أقســـــام : البكتريولوجى ، طب الحيوان ـ كليتى الطب ، الطب البيطرى ـ جامعة أسيوط.
رؤساء الأقسام : أ.د / عماد نافع، أ.د / ابراهيم سكـــر.

بعض الدراسات عن السل الكاذب فى الخراف فى صعيد مصـــر

اسماعيل صـــديق ، عبد الخالق الطمـــاوى ، سيد العمروســـــــى
ماهر مختـــار ، طه المـــلاوى ، جميل عزيـــــــز

يعتبر مرض السل الكاذب مرضا خطيرا بالنسبة للأغنام والماعز. وقد وجد أنه يسبب خسائر فادحه لهذه الصناعات فى مصر.

على أنه لم ينكشف حتى الان طريقة يعتمد عليها فى دقه تشخيص المرض ولا طريقه عمليه لاحداث مناعة فعالة ضـده.

تم دراسة وبائيات هذا المرض فى صعيد مصر واشتملت الدراسة على الآتـــى :
تم فحص الاغنام بعشر قرى ومزرعة حكومية واحدة اكلينيكية لمشاهدة أعراض المرض والظروف المحيطـــه باحـداث الاصـابة.
كما تم فحص الاغنام بمجزر أسيوط قبل وبعد الذبح بنفس الغرض. وقد وجد أن الغدة اللمفـــاويه النكافية هى أكثر الغدد اصابة بالمرض والتى منها تم عزل الميكروب.
وقد وجد ايضا أن الحملان أقل من ستة أشهر مصابة بنفس المرض رغم أنه لم يتم جز صوفها من قبـل هذا مما القى بعضا من الشك فى دور الجروح الناتجه عن جز الصوف فى أحداث الاصابة كما كـــان معتقـدا من قبل أنها الطريق الرئيسى لاحداث الاصابة.
وهكذا فأنه يرجح أن أكثر الطرق احداثا للأصابة فى صعيد مصر هو تناول الميكروب المسبب للمـــرض عن طريق الفم. وقد اخذت عينات من سطح التربية من بعض مساكن الأغنام. وعينات براز وكـــذا عينات من سطح الجلد ووسائد قطنية من الانف من الأغنام السليمة ظاهريا وكذلك التى يظهر عليها أعراض المرض الا أنه لم يتم عزل الميكروب المسبب للمرض من كل هذه العينات والاماكن. وقد تم احراء العدوى الصناعية فى الأغنام بالطرق المختلفة لدراسة امكان احداث الاصابة باى منها بحقـــن الميكروب تحت الجلد وفى سمك اوديم الجلد امكن عزل الميكروب المسبب من مكان الحقن فقط ولـــم يتم عزله من الغدد اللمفاوية المجاورة. بحقن الميكروب بطريقة التشريط والتى تشابه الى حد كبير الجروح الناجمة عن جز الصوف لم يتم عزل الميكروب لا من مكان الحقن ولا من الغدد اللمفاويـــه المجاورة. باحداث العدوى الصناعية عن طريق الانف امكن عزل الميكروب المسبب من الرئه فى حاله واحدة بينما لم يتم عزلها من الحالة الثانية رغم أن الرئتين كانتا بحالة غير طبيعية. فشلت العدوى الصناعية عن طريق الفم بالجرعة الصغيرة فى احداث اى افات ولم يتم عزل الميكروب بينما فى حالـــة العدوى الصناعية بالفم بجرعة اكبر ، حدثت تضخم فى الغدد اللمفاوية المعوية وتم عزل الميكروب منها احدثت العدوى الصناعية عن طريق الحقن بالوريد خراريج فى الاعضاء الداخلية وامكن عزل الميكروب من كل هذه الافات التشريحية غير أنه لم يتحدث الاصابة المثالية التى تشاهد فى الغدد الأخـــرى فى الاصابة الطبيعية لهذا المرض.

Dept. of Microbiology,
Faculty of Medicine, Assiut University,
Head Prof. Dr. E.K. Naffie.

SOME STUDIES ON CASEOUS LYMPHADENITIS OF SHEEP IN UPPER EBYPT

(With 4 Tables)

By

I. SEDDIK, A.A. EL-TIMAWY, S. EL-AMROUSI*, M.M. ZAKI, T. EL-ALLAWY*
and G.K. AZIZ**

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SUMMARY

Caseous lymphadenitis is a serius disease of sheep and goats. Sheep from ten villages and one governmental farm in Assiut governorate were examined for clinical manifestation of the disease and the isolation of the causative organism. Sheep from abattoir were also examined before and after slaughter for the same purpose. It was found that the parotid lymph node was the one that showed the highest percentage of infection.Moreover lambs below six months of age were found to be infected, although they were never been shown before.Ingestion was found to be the most predominant route of infection in Upper Egypt.Samples from surface soil of some sheep dwellings, faeces, surface skin and nasal swabs, from both apparently normal and clinically infected sheep failed to yield the Causative organism on culture. By experimental infection it was found that both intradermal and S/C inoculation only yielded the organism from the inoculation site and failed to isolate the organism by scarification method. Oral dosing was negative to any lesions in case of small dose, However, a bigger dose resulted in enlargement of mesentric lymph nodes and from which the organism was isolated. Intravenous inoculation produced lesions in parenchymatous organs, from which the organism was isolated.

INTRODUCTION

Caseous lymphadenitis is an insidious chronic disease of sheep and goats, which is of considerable concern to animal breeding on a world wide basis.

The etiologic factor has for a long time been accepted to be Corynebacterium pseudotuberculosis (C.ovis) since it was isolated for the first time from diseased sheep.

In Egypt, caseous lymphadenitis was found to affect about 10% of the sheep population and would cause sever losses to sheep industry which was estimated to stand for ten million Egyptian pounds anually.

The causative organism, C.ovis was found to cause different disease syndromes in sheep, other than caseous lymphadenitis. In equines and bovines it causes ulcerative lymphangitis. In bovine; particularly the water buffalo, a severe disease syndrome was attributed to such infection to which the name "oedematous skin disease" was given locally.

The earliest isolation of C.ovis from sheep was done as early as 1891 by Preisz and Guinard. They isolated the organism; C.ovis for the first time from sheep and related it to caseous lymphadenitis. NOCARD and MOHLOR (1899) as well as CAREE and BIOGTEOU (1908) isolated the Preisz-Nocard bacillus from affected sheep. They stated that it was Gram-positive diphtheroid capable of tissue invasion and production of powerful exotoxin.

Several authors thereafter (DAY, 1928, WOODROFF and GREFORY, 1929; JEWET, 1930; HUNTER, 1933) isolated C. ovis from diseased sheep and gave an account of caseous lymphadenitis.

Concerning the lymph node distribution of caseous lymphadanitis, MARCH (1958) stated that the most commonly affected lymph nodes are prescapulars and precrurals. Thereafter, mediastinals, bronchials and sublumbers. Finally all nodes of body may be affected. SMITH and JONES (1961) gave the impression that lesions were formed in lung, and in lymph nodes, especially prescapular, prefermoral and mediastinal lymph nodes. JUBB and KONNEDY (1971) also stated that the superficial nodes are only affected namely the prescapulars and precrurals being mostly infected.

In lower Egypt, NADIM et al. (1966) pointed out that in slaughtered sheep bronchials (31%), mediastinals (21.5%), submaxillaries (17.5%) and prescapulars (17.5%) were mainly found infected on meat inspection.

* Dept. of Medicine & Poultry Diseases, Faculty of Vet. Med., Assiut University.
** Dept. of Animal health care, Assiut Regional.

In the experimental studies HAMID and ZAKI (1973) artificially infected goat by C.ovis culture by both scarification and subcutaneous routes to study their immune response. The animals showed no clinical symptoms during the time of the experiment. Others tried the i.v. route; CARNE (1973) used a dose of 5×10^8 with the result of abscess formation in lungs and kidneys and death of some sheep within three days. Other concentrations were used by ADDO (1979) which amounted 4×10^8 with the aim of studying the pathology and bacteriology of abortion in experimentally infected ewes using I/V route.

In this work the distribution of infection in the lymph nodes in clinically infected animals and slaughtered sheep was studied. Some experimental studies were also tried, in order to clarify some aspects of the epizootiology of the disease in Upper Egypt.

MATERIAL and METHODS

1- Field cases: A total of 3822 sheep (768 under 6 months, 626 from 6 months to 1 year, 698 from 1 to 2 years and 1730 over 2 years of age) from 10 villages and one governmental farm in Assiut Governorate were examined clinically. This examination included the palpation of the external superficial lymph nodes, prescapular, precrural, submaxillary, parotid, supramammary in females and superficial inguinal in males.

The following samples were collected from both clinically healthy and apparently infected sheep for labotory examination and diagnosis:

a- Swabs from nostrils.
b- Swabs from hairless areas of surface skin (inner thigh).
c- Faecal pellets were secured in plastic bags.
d- Surface soil of some pens (sheep dwellings) were collected in plastic bags.
e- Swabs from lesions (abscessed lymph nodes or any other suppurative lesions).

2- Slaughtered sheep: A total of 33 sheep (24 males & 9 feamles) were examined at Assiut abattoir by antimortem and postmortem and postmortem examination. The following samples were collected for bacteriological examination:

a- Superficial lymph nodes; prescapular, precrural, submaxillary, parotid, superficial inguimal in males and supramammary in females.
b- Internal deeply seated lymph nodes that showed pathological lesions.
c- Swabs from any lesion in any organ.

3- Sheep for experimental infection: Fourteen Osimi sheep 1 - 3 years of age from Assiut Province were selected and their history and source indicated no previous infection. These were divided into seven pairs and were used for experimental infection.

4- Laboratory animals for pathogenicity test: Guinea-pigs, each weighing 200 - 300 gram were used.

5- C.ovis culture for experimental infection: A virulent strain of C.ovis was obrained from the central laboratory of Animal Health Research Institute which was standardized in broth culture to contain 10^{19} per one milliltre.

6- Culture and test media used:

a- Serum broth.
b- Blood agar.
c- Peptone water.
d- Christensen's medium.

All these media were prepared and used according to CRUICKSHANK et al. (1975).

Procedures adopted for sampling and culturing:

I - Clinically normal sheep: The samples were taken as mentioned before.

II- Clinically infected sheep:

The wool in the vicinity of the lesion was closely clipped, the area cleaned and disinfected by 5% tincture iodine. The swabs, were taken from inside the lesion after being incised under complete aseptic conditions.

These swabs were inoculated into MacCarteny's bottles containing serum broth. These bottles were incubated

at 37°C for 48 hrs. and were subcultured on blood agar plates. Plates were then incubated for 24 to 48 hrs. and were checked for bacterial growth, and the bacterial growth were identified as follows:

1- Direct smears were made, Fixed, stained by Gram's method and were examined for morphology and staining reaction.

2- Cultural characters in serum broth and on blood agar were noted and studied.

3- The following biological tests were done:

a- Sugar fermentation of dextrose, sucrose, maltose, lactose and salicin.

b- Reaction on litmus milk.

c- Gelatin liquefaction.

d- Catalase test.

e- Urease test.

f- Pathogenicity test for guinea pigs by S/C inoculation in the thigh region.

III- Slaughtered sheep:

The lymph nodes or lesions were scared by a heated spatula and then opened under complete aseptic conditions and inoculated into MacCartney's bottles containing serum broth. Similar further procedures for culture, isolation and identification were carried out.

IV - Experimental infection of sheep:

The dose, route and site of inoculation of the seven pairs Osimi sheep are given in Table 1. The last Pair (No. 7) was left as a control and was inoculated by different routes with peptone water only.

RESULTS

The clinical examination of 3822 sheep of different ages and sexes revealed the enlargement of lymph nodes of 167 heads (4.3%) as shown in Table II. The percentage of clinically infected sheep, site of lesion and C.ovis isolation as well as other isolates were recorded qlso in Table II.

Table III revealed C.ovis isolation from lymph nodes of 33 clinically normal slaughtered sheep and site of isolation. The clinical manifestations and lesions of sheep experimentally infected with C.ovis were recorded in Table IV.

DISCUSSION

In this study C.ovis was isolated from 20% os suckling lambs around six months of age which have never been shown before. This result agreed with the finding of MADDY (1953), who stated the possibility of isolation of C.ovis from four months old lambs.

The results of pathological findings before and after slaughtering sheep in this study showed that apparently normal sheep may harbour the organism. Culture of lymph nodes of slaughtered sheep (24 males and 9 females) resulted in the isolation of C.ovis from one lung and one parotid lymph node of male individuals, while those of female did not reveal the presence of the organism. Consequently it can be stated safely that the actual incidence of C.ovis infection should not be based only on the clinical examination of sheep since hidden latent infection may be overlocked on such examination. This constitutes a risk of the dissemination of the disease among healthy sheep.

Up till now there are no reliable methods to reach an accurate diagnosis of latent infection, AWAD (1960), FARID and MAHMOUD (1960), ZAKI (1968), SHIGIDI (1979) and BARAKAT et al. (1980).

Isolation of Staph. aureus, Strept. pyogenes and C.pyogenes from lymph nodes of clinically infected sheep were recorded in our research also RENSHAW et al. (1979) described cases from which they isolated other pyogenic organisms (C.pyogenes, C.equi, Staph. aureus and Pseudomonas aeruginosa) in association with C.ovis.

Laboratory examination of various lymph nodes of sheep pointed out that the parotid lymph nodes showed the highest percentage of C.ovis infection. NADIM et al. (1966) found that the bronchial and mediastinal lymph nodes showed the highest percentage of C.ovis infection.

Experimental infection in this study was carried out by different methods, one of which by scarification of the skin in an area adjacent to the right submaxillary lymph nodes. After the lapse of 200 days the organism was not recovered from the site of injection or the adjacent lymph node, which showed no abnormalities. Absence of infection by this route may threw some doubt on the role of shearing as a primary cause or way through which the causative organism may be introduced into the body.

Experimental intradermal infection resulted in the isolation of C.ovis only from the site of inoculation where it formed only a local suppurative focus.

Intranasal infection in this study resulted in the isolation of C.ovis from the lungs in one case, while in the other lung involvement without C.ovis isolation was manifested. Oral infection entailed a relatively small dose of C.ovis culture (10 mls. of 10^{19}/ml.) which may explain the reason of failiure to recover the organism from faeces, gut, and mesentric lymph nodes. This may be attributed to the lowered virulence of the organism so they produce no lesions or that the number of organisms in the inoculum was below that needed to initiate infection. CAHNE (1932) stated that sheep experimentally infected by the oral route suffered from lesions which were confined to the lymph nodes draining the buccal cavity and that the organism was not recorded from caudal gut.

In the 2nd experimental oral route,where a bigger dose of C.ovis culture (50 mls) was given,the possibility of intestinal infection was greater than small dose. The causative organism was recovered from the enlarged mesentric lymph nodes 200 days post infection similar to that reported by BELSCHNER (1959). Intravenous experimental infection of sheep with small C.ovis dose (1 ml.) resulted in abscess formation in the internal parenchymatous organs such as spleen, liver and lung as well as bronchial lymph nodes. C.ovis was recovered on culturing the lesions produced. It appears therefore that caseous lymphadenitis might have a bacteraemic phase in which the organisms are desseminated to parenchymatous organs, were they colonize and set up infection.

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Table (I): Experimental infection of sheep by C.ovis culture

Group No	Route of inoculation	Dose given	Site of inoculation
1*	Subcutaneous	3 ml.	Area adjacent to right axilla.
2*	Intradermal	3 ml.	0.2 ml. doses in different sites adjacent to right submaxillary l.n.
3	Scarification	3 ml.	In clipped area adjaoent to right shoulder.
4	Intranasal	3 ml.	1.5 ml. in each nostril by a dropper.
5	Oral	10 ml.	Oral dosing.
6a	Intravenous	1 ml.	Into the right jugular vein.
6b	Big oral	50 ml.	Oral dosing.
7	Different	---	Only peptone water by different routes.

* A week later these groups received a second identical dose by the same route.

Table(II) Percentage of Clinically Infected Sheep and C. ovis Isolation

Locality	Examined Sheep			Clinically Infected			Percent Inf.	Site of Lesions												C. ovis Isolation												Other Isolates		
	Fem.	Male	Total	Fem.	Male	Total		Pres.		Prec.		Subm.		Parot.		Sup.I		Sup.m		Abse.		Pres.		Prec.		Subm.		Parot.		Sup.I.		Sup.m		Abse.	Strep-ptoc.	C. pyog.	St. hy.
								R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L				
Om El Koseur	168	112	280	4	1	5	1.8%	-	-	1	-	-	-	4	1	-	-	-	-	—	-	-	-	-	-	-	-	2	-	-	-	-	-	—	1	-	2
El Mahmoudia	90	23	113	2	2	4	3.5%	-	-	-	-	-	-	3	1	-	-	-	-	—	-	-	-	-	-	-	-	-	-	-	-	-	-	—	-	1	2
Beni Ghaleb	279	186	465	-	-	-	0.0%	-	-	-	-	-	-	-	-	-	-	-	-	—	-	-	-	-	-	-	-	-	-	-	-	-	-	—	-	-	-
Baiout	530	70	600	5	2	7	1.2%	1	2	1	-	1	-	5	2	-	-	-	-	—	-	-	-	1	-	-	-	1	-	-	-	-	-	—	3	2	4
El Wadia	240	20	260	-	-	-	0.0%	-	-	-	-	-	-	-	-	-	-	-	-	—	-	-	-	-	-	-	-	-	-	-	-	-	-	—	-	-	-
Sidfa	80	3	83	2	-	2	2.4%	1	-	-	1	-	-	1	-	-	-	-	-	—	-	-	-	-	-	-	-	1	-	-	-	-	-	—	-	-	1
Beir Tasa	388	28	416	4	1	5	1.2%	2	-	1	1	-	-	-	1	-	-	-	-	—	-	-	-	-	-	-	-	-	1	-	-	-	-	—	-	2	1
El Sheikh Eea	196	23	219	7	2	9	4.1%	4	-	1	1	-	-	4	2	-	-	-	-	—	-	-	-	-	-	-	-	-	1	-	-	-	-	—	-	2	1
El Mekhaila	250	23	273	3	-	3	1.1%	-	-	-	-	1	-	1	1	-	-	-	-	—	-	-	-	-	-	-	-	-	-	-	-	-	-	—	2	4	4
El Hawatka Village	300	88	388	4	1	5	1.3%	1	1	-	-	-	-	1	2	-	-	-	-	—	-	1	-	-	-	-	-	1	-	-	-	-	-	—	1	-	2
El Hawatka Farm	600	133	733	111	16	127	17.3%	34	2	6	8	3	2	20	38	2	1	6	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	—	12	-	-
Total	3121	701	3822	142	25	167	4.3%	43	5	10	11	5	2	39	48	2	1	6	5	3	-	1	1	-	-	-	-	5	1	-	-	-	-	—	19	9	17

Fem. =Female
Pres. =Prescapular lymph node
Prec. =Precrural lymph node
R =Right
L =Left

Subm. =Submaxillary lymph node
Parot. =Parotid lymph node
Abse. =Abscess.
Sup.I. =Superficial inguinal lymph node
Supr. m. =Supramammary lymph node.

Table (III)

Isolation of C.ovis from Lymph Nodes Clinically Normal Slaughterd Sheep

Carcasses Examined	Lymph Nodes												Abscess Elsewhere	Lungs
	Pres.		Precr.		Parotid		Sub.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
C.ovis isol.	--	--	--	--	1	--	--	--	--	--	-	-	----	1*
Females (Number 9)	9	9	9	9	9	9	9	9	--	--	9	9	----	18
C.ovis isol	--	--	--	--	--	--	--	--	--	--	-	-	----	--
Total (Number 33)	33	33	33	33	33	33	33	33	24	24	9	9	----	1*
C.ovis isol.	--	--	--	--	--	--	--	--	--	--	-	-	----	--

Pres. = Prescapular
Precr. = Precrural
Sub. mx. = Submaxillary
Super. Ingu. = Superficial inguimal
* Staphylococci were isolated

Table IV: Clinical Manifestations and Lesions of Sheep Experimentally Infected with C. ovis.

Serial No.	Route of Infection	Clinical Manifestations		Period of Observation in days	P. M. Lesions	C.ovis 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. Petichael 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 xx	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	Septic foci left ant. L. of lungs. Conges. inter. lobe. Small caseated nodules of mediastinal l. node. liver fibrosis and patichae. Spleen slight congest. Bone marrow gelatinous. Abscess in righ thigh.	+ ve lungs only
6	Scarificat.	None	None	200	Petichael haemorrhages of left lobes of lungs. Petichael haemorrhages kidneys. Small scattered necrotic foci in liver.	- ve
7	Intranasal	None	Loss of weight	200	Lesions left diphragmatic lobes of lungs. Hepatization of right intermed. lobe of lungs. Small abscesses in right interm. lobe of lungs. Calcified area of ribs. Calcified foci in liver. Bone marrow gelatinous in long bones.	- ve
8	Intranasal	None	None	9 xx	Right ant. lobe of lungs hepatised 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 z	None	None	200	Large patches of congestion on both diaphragmatic lobes of lungs. Enlarged reteropharyngeal lymph node. Small scattered foci in liver.	- ve
10	Oral z	None	None	200	No pahtological lesions were observed.	- ve
11	Oral @ Big dose	Emaceation and staggering gait	None	77 xx	Small abscesses in left apical lobes of lungs. One medium sized abscess in portal lymph node. Enlargement of all mesenteric lymph nodes.	+ ve mes.l.nod. Staph.lungs
12	Intravenous	Emaceation	None	200	Internal abscesses in spleen, lungs, liver and bronchial lymph nodes.	+ ve from abscess

z 10 c.c. 24 hrs. C.ovis cul. (10^{19}/c.c.) @ 50 c.c. C.ovis culture xx Death.