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CLINICOPATHOLOGICAL AND VIROLOGICAL STUDIES ON CATTLE INFECTED WITH LUMPY SKIN DISEASE

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ABSTRACT

Lumpy Skin disease (LSD) is an infectious viral disease of cattle caused by *Lumpy Skin disease virus* (LSDV) of the family *Poxviridae* characterized by skin nodules covering all parts of the body. There are many aspects of LSD remaining unknown, thus immunological, hematological and biochemical parameters were estimated. During an outbreak of LSD in Ismailia governorate in Egypt, 131 Friesian cattle aging (2-4 years) were examined clinically for the presence of LSD lesions during the period from July to November 2016. Twenty five from them showed lesions suspected to be LSD. The animals were feverish, had multiple skin nodules and enlargement of superficial lymph nodes typical of LSD. Case history details such as changes in management and diet, previous drug administration, clinical findings and method of treatment were recorded. LSD revealed a macrocytic hypochromic anemia and granulocytic leucocytosis. Biochemical analysis revealed hypoproteinemia, hypoalbuminemia and hypoglobulinemia but raises in gamma globulins. Significant increase in serum alanine aminotransferase, aspartate aminotransferase activities, creatinine level and blood urea nitrogen was recorded. All these alterations showed improvement after medication. Rapid and accurate diagnosis of Lumpy Skin disease (LSD) is very important for its control. In this study, laboratory diagnosis of LSD was done by using polymerase chain reaction (PCR), isolation in specific pathogen free, embryonated chicken eggs (SPF-ECE) via chorioallantoic membrane (CAM)route, identification of the isolates with ager gel precipitation test (AGPT) as well as detection of neutralizing antibodies in paired serum samples.In conclusion, our study supports the use of PCR as a sensitive and rapid method for LSD diagnosis in addition to isolation in SPF-ECE and identification of isolates with AGPT. Moreover, serum neutralization test (SNT) must be used for measuring neutralizing antibodies in paired serum samples as a confirmatory aid for serological diagnosis. Sequencing for PCR product was recommended specially for samples negative in isolation.

Key words: Lumpy Skin disease; cattle; isolation; PCR; SNT; Hematology; biochemistry; virus.

INTRODUCTION

Lumpy Skin disease (LSD) is an infectious, eruptive, occasionally fatal disease of cattle caused by a double stranded DNA virus of the family *Poxviridea* and genus *Capripox virus.* The disease was first described in Northern Rhodesia *Capripox* which is also termed as Neethling virus (OIE, 2010; Salib and Osman, 2011). LSDV is the etiologic agent of an economically important disease of cattle in the Middle East and Africa (Fenner, 1996). In diseased cattle, the virus exists in skin nodules, crusts of skin lesions, blood, saliva, nasal discharge, semen and milk (Babiuk *et al.,* 2008). In Egypt, LSDV was first isolated and identified from cattle during two seen as an epidemic in Zambia in 1929 and has spread and affected cattle in Africa, including the outbreaks in Suez and Ismailia governorates during 1989 (House *et al.,* 1990; Davies, 1991). LSD was countries of South Africa, Egypt, and Sudan (Maclachlan and Dubovi, 2011). During the years 1989, 2006 and 2011, severe cyclic outbreaks were recorded in several Egyptian governorates. LSD was reported for the first time in Iraq and Turkey in 2013, indicating that the disease has a potential risk for further spread to the European Union and Caucasus Region, as well as to Asia (Salib and Osman, 2011; Abera *et al.,* 2015). The disease has significant economic importance to cattle industry due to reduction in milk production, abortion, temporary or permanent sterility, damaged hides and deaths (Anonymous, 2010; Tuppurainen and Oura, 2012). Radostits *et al.* (2007) reported that Lumpy Skin disease may be suspected whenever clinical signs indicate towards persistent fever, widespread of skin nodules, enlarged superficial lymph nodes,

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conjunctivitis, keratitis, corneal opacity, edema in the brisket and legs. Biting insects which are mainly mosquitoes and flies are the primary transmitter for LSD virus (OIE, 2005). LSDV can be maintained in ticks under over wintering condition (Lubinga *et al.*, 2014). Moreover, LSD is "list A" by the Office International des Epizooties because of its rapid spread and capability for causing great losses (Tuppurainen *et al.*, 2005). Rapid and accurate diagnosis of LSD is very important to control the disease by special measures, but there are no available methods for antigen detection and this complicates laboratory diagnosis based on clinical signs (Ireland and Binepal, 1998). LSD is rapidly spreading with eruption of lumps in skin after viraemic stage. This helps clinical diagnosis of the disease (Blood *et al.* 1983). However, sometimes animals show only few skin lesions which needs to differentiation from Pseudo-Lumpy Skin disease (Barnard *et al.*, 1994). Rapid diagnosis of LSD cannot be achieved by virus isolation specially cell cultures because it is time consuming where cytopathic effect (CPE) may take up to 14 day (Prydie and Coackley, 1959). Fluorescent antibody techniques (FAT) are hardly interpreted due to presence of occasional nonspecific fluorescence and absence of available monoclonal antibodies (Ireland and Binepal 1998). Polymerase chain reaction (PCR) could detect LSD virus in skin lesion time longer than virus isolation and it is rapid and accurate (Tuppurainen *et al.*, 2005). Serum neutralization test although it is time consuming, it is used for detection and titration of antibodies to LSD virus in infected or vaccinated animals due to its reliability (Tuppurainen, 2004). Abutarbush (2015) found that, inflammatory leukogram, anemia, thrombocytopenia, hyperfibrinogenemia, hyperproteinemia, decreased creatinine concentration, hyperkalemia and hyperchloremia in clinical cases of LSD in cattle. LSDV infected cows in early stages revealed leucopenia, Immunosuppressive effect was pronounced later. In late stage hemolytic anemia, leukocytosis, increase of serum CK and disturbance in liver and kidney function tests have been found. Neamat-Allah (2015) reported also hypoproteinemia, hypoalbuminemia and hyperglobulinemia especially gamma globulins. The aim of our study is to diagnosis of LSD using PCR, trial for isolation of the causative virus in specific pathogen free, embryonated chicken eggs (SPF-ECE) via chorioallantoic membrane (CAM) route, identification of the isolates with agar gel precipitation test (AGPT) and detection of neutralizing antibodies in serum samples of suspected animal to be infected with LSD virus. As there are many aspects of LSD that remain unknown, so the immunological, hematological, and biochemical parameters of LSDV in naturally infected cattle were estimated in this study.

MATERIALS AND METHODS

I. Animals:

One hundred and thirty one cattle aging (2-4 years) were examined during an outbreak of LSD in Ismailia Governorate in Egypt during the period from July to November 2016. The clinical investigations include examining temperature, skin, mucous membrane and superficial lymph nodes. Animals were classified according to the severity of the disease into three groups as follows: 25 apparently healthy cows group (1), 25 diseased animals group (2) and 25 treated animals group (3). Case history details such as changes in management and diet, previous drug administration, clinical findings and method of treatment were recorded.

II. Samples:

Samples were collected from Ismailia Governorate.

1. Blood samples:

Two blood samples were obtained from jugular vein. The first blood samples were taken in ethylene diamine tetra acetic (EDTA) tubes for hematological analysis. The second blood samples were taken in a sterile test tube for separation of serum that was used for biochemical measurements according to Jain (2000).

2. Tissues samples:

Twenty-five skin nodules which include epidermis, dermis and subcutis were collected and stored after their preparation at -80°C.

3. Serum Samples:

Twenty-five serum samples were collected twice from the same diseased animals. The first was taken when skin lumps began to appear and the second was taken after 3 weeks to detect neutralizing antibodies. Serum samples were stored at 20^{o} C until examined.

4. LSD virus:

It was obtained from Pox Departement, Serum and Vaccine Research Institute, Abbassia, Cairo. The virus was titrated according to Reed and Meunch (1938) and it was 10_6 $TCID_{50}$/ml.

5. Reference positive and negative serum:

Reference positive and negative bovine serum against LSD virus was obtained from Pirbrigh Laboratory (England).

6. SPF-ECEs:

They were obtained from NILSPF farm, Kom Osheim, Fayoum.

7. Cell culture:
Madin Derby Bovine Kindney (MDBK) cells were provided by Virology Departement, Animal Health Research Institute.

1. Diagnostic procedures:

a) Clinical examination:

b) All animals were subjected to clinical examination according to Rosenberger (1979).

c) Laboratory diagnosis:

1. Hematological examination:
Complete blood count was evaluated in an automatic cell counter at Hospital of Suez Canal University, Departement of Pathology (Horiba ABX SAS, Yumizen H500 OT, France).

2. Biochemical examination:
Serum samples were colorimetrically analyzed using test kits (Biomereux, France) for calcium (Glinder and King, 1972), total protein (Peters, 1968), albumin (Drupt, 1974) globulin which was calculated as the difference between total protein and albumin, blood urea nitrogen (Richterich, 1968), creatinine (Giorio, 1974) and serum transaminase "AST and ALT" (Reitman and frankel, 1957). The creatine phosphokinase (CK-MM) was measured in full automated biochemistry analyzer (Chemray 240. USSR). Some trace element values including zinc, copper and iron concentrations were determined in serum using atomic absorption spectrophotometry (Varley *et al.*, 1980).

3. Immunological examination:
Protein electrophoresis was done using SDS-polyacrylamide gel electrophoresis according to Laemmli (1970).

4. Virological examination:

a) Isolation in SPF-ECE:
Suspensions of skin nodules were subjected for inoculation of SPF_ECE, 10-12 days old embryo via CAM route according to Versteeg (1990) and eggs were examined daily for thickening and pock lesions from 3 to 7 days.

b) AGPT:
The test was carried out according to method described by Payment and Trudel (1993) using suspensions of CAM (isolates) showing pocks and thickening as unknown antigens against the reference positive and negative antiserum.

c) PCR:
Portions of skin nodules used in the current study were examined for LSD virus by PCR.

D) Serological examination:
SNT:
Collected serum samples were heat-inactivated at 56^{o} C for 30 minutes and tested for neutralizing antibodies to LSD virus using the OIE (2004) standard micro neutralization protocol using MDBK cell culture.

IV- Treatment trials of infected animals:
Animal's body was sprayed by equal volume of acetic acid 3% and lemon oil 0.1% as insect repellent. Oxytetracycline (1ml/10kg body weight) and Sulphadimidine (1ml/5kg body weight) were injected to the diseased animals for 3-5 days. Aspegic (100 mg/ kg B.W.) as antipyretic drug (vials) was injected by IV dribbling in saline solution daily for relief of fever and inflammatory conditions in feverish animals. Some cases received calcium borogluconate injection (1 liter/450kg) IV daily for five days.

V-Statistical analysis:
Data obtained from this investigation were statistically analyzed using the one-way analysis of variance using SPSS 16.0 for windows (Tamhane and Dunlop 2000).

RESULTS

The observed clinical manifestations were characterized by pyrexia (40-41^{o}C), anorexia, salivation, nasal discharge, depressions, decreased in milk yield at the beginning and stop the milk yield and enlargement of external lymph nodes (Figure 4). Skin nodules which ranged from a few to several hundred (Figure1-1) sometimes coalesced together. Later, these nodules appeared containing a clear serous or purulent exudates with furthermore ulcers formation (Figure 2). Some cases showed subcutaneous edema on fore legs (Figure 3), while others showed lameness and recumbency with severe edema.

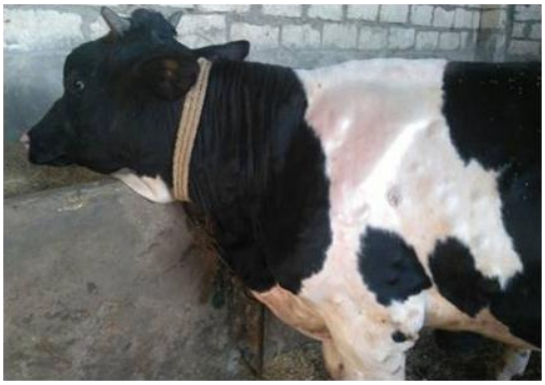

Figure (1): Lumpy Skin disease (LSD) in cow showing few scattered skin nodules. Multiples prominent nodules allover the side of forelegs vary in size.

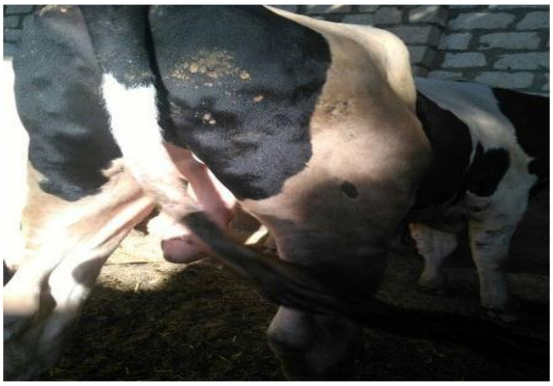

Figure (2): LSD in cow showing skin nodules leaving ulcer (14 days post-infection).

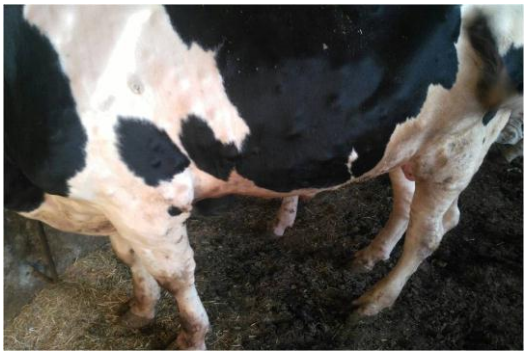

Figure (3): LSD in cow showing skin nodules and edema of the forelegs.

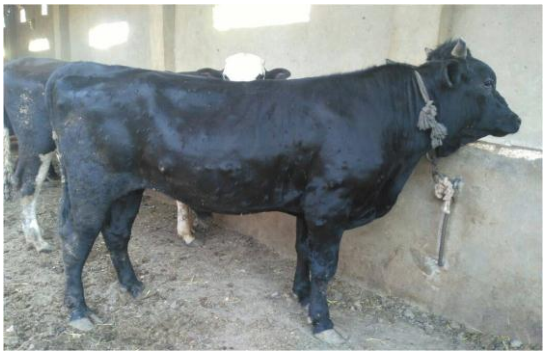

Figure (4): LSD in cow showing skin nodules cover all body parts and enlargement of precrural lymph node.

Hematological analysis revealed a highly significant decrease in RBCs count and Hb concentration in diseased groups comparatively with control. This appeared non-significant in PCV value. There were highly significant increase in MCV in diseased groups; and significant decrease in MCH, MCHC and a total leukocytes count associated with the presence of lymphopenia (Table 1).

Table 1: Alteration in erythrogram parameters in healthy and diseased animals suffering from LSD (mean ± SE).

Groups / **Parameters**	**Healthy control cows N= (25)**	**Infected and treated animals**	
		Infected animals by LSD N= (25)	**Treated animals N= (25)**
RBCs count (×10^6/µl)	7.81±0.92	6.45±0.11**	7.26±0.17
Hb (g%)	11.2±0.41	9.10±0.13**	10.99±0.29
PCV (%)	33.89±0.62	30.72±0.38	33.76±0.57
MCV (fl)	45.16±0.12	47.93±0.24**	45.13±0.31
MCH (pg)	15.14±0.35	13.42±0.21*	15.09±0.16
MCHC (%)	33.06±0.48	30.11±0.61*	31.91±0.37
WBCs (10^3/ µl)	9.10±0.61	13.79±0.27**	7.38±0.81
Neutrophil (10^3/ µl)	3.29±0.02	8.98±0.03*	4.51±0.05
Lymphocyte (10^3/ µl)	4.72±0.19	2.19±0.13*	3.56±0.27
Monocyte (10^3/ µl)	1.41 ± 0.28	1.29± 0.12*	1.31 ± 0.09
Esinophile (10^3/ µl)	0.16 ± 0.02	0.19 ± 0.03	0.18± 0.01
Basophile (10^3/ µl)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00

**Highly significant difference at p≤0.01, *significant difference at P≤0.05. SE=Standard error, PCV=Packed cell volume, RBCs=Red blood cell, MCV=Mean corpuscular volume, MCH =Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, WBC=white blood cell, LSD=Lumpy skin disease.

Regarding to results of biochemical analysis, (Table2) revealed significant decrease in total protein and albumin, however; there was a significant increase in globulin, especially gamma globulins in LSD infected cows. Also significant increase of CK in LSD infected cows clearer in late stage. In addition, significant increase in blood urea nitrogen, creatinine and AST and ALT" was found.

Table 2: Alteration in some biochemical parameters in healthy and diseased animals suffering from LSD (mean ± SE).

Groups / Parameters	Healthy control cows N= (25)	Infected and treated animals	
		Infected animals by LSD N= (25)	Treated animals N= (25)
T.proteingm/dl	7.45±0.81	6.01±0.52*	7.04±0.29
Albumin gm/dl	3.20±0.27	2.81±0.11*	3.01±0.18
Globulin gm/dl	4.25±0.52	3.20±0.16*	4.03±0.11
α globulin gm/dl	0.84±0.02	0.22±0.01*	0.76±0.01
β globulin gm/dl	1.18 ± 0.01	0.71±0.01	1.14±0.01
γ globulin gm/dl	2.23 ± 0.01	2.38 ± 0.05*	2.18 ± 0.05
ALT u/l	18.37±1.08	29.82±1.71**	20.92±1.23
AST u/l	48.95±1.65	65.72±1.91**	50.29±2.62
Blood urea nitrogen mg/dl	16.31±0.94	22.55±1.09*	17.72±0.98
Creatinine mg/dl	0.97±0.03	1.53±0.18*	1.01±0.09
CK-MM (U/L)	234.7± 5.44	281.5 ±3.97*	241.7±5.71
Calcium mg/dl	10.09 ± 0.59	8.35 ± 0.42*	9.62 ± 0.39
Zinc Mg/dl	138.62±5.31	130.51±4.12	136.76±4.90
Copper Mg/dl	97.71±2.69	79.84±2.30**	95.64±2.58
Iron Mg/dl	183.43±5.18	132.73±4.16**	180.62±4.93

* Significant P< 0.05 ** highly significant P <0.01 CK-MM=Creatine phosphokinase,

In group (3) the temperature of the animals returned to normal levels after treatment. The clinical signs were improved gradually and the skin condition improved with disappearance of nodules within 3 to 4 weeks in some cases. All parameters showed non-significant changes in all biochemical analysis in comparison to control groups and the value nearly returned to the normal levels.

PCR Results:
The twenty-five skin nodules were identified as PCR positive (Table 3).

Agar Gel Precipitation test (AGPT):
All the isolates (17) showed positive reaction (Table 3) where a clear precipitation lines appeared between isolates (unknown antigens) and control positive antiserum while not appeared in negative serum samples.

Table 3: Identification of LSDV with AGPT and PCR.

Test	Positive	Negative
PCR	25	_
Isolation and identification by AGPT	17	8

Isolation in SPF-ECE (CAM route):
The harvested CAM revealed the presence of pock lesion 4 days post inoculation and 17 out of 25 skin nodules appeared positive.

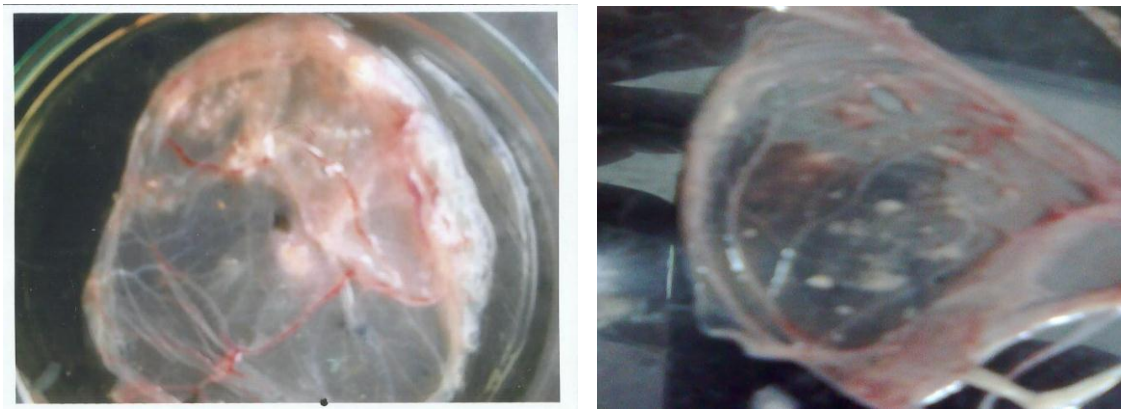

Figure (1): Inoculated CAM of SPF-ECE showed thickening and central necrosis.

Lesion of LSDV on CAM varied from thickening of membrane in 1st passage to white foci more pronounced by 2nd and 3rd passage.

Serum Neutralization test (SNT):

Neutralizing antibodies were detected less than 1/10 in first serum samples but were detected 1/80 (10 sera) and 1/160 (15 sera) as illustrated in (Table 4).

Table 4: Serum neutralizing antibodies in first and second serum samples (diseased animal's) using SNT.

Number of samples	Antibody titer	
	First serum samples	Second serum samples
10	>10	80
15	>10	160

DISCUSSION

LSD is a pox viral disease of cattle with a major socio-economic impact (Coetzer and Tuppurainen 2004; Ahmed and Dessouki 2013). 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 (Coetzer *et al.*, 1994). The importance of this disease increase gradually as the way of eradication and control is very difficult (Coetzer *et al.*, 1994). In our study, LSDV was isolated from local Egyptian cows and was confirmed by PCR. Whereas, it was reported that PCR is the very sensitive way to detect LSDV in blood and tissues of infected animals (Ireland and Binepal 1998).

Recorded clinical signs in this study were in agreement with the previous studies (Brenner *et al.*, 2006; Ismail and Yousseff 2006) who mentioned that LSD infected animals showed, pyrexia 40-41°C for large release and rapid clearance of pyrogens (Ismail and Yousseff 2006). The inappitance observed could be a natural squeal to fever (Radostits *et al.*, 2000).Ulceration lesion of leaving lumps attributed to the nodular lesions penetrating through the subcutaneous fasciae into the deeper fasciae layers and even into the musculature of the hind quarters (Vorster and Mapham 2008). These complications of LSD resulted from damage of skin or mucous membranes that were followed by secondary bacterial invasion in addition to stress induced immunosuppression, anorexia, persistent fever and severe debilitation. This finding close agreement with (Fayez and Ahmed 2011; El-Neweshy *et al.*, 2013). Lameness was a result of enlargement of prescapular and prefemoral lymph nodes (Aly *et al.*, 2006).

Regarding to hematological results, (Table1) showed a significant decrease in the number of total erythrocytic count and hemoglobin concentration in diseased groups which may be due to anemia and hemosidrosis of the lymph nodes and spleen (Jain 2000), how ever there was an increase in MCV in diseased groups, and decrease in MCH and MCHC which may indicated that macrocytic hypochromic anemia occurred due to infection by LSDV whichmight lead to hemolytic anemia. These results agreed with Douglas and Wardrop (2010) finding, that hemolytic anemia occurred with viral infection. Onthe other side, leucogram, investigations revealed granulocytic leucocytosis which could be due to secondary acute bacterial infections, especially pyogenic bacterial infections (Kumar *et al.*, 2007). Lymphopenia may be due to release of endogenous corticosteroid from viral infection.

Regarding serum biochemical analysis, there were highly significant increases in serum activities of ALT and AST in LSD infected animals when compared to healthy control and treated groups. These results may be due to liver function disturbance. These are in agreement with Abdalla and

Gawad (1992). The increase in AST also may be due to the breakdown of the heart muscle and or secondary bacterial infection (Agag *et al.,* 1989). Besides these, there was a highly significant increase in blood urea nitrogen in diseased animals in comparison with apparently healthy groups. This may be correlated to increase of protein breakdown which occurred in fever according to Kaneko *et al.* (1997) and the general tissue destruction caused by the virus. There was a highly significant increase in creatinine in diseased groups; due to the effect of LSD virus on the kidney (Radostits *et al.,* 2000).

On the other hand, a significant decrease in total protein and albumin could be attributed to two main factors; decreased synthesis and higher catabolic rate as well as damaged liver parenchma. While, increased γ globulins were mainly an immune response following infection and there is a genetic background (Agag *et al.,* 1992). Changes in trace elements in the serum, especially in copper, iron and copper may be related to decrease food consumption or to hypoproteinaemia, which hinder absorption of these elements. Moreover, infection was considered as a sort of stress on animals and is associated with increased level of prolactin and disturbed oxidant/ antioxidant status in the body (Ahmed, 2007). A significant decrease in calcium level in diseased groups may be attributed to hypoproteinemia (Coles, 1986). The significant increase of CK in LSD infected cows clearer in late stage could be due to muscle damage involvement (Kaneko *et al.,* 1997). A notice improvement was observed in cattle affected with LSD (group 3) after treatment. A similar result was reported by Hungerford (1990).

Reduction of losses caused by LSD could be achieved by control measures if accurate and rapid diagnosis of the disease is conducted (Carn, 1993).

PCR was performed as a rapid and confirmatory test where its results could be obtained at the same day of sample collection. Also, it could be used in spite of presence of virus- specific antibodies where these antibodies will be removed during the DNA extraction (Ireland and Binepal *et al.,* 1998). PCR could also be used in countries which have no endemic LSD and live virus is not available (Heine *et al.,* 1999).

Results of isolation in SPF-ECE is less time consuming than cell cultures and it is more productive than it due to cell cultures may have some latency with any virus which may interferes with LSD virus replication. The isolates (17) were all positive with AGPT which mean that they are caprpox viral isolates due to AGPT is a group specific test (OIE, 2004).

The response of antibody to LSD was detected and measured by SNT in our study where there is increase in their titer after 3 week in all samples and these results agreed with those obtained by Tuppurainen (2004) who found that the rise in titer were seen between days 21 and 24. So SNT is very important for epidemiological studies.

CONCLUSION

Lumpy skin disease is one of the major cattle diseases which have economic importance. Anemia, hypocalcaemia and hypoproteinemia, with a significant increase in gammaglobulin are the most common hematological and biochemical disorders in cattle infected with lumpy skin disease virus. Hygienic measures include eradication of arthropods and vaccination must be intensified for controlling of LSD. Treatment of the acute clinical syndromes of diseased cases is necessary. Our study supports the use of PCR as sensitive and rapid method for diagnosis of LSD in addition to the use of SPF-ECE to propagate LSD V. Moreover, SNT must be used for measuring neutralizing antibodies in paired serum samples as aid for diagnosis and we recommend DNA sequincing for PCR products specially for those which were detected as negative by isolation.

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دراسات باثولوجية أكلينيكية وفيروسية على الماشية المصابة بمرض الجلد العقدى

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مرض الجلد العقدى هو مرض فيروسي يصيب الماشية بسبب فيروس من عائلة بوكسفيريداي يتميز المرض بالعقيدات الجلدية التي تغطي جميع أجزاء الجسم. هناك العديد من جوانب المرض غير معروفه، وبالتالي تم عمل فحوصات مناعية، ودموية، والبيوكيميائية مع العزل الفيروسى للمرض. خلال أنتشار المرض في محافظة الإسماعيلية في مصر، لقد تم تسجيل عدد (٢٥) حالة جلد عقدي في مزارع أبقار فريزيان بالإسماعيلية خلال الفترة من يوليو إلى نوفمبر ٢٠١٦ وذلك أثناء الفحص لعدد (١٣١) حالة أبقار فريزيان (٢-٤ سنوات) بالمزارع وكذلك تم اخذ (٢٥) عينة من الحيوانات المريضة و(١٥) عينات من الحيوانات السليمة. كانت أهم الأعراض التي ظهرت في القطيع عبارة عن ارتفاع في درجة الحرارة تصل إلى ٤١.٥ درجة مئوية مع ضعف الشهية وقلة إنتاج اللبن مع ضعف عام و ظهور عدد كبير من العقد الجلدية مختلفة الأحجام والتي تغطي كل جسم الحيوان مع ظهور تضخم في الغدد الليمفاوية الأمامية والخلفية. وأسفرت النتائج للتحاليل الدموية عن وجود أنيميا حادة مع قلة عدد كرات الدم البيضاء. كما أظهرت التحاليل كذلك نقص في خلايا الليمفوسايت خاصة عند ظهور الأعراض. وأوضحت التحاليل الكيميائية وجود نقص في الكالسيوم والبروتين والالبيومين والجلوبيولين مع ارتفاع في الجاما جلوبيولين في الأبقار المصابة. وكذلك حدوث زيادة معنوية في اليوريا و الكرياتينين وأنزيمات الكبد. يعتبر التشخيص السريع والدقيق لمرض الجلد العقدى من اهم العناصر للسيطرة على المرض ونهدف فى دراستنا هذه الى التشخيص المعملى لمرض الجلد العقدى باستخدام اختبار انزيم البلمرة التسلسى، العزل فى البيض المخصب والخالى من اى مسببات مرضية. وقد تم تعريف المعزولات باستخدام اختبار الأجار جيل الترسيبى كما تم الكشف عن الأجسام المناعية المضادة لفيروس الجلد العقدى فى السيرم باستخدام التعادل فى خلايا الزرع النسيجى. وقد دعمت هذه الدراسة استخدام اختبار انزيم البلمرة التسلسى والعزل فى البيض المخصب والخالى من المسببات المرضية والتعريف للمعزولات باختبار الأجار جيل الترسيبى بالضافة الى الكشف والقياس للاجسام المناعية والمضادة للفيروس باستخدام اختبار التعادل التأكيدى والدقيق. وتتضح هذة الدراسة لعمل تتابع جينى للعينات الايجابية باختبار انزيم البلمرة التسلسى ولاسيما تلك التى كانت سلبية العزل.