Clinical, biochemical and pathological alterations during an Outbreak of Lumpy Skin Disease (LSD) among Cows in Egypt

By

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Abstract

An outbreak of lumpy skin disease (LSD) was distributed in dairy and beef cattle in Menoufia province- Egypt, during an outbreak of summer 2006. The disease infects both native Balady and severity the Holstein Friesian cattle. Lumpy skin disease was characterized clinically by pyrexia, anorexia, firm nodules covering the whole animal skin, subcutaneous edema of skin particularly of limbs, dewlap and ventral aspect of the abdomen with enlargement of superficial lymph nodes and thickness of lump vessel. Lumpy skin disease virus was detected by inoculation onto chorio-allantoic membrane of embryonated egg. LSD viruse antigen was detected by PCR assay. Agar gel precipitation test (AGPT) was used for detection of LSD antibodies in the collected sera. Animal under this study (Healthy control and affected cows) were injected by Amoxicillin Trihydrate 15% (7 my/kg B.W once daily) and Diclofenac Sodium 2.5% (1 ml/25 kg B.W) for 5 successive days. Whole blood samples with anticoagulant and sera was collected from 15 diseased cows at 1, 14, 30 days from beginning of illness, for hematological and biochemical analysis. Biopsy from Liver, kidneys and lung were collected for Histopathological investigation.

The result of this study revealed a great significant decrease in blood hemoglobin and highly significant increase in liver and kidney function parameters (AST, ALT, urea and creatinine) especially after 14 days from illness. It could be concluded that LSD have a severe harmful effect with a time-consuming course affecting productivity and vitality of affected cattle so that application of accurate vaccine programs and controlling of insect vectors were recommended to prevent the spreading of LSD among cattle in Egypt.

Introduction

Lumpy skin disease (LSD) is an acute, subactue or chronic severe systemic disease of cattle of all ages caused by capripoxvirus that belonging to the family poxviridae (*Tuppurainen et al., 2005*). LSD in an endemic in South Africa of the Sahara and the first appearance in Egypt was in the Ismailia province in May 1988 (*Ali et al., 1990*). The disease characterized clinically by 7 days persisted pyrexia (40-41°C), frequent lacrimation, nasal discharge, and salivation. Multiple dermal

nodules all over the body region (neck, brisket, back, thigh, muzzle, perineum and udder) were inspected. The swollen of superficial lymph nodes with thickness of lymph vessels, edema of the limbs and ventral abdominal wall were also observed. The lesions of the disease were also detected in mucous membranes of the mouth, nasal passages, rumen and abomasums, (*Ali and Obeid, 1977;; FAO 1963; Loses, 1986*). The pathological lesions of lumpy skin disease among Friesian and native breed cattle in Egypt was described by, *Hafez et al., (1989)*. LSD causes considerable economic losses due to emaciation, damage to hides mastitis, loss of milk production and mortality rate up to 40 % (*Khadr et al, 2006*) and morbidity in natural outbreaks might be 100% *(Barnard et al, 1994)*.

Laboratory diagnosis of LSD was most rapid by the demonstration of typical capripox virions in biopsy material using PCR assay and LSD virus could be grown on the chorio-allantoic membrane (CAM) of emberyonated hen's eggs. Maximum yield of the LSD virus were obtained in the CAM of 7-9 day embryos incubated at 33.5 and 35°C for 5 to 6 days *(Van Rooyen et.al .1969)*. The aim of the present study was to describe the clinical picture of the LSD among cattle during a natural outbreak of the disease in Egypt and to investigate the hematological pictures and serum biochemical changes in different stages of the disease that might accompany LSD outbreak in Egypt at summer of 2006.

Materials and Methods

During the period between May to September 2006 an outbreak of LSD appeared among cattle in Egypt. Symptoms, body temperature and clinical signs were registered in each case. Samples were collected from 15 affected cows and 5 healthy cows (used as control) blood samples and serum were collected at 1, 14 and 30 day from infection.

I -*Detection of lumpy skin disease virus (LSDV):*

A reference LSDV propagated on MDBK cell line was obtained from virology department, Animal Health Research Institute, Dokki, Giza. ELISA technique as well as used as a positive control for both virus isolation and PCR assay. Bovine anti-lumpy skin virus was obtained from pox department, serum and vaccine research, Abassia, Cairo.

Virus isolation and identification:

The collected samples were processed for virus isolation where 10% homogenates were prepared for PCR and inculcated into chorio-allantioc membrane (CAM) of fertile egg according to *Barleson, et al (1997)*. Three successive pass were done, were the harvested (CAM). were tested by immuno diffusion test (Agar Gel Precipitation Test, (AGPT) according to *Tantawi and Borzovich (1967)* to detect lumpy skin viral antigen. The DNA from each sample was extracted and

purified as described by *Sambrook et al. (1989)*. Extracted DNA from each sample was amplified using the protocol previously published by *Ireland and Binepal (1998)*. Briefly, each reaction mixture (50 µl) containing 250 ng of total DNA, 2 mM $MgCl_2$, 5 pmol of each primer (forward primer was 5' TGATTTTTCTTACTAT3' and reverse primer was 5' AAATTATATACGTAAATAAC3'), 200 µM of each dNTP and 2U of DNA polymerase Bristol, USA in a reaction buffer containing 75 mM Tris-HCl (pH 9), 2 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH4)_2$ SO4 and 0.001% BSA. Amplification was carried out in a MJ Thermal Cycler MJ incorporation, USA, programmed to perform a denaturation step of 95°C for 5 min, followed by 40 cycles consisting of 1 min at 94°C for denaturation, 1 min at 50°C for primer annealing and 1 min at 72°C for extension. The last extension step was 10 min longer. A 10 µl PCR products were mixed with 2 µl gel loading buffer (Sigma-Aldrich) and electrophoresed in 1.5% agarose gel, containing 1 µg/ml ethidium bromide in Tris-acetate buffer (0.04 M Tris-acetate and 0.001 M EDTA, pH 8). The resulting DNA fragments were visualized by UV transilumination and photographed *(Sambrook et al., 1989)*. A visible band of appropriate size 192 bp was considered as a positive reaction.

II - *Hematological Studies:*

The blood samples were collected from all investigated cows and taken from jugular vein on sterile tubes contain ethylene diamine tetracetic acid and (EDTA) as anticoagulant for evaluation of erythrocytic count (RBCs), hemoglobin concentration (Hb) packed cell volume (PCV), Total and differential Leucocytic count. The hematological parameters were determined according the methods described by, *Jain (1986)*.

III- *Biochemical evaluation:*

Another blood samples were taken without anticoagulant for separation of serum for determination of serum aspartate amino transferase (AST) and alanine amino-transferase (ALT) (*Reitman and Frankel, 1957*), Urea (*Patton and Crouch, 1997*), Creatinine (*Henry, 1974*), Calcium (*Gitelman, 1967*), Inorganic Phosphorus and Magnesium (*Burits and ashwood, 2001*) and total proteins, (*Peters, 1968*).

IV- *Histopathological studies:*

Tissue specimens from the liver and the lung were taken after the postmortem examination of the diseased cases and fixed in 10% neutral buffered formalin.

Histopathological technique.

Formalin fixed tissues were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate, embedded in paraffin wax and 3 u sections were obtained and stained by H & E stain for light microscopical investigation after (Bancroft and Marilyn, 2002).

V - *Therapeutic trial:*
Diseased animals were isolated in insect proof stables with complete rest. Symptomatic treatment was applied by antibiotic as Amoxicillin Trihydrate 15%, Bremer pharma GMBH, 27540. Bremer Haven-Germany at dose rate (7 mg/ kg B.W. once daily by Intramuscular injection for combating secondary infection. Antipyretic and anti-inflammatory was used Diclofenac Sodium 2.5%, El- Nasr Co. Egypt at dose rate (1 ml/15kg B.W). A local dressing of the skin lesions using antiseptics as Betadine Solution (Bovidene iodine u.s.p 10% w/v, the Nile. Co. Cairo) was done.

VI- *Statistical analysis:*
The data were analyzed using student T-test as described by, *Petrie and Watson (1999).*

Results

1-Virus isolation and identification
LSD viruses were detected in nodular samples by AGPT, where, lesions were observed in chorio-allantoic membrane 4 days post inculcation which have the typical pock's lesions of pox viruses group. Similar findings were recorded by *Rai (1986) and El–Rahim, et al., (2002).* PCR assay revealed the detection of genome for all examined nodules samples, Fig. (1), the same record was obtained by *Ireland and Binepal (1998)* and *Heine et al (1999).*

2- Clinical Findings
During this study the observed clinical manifestation were marked rise in the body temperature up to 40-41°C, anorexia, subcutaneous limbs, dewlap and ventral abdominal edema, The superficial lymph nodes particularly the prescapular and precrural ones were enlarged and lymph vessels were commonly thickened, appearance of multiple dermal nodules, scattered all over the body measuring about 1-3 cm in diameter and most of nodules were firm in texture. Some nodules disappeared spontaneously while others persisted for more than eight weeks and usually leafed scar, particularly on the buccal mucosa, muzzle, nostrils, eye lids, genitalia, perineum and conjunctiva, Fig (2). Dysponea and increase of respiratory rate were observed.

3-Hematological findings:
Table (1) showed a highly significant decrease in hemoglobin concentration, significant alteration in PCV %, WBCS count (leucopenia).

4- Biochemical analysis:

There is highly significant increases in liver and kidney function parameters (AST, ALT, Urea and Creatinine) especially 14 days post illness. No significant change in serum total protein, serum calcium, phosphorus and magnesium as shown in table (2).

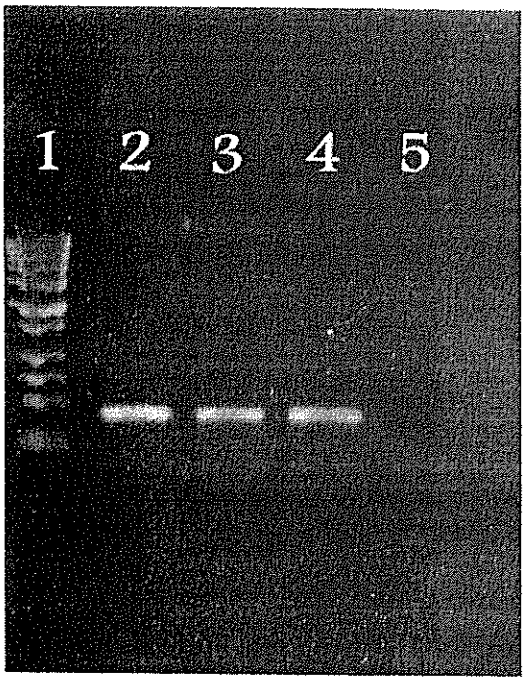


Fig. (1): Agarose – gel electrophoresis of PCR products (192 bp) Lane (1) bp ladder s a molecular DNA marker. Lane (2,3) were field lumpy skin disease virus, lane (4) reference +ve lumpy skin disease virus and lane (5) is control –ve tissue

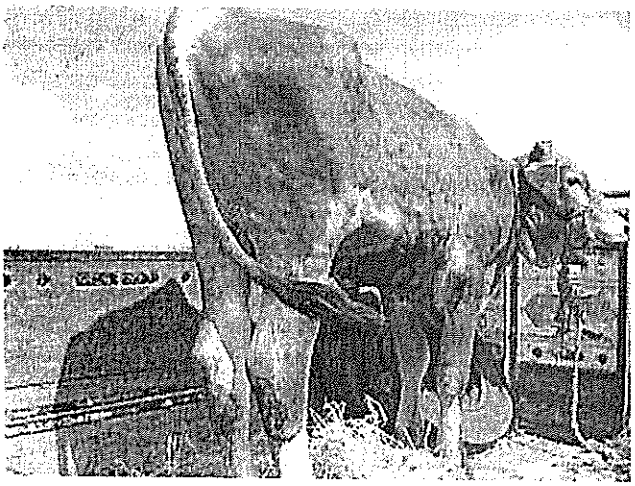


Fig (2): Clinical signs of lumpy skin disease showing lumps allover the skin of cow

Fig. (3) showing nasal discharge indicating pneumonia as complication of lumpy skin disease

Table (1): Hemogram of the healthy control and infected cows in different stages of LSD.

Group / Parameter	Healthy Control group	1st day of illness Pre treatment	14th days of illness Post treatment	30th days of illness Post treatment
Hb g/dl	14.7± 0.4	13.3 ± 0.9	7.2 ± 0.8**	9.6 ± 0.5*
PCV %	42 ± 1.9	43 ± 4	63.4 ± 3.7	35.6 ± 2.7
RBCs10^6/mm^3	10.17 ± 0.64	10.5 ± 0.47	7.89 ± 0.3*	9.34 ± 0.9
WBCs 10^3/mm^3	3.39 ± 0.195	3.61± 0.513	4.35 ± 163	4.02± 0.348
L %	75.2 ± 1.8	65 ± 1.6	46.8 ± 2.6*	64 ± 4
N %	30 ± 1.9	25 ± 0.46	21.6 ± 0.9	28.8 ± 1.8
M %	4.4 ± 0.7	3.4 ± 0.6	5.6 ± 1.7	6 ± 1.6
Ba %	0.6 ± 0.4	0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
E %	7.8 ± 1	6.0 ± 1	3 ± 1.3	5 ± 0.94

* Significant variation at (P ≤0.01)

* * Highly Significant Variation at (P ≤ 0.001)

Table (2): Serum biochemical parameters in Healthy and infected Cows in different stages of LSD

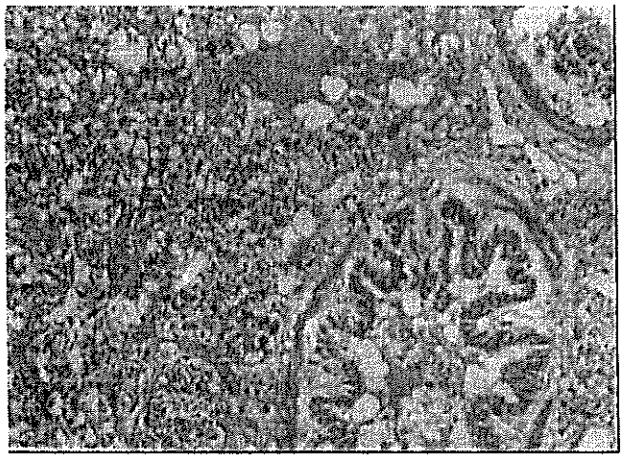
Group / Parameter	Healthy	1st day of illness	14th day of illness	30th days of illness
AST	48 ± 2	50.6 ± 3.6	121 ± 8.56**	57.6 ± 2.8*
ALT	16.6 ± 0.6	16.4 ± 1.7	29 ± 1.6**	24.4 ± 1.7*
T. Protein	6.5 ± 0.4	6.4 ± 0.2	82 ± 0.23	7.6 ± 0.46
Urea	26.7 ± 2.45	26.4 ± 2.2	39.8 ± 0.58 **	23.1 ± 0.9
Creatinine	1.1 ± 0.09	1.4 ± 0.2	2.60 ± 0.11**	1.1 ± 0.05
Calcium	7.8 ± 0.5	7.2 ± 0.4	7.2 ± 0.39	7.7 ± 0.4
Phosphorus	4.8 ± 0.7	43 ± 0.3	4.7 ± 0.4	4.4 ± 0.7
Magnesium	2.23 ± 0.3	2.4 ± 0.4	1.7 ± 0.3	2.1± 0.4

* Significant variation at (P≤ 0.01)

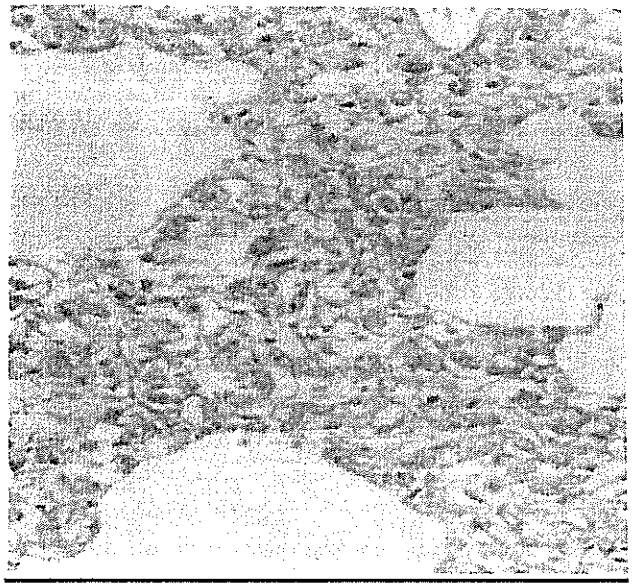
* * Highly Significant Variation at (P ≤0.001)

5 – Histopathological Findings:

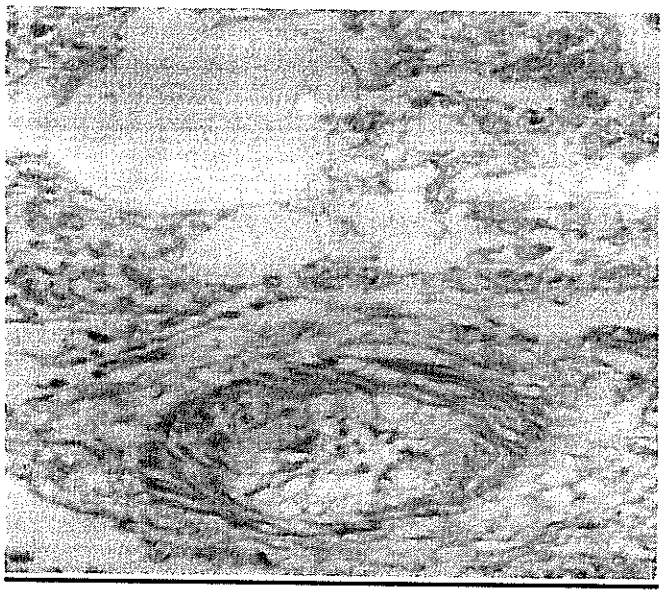
The histopathological investigation of the lung tissue showing catarrhal bronchitis (Fig.4), interstitial pneumonia and emphysema (Fig.5) and swelling of the intemal blood vessels (Fig.6). While the liver tissue showing congestion of the central vein and degenerative changes in hepatocytes (Fig.7).



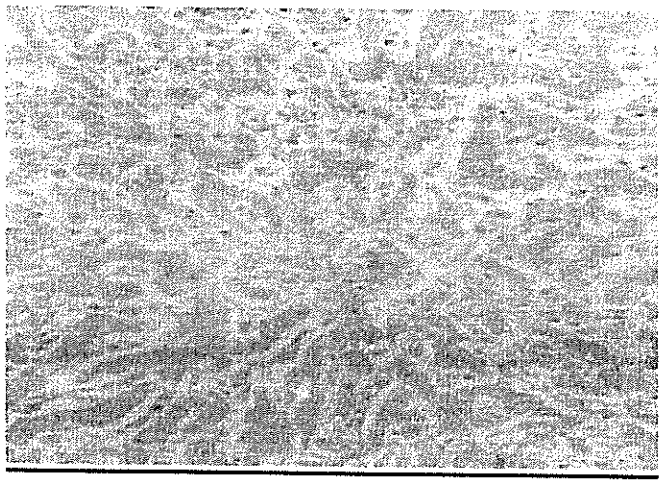
(Fig 4) lung showing Catarrhal bronchitis



(Fig 5) lung showing Interstitial pneumonia and emphysema in case of lumpy skin disease



(Fig 6)Lung showing swelling of blood vessels intima



(Fig 7) liver showing congestion of the central vein and degenerative changes in the hepatocytes

Discussion

The First out break of lumpy skin disease appeared among cattle in Egypt during summer 1988 *(Ali, et Al., 1990)*, but last one was reported among cattle in Menoufia province at summer 2005 by (*Khadr, et al., 2006*). The present study reported the re-occurrence of an out break of LSD among cattle in Menoufia province during the period from May to September 2006. In this study the most reported clinical manifestation of LSD in affected cows was fever 40-41°C with anorexia, sudden appearance of firm rounded skin nodules of about 1-3 cm in diameter on neck, shoulder, things, back of the affected animals. Subcutaneous edema on the limbs, dewlap and ventral abdomen, swollen of superficial lymph nodes, these symptoms have been well described before by *(Davis, 1991; Barnard et al., 1994;; Aly, et al., 2006 and Khadr ,el al, 2006*) which were similar to that reported in this study.

With regard to laboratory confirmations of the disease, they were depending on detecting of pock lesions on the inoculated chorio-allantoic membrane (CAM) 4 days post infection. LSD virus was inoculated by *(Van Rooyen et al.1969)* onto CAM of emberyonated eggs. Pock's lesions in CAM were only appeared in 7-9 day post embryos incubated at 33.5°C and 35°C for 5 to 6 days. AGPT was used in sero-diagnosis of the disease and most of the tested sera were showed precipitin lines in AGPT, similar result were obtained by *(El–Rahim, et al, 2002)*. The results concluded that PCR found to be rapid, sensitive, specific and could be applied on both blood and tissue samples regardless to any hazard of sample contamination, low virus titer and inability of virus to grow on tissue culture due to viral inactivation. The same conclusion previously reported by *Ireland and Binepal (1998) and Heine et al.(1999)*. The hematological findings revealed a highly significant decrease in RBCs count which may attributed to the stress condition and ulceration on mucous membranes and it's bleeding that leads to anorexia. Significance increase in lymphocytic percent and decrease in neutrophil due to the viral infections Kelly. W. R. (1984). Concerning the biochemical studies, there were a highly significant increase in the activity of both AST and ALT of infected cows compared by apparently healthy cows. The effect of LSD viral on liver enzymes activity was great and prolonged for mere than 30 days but kidney function were altered for a short period. The mineral and proteins metabolism seemed to be mildly influenced *(Agag, et al, 1989)*.

Concerning the Electrophortic analysis, the changes in the protein gram with an increase in gamma globulin fractions and total immunoglobulin indicated the ability of the body defense against this viral infections, these attribution also was reported by *(Agag, et al, 1989)*

from the previously study we can concluded that LSD was one of the major cattle diseases of economic importance, due to its harmful and long course of the disease that affecting on productivity.

This results were agreed with that recorded by *Agag, et al., 1989; Abdalla and Gawad 1992; Aly, et al., 2006*, how attributed the marked increase of ALT activity was to the hepatocellular damage caused by various agent. The highly significant increase in AST activity was rather inclusive with respect to the status of the liver, heart muscle and the general tissue breakdown caused by the viral or secondary invaders *(Agag et al., 1989)*. The high level of serum urea and creatinine values might be resulted from degenerative changes in kidney and liver. Moreover there was slight increase in serum total proteins in infected cows which was commonly observed in other virus affection *(Hafez and Agag, 1988)*.

The alterations that occurred in concentration of serum calcium, phosphorus and magnesium were of low significant values and almost might be due to anorexia that associated with the disease. The low incidence of illness with its low severity of the disease might among Balady cows compared by foreigner breeds might be back to high immunity and adaptation of the local cattle so that application of account vaccine programs and controlling of insect vectors were recommended to prevent the spreading of LSD among cattle in Egypt.

Concerning to the histopathological results, the gross lesions of LSD are well described Skin nodules have congestion, hemorrhage, edema, and vasculitis with consequent necrosis and involve all layers of the epidermis, dermis, subcutaneous tissue, and often adjacent musculature. Lymph nodes draining affected areas are enlarged up to 10 time's normal size with extensive lymphoid proliferation, edema, congestion, and hemorrhage, Prozesky and Barnard (1982). No data dealing with the hepatic and lung tissues histopathological alterations were available.

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