Clinico-biochemical and Electrocardiographic Changes in Cattle Naturally Infected with Lumpy Skin Disease.

Mahmoud A. Y. Helal1*, Marawan A. Marawan1, Halla E.K. El Bahgy2.

1Department of animal medicine, faculty of veterinary medicine, Benha university - Egypt
2Department of hygiene and veterinary care, faculty of veterinary medicine, Benha university - Egypt

Key words:
cardiac, cattle, electrocardiography, Troponin I, lumpy skin

ABSTRACT

Lumpy skin disease (LSD) is an economically important viral disease of cattle caused by Capripox virus. The disease is characterized clinically by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, edema of the skin and deaths in some cases. The aim of this study is to investigate the clinico-biochemical picture of LSD infected cattle with special reference to the cardiac abnormalities. This study was carried out on LSD infected cattle showing typical clinical signs of the disease in Kaliobia governorate (n = 50; and aged between 2 to 5 years old). Clinical and electrocardiographic examination (ECG) were performed firstly, then skin nodules were aseptically collected from infected cattle for molecular detection of LSDV using PCR, on the other hand, serum samples were collected for biochemical analysis. The results of the current study revealed that, all the examined tissue samples showed successful amplification of the viral attachment protein gene of LSDV with presence of positive bands at the target size (192 bp). Furthermore, troponin I (cTnI), urea, creatinine ALT, AST, ALP and potassium were significantly increased in LSD infected cattle compared to the control ones. In contrast, serum calcium, iron and sodium concentrations were significantly decreased. The ECG of LSD infected cattle showed abnormal P waves, shortening of P and T-waves amplitudes and decrease in RT interval compared to healthy cattle. The cardiac alterations in case of LSD infection could be related to the elevation of cTnI because of direct effect of the virus on the heart beside electrolyte imbalance, acidosis and hypocalcaemia.

1. INTRODUCTION

Lumpy skin disease (LSD) is an acute infectious viral disease of cattle and one of the most economically significant transboundary and emerging disease. The disease was firstly isolated and identified in Egypt in 1988 at summer in Suez Canal province then within 5 to 6 months, LSD rapidly spread to 22 out of 26 Egyptian governorates at the same year (Salem 1989).

LSD is caused by LSD virus (LSDV), which belongs to the Capripox virus genus of the Poxviridae family (Babiuk et al., 2008). The genomic sequence of LSDV, is about 151-kbp in length, consists of a central coding region bounded by identical 2.4 kbp-inverted terminal repeats and contains 156 putative genes (Tulman et al., 2001).

Regarding the host susceptibility, the disease has a limited host range as it affects cattle breeds of both sexes and all ages (young animals may be more susceptible to the severe form of the disease) and does not complete its replication cycle in non-ruminant hosts (Shen et al., 2011; Al-Salihi, 2014), however the virus was isolated from the skin lesions of Egyptian water
buffaloes under field condition (El-Nahas et al., 2011; Sharawi and Abd El-Rahim, 2011).

Clinically, LSD is characterized by fever, nodules on the skin, mucous membranes, emaciation, enlarged lymph nodes, edema of the skin, and sometimes death (Tuppurainen et al., 2005; Constable et al., 2017) such nodules can even be found in internal organs (Bowden et al., 2008).

Pathogenesis of LSD in cattle has been cleared before, and there is a good understanding of the way that the virus causes lesions in affected cattle. In addition, clinical signs and post-mortem lesions were well described. Previous reports (Zilva et al., 1988; Stockholm and Scott, 2013; Neamat-Allah, 2015; Sevik et al., 2016) about myocardial involvement in LSD infection as increased CPK together with AST activity were observed and may be a sign of skeletal and cardiac muscle injuries. Furthermore, histopathological changes in the heart, muscle fibers in clinical cases with LSDV infection were also recorded (Prozesky and Barnard, 1982; Woods, 1988).

Variation in the heart rate and rhythm can occur in normal animals due to strong or varying autonomic influence but can also reflect primary myocardial disease. Other factors such as acid-base and electrolyte imbalance, potassium, sodium, magnesium and calcium, can influence the cardiac rate and rhythm. Therefore, these factors must be taken into consideration in the assessment of apparent abnormalities detected on clinical examination of the cardiovascular system (Thakur, 1995; Cunningham, 1997; Smith, 2009). However, a little knowledge about electrocardiographic and serum biochemical findings associated with the effect of LSDV on the heart of infected cattle was found. Thus, early detection of cardio vascular disease might enable appropriate remedial action to be taken and a good prognosis of the disease can be achieved and subsequently rapid response to the treatment (Reddy et al., 2015).

In large animals, electrocardiography is confined to a simple base apex lead system to examine conduction disturbances and arrhythmias, which are detected by measurement of the various wave forms and intervals in the electrocardiography (ECG) that represent depolarization and repolarization in the heart, and by observation of their absence or abnormality (Rezakhani et al., 2004; Constable et al., 2017).

Herein, the aim of this study is to investigate clinical and biochemical picture of LSD infected cattle with special reference to the cardiac alterations.

2. MATERIALS AND METHODS

2.1. Samples

2.1.1. Tissue samples

Skin nodules were aseptically collected through surgical excision under local anesthesia from infected cattle showing typical signs of LSD in Kaliobia governorate (n = 50; and aged between 2 to 5 years old). These samples were placed in clean sterile tube containing glycerol and phosphate buffer saline (1:1) according to (Carn and Kitching, 1995) for molecular detection of viral DNA. The samples were pooled (totally 5 pooled samples, each one pooled sample for 10 animals) and finally were stored at – 70 °C until use.

2.1.2. Serum samples

Serum samples were collected from infected cases for biochemical analysis. Troponin I (cTnI) concentration was measured according to Collinson et al., (2001). Serum urea and creatinine was determined according to Patton and Crouch, (1977) and Hovet, (1985) respectively. Sodium and potassium were measured according to Henry et al., (1974). Estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to Reitman and Frankel, (1957). Alkaline phosphatase (ALP) according to Belfield and Goldberg, (1971). Calcium was determined according to Gindler and King, (1972). Inorganic phosphorus was determined according to Goldenberg et al., (1966). Iron was determined according to Bauer, (1982). Serum samples were also collected from healthy cattle (n = 15) in a control vaccinated farm where LSD was not reported. Control animals were clinically healthy free from any external, blood, and internal parasites.

2.2. Molecular detection of LSDV

2.2.1. DNA extraction from tissue samples

Wizard Genomic DNA purification kit, cat. # A1120. Promega, USA was used for DNA extraction of LSDV from the pooled tissue samples acc. to the manufacture instruction, then the DNA concentration (ng/ul) was measured using NanoDrop 8000 spectrophotometer. The eluted DNA was kept at -20 °C until use.
Table (1). Biochemical parameters in healthy and LSD infected cattle.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LSD infected cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin-I (U/l)</td>
<td>0.8±0.03</td>
<td>1.26±0.1*</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>25±1.5</td>
<td>47±4.8**</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>58.6±2.7</td>
<td>189.9±15**</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>32.8±2.2</td>
<td>190.8±37**</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>25±1.7</td>
<td>35±2.6</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9±0.03</td>
<td>1.97±0.06*</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>145±1.7</td>
<td>119.3±1.2**</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>3.5±0.17</td>
<td>5.3±0.27**</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>10.0±0.32</td>
<td>8.0±0.5*</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.5±0.2</td>
<td>5.07±0.4</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>90±3.2</td>
<td>65±1.4**</td>
</tr>
</tbody>
</table>

Means (± SE) are significantly different when (P<0.05) * and (P<0.01) **

Table (2). ECG traces in healthy and LSD infected cattle.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LSD infected cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-wave amplitude(mv)</td>
<td>0.20±0.01</td>
<td>0.10±0.01**</td>
</tr>
<tr>
<td>P-wave duration(second)</td>
<td>0.08±0.04</td>
<td>0.14±0.04**</td>
</tr>
<tr>
<td>QRS complex amplitude(mv)</td>
<td>1.0±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>QRS complex duration(second)</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>T-Wave amplitude(mv)</td>
<td>0.41±0.01</td>
<td>0.30±0.05**</td>
</tr>
<tr>
<td>T-Wave duration(second)</td>
<td>0.08±0.01</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>PR- interval(second)</td>
<td>0.18±0.01</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>RT- interval(second)</td>
<td>0.28±0.01</td>
<td>0.24±0.02*</td>
</tr>
<tr>
<td>QT- interval(second)</td>
<td>0.30±0.01</td>
<td>0.28±0.01</td>
</tr>
</tbody>
</table>

Means (± SE) are significantly different when (P<0.05) * and (P<0.01) **

Figure 1: Results of PCR after agarose gel electrophoresis, all the examined tissue samples showed positive bands at the target size (192 bp): L is 100 bp DNA ladder, - Ve lane is control negative, + Ve lane is control positive and the S lane represent the samples.
2.2.2. Polymerase chain reaction amplification and gel electrophoresis

PCR amplification was carried out using 5'-TTTCTGATTTTCCTAAT-3' as forward primer and 5'-AAATTATATACGTAAATAAC-3' as reverse primer (Ireland and Binepal, 1998) to amplify 192 pb fragments of viral attachment protein gene corresponding to (65136 to 65328) of LSDV whole genome sequence. The amplification reaction was carried out using thermal-cycler (Life ECO, China) according to the following cycles: Initial denaturation at 94 °C for 2 min for one cycle then 40 cycles consisting of denaturation at 94 °C for 50 seconds, annealing at 50 °C for 50 seconds, extension at 72 °C for 1 minute then one cycle final extension at 72 °C for 10 minutes then holding at 4 °C.

The PCR products were placed on a gel plate prepared using Tris EDTA buffer containing 1.5 % sea Kem® agarose, stained by GelRed stain then electrophoresed at 100 V for 30 minutes and finally visualized by ultraviolet light after using a 100 bp DNA Ladder RTÜ (GeneDirex) Cat No. DM012-R500.

2.3. ECG examination

LSD infected cases were examined by base apex lead system II was applied as; the right forelimb electrode was placed on the right side of the neck along the jugular groove one third of the way up the neck. The left forelimb electrode was placed on the ventral midline under the apex of the heart. The ground cables were placed on the left and right stifle joints. Alligator clips moisten with alcohol were used (Hiwing, 1977). ECG was evaluated for heart rate, rhythm, arrhythmias, variation in wave’s amplitude, and duration. Results were compared to healthy cattle as a control group.

2.4. Statistical analysis

The obtained results from the experiments were expressed as mean ± SEM and were analyzed using (SPSS Statistics for Windows, version 23.0. Armonk, NY: IBM Corp). Differences were declared significant at (P < 0.05) and (P < 0.01).

3. RESULTS

3.1. Clinical investigation of cattle

Clinical examination of LSD infected cattle in the current study revealed presence of recurrent fever (40 °C and some cases reached to 41.5 °C) lethargy, anorexia, tachycardia (120/ minute), cardiac arrhythmia, enlargement of prescapular and precrural lymph nodes and serous ocular and nasal discharges. Characteristic skin nodules of the disease were observed and located particularly on the head and neck, these nodules included the dermis and epidermis of the skin. Moreover, the clinical follow up of the diseased animals showed that, some animals exhibited edema in the dewlap and legs two days preceding the eruption of characteristic lumps and nodules on the skin.

3.2. Molecular detection of LSD viral DNA using PCR

All pooled tissue samples showed successful amplification of the viral attachment protein gene of LSDV with presence of positive bands at the target size (192 bp) using PCR after gel electrophoresis that confirmed infection by LSDV (Figure 1).
3.4. Biochemical findings

Serum biochemical values obtained from both healthy and naturally infected cattle suffering from LSD were shown in table 1. The results showed group showed significant increase \((P < 0.05)\) of cTnI, urea and creatinine. Moreover, ALT, AST, ALP and potassium were also significantly increased \((P < 0.01)\). On the other hand, iron and sodium concentrations were significantly decreased \((P < 0.01)\) together with serum calcium concentration \((P < 0.05)\) in LSD group compared to the control ones.

3.5. ECG findings

ECG of LSD infected cattle showed abnormalities in 60 % of cases (30 out of 50). These alterations included abnormal P waves, shortening of P and T-waves amplitudes and decrease in RT interval compared to ECG of healthy cattle. Additionally, there were a significant decrease \((P< 0.01)\) in P wave amplitude and T wave amplitude. While significant increase \((P< 0.01)\) in P wave duration in LSD group was observed. Moreover, there was significant decrease \((P < 0.05)\) in RT intervals in LSD infected cattle than that of healthy ones. (Figure 2 and table 2).

4. DISCUSSION

Lumpy skin disease (LSD) is one of the most important endemic infectious viral disease of Africa including Egypt (Elhaig et al., 2017) and can be transmitted to the susceptible host by haematophagous insects, such as mosquitoes and stable flies (Lubinga et al., 2014). Both sex and all ages of cattle are the natural host; both zebu and exotic breeds are susceptible and are severely affected (Al-Salihi, 2014).

LSD can be speculated easily in the affected animal from the clinical picture of the disease. In the current study the typical clinical signs of LSD were observed ranging from acute disease to severe clinical manifestations including fever, enlarged superficial lymph nodes and edema in the dewlap and legs passing on appearance of the quintessential skin nodules which are very characteristic for the disease which is attributed to the virus tropism to skin tissues and internal organs (Tuppurainen et al., 2005; Gari et al., 2010; Constable et al., 2017).

Molecular detection of LSD viral attachment protein gene was carried out on nodular tissue samples using PCR which is quick, sensitive technique (El-Kenawy and El-Tholoth, 2011). The results of PCR in the present study certified infection of the cattle by LSDV through observation of PCR positive band at the expected size of 192 bp fragments. These findings were consistent with the results of El-Nahas et al., (2011), Sharawi and Abd El-Rahim (2011) and EL-Khabaz (2014).

Biochemical profiles can be utilized to elucidate the pathogenesis of the disease, improve its prognosis, management, and treatment methods (Şevik et al., 2016). cTnI is a cardiac biomarker that has been reported to be increased with myocardial damage. There were significant increase in serum cTnI and AST in LSD infected cattle. This results was agreed with those reported by Neamat-Allah, (2015) and Şevik et al., (2016). The increase in the activity of AST may be related to the inflammation of skeletal or cardiac muscles; therefore, AST elevation together with cTnI in our study may have been related to injury or inflammation of cardiac muscle due to the presence of LSDV in the heart (Zilva et al., 1988; Agag et al., 1992; Stockham and Scott, 2013; Helmy et al., 2017). Other researchers recorded histopathologic changes in the heart, muscle fibers, lymph nodes, and skin (Prozesky and Barnard, 1982; Woods, 1988; Zilva et al., 1988; Stockham and Scott, 2013). El-Mandrawy and Alam, (2018) reported the occurrence of heart injury in LSD infected cases, these manifestations may reflect myocardial damage.

The increase in the level of liver enzymes (AST, ALT, and ALP) in LSD infected cattle can be related to the hepatic injury produced by the presence of the virus in the liver. This finding is in agreement with a previous study that reported that pox lesions that caused by capri pox virus can be seen in the liver (Bowden et al., 2008; Awad et al., 2010; Hassan et al., 2011; Şevik et al., 2016; Helmy et al., 2017) Regarding to the decrease in serum sodium and increase in serum potassium, urea and creatinine levels. This may be attributed to the effect of virus on kidney tubules causing renal impairment or reduction of glomerular filtration and increase catabolic rate of protein, Neamat-Allah, (2015) attributed the increase in level of urea to the increased protein breakdown during hyperthermia. However, infected animals are generally suffering from malnutrition and low energy status following loss of appetite and fever with
subsequent disturbance of all the metabolic processes (Rasby et al., 1991; Ahmed, 2007 and El-Mandrawy and Alam, 2018). Disturbance in sodium and potassium levels might be related to decrease food consumption or decreased absorption of these elements. Moreover, infection was considered as a sort of stress on animals and is associated with increased level of disturbed oxidant/antioxidant status in the body (Ahmed, 2007). Hyperkalemia may have been caused by the metabolic acidosis or the muscular damage that is associated with LSD infection (Carlson, 2002). Hypocalcaemia observed in the present study could be attributed to off food and renal infiltration impairment. Stockham and Scott, (2013) and Awad et al., (2010) reported that hypocalcaemia could be attributed to hypoproteinemia resulting in decrease protein bounded calcium. Furthermore, Hassan et al., (2007) denoted that hypoalbuminemia and hypoproteinemia could be resulted from severe anorexia, off food and liver disease which unable to synthesis protein. The significant decrease in iron in the present study is attributed to the viral infection that can affect iron metabolism and regulation in the body (Drakesmith and Prentice, 2008).

Serum biochemical changes in the present study were consistent with clinical findings. These changes are mainly caused by the primary disease process, and the differences seen within the same parameter are likely due to the change in the stage of diseases and the complexity of the case (Abutarbush, 2015).

ECG is considered as one of the most important parameters for an animal suffering from cardiovascular problems. Electrocardiography also useful as prognostic indicator in large animal medicine, and it provides a record of the varying potential difference that occurs over the surface of the body as the result of electrical activity within the heart. In large animals, including cattle, a base apex lead has been shown to be an ideal lead system for monitoring cardiac arrhythmias (Rezakhani et al., 2004)

ECG of LSD infected cattle showed P wave abnormalities, shortening of P and T-waves amplitudes and decrease in RT interval compared to healthy cattle. Heart rate and rhythm may vary among normal animals but can also be a reflection of primary myocardial disease. Other factors such as acid-base and electrolyte imbalance, potassium, sodium and calcium, can influence cardiac rate and rhythm. Therefore, these factors must be taken into consideration in the assessment of apparent abnormalities detected on clinical examination of the cardiovascular system (Thakur, 1995; Cunningham, 1997; Smith, 2009). Taken together, it was noted that the results of biochemical profile were consistent with the results of clinical and electrocardiographic findings.

Based on the results of this study we concluded that the cardiac alterations in LSD infected cattle could be attributed to the elevation of cTnI due to direct effect of the virus on the heart beside electrolyte imbalance, acidosis and hypocalcaemia.

5. REFERENCES


