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SOME EPIDEMIOLOGICAL STUDIES ON OVINE PSEUDOTUBERCULOSIS

(With 3 Tables & 7 Fig.)

By

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بعض الدراسات الوبائيه على السل الكاذب في الأغنـــام

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خلال الفتره من يوليو الى ديسمبر ١٩٩٣ تم فحص عدد (٩) قطعـــان من الأغنـــام المحليـــه (متوسط القطيع = ١٢٣ ، مجموع الأغنام = ١١٠٧) بقرية بنى عدى بمحافظة أسيوط اكلينيكيا لمعرفة حالات الاصابه بالسل الكاذب . ووجد أن ١٧ ر ٣٠ ٪ من الأغنام التى تم فحصها كانت مصابه بالعلامات المرضيه المميزه لهذا المرض (تضخم فى الغدد الليمفاويه السطحيه). وكانت كلا من الغده الليمفاويه قبل عظمة اللوح (Superficial cervical) والغده الليمفاويه امام عظمة الفخـــذ(Subiliac) تمثلان أعلى نسبة أصابه على عكس الغده الليمفاويه النكافيه وخلف البلعوم الخارجيه (Parotid and Lateral retropharyngeal) كانت أقل اصابه . هذا ولم يشاهد أى اصابه فى الغدد الليمفاويه السطحيه الأخرى . وكان الجلد فوق هذه الغدد المصابه خالى من الصوف . هذا وقد ذكرت الفحوص الاكلينيكيه والباثولوجيه والهستوباثولوجيه . وقد تم عزل ميكروب السل الكاذب من كل العينات المجمعه . وعزل كلا من الميكروب العنقودى الذهبى مع ميكروبات سالبه لصبغة الجرام مع ميكروب السل الكاذب من ثلاث عينات . لم يكن هناك أى اختلافات معنويه بين الاناث أو الذكور المصابه . ووجد أن هناك ارتباط معنوى بين عمر الأغنام ومعدل الاصابه بالمرض وقد أظهرت معادلة الدرجه الرابعه ان معدل الاصابه بالسل الكاذب فى الأغنام يزداد بزيادة العمر حتى ٥٠ ر ٣٢ شهر ثم بعد ذلك يقل بالزياده فى العمر . سحجات او جروح الجز ربما تلعب دوراً محورياً فى انتشار المرض فى قطيع الغنم .

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SUMMARY

During period of July- December, 1993, nine flocks (n=1107- x= 123) of native breed sheep in Bani-Adi village, Assiut Governorate, were examined clinically for ovine pseudotuberculosis. 30.17% of the examined sheep showed the characteristic lesions of pseudotuberculosis (enlargement of the superficial lymph nodes). The superficial cervical (61.53%) and the subiliac (50.89%) lymph nodes showed the highest percentage of infection. In contrast, the parotid and lateral-retropharyngeal lymph nodes were less infected (5.24%). Occurrence of the lesions in the other superficial lymph nodes were not observed. The skin above the enlarged lymph nodes of diseased sheep was free from wool. Clinical, pathological and histopathological examinations were described. All collected lymph nodes samples yielded *Corynebacterium pseudotuberculosis*. *Staphylococcus aureus* coupled with Gram's negative bacteria were isolated from three lymph nodes in association with corynebacterium organism. There was no significant variation between infected females and males sheep. A significant correlation seems to be exist between age of the examined sheep and prevalence of the disease. The fourth-order polynomial [the coefficient of determination (R) was high ((98%)] showed that the morbidity percentage of ovine pseudotuberculosis increased with increasing the age till 32.05 months, thereafter decreased by further increasing in age of the animal. Shearing abrasions or wounds appeared to play a pivotal role in spreading of the disease among sheep flock.

Keywords: Epidemiology, ovine pseudotuberculosis.

INTRODUCTION

Ovine pseudotuberculosis (caseous lymphadenitis), chronic disease of sheep caused by *Corynebacterium pseudotuberculosis*, is considered one of the most important diseases of sheep flocks causing considerable economic losses in a variety of ways. These ways include unthriftiness and death of some sheep *JENSEN and SWIFT, 1982)*, partially or entire carcass condemnation particularly when multiabscessiation in

superficial and visceral lymph nodes were presented (STOOPS et al ., 1984), the wool production may be reduced (PATON et al., 1988) and decrease in the reproductive efficiency (ALONSO et al., 1992).

Ovine Pseudotuberculosis is characterized by caseous necrosis, mainly of superficial lymph nodes (external form) and occasionally of internal lymph nodes (visceral form). The later form was more serious causing emaciation and death of sheep (AUGUSTINE and RENSHAW, 1986). After external form (clinical form) of ovine pseudotuberculosis occurred, there may be instances that microorganism (Corynebacterium pseudotuberculosis) subsequently moves to the lungs and bronchial lymph nodes of the infected sheep by haematogenous and lymphogenous routes producing respiratory disorder (STOOPS et al., 1984). Abscessiation of the superficial lymph nodes (clinical form of pseudotuberculosis) of sheep were among the major causes of decrease in hide value (BROWN and OLANDER, 1987). Flaws in sheep skin can significantly lower the market value.

Shearing abrasions or wounds with tools contaminated with infected discharge of bursted lesions were considered the commonest mode of infection and transmission of the disease among sheep flock (JENSEN and SWIFT, 1982). Sheep may also gained the infection through ingestion (SEDDIK et al., 1983) or inhalation (STOOPS, et al., 1984).

Due to the insidious, endemic and often subclinical nature of ovine pseudotuberculosis, reliable information on the incidence and prevalence of the disease was scanty (BROWN and OLANDER, 1987). However, ovine pseudotuberculosis appears to be prevalent in Egypt (AWAD, 1960; NADIM et al., 1966; EL-ABDIN et al., 1976; MOTTELIB et al., 1976; and SEDDIK et al., 1983) but, apparently, little informations were available regarding the epidemiological aspect of the disease; like the age and sex susceptibility, and distribution of the lesions on the body of the infected sheep. Consequently, this study was carried out to provide some informations on the epidemiology of pseudotuberculosis in sheep at Bani-Adi village of Assiut Governorate.

MATERIAL and METHODS

Bani-Adi village is one of the biggest villages of Assiut Governorate located in Egyptian west desert. During period of July-December, 1993, 1107 head of native breed of sheep in Bani-Adi village were examined clinically for ovine pseudotuberculosis. This examinations included the palpation of the superficial lymph nodes; parotid and lateral

retropharyngeal, mandibular, superficial cervical, and subiliac (Fig. 1). The supramammary lymph nodes in ewes and the superficial inguinal nodes of rams were also palpated. The investigated sheep were bred in nine flocks (x=123). These sheep were grazed in open yard at day while at night they kept in unhygeinic barn. During the time of shearing (the first time of shearing usually occurred after five or six months of the first parturition), all respective sheep were dipped in water canal for clearing and ripening the wool fibers, and were thereafter dried* under the sun and clipped.

The examined sheep were in different ages and sex as given in table 1&2. Data on prevalence, sex distribution and existence of lesion characteristic (enlargement of the superficial lymph nodes) of ovine pseudotuberculosis were collected and tabulated. These collected data were thereafter analyzed statistically according to the methods described by *MILTON and TSOKOS (1983)* to determine the significant differences.

The following fourth-order polynomial *(BURDEN and FARIES, 1985)* was used to describe the relationship between the morbidity percentage of ovine pseudotuberculosis and age of the investigated sheep:

$$M(X) = A + Bx + Cx^2 + Dx^3 + Ex^4 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where M(X) is the polynomial value, x is the age per months, and A,B,C, and D, are the polynomoal coefficients. This polynomial was fitted to the morbidity percentage of the disease for estimating the polynomial coefficients.

The first derivative of the polynomial represents the response of the morbidity percentage to the age. Its value can be calculated from the following:

$$\frac{dM}{dX} = B + 2Cx + 3Dx^2 + 4Ex^3 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

when the derivative $\frac{dM}{dX}$ equal to zero, a maximum or minimum value of the morbidity percentage can be calculated. The maximum or minimum value depends on the second derivative of the polynomial (1). In this work, the maximum value was calculated.

Thirteen head of clinically diseased sheep were selected according to the severity of the characteristic lesions and subjected to bacteriological analysis. On the other side, three

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* Dryness of the dipped sheep usually incomplete to facilitate movement of the clipping tool among the wool.

enlarged lymph nodes (superficial cervical lymph nodes) of three affected cases were subjected to histopathological examination.

For bacteriological analysis, one of the enlarged lymph nodes of each selected case was surgically excision as a whole unit and transferred to the laboratory with minimum of delay. The obtained lymph node was incised with a sterile scalpel and the caseous material at the periphery of the lesion was picked up with bacteriological loop and streaked onto a petri dish containing brain heart infusion agar supplemented with 10% citrated horse blood. The streaked plates were incubated aerobically at 37°C for 48h. Bacterial isolates were identified morphologically and biochemically on the basis of criteria of *CARTER and CHENGAPPA (1991)*. Direct smears from ruptured lesions of some affected cases were also made and stained by Gram's stain.

For histopathological examinations, caseous material and adjacent tissue from the enlarged lymph nodes were incised. These specimens were fixed in 10% neutral buffered formaline, trimmed in paraffin and sectioned. The paraffin sectioned slides were stained with hematoxylin and eosin, and were thereafter examined.

RESULTS

Of 1107 clinically examined sheep, 334 (30. 17%) heads showed the characteristic lesions of ovine pseudotuberculosis. The characteristic findings of clinically diseased sheep were enlargement of lymph nodes (one or more lymph nodes were involved in each animal). The skin above the enlarged lymph node of clinically diseased sheep was free from wool (Fig. 5). By simple squeezing, some of these enlarged lymph nodes were easily ruptured discharging greenish coloration of pus like material. In other cases, the enlarged lymph nodes were ruptured spontaneously at the central point of the lesion discharging a similar color of pus like material into the wool. No systemic reactions were observed.

The obtained lymph nodes by surgical interference had characteristic appearance of ovine pseudotuberculosis when incised viz. multiple layers of caseous material surrounded by thick walls of connective tissues (laminated caseous necrosis) (Fig. 6). Histopathological examinations of the collected specimens revealed that the main lesion was chronic alterative lymphadenitis (Fig. 7) caseous necrosis which appeared as eosinophilic structurless mass contained basophilic remnants of nuclear fragmentation and probably bacterial colonies.

Inflammatory cells included lymphocytes and epithelioid cells were seen. Older lesions showed signs of encapsulation represented in fibroblastic proliferations. In chronic lesions concentric connective tissue rings separated the necrotic areas.

Bacteriological analysis revealed the existence of *Corynebacterium pseudotuberculosis* in all collected samples. *Staphylococcus aureus* coupled with Gram's negative bacteria was isolated from three samples in association with corynebacterium organism.

The sex distribution of the clinically diseased sheep (Table 2 and Fig. 2) revealed that the morbidity percentages in both females and males were 30.05 and 30.86, respectively.

On the other hand, the age distribution of clinically diseased sheep in this work was given in Table 1. A significant correlation presents between the age of infected sheep and the prevalence of the disease (Fig. 3). The fourth-order polynomial described well the morbidity percentage of the disease among the investigated sheep as a function of age. The coefficient of determination (R) was high (98%). The maximum morbidity percentage of the disease occurred at age of 32.05 months (Fig. 3).

Table No. 3 and Fig. 4 summarizes distribution of the enlarged lymph nodes of clinically infected sheep (334), where the superficial cervical (61.53%) and the subiliac (50.89%) lymph nodes were predominant lesions. In contrast, the parotid and lateral retropharyngeal lymph nodes were less affected (5.24%). Occurrence of the lesions in the mandibular, superficial inguinal and supramammary lymph nodes were not noticed.

DISCUSSION

Due to the insidious nature and subclinical form of ovine pseudotuberculosis *(STOOPS et al., 1984 and BROWN and OLANDER, 1987)*, some distortion of the actual prevalence of the disease is possible. However, the obtained results in this work may indicate a considerable morbidity rate of the clinical form of ovine pseudotuberculosis among the investigated sheep flocks in Bani-Adi village.

Enlargement of the superficial lymph nodes of the diseased sheep without systemic illness was the predominant observed clinical findings. The agreed with those previously reported by *JENSEN and SWIFT (1982); SEDDIK et al. (1983) and STOOPS et al. (1984)*. The characteristic wool-free skin areas above the

lesions of the diseased sheep can be explained on opinion of *CARTER and CHENGAPPA (1991)* who reported that *Corynebacterium pseudotuberculosis* organism liberated a relatively weak exotoxin responsible for dermonecrosis.

The observed gross and microscopic lesions of this study gave resemblance to those described in natural and experimental studies *(SCHECKMEISTER, 1956 and BROWN and OLANDER, 1987)*. On gross examination caseopurulent inspissiated putty like material were seen in the affected lymph nodes. In addition, diffuse chronic alterative lymphadenitis were noticed in the examined specimens. Such gross and microscopic alterations could be probably assumed to the soluble exotoxin produced by the organism and the surface lipid which is leukotoxic in nature *(JOLLY, 1966 and CAMERON and FULS, 1973)*.

There was no significant differences in the morbidity percentages of ovine pseudotuberculosis in both diseased females and males sheep ($P>0.05$). This may indicate that ovine pseudotuberculosis is non sex-linked disease.

A significant correlation seems to be exist between age of the examined sheep and the prevalence of the disease. The fourth-order polynomial showed that the values of the morbidity percentages of ovine pseudotuberculosis increased with increasing the age till 32.05 months, thereafter decreased by further increasing in age of the animal. The decreased morbidity percentages of the disease after the sheep reach three years of age may be attributed to a build-up of acquired immunological state. Acquired immunity after primary infection of ovine pseudotuberculosis was reported by *PEPIN et al. (1993)*. On the other side, the morbidity percentages of ovine pseudotuberculosis in the suckling and yearling lambs were low. This may be attributed to two reasons; the first, shearing of sheep (if shearing abrasions or wounds are of prime importance in infection) usually occurred in the 5^{th} month after the first parturition. The second, it was possible that lambs in the infected flocks had maternal antibodies but such lambs may not be infected. When lambs were separated from their ewes immediately after birth, maternal antibiodies would not be available *(LAAK, et al., 1992)*. However, *SEDDIK et al. (1983)* isolated *Corynebacterium pseudotuberculosis* from 20% of suckling lambs around six month of age.

Corynebaterium pseudotuberculosis bacteria on skin of the animal enter afferent lymphatic vessels through shearing abrasions or wounds and gradually moves into the regional lymph node under the influence of its exotoxin, where they continue to grow and multiply, and thereafter the characteristic lesion was developed *(JENSEN and SWIFT, 1982 and BROWN and OLANDER,*

1987). In sheep the superficial cervical and the subiliac lymph nodes were approximately received all superficial lymph flow particularly in the parts of wool shearing (shoulder regions, lateral sides of the thorax, dorsal back and lumber regions) where the afferent lymphatic vessels of the superficial cervical lymph nodes come from the skin of the caudal part of the head, external ears, neck, thoracic limb and from the skin of the dorsal and lateral sides of the thorax, cranial to a transverse line drawn through the seventh or eighth rib while the afferent lymphatic ducts of the subiliac lymph nodes received lymph flow from the skin and subcutis of the pelvic and cranial part of the thigh areas and from the skin and subcutis caudal to the transverse line drawn through the seventh or eighth rib, except from the caudoventral regions of the abdomen (Fig. 1) *(SAAR and GETTY, 1975).* This may interpret the high infection percentage of the superficial cervical and the subiliac lymph nodes of clinically diseased sheep rather than other superficial nodes. However, *SEDDIK* et al. *(198*) reported that the parotid lymph node was the one that showed the highest percentage of infection.

Due to inadequate prophylaxis, difficult treatment and inaccurate diagnostic technology, retention of clinically diseased sheep should be discouraged because they act as a reservoir of infection *(STOOPS* et al., *1984).* Complete drying of the sheep skin before shearing is urgently needed because probability of infection was enhanced if the skin was wet *(NARIN and ROBERTSON, 1974).*

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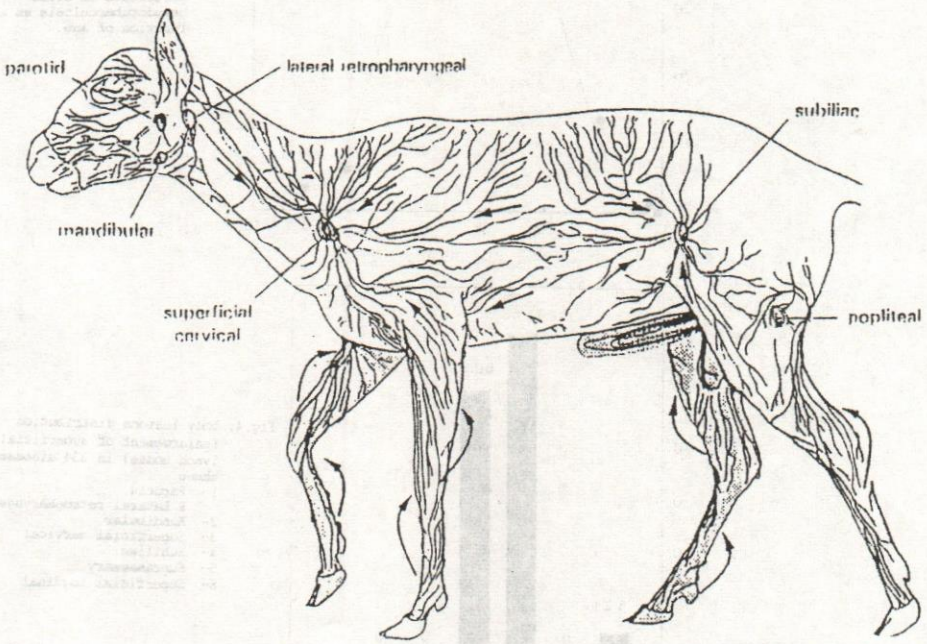

Fig.1: Superficial lymph nodes of sheep and their lymphatics (Saar and Getty, 1975)

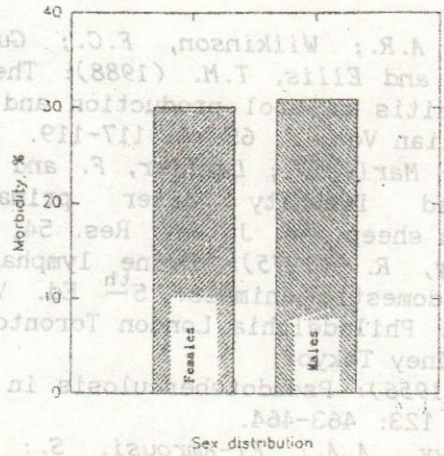

Fig.2: The prevalence of ovine pseudotuberculosis in females and males sheep.

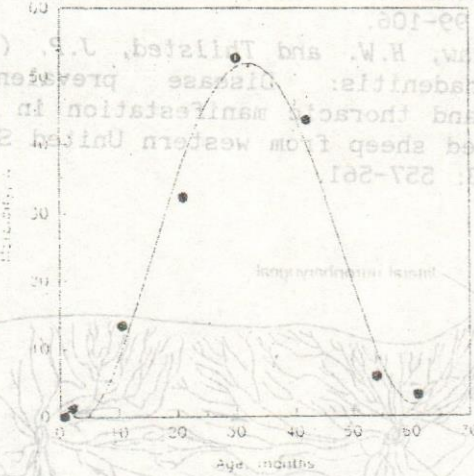

Fig.3: The observed (circles) calculated (solid line) values of the morbidity percentage of ovine pseudotuberculosis as a function of age.

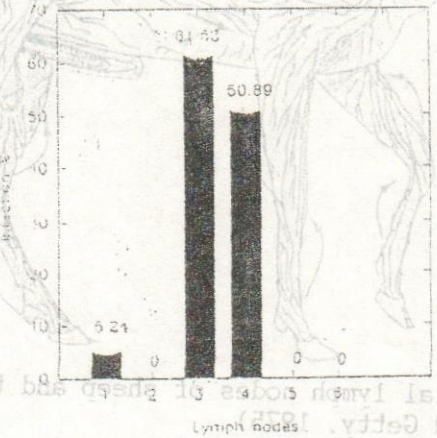

Fig.4: body lesions distribution (enlargement of superficial lymph nodes) in 334 diseased sheep.
1- Parotid & Lateral retropharyngeal
2- Mandibular
3- Superficial cervical
4- Subiliac
5- Supramammary
6- Superficial inguinal

Fig. 5 : Enlargement of the superficial cervical lymph nodes of the diseased sheep. Note: the skin above the enlarged node is free from wool.

Fig. 6 : Laminated caseous necrosis of the infected lymph node. Note: the onion appearance.

Fig. 7: Chronic alterative lymphadenitis H&E stain (X 250)

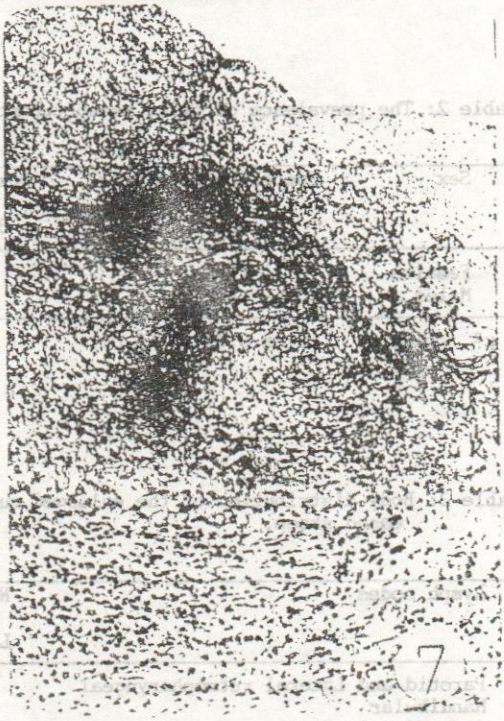

Table 1: The prevalence of ovine pseudotuberculosis in examined sheep of different ages.

Age	Number of examined Sheep	Number of clinically diseased sheep	(%)
7 - 30 days	80	0	0
> 30 - 90 days	78	1	1.28
> 3 - 18 months	112	15	13.39
> 18 - 24 months	186	60	32.25
> 2 - 3 years	269	142	52.78
> 3 - 4 years	250	109	43.60
> 4 - 5 years	100	6	6.00
> 5 years	32	1	3.12
Total	1107	334	30.17

Table 2: The prevalence of ovine pseudotuberculosis in examined females and males sheep.

Sex	Number of examined sheep	Number of clinically diseased sheep	(%)
Females	945	284	30.05
Males	162	50	30.86

x^2 females . males 0.04 (P >0.05)

Table 3: Body distribution of the enlarged superficial lymph nodes in 334 clinically diseased sheep.

Lymph nodes	Number of swelled Lymph nodes	(%) of infection
Parotid and Lateral retropharyngeal	35	5.24
Mandibular	0	0
Superficial cervical	411	61.53
Subiliac	340	50.89
Supramammary	0	0
Superficial inguinal	0	0