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RESEARCH

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Molecular, clinico-pathological and sero-diagnosis of LSDV in cattle at Sharkia and Fayoum Governorates

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ABSTRACT

***Background*:** A total of 30 biopsy samples from cutaneous nodules were obtained from infected animals at different stages during the course of the Lumpy skin disease (LSD), and (100) Peripheral blood samples without anticoagulant were drawn from apparently healthy non vaccinated cattle against LSDV and (100) serum samples were drawn from cattle 4 weeks post vaccination with local attenuated sheep pox virus vaccine located in Sharkia and Fayoum governorates.
***Methods*:** Lumpy skin disease virus (LSDV) was isolated from skin biopsies collected from clinically infected cattle. The virus was isolated on MDBK cell line and identified by agar gel precipitation test (AGPT) and indirect fluorescent antibody technique (IFAT) using specific hyper immune serum against LSDV. Further identifications were carried out by polymerase chain reaction (PCR) and clinco-pathological investigation.
***Results*:** The results showed that 11/30 biopsies were positive by AGPT, 19/30 by IFAT and 30/30 by PCR. While results of sero-diagnosis showed that 45/100 from apparently healthy non vaccinated cattle and 68/100 from vaccinated cattle were positive by SNT respectively and in general 90/200 of tested cattle give protective antibody titer, while 23/200 gave non protective titer and 87/200 have no antibody againest LSDV. The results of clinco-pathological revealed highly significant increase in ALT, AST, ALP, GGT, urea and uric acid, while the level of total protein, albumin and calcium showed significant decrease and non-significant reaction in creatinine and non-organic phosphorus in infected cattle. The results of antioxidant both malondialdehyde (MDA) and catalase enzyme (CAT) showed significant increase while level of gloutathion (GSH), total antioxidant capacity (TAC) and gloutathion peroxidas (GPX) showed significant decrease in infected cattle.
***Conclusion*:** Sero-survey, conventional techniques and PCR assay should be applied besides clinco-pathological for any cases with skin lesions as early as possible to diagnosis and apply adequate control measures. The results encountered in the present study revealed that cattle infected with LSD exposed to strong oxidative stress so recommended to use antioxidants in infected animals during treatment.

Keywords: IFAT ; LSDV; PCR .

BACKGROUND

Lumpy skin disease is caused by a virus in the genus Capri Poxvirus of the family poxviridae. It is an acute, sub-acute or in apparent disease in cattle and affects all ages and breeds. The disease characterized by mild to severe signs including fever, enlarged lymph nodes, firm rounded nodules in the skin (1-4cm) which may extend to mucous membrane and internal organs. In severe cases edema, lymphadenitis and death may be occurred **Davies, (1991)**. Lumpy skin disease (LSD) is an economically important disease of cattle and can produce a chronic debility in infected cattle comparable to that caused by foot-and-mouth disease (FMD) (**Davies and Otema 1981**).

Aedes aegypti mosquitoes were capable of the mechanical transmission of LSDV over a period of 2–6 days post-infective feeding **Chihota et al., (2001)**. The impacts of global climate change on insect vectors, established as a route of transmission for LSD suggesting that there

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were real risks of further spread of these diseases into other geographic regions **Hunter and Wallac, (2001)**.

In Egypt, LSD virus (LSDV) was isolated for the first time from cattle in two disease outbreaks in 1988. The virus was detected in a pooled sample from the first outbreak in Suez and was isolated subsequently from the second outbreak Ismalia. The capripox viruses were identified as LSDV by neutralization with specific antiserum and by their ability to produce generalized LSD in experimentally inoculated cattle **House et al., (1990)**. In 1988 and 1989 an outbreak of confirmed LSD occurred among cattle in Egypt **Ali et al., (1990**). In 1998, an outbreak of LSD was reported among cattle in El-Menia Governorate in Upper Egypt **Abd El-Rahim et al., (2002**). Recurrent LSD outbreaks among cattle were recorded in 2006 in Banisuef, Behera, Ismalia and New Valley provinces in Egypt **OIE, (2006**). The first report of LSD outside Africa was described in Kuwait in 1986, where 642 cases were reported in cattle **Ordner and Lefevre, (1987**).

The World Organization for Animal Health (**OIE**) categorizes LSD as a notifiable disease because of the substantial economic impact of an outbreak. The disease is more severe in cows in the peak of lactation and causes a sharp drop in milk yield because of high fever caused by the viral infection itself and secondary bacterial mastitis. Temporary or permanent infertility may occur in cows and bulls. Diagnosis of LSDV depends on the characteristic lesion and laboratory confirmation of the isolated virus. Cultures are examined for the characteristic cytopathic effect, with the final identification by AGIT and IFAT **OIE, (2008**).

Pathogenic mechanism of viral disease involves implantation of virus at the site of entry. Replication at this site, spread to target organs and spread to site of shedding of virus into environment. Viral disease occurs if the virus replicates in essential cells sufficiently and damage directly the organs or indirectly functionally as result of host immune response. Significant changes can be observed in serum biochemical values when damage of cellular organs occur **Muart et al., (2016**). Few studies conducted on pathogeneses of LSD in cattle **EL Neweshy, (2013)**, additionally; there is limited information in literatures about serum biochemical findings in cattle infected with LSD virus **Muart et al., (2016).** Oxidative stress is extremely dangerous as it does not exhibit any symptoms and is recognizable with great difficulty by means of laboratory methods. Measurement of oxidative stress allows the assessment of real status of physiological defense and prevention of the appearance of correlated pathogeneses **Piccione et al., (2009**). Serum biochemical parameters can be useful tool for assessing animal health and help better understanding and pathogeneses of the disease.

The conventional gel-based PCR method described below is a simple, fast and sensitive method for the detection of capripox virus genome in EDTA blood, biopsy, semen or tissue culture samples. Recently, quantitative real-time PCR methods have been described that are reported to be faster and have higher sensitivity **Balinsky et al., (2008**). Real-time PCR assays have been widely utilized for early diagnosis of many other animal viral diseases. In this study a real-time PCR assay was developed and evaluated for detection of LSD in field samples.

The objective is to diagnose lumpy skin disease virus by real time PCR in comparison to conventional techniques and investigates the effect of LSDV in biochemical and oxidative status in infected cattle.

MATERIALS AND METHODS

Virus Isolation and Identification:

Samples: 30 biopsy samples from cutaneous nodules were obtained from infected animals at different stages during the course of the disease, and 100 Peripheral blood samples without

anticoagulant were drawn from apparently non vaccinated cattle against LSDV and 100 serum samples were drawn from cattle 4 weeks post vaccination with local attenuated sheep pox virus vaccine located in Sharkia and Fayoum governorates.

Sample preparation:

10% suspension was prepared from each skin nodules following the method of **Burleson et al., (1997)** for virus isolation and identification by agar gel precipitation test (AGPT), IFAT and PCR. The sera samples were separated by centrifugation for biochemical examination and examined for the presence of LSDV antibodies by SNT.

Virus Isolation:

0.2 ml from each prepared skin nodules suspension was inoculated for three passages into MDBK cell line supplied from Tissue culture Unit, department of virology, Animal Health Research Institute, Dokki, Giza and examined daily for the presence of cytopathic effect according to the method described by **Carn and Kitching, (1995).**

Detection and Identification of LSDV antigen:

The AGPT and IFAT were performed for the detection and identification of LSDV. The techniques were performed according to the method described by **OIE, (2010**), standard reference LSDV (Neethling strain) and standard reference LSDV antiserum was obtained from department of virology, Animal Health Research Institute, Dokki, Giza. It was used for identification by in AGPT and IFAT.

Serum biochemical analysis:

Estimation of ALT and AST according to **Reitman and Frankle, (1957),** Alkaline phosphatase according to **Belfied and Goldenberg, (1971),** Gamma Glutamyle Transpeptiase according to **Psijin, (1971**), Total Protein and Urea according to **Henry et al 1974),** albumin according to **Dumas et al., (1971),** uric acid according to **Artiss and Entuistle, (1981),** Serum creatinine according to **Hovet, (1985)**, calcium according to **Gundler and Kin, (1972)**, inorganic phosphrous according to **Goldenberg et al., (1966),** iron, copper and zinc according to **Bauer, (1982),** Total antioxidant according to **Karecevic, (2001),** malondi aldehyde according to **Ohkawa et al., (1979),** catalase enzyme according to **Aebi, (1974)**, Glutathione according to **Owens and Belcher, (1965) and** Glutathione peroxidase according to **Paglia and Valentine, (1967)**

Statistical Analysis:

The obtained data were statistically analysed according to **SPSS14 (2006)**

Detection of LSDV by polymerase chain reaction:

DNA extraction: Molecular identification of LSDV was applied on the 30 collected nodules according to method described by **Gubbels et al., (1999).** DNA of tissue samples were extracted by using Thermo scientific kits (Gene JET Genomic DNA Purification Kit #K0721, #K0722)

Amplification of DNA:

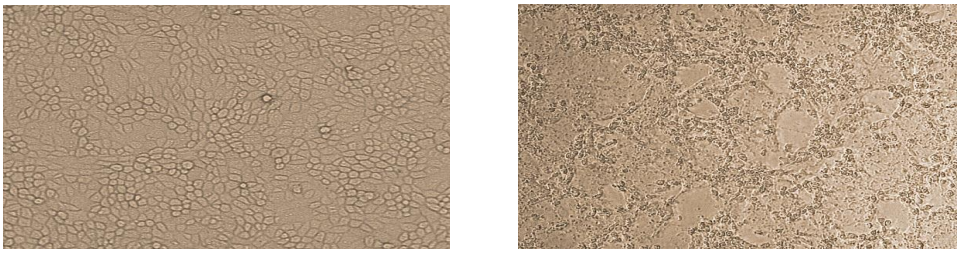
DNA amplification was done by using GPS kits for genetic detection of LSDV in 25 ul reaction volume containing 12.5 ul of 2× hotstart qPCR mix, 1 ul of RieAna dtec-qPCR-mix, 5 ul of DNA template and fill up to 25 ul with DNAse and RNAse free water. In the case of positive control or negative control, only 5 µl of standard template RieAna or DNAse and

RNAse free water were added to the reaction volume respectively instead of DNA. The optimized cycle program for real time- PCR was as mentioned in table (1).

Table (1): showing the optimized condition for real time PCR

Temperature		Time	Step
95°C		5 minutes	Activation
45 cycles	95°C	20-30 seconds	Denaturation
	60°C	45-60 seconds	Hybridization, extension, data collection

RESULTS



(A) (B)

Photo (1): A-Control non infected MDBK cell line. B- Characteristic CPE of LSDV by cell rounding, cell aggregation, coalesce together to form clusters within 72 hr. post inoculation (Magnified 400 x)

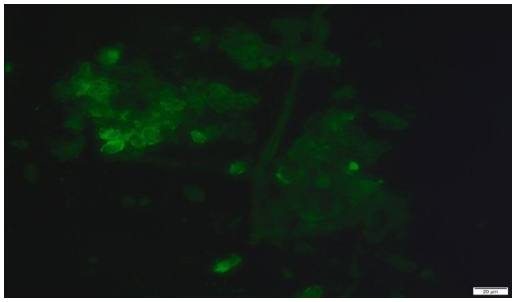


Photo (2): MDBK cells infected by suspected local LSDV isolates and stained by fluorescence isothiocynate showed intracytoplasmic yellowish green fluorescent granules (Magnified 400 x)

Table (2): detection of LSDV in samples from skin nodules of infected cattle by AGPT, IFAT and PCR

Governorates	No of samples	No / % of positive by AGPT	No / % of positive by IFAT	No / % of positive by PCR
Sharkia	13	5/38.5%	8/61.5%	13/100%
Fayoum	17	6/35.3%	11/64.7%	17/100%
Total	30	11/36.7%	19/63.3%	30/100%

Table (3): detection of LSDV antibodies in cattle sera by SNT

Governorates	Cattle status	No of samples	No. of +Ve	SNT titer			
				8	16	32	64
Sharkia	Apparently healthy non vaccinated	58	20	3	11	5	1
	Vaccinated cattle	42	35	5	11	15	4
Fayoum	Apparently healthy non vaccinated	55	25	8	9	6	2
	Vaccinated cattle	45	33	7	9	13	4
-	Total	200	113	23	40	39	11

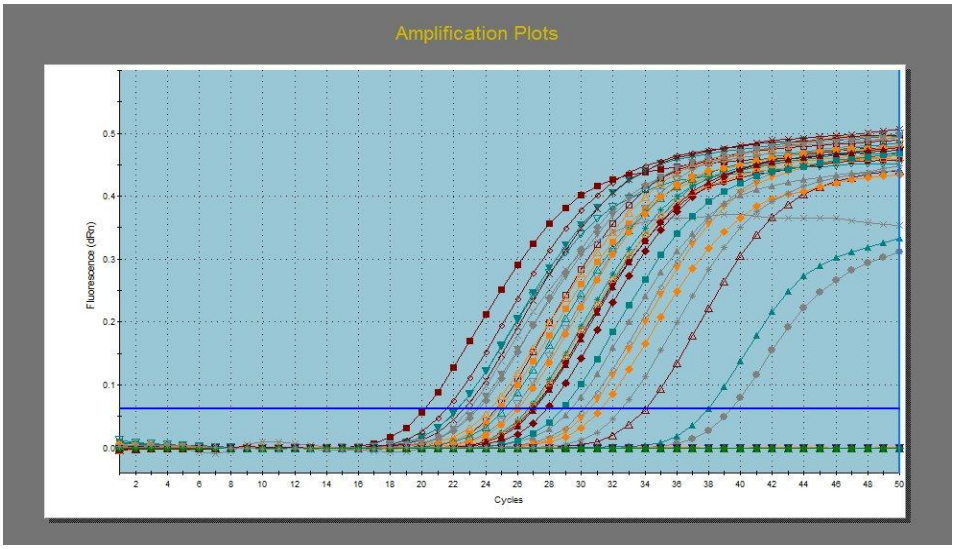


Fig. (1): Amplification plot of real time PCR results performed on tissue samples of infected cattle by using GPS real time PCR Kit specific for genetic detection of LSDV.

Table (4): Alteration in some biochemical and mineral in infected cattle with LSD virus (Means ± SE)

Infected	Control	Parameters
31 ± 1.8***	17.6 ± 1.8	ALT(UL)
48.2 ± 2.7***	31 ± 1.8	AST(UL)
29 ± 1.2***	23.2 ± 2	GGT(UL)
93 ± 2**	70.6 ± 3.9	ALP(UL)
6.48 ± 0.29*	7.9 ± 0.18	T. P (gm /dl)
3.11 ± 0.11 **	3.88 ± 0.18	Alb (gm / dl)
33.8 ± 3**	20.4 ± 1.5	Urea (mg /dl)
5.46 ± 0.29**	4 ± 0.2	Uric acid (mg /dl)
1.1 ± 0.1	0.94 ± 0.1	Creatinine (mg /dl)
8.8 ± 0.25*	9.7 ± 0.4	Ca (mmol)
5.1 ± 0.18	4.9 ± 0.18	Ph (mmol)

Significant *P ≤ 0.05 **P ≤ 0. 01 ***P ≤ 0.001

Table (5): Alteration in some trace element and antioxidants in infected cattle with LSD virus (means ± SE)

Infected	Control	Parameter
114.8 ± 2 ***	133 ± 3.2	Iron (µg/dl)
33.6 ± 1.7 *	40.4 ± 2.2	Cupper (µg/dl)
34.6 ± 3.7	37.6 ± 2.23	Zinc (µg/dl)
5.24 ±0.3 **	3.12 ± 0.34	MAD n.mol/l
88.6 ± 2*	68.2 ± 2.39	CAT(u/ l)
13.37 ± 0.8***	21.4 ± 1	GSH (u ml)
0.61 ± 0.09***	1.38 ± 0.06	TAC (m mol/l)
5.45 ± 0.43***	7.97± 0.7	GPX (u ml)

Significant *P ≤ 0.05 **P ≤ 0. 01 ***P ≤ 0 . 001

DISCUSSION

In many areas of the world, especially in Africa and Asia, LSD is a subacute to acute cattle disease which is characterized by extensive cutaneous lesions and signs typical of generalized poxvirus diseases. However, the transmission of the disease among cattle is inefficient and arthropod-vector **Coetzer et al., (1994).** The importance of this disease increase gradually as the way of eradication and control is very difficult.

The present study concerned with serosurvey and characteristic trials for isolation of LSDV from skin nodule samples from infected cattle on tissue culture with further identification by means of conventional serological tests as AGPT and IFAT in addition to advanced molecular characterization of virus isolate using PCR as well as serum biochemical changes.

LSDV was isolated from samples collected from naturally infected cattle by inoculation on MDBK. CPE was characterized by cell rounding, cell aggregation, coalesce together to form clusters that scattered all over the monolayer within 72 hr. post inoculation and gradually increased till 70-80 % of sheet was completely detached (Photo 1). These findings agree with those of **Woods, (1988).** Isolated LSDV was identified by serological tests (Table 2), 11/30 of samples give clear precipitation lines by AGPT using reference LSDV antisera and clear precipitation lines were appeared using reference LSDV antisera the same results were recorded by **Ahmed and Zaher (2007).** 19/30 of samples show characteristic specific Intracytoplasmic yellowish green fluorescent granules by IFAT (Photo 2) as observed by Isolated LSDV was identified by serological tests **Davies, (1991).** While results of ser-diagnosis (Table 3) showed that 45/100 from apparently healthy non vaccinated cattle and 68/100 from vaccinated cattle were positive by SNT and antibody titer ranged from 8 to 64. The protective titer were consider ≥ 16 **Cottral, (1978)**, 90/200 of tested cattle give protective antibody titer, while 23/200 gave non protective titer and 87/200 have no antibody to LSDV.

Serological methods are useful for confirming retrospectively LSD but are too time consuming to be used as primary diagnostic methods **Davies, (1991).** Serological assessment of antibodies to a capripox virus may sometimes be difficult due to the cross-reactivity encountered with other poxviruses as well as to the low antibody titers elicited in some animals following mild infection or vaccination **Kitching and Hammond, (1992)**. Therefore, PCR was the test of choice for rapid detection and identification of the LSD outbreak causative agent. A total of 30 samples tested by real-time PCR for the presence of LSDV give positive with CT value ranged from 17 to 36, suggesting higher concentrations of virus (Fig 1).

The high sensitivity of PCR (100%) in detecting the LSDV DNA in skin nodular samples correlate with **Tuppurainen et al., (2005), Sharawi and Abd El- Rahim, (2011),** this may be attributed to the viral tropism to skin tissues and its persistence in high concentration. **Tuppurainen et al., (2005)** and **Zeynalova et al., (2016)** reported that LSD viremia is relatively short-lived – blood samples were positive for PCR for 4–11 days post-infection, while virus could be detected in skin lesions up to 92 days post-infection. A more effective technique is real time PCR because it is fast, is performed in closed vessel systems, avoids post PCR-processing, thereby reducing risks from contaminations, and thus is suited ideally for rapid, specific and highly sensitive diagnosis **Zeynalova et al., (2016**).

Since sensitive viral DNA-detection technologies can overcome the technical, time-related problems and costs associated with virus isolation for the diagnosis. The nucleic acid detection results confirmed our conclusions pertaining to the relatively low virus isolation rates because PCR testing was able to detect viral nucleic acid in 100% of skin and blood samples collected from clinically infected cows **Awad et al., (2010).**

The results of biochemical analysis as regarding in Table (4 and 5), revealed highly significant increase in ALT, AST, ALP, GGT, urea and uric acid. While the level of total protein, albumin and calcium showed significant decrease and non-significant reaction in creatinine and non-organic phosphorus in infected cattle. The increase in level of ALT and AST may be attributed to liver injuries, **Aly et al., (2006)**, who attributed the marked increase in level of AST and ALT activity to hepatocellular damage .The increase in AST activity may be also due to heart muscle and general tissue break down caused by viral infection or secondary bacterial infection **Agag et al., (1992)**. The increase in level of ALP and GGT due to the injury of cell lining the biliary ducts of liver. Also this increase may attributed to the biliary disease intrahepatic cholestasis and infiltrative disease of liver **Stockhom and Scott, (2008)**.

Hypoalbuminemia and Hypoproteinemia could be resulted from severe anorexia, off food and liver diseased which unable to synthesis protein. **Hassan et al., (2011)** reported that these results attributed to decreased synthesis and higher catabolic rate of protein as well as damaged of liver parenchyma. The partial explanation of albumin changes to deposition of albumin, potentially derived by subepithelial eosinophilic inflammation, seems to be an early and key pathogenic factor in skin necrosis development **Van Cauwenberge et al., (2005).**

Concerning to increase level of urea and uric acid, this increase attributed to the effect of virus agent in kidney tubules causing renal impairment or reduction of glomerular infiltration and increase catabolic rate of protein. **Neamat-Allha, (2015)** explained the increase level of urea in may be due to increase protein breakdown in hyperthermia.

Hypocalcaemia could be attributed due to animal off food, renal infiltration impairment. **Stockhom and Scott, (2008)** reported that hypocalcaemia could be attributed to hypoproteinemia resulting decrease protein bounded calcium. Regarding to results of trace element (Table 5) showed significantly decrease in iron, cupper and nonsignificant in zinc in serum of infected animal with LSDV. These results could be attributed to reduce food intake during time of infection. **Kocyigit et al., 2002** mentioned that LSD virus infection caused alteration in trace elements and redistribution as a result of factor released from stimulating phagocytic, while **Ahmed, (2007)** mentioned that changes in trace element in the serum may be related to decrease food consumption or to hypoprotienaemia, which hinder its absorption.

Regarding to results of antioxidant (Table 4), both malondialdehyde (MDA) and catalase enzyme (CAT) showed significant increase while level of gloutathion (GSH), total antioxidant

capacity (TAC) and gloutathion peroxidas (GPX) showed significant decrease in infected cattle. Oxidative stress results when reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanism **Sies, (1991)** and / or from decrease in antioxidant defense, which lead to damage of biological macromolecule and disruption of normal metabolism **Trevison et al., (2001)**. This condition can contribute and / or lead to the onset of health disordered in animals. The activation of free radical formation judged by the dynamics of lipid peroxidation malondialdehyde (MDA) is commonly used as monitor lipid peroxidation which occur when reactive with poly unsaturated fatty acid and has been considered as a major mechanism of cell damage **Boobis et al., (1989)**. High significant increased in MDA suggested that enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of free radical **Rantnam et al., (2006).** The non-enzymatic antioxidant gloutathione (GSH) is the one of the most abundant tripeptides present in cell . Its function mainly concerned with the removal of free radical and maintenance of membrane protein Thiol and as substrates for glutathione peroxidase. The significant decrease in glutathione has been associated with enhanced lipid peroxidation . A major product of membrane peroxidation can also react with GSH to yield inactive thioether derivatives **Esterbauer et al., (1991).**

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissue and its highest activity were present in red cells and in liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Significant increase in catalase enzyme in affected animal indicated the higher formation of hydrogen peroxide, this indicate the body's response to compete the oxidative stress **Kataria et al., (2010).** Total antioxidant capacity (TAC) are a measurement of exogenous and endogenous antioxidants in large spectrum **Karecevic (2001).** Significant decrease of TAC in affected animal attributed to excessive production of free radicals leading to occurrence of oxidative stress **Hanan et al., (2016).**

Glutathione peroxidase (GPX) is a member of a family enzyme whose function is to detoxify peroxidase in the cell. Because peroxidase can decompose to form highly reactive radical .The GPX enzymes play critical role in protecting the cell from free radical damage, particularly lipid peroxidation .The significant decrease of GSX in affected cattle indicated its use in an attempt to reduce oxidative stress **Kataria et al., (2010).**

CONCLUSION

In conclusion, LSDV antibodies were prevalent throughout the 2 Egyptian governorates and PCR assay should be applied besides conventional techniques for any cases with skin lesions as early as possible to diagnosis and apply adequate control measures. The results encountered in the present study revealed that cattle infected with LSDV exposed to strong oxidative stress so recommended to use antioxidants in infected animals during treatment.

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REFERENCES

Abd El-Rahim, I. H. A.; Ballal, S. E. and Hussein, M. (2002): "An outbreak of lumpy skin disease among cattle in upper Egypt (El-Menia Governora-te) Minufyia Vet. J., 2(1):185-195.

Aebi, H. (1974) :" Catalase in : H U Bergmeyer and H. U lrich : Methods of enzymatic analysis , (2nded), Academic press, Verlog Chemic Weinheim, New york, Vol. 2,pp. 673-678 .

Agag, B.; Mousa, Sh.; Hassan, H.; Nabila, S. and Abd El-Aziz (1992): Clinical, serological and biochemical on lumby skin disease. J. of App. Anim. Res. 1:1, p, 13-23.

Ahmed, W. (2007): Overview on some factors negatively affecting ovarian activity in large farm animals. Global Vet. 1, P: 53-66.

Ahmed, W.M. and Zaher, S.K. (2007): Observations on lumpy skin disease in local Egyptian cows with emphasis on its impact on ovarian function. African Journal of Journal of Microbiology Research, (2): 252-257.

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. The Veterinary Record, 127: 549-550.

Aly, A; Ibtisam, M; Mohammed,M.(2006): " Lumpy skin disease as afiled program of cattle in Shrkia Gavernorate Egypt. " J.Com .Path .Clinic Path 14(1) 162-173

Artiss, J. and Entuistle (1981):"Enzymatic determination of uric acid "Clin.Chem" Acta. 116; 301-309

Awad, W. S.; Ibrahim, A. K. and Moniem, M. A. (2010): Evaluation of different diagnostic methods for diagnosis of Lumpy skin disease in cows. Trop Anim. Health Prod. Vol. 42, P: 777–783.

Balinsky C.A, Delhon G, Smoliga G, Prarat M, French R.A, Geary S.J, Rock D.L & Rodriguez L.L. (2008). Rapid preclinical detection of sheep pox virus by a real-time PCR assay. J. Clin. Microbiol, 46, 438–442.

Bauer, J .D.(1982): Clinical laboratory methods 9thed . Tne C.V. Mosby Company, 1180 USA

Belfied, A. and Goldenberg, D. (1971): "Caloremetric determination of Alkaline phosphatase activity'. Enzyme 12: 561-568

Boobis, A, Fawthrop, D and Davis, D. (1989): "Mechanisms of cell death". Sci. 10: 175-280.

Burleson F., Chambers T. & Wiedbrauk, D. (1997): Virology: a laboratory manual. Academic Press, Harcourt Brace Jovanovich, San Diego.

Carn V. and Kitching R. (1995): An investigation of possible routes of transmission of lumpy skin disease virus (Neethling). Epidemiol. Infect., 114, 219–226.

Chihota, C.; Rennie, R.;Kitching and Mellor, P. (2001): Mechanical transmission of lumpy skin disease virus by *Aedes aegypti* (Diptera: Culicidae) Volume126 / Issue 02 / April 2001, pp 317-321

Coetzer, J.; Thomson, G. and Tustin, R. (1994). Poxviridae. In Infectious diseases of livestock (ed.), Vol. 1Oxford University Press, Cape Town, South Africa, : 601-603.

Cottral, G. (1978): Manual of standardized methods for veterinary microbiology. Cornell University Press, Ithaca, NY.

Davies, F. (1991): Lumpy skin disease, an African University. Br. Vet. J., 19. Woods, J.A., 1988. LSD, A review. Trop. Anim. Health. 147: 489-503.

Davies, F.G. and Otema, C. (1981): Relationships of capripoxviruses found in Kenya with two Middle Eastern strains and some orthopoxviruses. Research in Veterinary Science, 31: 253-255.

Dumas, B., Waston, W. and Bigg, S.H. (1971): Quantitative calometric determination of albumin in serum or plasma. Clin. Chem. Acta, 31: 87-90.

EL-Neweshy, M; EL-Shemey, T. And Yousif, A. (2013): A pathogenic and Immunochemistry finding natural Lumpy Skin Disease in Egypt cattle. Pakistan veterinary journal: 33(1) - 60-64.

Esterbauer, H; schrauar, R and Zollner, H. (1991): "chemistry and biochemistry malondialdehyde and related aldehydes "

Goldenberg, H.; Novelli, L. and Vallee (1966): Determination of inorganic phosphorus. Clinc.Chem, 12: 871 -873

Gubbels, M., De Vos, M.,Van Der Weide, J. Viseras, L.M., Schouls and E. De Vries (1999): "Simultaneous detection of bovine Theileria and Babesia species by reverseline blot hybridization." J. Clin. Microbiol. 37, 782-789.

Gundler, E. and Kin,J. (1972): Rapid Calorimetric determination of calcium in biologic fluids with methyl thymol blue . Am.J. Clin. Pathol. 58 (4): 376-382

Hanan, K; Heba,G ; Norhan ,N and Mohamed ,R. (2016): 13th sci. cong. Egyptian Society for cattle disease 1-4 Feb, Hurggada, Egypt .

Hassan, H; Elkhrdosy A and ALI, M. (2011): Immunobiochmical profile in cattle infected with lumpy skin disease. J. Basi .chem 1:21-25

Henry,R.,J.; Canon D.C.and Winkerlmar, J.W.(1974): Clinical chemistry principals and techniques .2nd. Ed. Hurpar and Row puplisher, Hagers town.

House J.; Wilson T.; El Nakashly S.;Karim I. ; Ismail I.; El Danaf N.;Moussa A. and Ayoub N. (1990). The isolation of lumpy skin disease virus and bovine herpesvirus-4 from cattle in Egypt. J. Vet. Diagn. Invest. , 2 (2):111-115.

Hovet, O. (1985): Interpretation of clinical laboratory tests. Determination of creatinine pp: 220-234, edited by sest, G.; Schiele, F. and Yuong, D. Biochemical Publication.

Hunter, P. and Wallac, D. (2001): Lumpy skin disease in southern Africa: a review of the disease and aspects of control Tydskr. S. Afr. vet. Ver. 72(2): 68-71.

Karecevic, D. (2001): Calorimetric method for determination of total antioxidant. J. Clinc. Patholo; 19-25

Kataria, N.; Kataria, A.; Moan, R.; and Gahlot, A. (2010): Evaluation of Oxidative stress in brucella infected cow. J. of Stress Physio., Biochem.6 (2).54:356-361.

Kitching, R. and Hammond, J. (1992): Poxvirus, infection and immunity. In: Encyclopaedia of immunology 3 (I.M. Roitt and P. Delves, 1, 3rd edition): 1261-1264. Academic press, London.

Kocyigit, A Quval, S; Eroel, O;and Aktepe ,K (2002): "Association among selenium ,zinic ,copper and iron concentration and immuno regulatary cytokine levels inpatients with cutaneous Leshmaniasis :Biol.Trace Elem .Res ,90 (1-3):47-55

Muart, S; Oguzhan A, Muge, D and Omer, B (2016): Serum Biochemistry of lumpy skin disease virus - infected cattle. BioMed Research International Volume 2016, Article ID 6257984, 6 pages.

Neamat-Allha, A. N. (2015): Immunological, heamatological, biochemical histopathological studies on cows naturally infected with Lumpy Skin Disease .Veterinary world, Eissue 2231-0916 11:1131-1136

Ohkawa, H; Ohishy, W and Yoogi, K (1979): "Calometeric method for determination of malondialdhyide in serum. Anal .Biochem, 95-351.

OIE (2006): "Annual Reports on the animal health status and disease control methods and list A disease outbreaks." Statistics. OIE, Paris, France, P.4.

OIE, (2008): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Office International des Epizooties, Paris, France: World Organization for Animal Health.

OIE (2010): Chapter 2.4.14. Lumpy Skin Disease

Ordner, G. and Lefervre, P. (1987): La dermatose nodulaire contagieuse des bovines. Etudes et sytheses de l'Institut d'Elevage et de Medicine Veterinarie Tropicale, Maison-Alfort, Paris, pp 92.

Owens, C and Belcher, R. (1965): ''Calometeric method for the determined glutathione." Biochem. J 94(3): 705-711

Paglia, D; and Valentine, N. (1967): "Studies on quantitative and qualitative characterization of glutathione peroxidase". J .Lab .Clin .Med; 70: 158 -169

Piccione, G; Caola, G. and Giannettio, C. (2009):selected biochemical serum parameters in ewes during pregnancy, post-parturation, lactation and dry period. Animal Science Papers and Reports, 27 (4): 321-330.

Psijin, J .P. (1971): "Kinetic determination gamma glutamyl traspeptidase activity". Clin. Chem .Acta, 35:329-

Rantnam,D ; Ankola, D; Bhardy ,Vand Sahara ,D (2006): "Role of antioxidants in prophylaxis and therapy : J control release(20) 113(3) 189-207 .

Reitman,A.and Frankle,S. (1957): Acalorimetric method for determination of sera glutamic oxalo acetic acid and glutamic pyruvic traminases. Am. J. Clin.Path.28:56:67

Sharawi S. and. Abd El-Rahim, I. (2011): The utility of polymerase chain reaction for diagnosis of lumpy skin disease in cattle and water buffaloes in Egypt Rev. sci. tech. Off. int. Epiz., 2011, 30 (3), 821-830

Sies, H. (1991): Oxidative Stress: oxidants and antioxidants. Academic Press, London, and San Diego

Statistical Package for social science (SPSS) for Jane 2006, slandered version SPSS14 (2006) copyright SPSS INC., 1989-2006

Stockham, S.L .and Scott, M.A.(2008): Fundamentals of veterinary clinical pathology.2ndEd.Ames,IA,USA.Blakwell publishing pp.639-674

Trevison,M ;Browan ,R;Ram,M ;and Armslong ,D .(2001): correlates markess of oxidative status in the general population :Am .J of Epidemiology,145 :384-356.

Tuppurainen, E., Venter, E., and Coetzer, J. (2005): The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. Onderstepoort J. Vet. Res. 72, 153–164. doi: 10.4102/ojvr.v7202.213

VanCauwenberge, P; Geveart, H and Vanzeled, T (2005): New insights into the pathology of nasal polyposis:The Role of suparantigens and IgE.Verk K A cod Gen eeskd Belg; 67(1) 5-28

Woods, J.A., (1988): LSD, A review. Trop. Anim. Hlth.. Prod., 20: 11-17.

Zeynalova, S.; Asadov, K.; Guliyev1, F.; Vatani1, M. and Aliyev, F. (2016): Epizootology and Molecular Diagnosis of Lumpy Skin Disease among Livestock in Azerbaijan. Frontiere in microbiology, vol. (7), article 1022, P: 2-7.

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