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CASEOUS LYMPHADENITIS OF SHEEP AND GOATS IN ASSIUT FARMS AND ABATTOIRS

(With 4 Tables and 11 Figures)

By

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مرض السل الكاذب في الأغنام و الماعز بمزارع ومجازر أسيوط

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أجريت هذه الدراسة على عدد ١٢٥٢ , ٥٢٠ رأسا من الأغنام والماعز من مزارع الحكومة والقطاع الخاص وكذا قطعان خاصة بالأهالي من أربع مناطق بمحافظة أسيوط ومن خلال الفحص الأكلينيكي وجد أن ٦,٧٩% , ٤,٨١% من الأغنام والماعز التي تم فحصها كانت مصابة بالعلامات المرضية المميزة لمرض السل الكاذب " تضخم في الغدد الليمفاوية الخارجية" وكانت الغدد الليمفاوية النكافية تمثل أعلى إصابة في الأغنام والماعز وعند فحص عدد ٢٩٥ رأسا من الضأن البلدي و ٨٨ رأ سا من الماعز التي تم ذبحها في بعض مجازر محافظة أسيوط وجد أن ٣٧ (١٢,٥٤%) , ٦ (٦,٨٢%) رأسا من تلك الحيوانات مصابة بخراريج داخلية في الرئة, الكبد والغدد الليمفاوية الرئوية والكبدية كذا تضخم في بعض الغدد الليمفاوية السطحية, هذا وقد تم عزل عدد ثلاثون عترة (٢٧ من الأغنام , ٣ من الماعز) من عترات الميكروب كورينى السل الكاذب من العينات التي تم أخذها سواء من الحيوانات الحية أو المذبوحة وكانت هذه الميكروبات متشابهة الى حد كبير في خواصها المورفولوجية وفي بيئات الزرع المختلفة وفي الاختبارات البيوكيميائية فيما عدا بعض الاختلافات ولقد تم عزل ميكروب كوريني السل الكاذب باعلى نسبة من الغدد الليمفاوية النكافية من الحيوانات الحية ومن خراريج الرئة من الحيوانات المذبوحة ولقد تم اجراء اختبار الحساسية بالمضادات الحيوية عل العترات المعزولة كذا سلوك هذه الميكروبات عند حقنها في حيوانات التجارب "الفأر الابيض و الأرنب الهندي " ولقد ذكرت نتا ئج الفحوص الاكلينيكيةو الباثولوجية وقد نوقشت الاهمية الاقتصادية والصحية لهذه الميكروبات وكذا الاشتراطات الواجبة للسيطرة على هذا المرض.

SUMMARY

This study was conducted on 1252 sheep and 520 goats from governmental and private sector farms and also from owner's flocks in 4 districts at Assiut locality. The surveyed cases were examined clinically for ovine and caprine pseudotuberculosis. Incidence of infection was 6.78% in sheep flocks, while that of goats was 4.81%. The parotid lymph nodes showed the highest percentage of infection. On the other hand, out of 295 sheep and 88 goats examined during routine meat inspection in some abattoirs at Assiut Province, 37 sheep and 6 goats showed post–mortem (P.M.) lesions characteristic of caseous lymphadenitis. 30 strains of Corynebacterium *ovis* (*C. ovis*) were isolated, 27 of ovine origin and 3 isolates of caprine origin. All isolates of ovine or caprine origins were to a great extent similar in their morphological, cultural and even biochemical characteristics with certain variations. The parotid lymph nodes being the most common site of *C.ovis* in sheep (11.76%) and goats (8%), while the most commonly affected organ in ovine visceral caseous lymphadenitis (CLA) was the lung. Experimental infection of Guinea-pigs and adult mice as well as antibiogram of local isolates were also performed. Results of clinical, and pathological examinations were described. The economic and public health importance of these organisms, as well as the recomended control measures were also discussed.

Key words: *Lymphadenitis – C.ovis – Bacteriology – Pathology.*

INTRODUCTION

Corynebacterium ovis (C.ovis) synonyms: *Corynebacterium pseudotuberculosis,* "Preiz-Nocard bacillus" is the aetiological agent of caseous lymphadenitis (CLA) of sheep and goats. The disease is characterized by discrete, chronic abscesses containing a caseous pus, particularly in superficial lymph nodes and also in visceral nodes and organs (Batey, 1986). The lesions consist of a central mass of thick and sometimes dry greenish white necrotic material surrounded by a connective tissue capsule (Jensen, 1974), and on gross examination, CLA lesions are considered unique and diagnostic (Ayers, 1977).

C.ovis is sensitive to most antibiotics (Soltys,1963), but unfortunately the lesion is so encapsulated that any antibiotic is unlikely

to penetrate the capsule and kill the organism. Antibiotics give only good results in the very early stages of the disease (Maddy, 1953), but this stage usually passes undetectable due to the lack of any diagnostic test.

The purpose of the present study was to determine the prevalence of the disease and to elucidate the distribution of lesions together with organism isolation. The correlation between *C.ovis* and their sites of isolation among diseased, living and slaughtered sheep and goats was also emphasized. Experimental infection of mice and Guinea-pigs as well as antibiogram of local isolates were also performed.

MATERIALS and METHODS

Animals:

(A) Field cases:

(a) Sheep flocks: A total of 1252 sheep of native breeds aging 2-5 years were examined for caseous lymphadenitis. 872 cases were surveyed from El-Hawatka governmental farm and 380 cases in some villages and areas at Assiut Governorate (Table 1).

(b) Goat herds: A total of 520 goats belonging to owners' flocks aging 1-3 years were examined for caseous lymphadenitis (Table 1).

(B) Slaughtered animals:

Specimens from superficial and deep lymph nodes and parenchymatous organs were obtained from slaughtered 37 sheep and 6 goats which showed P.M. lesions suspected to be caseous lymphadenitis during routine meat inspection in some abattoirs at Assiut Governorate. These samples were picked out of 295 sheep and 88 goats carcasses.

Methodology:

A) Processing of specimens and pus swabs:

Pus samples and swabs were taken either from field or slaughtered cases as follows

(1) Field cases:

The wool over and surrounding the swollen superficial lymph nodes was clipped by a clean curved disinfected scissor and the clipped area was cleaned with 70% ethyl alcohol. The swollen lymph nodes were incised surgically by using a sterile scalpel. Caseated materials were evacuated and pus swabs were taken from the peripheral of lesions. The incised lymph nodes were washed by hydrogen peroxide and touched with tincture iodine.

(2) Slaughtered cases:

The affected organs and lymph nodes were immeresed in 70% ethyl alchol, then flammed to get rid of any surface contamination. Consequently, searing of the surface was performed by the aid of a hot spatula to assure complete sterile surface and incised by sterile scalpel. Caseated materials were evacuated and pus swabs were taken from the peripheral of lesions.

(B) Line of diagnosis:

(1) Direct microscopic examination:

Direct pus smears were made, stained with Gram's method and by Ziehl Neelsen's stain to exclude acid-fast bacilli and then examined microscopically.

(2) Isolation technique:

Pus swabs either from field cases or slaughtered carcasses were sawn in 10% serum broth for 24 hours at 37°C as this procedure resulted in an enrichment of the cultures and made primary isolation much easier (Mostafa et al., 1973). Then, subcultured on 10% sheep blood agar, MacConkey agar and Loffler's serum media which were incubated at 37°C for 24 and 48 hours. The characteristic colonial appearance of *C.ovis* on blood agar plates were selected, transfered to slants of nutrient agar and incubated at 37°C for 48 hours. All isolates were identified morphologically, culturally and biochemically according to Quinn et al. (1994) and Collee et al. (1996).

(3) Experimental infection:

To assess the pathogenicity of *C.ovis,* selected strains from each of sheep and goats were used:

(a) Pathogenicity of isolated *C. ovis* to guinea-pigs

(1) Strains for investigation.

Two isolates of biochemically identified *C.ovis* isolated in this investigation were selected for pathogenicity studies. One isolate from each species of animals examined for caseous lymphadenitis

(2) Laboratory animals.

Guinea-pigs (about 250 g) obtained from Animal-house, Assiut University.

(3) Bacterial suspension inoculum.

The selected strains of *C.ovis* were subcultured on 10% sheep blood agar plates, then grown in 10% serum broth and incubated at 37°C for 24 hrs (Awad et al., 1977).

(4) Methodology (Awad et al., 1977 and Khater et al., 1984):

1.0 ml of 24 hrs broth culture was inoculated subcutaneously in first group of male guinea-pigs while the second group were injected intradermally at a dose of 0.2 ml of the same broth culture. For each strain 2 guinea pigs were used. A group of 2 guinea-pigs served as control where the animals were inoculated by sterile broth (1.0 ml, 0.2 ml for subcutaneous and intradermal injection respectively). The experimentation period lasted 36 days. Post mortem examination was carried out on dead animals and those survived were sacrificed at the end of the experiment. Bacteriological reisolation was recorded.

(b) Pathogenicity of C. ovis to mice: (Carter and Cole, 1990)

White mice of 16 to 20 g. weight were used. The first group was injected intraperitoneally (I/P) and the infective dose was 0.2 ml of the organism suspension with a viable count of 5×10^7. The second group was injected subcutaneously (S/C) at a dose of 0.1ml of 7×10^9 of the same organism. Three mice were used for each strain (two inoculated with the organism and one with sterile broth and left as a control). All mice were kept under observation for 30 days. Clinical, post mortem findings and bacteriological reisolation were recorded.

(c) Antimicrobial susceptibility testing:

All isolates of *C.ovis* obtained in this study were tested for their sensitivity to some antimicrobial agents by disc diffusion method as described by Finegold and Martin (1982).

Gross pathology:

Organs and tissues from slaughtered and experimental animals were examined for lesions suggestive of caseous lymphadenitis.

Histopathology:

Tissue samples from slaughtered animals and representative skin tissues at the sites of injection of C.ovis from all dead experimental animals (guinea-pigs & mice) were obtained and fixed in 10% neutral buffered formalin. In addition, tissue samples from all internal organs, including liver, lung, spleen and kidneys, as well as other tissues from exprimental animals were also represented. Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissue samples were sectioned at 3-4μ and stained with haematoxylin and eosin (HE) according to the method described by Bancroft and Stevens (1982).

RESULTS

The obtained data were presented in Tables 1, 2, 3 & 4.

Pathological findings:

Gross pathology:

Greenish yellow pus was released when the affected lymph nodes from sheep cases were incised. Affected lymph nodes from goat cases contained yellowish cheesy material. Some of the enlarged superficial lymph nodes from the slaughtered animals contained dry caseated material and showed the characteristic onion-shaped lesions (Fig. 1). Lungs of the slaughtered sheep and goats had circumscribed lesions which contained caseated pus. Edematous material (gelly-like) admixed with blood-tinged exudate was noticed at site of injection of *C.ovis* (subcutaneous and intra-dermal) in all experimental animals. Gross examination of intrenal organs of experimental animals revealed congestion and edema. Haemorrhages were seen in the regional lymph nodes. In animals injected intra-peritonealy, the peritoneal surface was haemorrhagic and edamatous.

Histopathology:

Examination of the enlarged superficial lymph nodes from slaughtered animals revealed alternated layers of caseous necrosis and fibrous tissue (Fig. 2). The process of caseous necrosis was massive and involved the whole lymph node tissue. Areas of caseous necrosis contained foamy phagocytes and showed scattered cholestrol clefts (Fig. 3). Calcium deposits were seen on background of the necrosed tissue (Fig. 4). Pulmonary lesions in slaughtered goats and sheep showed caseated content and which was surrounded by a fibrous capsule. Guinea pigs and mice injected subcutaneously and intra-dermally showed prominent cutaneous lesions. At site of injection, there were infiltrates of neutrophils which formed dense cellular masses (Fig. 5). In all skin tissues examined, most of the infiltrating neutrophils showed rhexis of their nuclei. Increased basophilic staining of the affected cutaneous tissues was due to the presence of clumps of the fragmented nuclear chromatin (Fig.6). The rhectic neutrophils were frequent at the central patrs of the lesions. In animals injected subcutaneously, the cellular infiltration involved epidermal and dermal tissues. The epidermis was completely destroyed and the underlying dermis (especially at the dermal papillae) was edematous (loose CT) and haemorrhagic (extravasated erythrocytes). The dense cellular infiltrates masked most of the dermal tissue. In animals injected intra-dermally, the neutrophilic cellular reaction was mainly observed in dermal tissue and also extended to hypodermis (panniculus) and subcutaneous muscles. In these animals, panniculitis was

evidenced by the neutrophilic cell infiltration of adipose tissue associated with macrophage cell reaction (Fig. 7). Subcutaneous muscles showed degenerative and necrotic changes. In general, the encountered picture of cellulitis was more marked in animals injected intra-dermally.

Regional lymph nodes (homolateral to site of injection) showed congestion, edema and haemorrhages in association with necrotic changes in the cortical lymphoid follicles. Also, there was neutrophil cell infiltration in the paracortical areas. In animals injected intra-peritoneally, dense aggregates of neutrophils, showing karyorhexis, and numerous extravasated erythrocytes were noticed on the peritoneal surface (Fig. 8).

Histological changes of internal organs of animals injected subcutaneously and intra-dermally were represented by congestion and edema in association with degenerative changes in the constituting cells of parenchymatous organs. In animals injected intraperitoneally, the histological changes in the internal organs were more pronounced. Liver showed hydropic degeneration of hepatocytes (Fig. 9) and also focal necrosis. Bronchiolitis (Fig. 10) and peribronchitis were noticed in the examined lung tissues. The examined kidney tissues revealed tubular degenerative changes and occasional hyaline glomerular thrombi in association with interstitial haemorrhages. Myocardial tissues disclosed haemorrhages between myofibers which showed hydropic degeneration. Subendocardial haemorrhages were also seen. Spleen manifested necrotic changes in the lymphoid sheaths which had "starry-sky" appearance (Fig.11) in association with moderate degree of haemosiderosis. Adrenal tissues revealed congestive edema and scattered haemorrhages. Aggregates of neutrophils showing karyorrhexis were detected in the lungs, kidneys and spleen. However, these cellular aggregates were not comparable to those noticed in the cutaneous lesions. Blood vessels in most of the examined organs had hypertrophic and proliferated endothelial cells.

DISCUSSION

Incidence of caseous lymphadenitis among living sheep and goats
[A] Field cases:

The summarized results in Table (1), verify that out of 1252 sheep and 520 goats belonging to governomental, private sector farms and owner's flocks, 85(6.79%) and 25 (4.81%) of sheep and goats, respectively showed the characteristic lesions of caseous lymphadenitis.

The swollen lymph nodes were hard to touch and when they were surgically opened a thick caseous greenish-yellow pus came out in sheep cases. In goat cases the nature of pus was significantly different from that encountered in sheep, being yellowish in colour and of the consistancy of soft cheese. The greenish tinge is a product of sheep pus irrespective of the oganism causing it (Abd-El Ghaffar et al., 1966).

The prevalence by geographic area and CLA lesion distrbution for 1252 and 520 sheep and goats originating from 4 different regions at Assiut Governorate were summarized in Table (1). It seems likely that significant differences in prevalence between regions are actually reflective of real difference resulting from ecologic variations between regions.

Out of 85 and 25 sheep and goats with enlarged lymph nodes, 62 and 16 showed enlargement of one lymph node while 23 and 9 sheep and goats respectively showed more than an enlarged one (Table 2).

It is worthnoting that, the incidences of caseous lymphadenitis among live sheep and goats in the present study was 6.79% and 4.81% respectively (Table 1). Higher incidences were reported by Awad et al. (1977) and Moustafa and Afifi (1996). Such variation may be attributed either to the varied locality or habitate in which the animals live.

As illustrated in Table (1), it appears that the percentage of caseous lymphadenitis in goats is lower than that of sheep. This is probably due to the fact that goats are not sheared and accordingly, the chance of their infection are not as frequent as that of sheep (Soliman et al., 1970). This is in contrast to the work of Awad et al. (1977) and Moustafa and Afifi (1996) who found that the highest incidence of CLA was recorded in goats rather than sheep. The latter authors added that the higher incidence of infection in goats examined was attributed to cut wires in the skin and browsing.

Information derived from Table (2) declars that incidence of infection was the highest in the parotid lymph nodes as 66 nodes were infected clinically, while the incidence was the lowest in superficial inguinal lymph nodes as only 5 nodes were found infected clinically. These findings agree to a certain extent with those reported by Aziz et al. (1982) and Zaitoun and Bayoumi (1994). Contradictory findings were given by Moustafa and Afifi (1996) and Abd-El-Ghani et al. (1998). In this respect, it is assumed that way of natural infection is through ingestion as the parotid nodes situated away from shearing areas. Lesions in parotid lymph nodes might represent both cutaneous and mucosal

modes of entry of the organism and to some extent reflect the peculair browsing habit of goats. It is also possible that parotid involvement is resulted from fighting among billies (Batey 1986).

[B] Slaughtered animals:

Investigation of the prevalence of caseous lymphadenitis in slaughtered sheep and goats revealed that out of 295 and 88 sheep and goats examined, 37 (12.54%) and 6 (6.82%) sheep and goats carcasses showed lesions of caseous lymphadenitis (Table 3). Awad et al. (1977) recorded higher incidence of lesions of caseous lymphadenitis in sheep (24.59%). However, our findings nearly agree with the observation of Batey (1986) who found that out of 2920 slaughtered goats in Australia, 227 (7.8%) had lesions of caseous lymphadenitis.

In the present work as shown in table (3), out of 37 and 6 slaughtered sheep and goats which showed P.M. lesions characteristic for caseous lymphadenitis, 26 and 3 had lesions in the internal lymph nodes and/or organs alone with no evidence of infection in the superficial lymph nodes. 5 sheep and 2 goats had lesions in the superficial lymph nodes alone. On the other hand, the remaining 6 and 1 sheep and goat showed involvement of the superficial and /or internal lymph nodes. These findings tend to agree with that reported by Hein and Cargill (1981) and Loot et al. (1984).

Incidence of *C. ovis* recovered from cases showing the characteristic lesions of caseous lymphadenitis.

(1) Field cases:

As shown in Table (2), it was found that out of 117, and 36 pus swabs from lymph nodes of sheep and goats, respectively showing the characteristic lesions of caseous lymphadenitis, 20 (17.09%) and 3 (8.33%) specimens revealed *C. ovis* infection.

From the results achieved, the incidence of *C. ovis* in goats was significantly lower than that found among sheep. These findings agreed with the observations of Abd-El- Ghani et al. (1998). The lower incidence of *C. ovis* in goats may be due to the fact that the lymph nodes abscesses in goats are not commonly caused by *Corynebacterim ovis* but may be attributed also to other pyogenic micro-organisms such as Staphylococci, Sterptococci, *Pseudomonas aeruginosa* and other organisms (Pepin et al., 1989).

(2) Slaughtered cases:

Investigation of the prevalence of *C. ovis* infection in slaughtered sheep and goats revealed that out of 37 sheep and 6 goats with lesions in

their parenchymatous organs, internal and external lymph nodes, in only 7 (18.92%) sheep cases the condition was associated with *C. ovis* (Table,3). The lesions did not appear to affect the well-being of the infected sheep and the significance of infection was mainly confined to the presence of abscesses in visceral nodes and organs of animals slaughtered for human consumption. Batey (1986) found that there is no significant difference in body weight between affected and non-affected sheep and losses due toxaemia do not occur. Moreover, Batey (1988) noticed that no correlation was found between condemnation of sheep for emaciation and the occurrence of caseous lymphadenitis and the disease does not have sufficient effect on animal health to warrant special attention at post-mortem inspection. On the other hand, some investigators have suggested a possible role for the caseous lymphadenitis in the causation of a debilitating condition referred to as "thin ewe syndrom" (Stoops et al., 1984).

The visceral lesions of caseous lymphadenitis are the result of haematogenous and lymphogenous spread of infection (Stoops et al., 1984). Indeed, inflammation in sheep has been shown to increase vascular permeability and thereby increase lymph flow, prostaglandin levels and polymorphonuclear cells efferent from the local lymph node which could be expected to facilitate the dissemination of an organism such as Corynebacterium via the lymphatics (Johnston et al., 1979).

No *C.ovis* could be revealed from lymph nodes of goats from abscesses of lung parenchyma or liver specimens (Table, 3) and therefore, one can easily conclude that the visceral form of caseous lymphadenitis in goats has not been recorded in the present study. These findings substantiate what has been reported by Batey (1986) and Abd-El-Ghani et al. (1998).

C.ovis amounting 30 strains were isolated from ovine and caprine specimens. So, the isolation of the causative organism of caseous lymphadenitis (*C.ovis*) is not a simple procedure. As a whole, this low success percentage in the isolation is attributed mainly to the prevalence of some secondary organisms, such as staphylococci and streptococci, in the lesions which might overshadow the sensitive *C.ovis*. Failure to isolate *C.ovis* on solid media was probably a consequence. Furthermore, *C.ovis* can be more easily isolated from lesions in the early pathological condition "pus formaion" but mostly if calcification is found the organism cannot be isolated.

All strains of *C. ovis* isolated in the present study were to a great extent similar in their morphological, cultural and biochemical characteristics except for some variations in sugar fermentation. The recovered strains of *C. ovis* in this investigation agreed in its behaviour with those isolated by Abd-El-Ghaffar et al. (1966) and Mostafa et al. (1973).

Correlation between *C.ovis* and their sites of isolation from sheep and goats:

(1) Field cases:

As shown in Table (2), out of 20 strains of *C. ovis* recovered from affected lymph nodes of sheep, ten strains were isolated from the parotid lymph nodes, five strains from prescapular lymph nodes, two strains from reteropharyngeal lymph nodes and one strain from supramammary, superficial inguinal and perfemoral lymph nodes. On the other hand, 3 strains of *C.ovis* were recovered from goats, 2 from the parotid lymph node and 1 from the prescapular lymph nodes.

From the results obtained, it is obvious that the parotid lymph nodes were the most common site of infection in living sheep and goats. These findings substantiate what has been reported by Aziz et al. (1982) and Batey (1986). This is in contrast to the work of Abd-El-Ghani et al. (1998) who found that abscessation due to *C. ovis* was commonest in the prescapular lymph nodes.

(2) Slaughtered cases:

Concerning slaughtered sheep, *C. ovis* was isolated from 7 cases (Table 3). Out of 7 strains recovered, 3 strains were isolated from abscesses in lungs and one strain each from liver tissue and hepatic, bronchial and mediastinal lymph nodes.

Information derived from the achieved results declars that lung seem to be the predilection seat of *C. ovis* infection in sheep carcasses followed by the thoracic lymph nodes as represented by the bronchial and mediastinal lymph nodes as well as liver and hepatic lymph nodes. These findings are in line with the observations reported by Stoops et al. (1984) and Moustafa and Afifi (1996).

Thoracic lymph nodes (bronchial and mediastinal lymph nodes) in Egyptian sheep are more liable to *C. ovis* infection due to managmental conditions. These animals are usually reared mostly on the banks of water ways and in the fields after crops where dust contaminated with infection is inhaled and thus the infection is finally trapped in the thoracic lymph nodes.

In this study, no *C. ovis* could be revealed from caprine internal lymph nodes or organs such as lungs and liver (Table 3) and therefore, one can easily conclude that the visceral form of caseous lymphadenitis in goats has not recorded in the present work. These findings substaniate what has been reported by Batey (1986) and Abd-El-Ghani et al. (1998). A contradictory finding was reported by Hein and Cargill (1981) who recorded higher relative occurrence of visceral involvement in goats.

The studied pathogenicity of *C.ovis* in guinea pigs in the present work, reaveled that the selected strains of *C.ovis* caused death of the inoculated guinea pigs within 4-5 days irrespective of route of infection. The inoculated organism either of ovine or caprine origin could be recovered from the site of inoculation and from precrural and prescapular lymph nodes of the guinea-pigs. On the basis of these results it is apparent that the cultures of *C.ovis* isolated from sheep and goats were identical.

Mice injected with selected strains of ovine and caprine origin either I/P or S/C routes lost their condition after a few hours and refused food. Consequently, the mice were unable to move, rested their abdomens on the bedding and their backs were arched and their eyes closed. Death occurred 30 "hrs" and 50 "hrs" after I/P and S/C injection ,respectively, with gross post mortem picture similar to that previously recorded in *C.ovis* infection of mice (Abd-El-Ghani et al., 1998). The organism could not be isolated from the heart blood or from enlarged liver or spleen of mice. Little is known about the pathogenesis of *C.ovis* in adult mice but deaths are probably attributed to systemic effects of the exotoxin. Our findings agree to a certain extent with those reported by Abd-El-Ghani et al. (1998).

Pathology:

Gross and microscopic appearances of the examined lymph nodes of slaughtered animals were similar to those reported in caseous lymphadenitis (Jensen, 1974). The basic nodal histological finding was massive caseous necrosis which was alternated with proliferated CT. Additionally, cholestrol clefts were consistently observed in the necrosed nodal tissues. Thomson (1984) described cholestrol clefts as an occasional feature accompanying tissue necrosis. As far as we aware, the report of Ibrahim and Mahmoud (1987) was the first to describe cholestrol clefts as a constant histological finding in caseous lymphadenitis. Accordingly, it may be reasonable to presume that

cholestrol clefts are one of the specific features of tissue necrosis induced by *C.ovis*.

Cutaneous lesions described herein in experimentally inoculated animals bear a similarity to those reported in previous studies (Minett, 1922 and Tobin & Morse, 1957). The encountered cutaneous lesions were severe which may indicate that the high virulence of the inoculated *C.ovis*. The post mortem findings in guinea-pigs depended on the route of injection,the time elapsed between injection and death of the animal and on the virulence of the inoculated strain (Soliman et al., 1970). The main histological feature detected in the cutaneous lesions in the present study was the dense aggregates of neurtophils which showed rhexis of their nuclear chromatin (caseous necrosis). However, no liquifaction necrosis of these cells was observed. This finding may substantiate the suggestion that *C.ovis* produces leucocidin material which causes death of leukocytes in a non-lytic manner. Despite the isolation of the organism from the lymph nodes of the inoculated Guinea pigs, no comparable nodal lesions to those found in sheep and goat cases were detected. This may be related to species variations concerning the behaviour of *C.ovis* infection.

Degenerative changes of parenchymatous organs in the presently inoculated animals may be related to the effect of *C.ovis* exotoxins. Thus, it can be concluded that *C.ovis toxins* have pyogenic and toxigenic effects. Both effects were evidenced by the cutaneous histological changes at site of inoculation and the latter effect was represented by the widespread degenerative changes in the parenchymatous organs. The present histological changes of parenchymatous organs in the animals inoculated intraperitoneally were relatively more severe. This may be ascribed to the higher level of exotoxin absorbed from the peritoneal surface. Congestion and edema observed everywhere in the present animals may also reflect the effect of *C.ovis* exotoxins on the vascular walls. Carne and Onon (1978) proposed indirect effect of *C.ovis* toxin on the vascular walls through release of histamin and serotonin from blood vessel-associated mast cells. The present histological changes in the inoculated mice and Guinea pigs confirm the previous findings that *C.ovis* is highly pathogenic for laboratory animals (Khater et al., 1977 and 1984).

Antibiogram:

The antibiogram study conducted on the isolated strains (Table 4) showed that penicillin-G, Chloramphenicol, Gentamycin and Rifampicin

were the most effective antibiotics, while Nalidixic acid and Colistin-sulphate were not effective. These findings agree to a certain extent with those reported by Olander and Brown (1987) and Abd-El-Ghani et al. (1998). Information derived from Table 4 revealed that no much difference was noticed between the behaviour of ovine and caprine strains of *C.ovis* to various chemotherapeutic agents. These findings conform to that recorded by Abd-El-Ghani et al. (1998).

Economic losses due to CLA are varied, in Egypt, Afify (1978) estimated the economic losses due to decrease in meat "Condemnation of infected carcasses or parts of carcasses", milk, wool and offsprings. Furthermore, other losses occur due to decrease in the reproductive efficiency of many affected animals (Mostafa et al., 1973). Finally, spreading of CLA can lower the market value of an animal with an external abscess (Williams, 1980).

The public health importance of *C.ovis* has been emphasized by many authors, as it has been implicated in a case of an eosinophilic pneumonia and abscessation of either axillary or superficial inguinal node or in the cervical lymph nod due to ingestion of raw goat's milk (Goldberger et al., 1981). Furthermore, several cases of occupational infection have been documented in sheep herders and abattoir workers (Blackwell et al., 1974 and Henderson, 1979).

Due to inadequate prophylaxis, difficult treatment and inaccurate diagnostic technology, strict control measures must be taken in consideration to eradicate the disease by isolation or culling all infected animals. Retention of clinically diseased animals should be discourged because they act as a reservoir of infection (Stoops et al., 1984). All shearing tools should be dipped in strong disinfectant before usage. Complete dryness of the sheep skin before shearing is urgently needed because probability of infection is enhanced if the skin is wet. Finally, younger age group should be shorn firstly.

From the results achieved, one can safely conclude that CLA has been and continue to be a serious problem for sheep and goats at Assiut Governorate.

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LEGENDS FOR FIGURES

Fig. 1: Prescapular lymph node from a slaughtered sheep showing the onion-shaped appearance characteristic for caseous lymphadenitis. There is laminated pattern in the affected node made by the alternated layers of caseous necrosis and fibrous tissue.

Fig. 2: Fibrous tissue (F) alternating with caseous necrosis (*) in a prescapular lymph node of sheep. HE. x 125.

Fig. 3: Cholestrol clefts (arrow) at the site of caseous necrosis. Prescapular lymph node of sheep. HE. x 125.

Fig. 4: Calcium deposits on background of caseous necrosis. Prescapular lymph node of sheep. HE. x 125.

Fig. 5: Dense aggregates of neutrophils (N) in the dermal tissue. Most of the neutrophils are necrosed. The surrounding tissue is edematous and has less number of infiltrating neutrophils. Mice died 50 hrs after subcutaneous injection.HE. X200.

Fig. 6: Heavy and massive dermal neutrophilic cell infiltration. Most of the neutrophils show karryorhexis of their nuclei. Chromatin debris accumulated at site of necrosed cells gives the lesion more basophilic affinity. Guinea pig died 5 days after intra-dermal injection. HE. X200.

Fig. 7: Panniculitis in a guinea pig died 5 days after intradermal injection. The subcutaneous adipose tissue (T) is infiltrated by neutrophils and shows macrophage cell reaction. Adjacent dermal tissue (D) is edematous and has cellular infiltration. HE. X280.

Fig. 8: Aggregates of karyorrhectic neutrophils in the peritoneal tissue of a mice injected intraperitoneally. Numerous extravasated erythrocytes are admixed with the necrosed neutrophils. HE. X200.

Fig. 9: Hydrobic degeneration of hepatocytes which have reticulated and granular cytoplasm. Liver of a mice injected intra-peritoneally. HE. X280.

Fig. 10: Leukocytes admixed with mucus in the lumen (L) of a bronchiole. The bronchiollar lining epithelium is vacuolated and goblet cells are hypertrophied. The peribronchiolar tissue is infiltrated by neutrophils. Mice injected intra-peritoneally. HE. X280.

Fig. 11: Spleen of a mice died 30 hrs after intra-peritoneal inoculation. The splenic follicle (F) shows necrotic changes of their lymphoid cells. The follicle has "starry sky" appearance due to the presence of vacuolated reticular phagocytes (arrows). HE. X280.

Table (1): Prevalence of Caseous lymphadenitis [CLA] in living sheep and goats from some geographic origin in Assiut Governorate

Locality	Species of animals													
	Sheep							Goats						
	No. of examined sheep			No. of clinically infected sheep				No. of examined goats			No. of clinically infected goats			
	M	F	Total	M	F	Total	% of CLA	M	F	Total	M	F	I	% of CLA
EL-Nekheila	5	54	59	-	9	9	15.25	4	179	183	-	2	2	1.09
Balout	15	97	112	1	22	23	20.54	9	117	126	2	5	7	5.55
Bani-Adi	23	186	209	2	47	49	23.44	18	193	211	2	14	16	8.29
El-Hawatka farm	14	858	872	-	4	4	0.46	-	-	-	-	-	-	-
Total	57	1195	1252	3	82	85	6.79	31	489	520	4	21	25	4.81

M = Male F = Female

Table (2): The number of affected animals, distribution of caseous lymphadenitis lesions and prevalence rate of C. ovis among living sheep and goats.

Breed	A	Infected animal			Site of lesions and site of isolation of *C. ovis* from clinically infected cases																				C	D	% *
		B1	B2	B3	prescapular L. N			parotid L. N.			Retropharengeal L. N.			Supramammary L. N.			Superficial-inguinal L. N.			prefemoral L. N.			Submaxillary L. N					
					No Ex	No + Ve	%	No Ex	No + Ve	%*	No Ex	No + Ve	%	No Ex	No + Ve	%	No Ex	No + Ve	%	No Ex	No + Ve	%	No Ex	No + Ve	%			
Sheep	1252	62	23	85	30	5	5.88	50	10	11.76	12	2	2.35	7	1	1.18	5	1	1.18	7	1	1.18	6	-	0.00	117	20	17.09
Goats	520	16	9	25	11	1	4	16	2	8	5	-	0.00	-	-	-	-	-	-	2	-	0.00	2	-	0.00	36	3	8.33
Total	1772	78	32	32	41	6	5.45	66	12	10.91	17	2	1.82	7	1	0.91	5	1	0.91	9	1	0.91	8	-	0.00	153	23	15.03

A = No. of animals examined
B1 = No. of animals showed one enlarged lymph node
B2 = No. Of animals showed more than one enlarged lymph node
B3 = Total No. of clinically infected animals
C = Total number of lymph nodes examined
D = Number of positive isolation (No. of C. ovis isolates)
E = Percentage in success of isolation
* % percentage was calculated in relation to the total number of animals showing enlarged lymph nodes

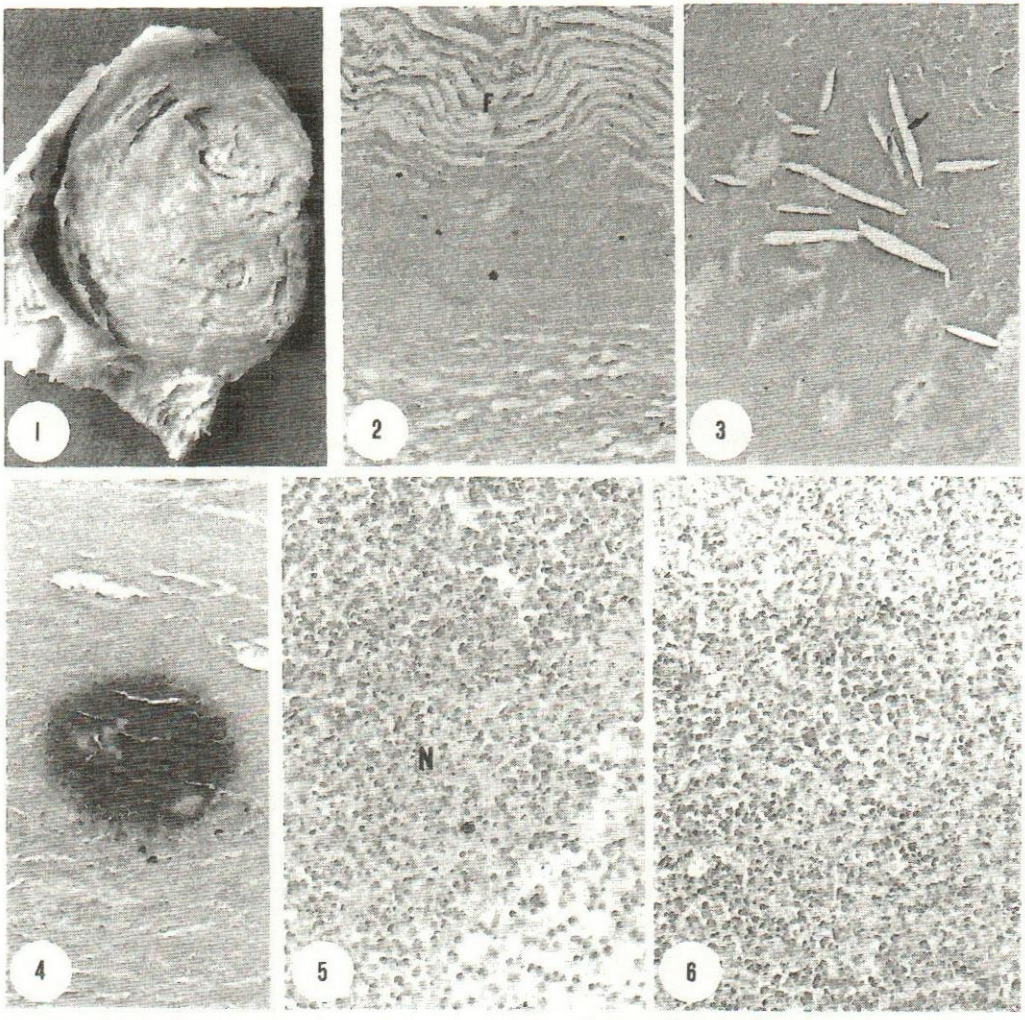
Table (3): Number of affected carcasses, distribution of CLA lesions and prevelance rate of C.ovis among slaughtered sheep and goats.

Cases	Slaughtered sheep			Slaughtered goats		
Number of carcases examined	295			88		
Number of caracses and percent showing CLA lesions	37 (12.54 %)			6 (6.82 %)		
Distribution of CLA lesions						
Superficial lymph nodes alone						
	No. Ex	No. + Ve	% *	No. Ex	No. + Ve	% *
Parotid lymph nodes	3	-	0.00	2	-	0.00
Prescapular lymph nodes	2	-	0.00	-	-	-
Total	5	-	0.00	2	-	0.00
Internal lymph nodes or organs alone						
Bronchial lymph nodes	5	1	2.70	-	-	-
Mediastinal lymph nodes	6	1	2.70	-	-	-
Lungs	11	3	8.11	3	-	0.00
Livers	3	1	2.70	-	-	-
Hepatic lymph nodes	1	1	2.70	-	-	-
Total	26	7	18.92	3	-	-
Superficial and / or internal lymph nodes or organs						
Retropharengeal lymph nodes	2	-	0.00	1	-	-
Mesenteric lymph node	2	-	0.00	-	-	-
Muscles (fleshy part of diaphragm)	2	-	0.00	-	-	-
Total	6	-	0.00	1	-	-
Total number of lymph nodes or organs examined	37			6		
Number of positive isolation	7			-		
Percentage in success of isolation	18.92			0.00		

%* Percentage was calculated in relation to the total number of animals showed pathological lesions suspected to be caseous lymphadenitis

Table (4): In vitro antimicrobial drug sensitivity of C. ovis isolated from sheep and goats.

Content / disc	Ovine strains [27]				Caprine strains [3]			
	Sensitive		Resistant		Sensitive		Resistant	
	No.	%	No.	%	No.	%	No.	%
Penicillin-G (10 I U)	27	100	0	0.00	3	100	0	0.00
Streptomycin (10 μg)	21	77.78	6	22.22	2	66.67	1	33.33
Nalidixic acid (30 μg)	0	0.00	27	100	0	0.00	3	100
Chloramphenicol (30 μg)	27	100	0	0.00	3	100	0	0.00
Colistin sulphate (10 μg)	0	0.00	27	100	0	0.00	3	100
Neomycin (10 μg)	11	40.74	16	59.26	1	33.33	2	66.67
Gentamycin (10 μg)	27	100	0	0.00	3	100	0	0.00
Cephalothin (10 μg)	13	48.15	14	51.85	0	0.00	3	100
Rifampicin (10 μg)	27	100	0	0.00	0	0.00	3	100
Trimethoprim sulphamethoxazol [1.25 + 23.75 μg]	15	55.56	12	44.44	2	66.67	1	33.33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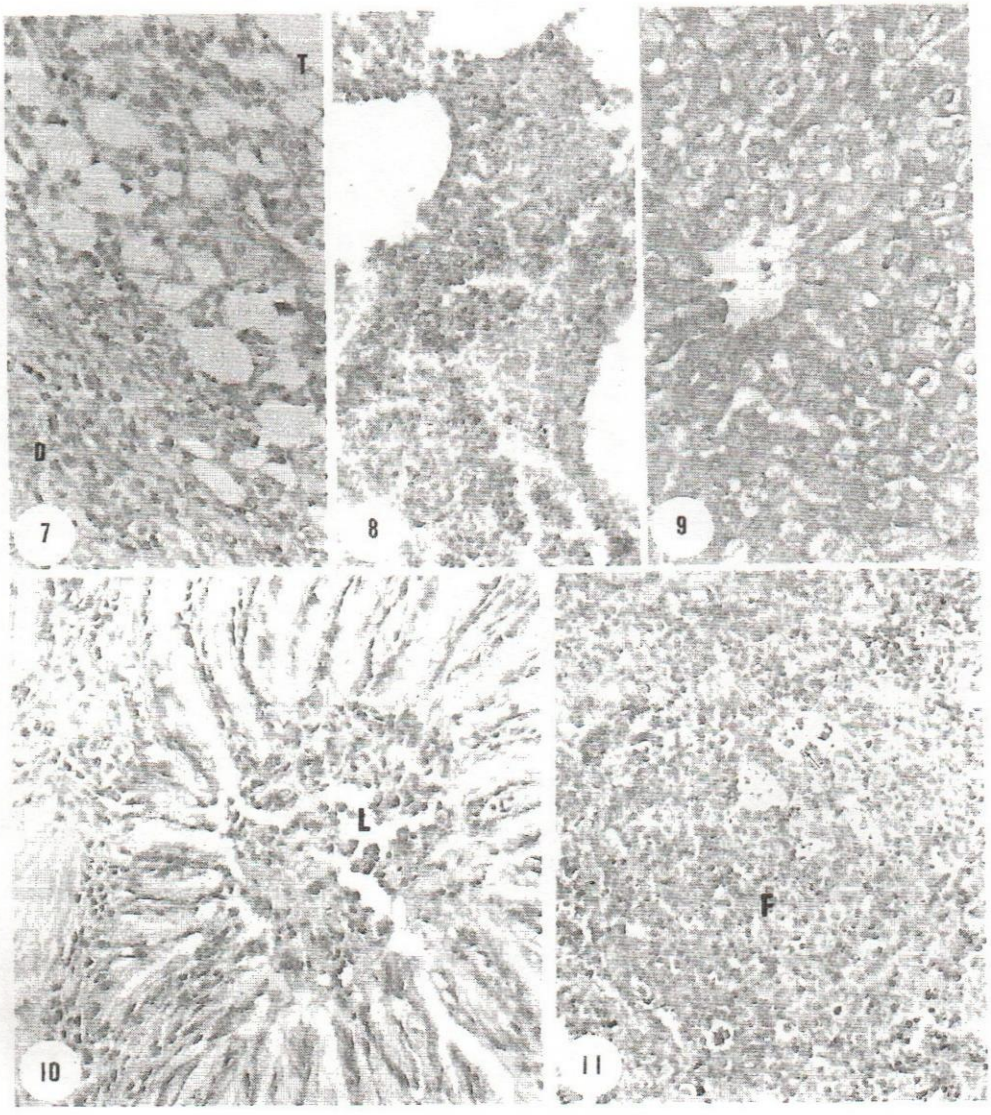
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