل البلمرة المتسلسل PCR التقليدي لتحديد LSDV باستخدام اثنين من الجينات المحافظة ، جين Fusion وجينات الترميز P32. أظهرت الحيوانات المصابة أعراضًا نموذجية لعدوى LSD. كانت معدلات المرض في الماشية مرتفعة عند الذكور (43%) ، وفي الفئة العمرية 6-12 شهرًا (44.4%). أظهرت تحاليل الدم انخفاضًا معنويًا (P < 0.05) في عدد كرات الدم الحمراء (0.51 ± 6.13) و الهيموجلوبين (0.15 ± 9.07) في الحيوانات المصابة. أظهرت الكريات الدم البيضاء زيادة معنوية (P < 0.05) (0.03 ± 15.13) في عددها في الحيوانات المصابة. أظهرت الكيمياء الحيوية في مصل الحيوانات المصابة زيادة معنوية (P < 0.05) في جميع المتغيرات البيوكيميائية ماعدا ألبومين الدم. (2.61 ± 0.35). تم تأكيد العدوى بفيروس الجلد العقدي بواسطة تفاعل البلمرة المتسلسل PCR لكل من الجينات وتم إيداع التسلسلات في بنك العترات GenBank بأرقام MN699855 و MN699856. و قد أوصت الدراسة بضرورة توجيه الانتباه إلى سلالة LSDV المنتشرة في الحقل ومقارنتها بالسلالات المعزولة سابقًا. يجب أن يعتمد الممارسون الميدانيون على معاملات الدم للحيوانات المصابة لاختيار العلاج المناسب.