Some clinicopathological and pathological studies of *C. ovis* infection in sheep

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**SUMMARY**

This study was conducted on 480 male sheep from three private farms in Sharkia Governorate. The surveyed cases were examined clinically for *caseous lymphadenitis*, 60 male sheep gave positive result for isolation of *C. ovis*.

The diseased sheep showed depression, loss of appetite and abscesses spread all over the body.

Blood picture of diseased sheep revealed significant drop in erythrocytic count, haemoglobin concentration and packed cell volume (macrocytic hypochromic anemia) with significant leucocytosis; neutrophilia, monocytosis and lymphopenia. Serum chemistry revealed significant increase in the activities of ALT and AST as well as urea and creatinine levels, while total protein and albumin revealed significant decrease. Globulin fraction revealed significant increase in $\gamma_1$ and $\gamma_2$ and decrease in $\beta_1$ and $\beta_2$ and serum testosterone concentration showed significant decrease.

Gross examination showed enlargement and abscess formation of variable size in lymph nodes. Severe congestion of most internal organs was noticed. The histopathological examination revealed degeneration and necrosis in the different layers of spermatogonial cells *C. ovis* caused pathological changes in the testes and decreased testosterone level, therefore, *C. ovis* could be expected to decrease the reproduction in sheep.

**INTRODUCTION**

*Corynebacterium pseudotuberculosis* is a Gram positive bacteria that causes caseous lymphadenitis (CLA, *C. ovis* or Cheesy gland) in sheep and goats, ulcerative lymphagitis in horses and necrotic dermatitis in cattle. Clinical diagnosis of CLA in sheep possesses many problems where palpation of lymph nodes is unreliable and it is not specific (Brown and Olander, 1987).

Caseous lymphadenitis in sheep is characterized by abscessation of one or more superficial lymph nodes. The lesions consist of a central mass of thick and sometimes dry greenish white necrotic material surrounded by a connective tissue capsule. Currently, diagnosis of CLA is based on the cha-
racteristic clinical symptoms and the detection of *C. pseudotuberculosis* in pus taken from the abscess. It has been suggested that this organism is a saprophyte (Sting *et al.*, 1998).

*C. ovis* in sheep caused leucocytosis with significant drop in both red blood cells count and haemoglobin concentration (severe anaemia). Marked drop in the level of total serum protein, elevation of serum creatinine and activities of aminotranferases (AST & ALT) (Musa, 1998).

The male gonads have a double function, producing testosterone and the spermatozoa which are essential for fertilization of the ovum in the reproductive process. The pituitary gonadotropin (LH), stimulates interstitial cells in the testis to produce testosterone and FSH promotes spermatogenesis by the germinal cells (Kaplan and Szabo, 1979).

Testosterone is secreted primarily by the leydig cells of the testis and maintain the miotic division and spermatogenesis (Adam *et al.*, 1994).

Caseaus lymphadenitis can be endemic in a herd flock and it causes significant economic impact on the small ruminant industry through decreasing meat yield, damaging wool and leather and decreas ing the reproductive efficiency of the animal (Williamson, 2001).

This study was carried out to determine the effect of *C. ovis* on the general health and reproduction of sheep through studying serum biochemical changes, bacteriological isolation, post-mortem and histopathological findings in sheep suffering from caseous lymphadenitis.

**MATERIALS AND METHODS**

A total of 480 male sheep were clinically examined from three private farms in Sharkia, of which 60 male sheep (1-4 years old) were suffering from pinhead to about 5 cm abscesses. The abscesses spread all over the body especially at the prescapular and precrural lymph nodes and also at the parotid lymph nodes. Pus appeared whitish and semisolid in texture. In addition 20 apparently healthy sheep were used as control animals.

1. **Bactriological samples:**

Two swabs were taken from each enlarged lymph node under complete aseptic conditions. The wool over and surrounding the swollen lymph node was clipped, and the area of abscess was disinfected with 70% alcohol, the abscess was open and swabs were taken from periphery of the lesion. Swabs were incubated at Brain heart infusion broth “BHIB” (Oxoid) media. The culture media was sheep blood agar
at 37°C for 48 hours. Smears were prepared from the grown colonies and stained with Gram’s stain and examined microscopically according to Cruickshank et al. (1975).

Gram positive non-spore forming pleomorphic organisms were identified biochemically according to Carter and John (1990).

2. Blood samples:
Two blood samples were taken from jugular vein from apparently healthy and diseased sheep. The first sample was collected into a clean dry bottle containing EDTA anticoagulant for haematological studies (Jain, 2000). The second blood sample was placed into clean centrifuge tube to separate serum. Serum was obtained by centrifugation at 3000 rpm for 15 minutes.

Fresh serum was used for electrophoresis which was done on cellulose-acetone membranes in a microzone cell according to Osbalaiston (1972). Another part of serum was used for biochemical analysis and estimation of testosterone hormone.

3. Assay of serum biochemical parameters:
Total protein was assayed according to Dounas et al. (1981), albumin according to Drupt (1974), serum protein electrophoresis according to Osbalaiston (1972), AST and ALT activities according to Reitman and Frankel (1957), Urea according to Tabucco (1979), creatinine according to Fabiny and Eringhausen (1971) and testosterone by radioimmunoassay according to Adam et al. (1994).

4. Tissue samples:
Specimens from organs of 5 animals such as lung, heart, liver, kidney, testis and regional lymph nodes were collected from diseased sheep and kept in 10% buffered formalin for the histopathological studies according to Bancroft et al. (1994).

5. Statistical analysis:
Data were analyzed statistically according to Petrie and Watson (1999).

RESULTS
Clinical examination of 3 farms of sheep in Sharkia Governorate revealed that 60 male sheep were suffering from enlargement of lymph nodes and many abscesses spread all over the body. There was loss of appetite and body weight. Clinically infected sheep and the rate of isolation of C. ovis were illustrated in table (1).

Bacteriological examination and biochemical tests performed on the isolated organism revealed the presence of Corynebacterium ovis as the cause of the disease. Colonies
were small, white and after several days incubation the colonies appeared dry crumbly and creamy in color. *C. ovis* is strong urease positive and is able to ferment sugar, hydrolysed gelatin and reduce nitrate.

The haematological studies (Table, 2) showed significant decrease in RBCs count, PCV %, Hb concentration, MCH and MCHC % and significant increase in MCV.

Results of leucogram of diseased sheep with caseous lymphadenitis are shown in table (3). The pattern of total leucocytic count showed significant increase due to significant increase in neutrophils and monocytes.

Results obtained from table (4) and Figs. (1 & 2), showed a significant decrease in total protein, albumin, $\beta_1$ and $\beta_2$ globulin levels in diseased sheep whereas a significant increase in $\gamma_1$ and $\gamma_2$ globulin levels was seen. Statistical analysis revealed insignificant variations of A/G ratio.

Activities of serum enzymes aspartate and alanine aminotransferases revealed a significant elevation (Table, 5). The same pattern was observed in of blood urea nitrogen and creatinine. Serum testosterone concentration showed significant decrease in diseased sheep.

Greenish yellow pus was released when the affected lymph nodes from diseased sheep were incised. Some of the enlarged superficial lymph nodes from the slaughtered animals contained dry caseated material and showed the characteristic onion shaped lesions. Abscesses in the lung, severe congestion of most internal organs including lung, liver, kidneys, heart, testis and lymph nodes were observed.

Microscopical examination revealed that pulmonary congestion was a generalized feature. There was vasculitis, other cases showed degeneration of the wall of blood vessels and in some cases thrombus formation of most of pulmonary arterioles were noticed. Lung showed interstitial pneumonia and there was hyperplasia of epithelial lining of the bronchiol. Thickening of the pleura (Fig., 3) was observed. Lung also showed abscesses with caseous necrotic material in the center surrounded by a zone of living and dead neutrophils (Fig., 4).

The liver was severely congested with degeneration of hepatic cells, hyperplasia of the bile ductules, granular and vacuolar degenerative changes. Some hepatocytes showed necrosis (Figs. 5 & 6).

The heart showed haemorrhages inbetween the muscles, also hemolysed blood was noticed.
Degeneration and necrosis of myofibers with infiltration of inflammatory cells were seen (Figs. 7 & 8). Kidneys showed congestion, some cases showed haemorrhage and few cases showed thrombus formation. Degeneration of the epithelial lining of renal tubules was observed (Fig., 9), atrophied glomerular tuft capillaries with interstitial mononuclear cells aggregation (Fig., 10) were noticed.

Thickness of tunica albugina of the Testis, severe edema and degeneration of the leydig cells were observed. Degeneration and necrosis were seen in the different layers of spermatogenic cells (Fig., 11).

Congestion with perivascular edema were seen.

Lymph nodes revealed dilatation and congestion of most of the blood vessels of both cortex and medulla. Severe haemorrhages were seen. Variable degrees of lymphocytic depleion of lymphoid follicles and necrosis were observed (Figs. 12 & 13). Dilatation of sinusoids. Large abscess formation was seen. Abscess was formed of central caseous necrotic material surrounded by heavy zone of living and dead neutrophils and lymphoid cells infiltration.

Table (1): Prevalence of *C. pseudotuberculosis* among diseased sheep.

<table>
<thead>
<tr>
<th>Farms</th>
<th>No. of examined male sheep</th>
<th>No. of diseased male sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm (1)</td>
<td>150</td>
<td>19</td>
</tr>
<tr>
<td>Farm (2)</td>
<td>190</td>
<td>27</td>
</tr>
<tr>
<td>Farm (3)</td>
<td>140</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>480</td>
<td>60</td>
</tr>
</tbody>
</table>

Table (2): Mean values of erythrogram of healthy and diseased sheep with caseous lymphadenitis.

<table>
<thead>
<tr>
<th>Groups of sheep</th>
<th>RBCs (x 10^6)</th>
<th>P.C.V. (%)</th>
<th>Hb (g/dl)</th>
<th>M.C.V (fl)</th>
<th>M.C.H. (pg)</th>
<th>M.C.H.C. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy sheep</td>
<td>13.2 ±0.92</td>
<td>35.5 ±1.6</td>
<td>12.0 ±0.66</td>
<td>34 ±0.19</td>
<td>10 ±1.1</td>
<td>31.5 ±1.1</td>
</tr>
<tr>
<td>Diseased sheep</td>
<td>11.3* ±0.86</td>
<td>31.6* ±1.9</td>
<td>10.8* ±0.23</td>
<td>37* ±2.1</td>
<td>8* ±0.9</td>
<td>28.2* ±1.3</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.
Table (3): Mean values of leucogram of healthy and diseased sheep.

<table>
<thead>
<tr>
<th>Groups of sheep</th>
<th>T.L.C. (x 10^3/μl)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Monocytes (%)</th>
<th>Eosinophils (%)</th>
<th>Basophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy sheep</td>
<td>15.13 ±0.35</td>
<td>34.0 ±0.1</td>
<td>62 ±0.1</td>
<td>3 ±0.01</td>
<td>1 ±0.01</td>
<td>-</td>
</tr>
<tr>
<td>Diseased sheep</td>
<td>18.11* ±0.29</td>
<td>45* ±0.4</td>
<td>40* ±0.2</td>
<td>4* ±0.01</td>
<td>1 ±0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (4): Mean values of total protein, albumin and electrophoretic pattern of proteins of healthy and diseased sheep.

<table>
<thead>
<tr>
<th>Groups of sheep</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>Total globulin (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha</td>
<td>Beta</td>
<td>Gamma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>α_1</td>
<td>α_2</td>
<td>β_1</td>
</tr>
<tr>
<td>Healthy sheep</td>
<td>7.24 ±0.4</td>
<td>1.76 ±0.1</td>
<td>0.71 ±0.02</td>
<td>0.45 ±0.02</td>
<td>0.65 ±0.02</td>
</tr>
<tr>
<td>Diseased sheep</td>
<td>7.01* ±0.12</td>
<td>1.58* ±0.07</td>
<td>0.64 ±0.05</td>
<td>0.51 ±0.05</td>
<td>0.59 ±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as absolute values ± S.E.
* Significant at P < 0.05.
Table (5): Mean values of some biochemical parameters of healthy and diseased sheep.

<table>
<thead>
<tr>
<th>Group of sheep</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy sheep</td>
<td>13.2 ±1.3</td>
<td>66.1 ±3.4</td>
<td>20.3 ±0.5</td>
<td>0.5 ±0.03</td>
<td>3.42 ±0.82</td>
</tr>
<tr>
<td>Diseased sheep</td>
<td>37.6* ±1.9</td>
<td>87.6* ±3.3</td>
<td>22.2* ±0.1</td>
<td>0.9* ±0.02</td>
<td>2.11* ±0.63</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.

Fig. (1): Densitometer tracing of electrophotogram of serum proteins of apparently healthy sheep.
Fig. (2): Densitometer tracing of electrophotogram of serum proteins of *C. ovis* infected sheep.

Fig. (3): Lung of diseased sheep showing increase in pleural thickening. (H & E. X 200).
Fig. (4): Lung of diseased sheep showing abscesses with caseous necrotic material in the center surrounded by a zone of living and dead neutrophils (H & E. X 400).

Fig. (5): Liver of diseased sheep showing congestion of blood vessels with vacuolar degenerative change of hepatocytes (H & E. X 100).
Fig. (6): Liver of diseased sheep showing congestion of blood vessels and necrosis of some hepatocytes (H & E. X 200).

Fig. (7): Heart of diseased sheep showing hemorrhage with severe necrosis of myocardial fiber (H & E. X 400).
Fig. (8): Heart of diseased sheep showing congestion of the blood vessels. (H & E. X 200).

Fig. (9): Kidney of diseased sheep showing haemorrhage and degeneration of the cells lining the renal tubules (H & E. X 125).
Fig. (10): Kidney of diseased sheep showing interstitial mononuclear cells aggregation. (H & E X 125).

Fig. (11): Testis of diseased sheep showing necrosis in spermatocytes and the semineferous tubules were devoid of sperms. (H & E. X a: 100, b: 400).
Fig. (12): Lymph node of diseased sheep showing depletion of lymphocytes and neutrophils infiltration (H & X 200).

Fig. (13): Lymph node of diseased sheep showing depletion of lymphocytes of lymphoid follicles with edema (H & E. X 200).
DISCUSSION

Caseous lymphadenitis (abscission of the lymph nodes) is an important disease of sheep and goats in Egypt (Williamson, 2001). The disease is characterized by the presence of cutaneous suppurative lymphadenitis with characteristic onion-like appearance of the affected lymph node and isolation of *C. ovis* in the respective flock (Jubb et al., 1993). Lavean et al. (1997) concluded that the isolation of *C. pseudotuberculosis* from the affected cases by bacteriological culturing was the appropriate confirmatory method for diagnosis of caseous lymphadenitis.

Haematological evaluation revealed significant decrease in red cell count, haemoglobin concentration, PCV, MCHC and an increase in MCV (macrocytic hypochromic anaemia). These changes may be due to the harmful effect of bacterial toxin on the haemopoietic system and also the effect of toxin on the endothelial cells of the blood vessels. Similar explanation was reported by Jain (2000) who stated that *C. ovis* exotoxin cause massive removal and degradation of red cell membranes by the reticuloendothelial system.

The diseased sheep showed significant leucocytosis, neutrophilia, monocytosis and significant lymphopenia. Results are attributed to *C. ovis* infection which cause tissue destruction, abscessation and stimulation of leucoipoiesis by the nucleic products. Similar results were obtained by Soucek and Souckova (2001).

Significant increase in ALT and AST activities were observed in diseased ram. This elevation may be due to *C. ovis* exotoxin effects on the liver. In the present work the histopathological alterations showed degenerative changes in the liver and supported the previous findings. Similar results were obtained by Attia and Aziza-Essa (1997).

Serum level of total protein and β1 and β2 globulins were significantly decreased in diseased sheep. This probably due to liver damage which confirmed by results of histopathological examination and loss of appetite. Significant elevation was observed in the percent of γ1 globulin. Our results agree with Attia and Aziza-Essa (1997) who mentioned that the drop in serum total protein may be due to malnutrition and reduced appetite of infected sheep. They added that such reduction of albumin happened due to increase catabolism. It is suggested that the decrease may be due to the bacterial infection which caused increase the capillary permeability and permitted escape of plasma protein into tissue, hence total protein was decreased in blood.
The significant decrease in $\beta_1$ and $\beta_2$ globulin may be attributed to the liver damage because these globulin fractions are synthesized by liver. The increase in gamma globulin in this work was due to increased production of immunoglobulins as a result of Corynebacterium pseudotuberculosis infection.

Marked increase was noticed in serum urea and creatinine in diseased sheep. This change may be attributed to degenerative changes in the kidneys which were observed histopathologically and indicated renal impairment by the exotoxin of C. ovis bacteria. Also the decreased renal blood flow tended to increase serum creatinine levels. Similar findings were reported by Williamson (2001).

Significant decrease was found in serum testosterone concentration in diseased sheep. This decrease may be attributed to the bacterial toxin effect on leydig cells of the testes of diseased sheep. Leydig cells are responsible for testosterone production (Shan and Hardy, 1992).

Regarding to the histopathological results, the lung of diseased sheep showed pulmonary congestion, vasculitis of the blood vessels and other cases showed thrombus formation of most of pulmonary arterioles. Hyperplasia of epithelial lining of bronchi. Theses findings are in agreement with the findings of Lavean et al. (1997) who reported that C. ovis in sheep cause hyperplastic bronchial epithelium and congested capillaries (Bronchiolitis).

The liver showed several pathological changes in the diseased sheep. These changes were in the form of vascular damage, granular degeneration of hepatocytes. These findings were in agreement with the findings of Carter et al. (1990) who reported that degenerative and necrosis of the wall of the portal veins was observed and some hepatic cells were necrosed and detached.

Examination of the heart showed degeneration and necrosis of myofibers. These findings agree with Soucek and Souckova (2001). The kidneys of diseased sheep showed haemorrhage, thrombus and vasculitis. The glomerular tuft in some cases showed atrophy while other showed hypercellularity. The present findings agree with Eman et al. (2003).

The testis showed thickening of the tunica albugina, severe edema and degeneration of the leydig cells. These findings agree with that of Carter et al. (1990) who reported that C. ovis caused orchitis in the male guinea pigs. In the present work, the histopathological alterati-
on of testis support the finding of the decrease of testosterone hormone which lead to decrease of the reproductive efficiency (Sempere et al., 1999).

The examination of lymph nodes showed thickening of the capsule with congestion of the blood vessels of both cortex and medulla, variable degrees of lymphocytic depletion of lymphoid follicles and necrosis were observed. The present finding agreed with Soucek and Souckova (2001).

From the present study, C. ovis affected the general health caused histopathological changes in the tests and decreased the level of testosterone which decrease the reproductive efficiency. The disease causes significant economic impact on the small ruminant industry. Periodic examination of sheep farms and early treatment of affected cases is recommended.

Acknowledgement:
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REFERENCES


sheep with special reference to its immunization potency." Egypt. J. Comp. Pathol and Clinic Pathol, 16(2):100-122.


