

قسم : طب الحيوان وامراض الدواجن - كلية الطب البيطرى - جامعة أسيوط .  
رئيس القسم : أ. د. / ابراهيم محمد حسن سكر .

دراسات تجريبية ومسح شامل عن مرض السل الكاذب  
فى الأغنام فى مصر العليا

جميل عزيز ، طه العلاوى ، ماهر زكى \* ، سيد العمروسى

تم فحص ٣٨٢٢ خراف فحصا اكلينيكيًا للغدد الذى أظهر وجود ١٦٧ خروف به  
تضخم فى هذه الغدد .

أما عن العدوى بواسطة الحقن بالميكروب تحت الجلد وفى الجلد فقد أمكن عزل  
الميكروب من هذه الأماكن فقط ولم يعزل فى حالة تجريح الجلد .

وفى حالة العدوى عن طريق الأنف أمكن عزل الميكروب من الرئتين لحيوان واحد  
بينما فى حالة الغدد وفى عن طريق الفم فقد أمكن عزل الميكروب من غدد الأمعاء .

وبالنسبة للعدوى عن طريق الوريد فقد ينتج عن ذلك وجود خواريج فى كسل  
من الطحال والكبد وغدد الرئتين .

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INCIDENCE AND EXPERIMENTAL STUDIES OF CASEOUS LYMPHADENITIS IN UPPER EGYPT.  
(WITH 2 TABLES)

BY

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(Received at 14/6/1981)

SUMMARY

Clinical examination of 3822 sheep revealed enlargement of lymph nodes in 167 heads (4.3%), higher percentage of infection in parotid gland (49.4%).

*Corynebacterium ovis* was isolated from one enlarged parotid lymph node of one sheep out of 13 slaughtered ones.

Experimental infection by subcutaneous and intradermal inoculation revealed isolation of *C. ovis* from site of inoculation only, it was not isolated in case of scarification method.

Interanasal infection resulted in isolation of *C. ovis* from lungs of one animal only while in case of oral infection, *C. ovis* was isolated from the mesenteric lymph nodes.

Intravenous infection resulted in abscess formation in spleen, liver and bronchial lymph nodes.

INTRODUCTION

Caseous lymphadenitis is a chronic disease of sheep and goat causing great economic losses in animals either for the affected animals or in wool production. The difficulty in early diagnosis makes it as a complicated problem. In Egypt, caseous lymphadenitis affects about 10% of sheep population (The academy of scientific research and technology, Egypt).

Concerning distribution of the disease in lymph nodes, MARCH (1958) reported that prescapular and precural lymph nodes of affected animals are most commonly affected, then mediastinalis, bronchialis and sublumbers. Finally all lymph nodes are affected. JONES (1961) stated that lesions were found in lungs and lymph nodes and also kidneys as well as other viscera are affected.

For the diagnosis of the disease, AWAD (1960) in Sudan used the agglutination test to investigate pseudo-tuberculosis infection in sheep and reported promising results. Other serological tests were tried by ZAKI (1968) and ZAKI and ABDEL HAMID (1974).

In the field of epizootiology of caseous lymphadenitis, CARNE (1932) described ingestion as a possible method of infection and the organism was recovered from ovine faeces.

With respect of experimental studies, many investigators tried the (I/V) inoculation. CARNE ET AL. (1972) used a dose of  $5 \times 10^8$  that resulted in abscess formation in lungs and kidneys and death of some sheep. Another concentration was tried by ADDE (1979) who used  $4 \times 10^8$  aiming to study the pathological lesions by (I/V) route.

The aim of the present study was designed to study the distribution of infection in lymph nodes of clinically infected and slaughtered sheep as well as experimental infection to clarify the aspects of the epizootiology of the disease in upper Egypt.

MATERIALS AND METHODS

Animals:

- a- Field cases: 3822 sheep between 6 months to 2 years in Assiut Governorate were examined clinically. Swabs were made from nostrils, hairless areas of skin surface and also from lesions (i.e) abscessed lymph nodes or any other suppurative lesions. Water samples of some pens were also collected.
- b- Slaughtered sheep: A total of 33 sheep (22 males and 9 females) were examined at Assiut abattoir for both anti and post mortem. Samples were collected for bacteriological examination from superficial and internal deeply lymph nodes that showed pathological lesions.

- c- Laboratory animals: Guinea pigs weighing 200-250 gms were used for carrying out the pathogenicity test.
- d- Sheep for experimental infection: 14 Oseemy sheep (1-3 years old) were obtained from a private farm, Assiut province, where history indicated no previous infection, were used for experimental infection. The animals were divided into 7 pairs.
- e- C. ovis culture for experimental infection: A virulent C. ovis broth culture was obtained from Central Laboratory of Animal Health and Research, Dokki, Cairo. It was standardized to contain  $10^{19}$ /Iml.\*

## II- Methods:

- a- Procedures adopted for sampling and culture: Swabs were taken from nasal, faecal matter, surface soil of some pens and also from inner thigh of both clinically normal and infected sheep. Bacteriological studies were carried out with special reference to specific methods of Coryne ovis isolation and identification. These were carried out according to CRUICKSHANK (1952).
- b- Experimental infection of sheep: 7 pairs of oseemy sheep were experimentally infected by a 48 hours virulent C. ovis broth culture (contained  $10^{19}$  organisms /Iml). The first 5 pairs were inoculated by 3 ccs subcutaneously, 3 ccs intradermal, 3 ccs intranasal, 3 ccs by scarification and 10 ccs by oral route. One animal of the 6th pair received (I.V) inoculation of C. ovis broth culture, the other received 50 ml broth culture. The last pair (7th) was left as a control, and inoculated with peptone water by different routes. A week later these groups were given a second identical dose by the same routes.

## RESULTS

### I- Field cases:

The clinical examination of 3822 sheep of different ages revealed enlargement of lymph nodes in 167 heads (4.3%). Higher percentage of infection was noticed in the parotid lymph nodes of 87 heads (49.4%). Cultures of swabs from affected lymph nodes resulted in isolation of C. ovis from 6 lymph nodes out of eight nodes (75%) (Table I). Other isolates as Strept. Spp. 37.7%, Staph. 42.3% and Coryne pyogenes 20%.

Coryne. ovis was not isolated from faeces, surface soil, nostrils, surface skin of apparently normal animals. It was only isolated from lymph node of those showing enlargement or swelling.

### II- Slaughtered sheep:

One enlarged prescapular, parotid and 2 submaxillary lymph nodes were detected before slaughter out of 33 cases examined (Table 2). Coryne. ovis was isolated from one enlarged parotid lymph node as well as from one of the two lungs which showed lesions.

### III- Experimental infection:

Subcutaneous and intradermal inoculation resulted in isolation of C. ovis only from inoculation site while it was not isolated from neither scarification site nor the adjacent lymph nodes which showed no abnormalitis. Intranasal inoculation resulted in isolation of C. ovis from the lungs of one case only. Oral dosing with smaller dose gave negative results while the bigger oral dose (50 ml) resulted in isolation of C. ovis from enlarged mesyentric lymph nodes but neither from faeces nor from intestine.

Intravenous inoculation resulted in abscess formation in spleen, lungs, liver and bronchial lymph nodes. This animal showed progressive emaciation.

## DISCUSSION

The clinical examination of 3822 sheep in this study revealed percentage of 4.3% of affected animals. Several authors reported that the main way of infection is through cutaneous wounds as a result of shearing process (SIGMUD, 1973 and JENSEN, 1974). However, in the present study it was observed that suckling lambs of months old were also found infected and C. ovis was isolated from lambs (20%) which have never been shorn before. Such observation was in agreement with that reported by MADDY (1953).

\* : Kindly provided by Prof. Dr. A.A. Barakat. Ani. Heal. Res. Inst. Dokki, Cairo.

The results of anti and postmortem examination of slaughtered sheep showed that apparently normal sheep may harbour the micro-organism as cultures made from lymph nodes and organs of 24 males and 9 females slaughtered sheep resulted of isolation of *C. ovis* from one lung and one parotid lymph node of male animal, while that of females did not reveal the presence of the organism. It seems that incidence of *C. ovis* infection should not depend only upon clinical examination of sheep as such infection may be overlooked during clinical examination. However, there are no reliable method of diagnosis of latent infection (AWAD, 1960, ZAKI, 1968; SHIGIDI, 1979 and BARAKAT, ET AL, 1979). In Cairo abattoir reported NADIM (1966) a percentage of 2.22% infected cases.

During examination of lymph nodes of slaughtered sheep, some of them were enlarged and contained pus which did not reveal *C. ovis* isolation. This may be due to presence of old lesions and the organisms in this case may be in declined phase. Similar interpretations were also discussed by WILSON and MILES (1978).

LOTFI ET AL, (1977) isolated *Pasteurella multocida* from lesions simulating caseous lymphadenitis in sheep. These observations were in agreement with our findings where *Staphylococci* were isolated from slaughtered sheep. *Streptococci*, *Coryne pyogenes* were also isolated from lymph nodes of clinically infected sheep.

Laboratory examination of various lymph nodes of sheep revealed that parotid lymph node showed highest percentage of infection, however, NADIM ET AL, (1966) found that bronchial and mediastinal lymph nodes showed the highest percentage of *C. ovis* infection from cases of slaughtered sheep.

Experimental infection in the present study was carried out by different methods. In scarification area, adjacent to right submaxillary lymph node, 200 days later the organism was not recovered from both scarified area or adjacent lymph node. Absence of infection by this route may consider the role of shearing as a way of infection is doubtful.

Experimental intradermal inoculation resulted in isolation of *C. ovis* only from site of inoculation where it caused a local suppurative focus. CARNE (1948) mentioned that natural infection caused initial suppuration of skin. This is in agreement with our results of experimental infection.

Nasal infection of sheep may cause a typical form of caseous lymphadenitis. BELONGE (1954) reported that bronch-pneumonia in a flock of sheep from which the same organism was isolated. In the present study, intranasal infection in 2 sheep resulted in isolation of *C. ovis* from lungs of one animal, the other animal showed lung lesions with absence of the organism. In our country inhalation of dust may constitute a principle way of infection. However, failure of isolation of the organism from nostrils of both clinically normal and infected sheep dwellings was observed.

Oral experimental infection was not succeeded in this study as it may be attributed to the lowered virulence of the organisms in the inoculum was below the potential number needed to initiate infection. However, isolation of *C. ovis* from faeces of both healthy and infected sheep was reported by BELSCHNER (1954).

In the second experimental oral infection where a bigger dose of *C. ovis* culture (50 ml) as given orally, the possibility of intestinal infection was greater than small dose as the isolation of organisms from mesenteric lymph nodes occurred 200 days post infection. BELSCHNER (1959) suggested that oral infection might be introduced through abrasions of the lips and gums or injury to intestinal wall.

The intravenous inoculation of sheep with a small dose (1 ml) resulted in abscess formation in parenchymatous organs as spleen, liver and lungs.

It appears that *C. ovis* might have a bacteraemic phase in which the organisms are disseminated to parenchymatous organs where they congregate and set up infection beginning with organs rich in lymphoid tissue since *C. ovis* has a lymphoid affinity. However, CAMERON (1972) reported that *C. ovis* culture in doses of  $2 \times 10^{7-10}$  seldom caused lesions where doses of  $10^{9-10}$  resulted in death of some animals within 3 days.

In the respect with infection of parotid lymph node, it is assumed that way of natural infection in upper Egypt is through ingestion as these nodes situated away from shearing areas. Furthermore, very important character of the disease is that infected sheep are deprived from their immunity system as lymph nodes are converted to abscesses. The outcome is not only depletion but also lowered immune response to vaccines.

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TABLE (2)  
Isolation of C. ovis from Lymph Nodes Clinically Normal  
Slaughtered Sheep.

Carcasses Examined	Lymph Nodes												Abscess EI sewhere	Lungs
	Pres.		Precr.		Parotid		Su b.mx		Super. Ingu.		Supra mammary			
	R	L	R	L	R	L	R	L	R	L	R	L		
Males (Number 24)	24	24	24	24	24	24	24	24	24	24	-	-	-----	48
<u>C. ovis</u> isol.	--	--	--	--	1	--	--	--	--	--	-	-	-----	1*
Females (Number 9)	9	9	9	9	9	9	9	9	-	-	9	9	-----	18
<u>C. ovis</u> isol.	-	--	--	--	--	--	--	--	--	--	-	-	-----	----
Total (Number 33)	33	33	33	33	33	33	33	33	24	24	9	9	-----	1*
<u>C. ovis</u> isol.	--	--	--	--	1	--	--	--	--	--	-	-	-----	----

Pres. = Prescapular.

Super. Ingu. = Superficial inguinal

Precr. = Precrural.

Sub. mx. = Submaxillary.

\* : Staphylococci were isolated.

Table 3 Clinical Manifestations and Lesions of Sheep Experimentally Infected with *C. Ovis*

Serial No.	Route of Infection	Clinical Manifestations		Period of Observation in days	P. K. Lesions	<i>C. Ovis</i> Isolation
		First Infection	Second Infection			
1	Subcutaneous	Abscess at site of inoculation in 7 days.	Abscess at site of infection in 7 days.	200	Slight haemorrhage in trachea and lungs. Small patches of congestion on left diaphragmatic lobe of lungs. Petichaeal haemorrhages on kidney.	+ ve site of in.
2	Subcutaneous	Abscess 7 days	Abscess 7 days	200	No pathological lesions were observed	+ ve site of in.
3	Intradermal	Abscess 6 days	Abscess 6 days	10 <sup>IX</sup>	Flabby heart and excess pericardial fluid.	+ ve site of in.
4	Intradermal	Abscess 6 days	Abscess 4 days	200	Slight haemorrhages in trachea and lungs. Congestion of both diaphragmatic lobes of lungs.	+ ve site of in.
5	Scarificat.	None	None	200	Right foot left ant. l. of lungs. Conges. inter. lobe. Small caseated nodules of mediastinal l. node. Liver fibrosis and petichae. Spleen slight congest. Bone marrow gelatinous. Abscess in right tibia.	+ ve lungs only
6	Scarificat.	None	None	200	Petichaeal haemorrhages of left lobes of lungs. Petichaeal haemorrhages kidneys. Small scattered necrotic foci in liver.	- ve
7	Intranasal	None	Loss of weight	200	Abscesses left diaphragmatic lobes of lungs. Metastatic of right interm. lobe of lungs. Small abscesses in right interm. lobe of lungs. Folded ribs. Calcified foci in liver bone marrow gelatinous in long bones.	- ve
8	Intranasal	None	None	9 <sup>IX</sup>	Right ant. lobe of lungs hepatized and numerous scattered abscesses inside lung tissue. Enlargement of mediastinal lymph node enlarged heart. Congestion of right and left ventricles.	+ ve lungs only
9	Oral	None	None	200	Large patches of congestion on both diaphragmatic lobes of lungs enlarged retropharyngeal lymph node. Small scattered foci in liver.	- ve
10	Oral	None	None	200	No pathological lesions were observed.	- ve
11	Oral* M.G. Dose	Abscession and abscessing foci	None	7 <sup>IX</sup>	Small abscesses in left apical lobe of lungs. One medium sized abscess in portal lymph node. Enlargement of all mesenteric lymph nodes.	+ ve mes. l. nod Staph. lungs
12	Intravenous	Abscession	None	200	Internal abscesses in spleen, lungs, liver and bronchial lymph nodes.	+ ve from abscess

10 c.c. 24 hr. *C. Ovis* cal. (10<sup>IX</sup> / c.c.) \* 50 c.c. *C. Ovis* cal. 10<sup>IX</sup> Death.