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BACTERIOLOGICAL STUDIES ON OVINE VISCERAL CASEOUS LYMPHADENITIS (PSEUDOTUBERCULOSIS)

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SUMMARY

The relationship between the visceral form of caseous lymphadenitis (pseudotuberculosis) and a chronic debilitating condition of emaciated mature sheep was investigated. Internal abscesses were dound during necropsy in 80 %. of these animals and *Corynebacterium pseudotuberculosis* was isolated from 87.5 % of the animals with internal abcesses. Other pyogenic bacteria including *A. pyogenes, R. equi, S. epidermidis, S. aureus* and *P. aeruginosa* were also isolated in association with *C. pseudotuberculosis. Moraxella spp.* was isolated in 37.5 % of the animals with internal abscesses. In some abscesses, *Moraxella spp* was the predominant microorganism isolated and in others, they were asociated with *C. pseudotuberculosis* . The results indicated that the visceral form of caseous lymphadenitis is either an important contributing factor to the development of debilitation and emaciation of sheep or that the debilitating condition and emaciation of sheep may be act as a predisposing factor for development of visceral caseous lymphadenitis. A skin test (allergin) prepared by sonicating *C. pseudotuberculosis* proved to be of limited value in detecting animals infected with visceral caseous lymphadenitis. Only 52.4 % of the animals with abscesses caused by *C. pseudotuberculosis* gave positive delayed type hypersensitivity skin test responses.

The susceptibility of most predominant isolates to various chemotherapeutic agents were briefly discussed.

INTRODUCTION

Corynebacterium pseudotuberculosis (C. ovis) is the aetiological agent of caseous lymphadenitis (CAL) which is a chronic disease of sheep

comprising a considerable concern to the sheep industry world wide (3, 4). The disease causes annual lossess of about $ 17 million in wool production to the Australian wool industry (18) and meat losses due to carcass condemenation. The disease is characterized by abscessation and suppurative infection of either or both lymph nodes (especially superficial lymph nodes such as prescapular, prefemoral and precrural) and visceral organs. Occasionally the disease becomes generalized and abscess formation occurs in many organs, including internal lymph nodes, lung, liver, kidney, brain and spinal cord (13, 15, 17). The visceral form of caseous lymphadenitis has been suggested as a factor involved in the occurrence of debilitating condition referred to as thin ewe syndrome (12). The caseous lymphadenitis lesion consist of a central mass of thick cheesy and sometimes dry greenish-white necrotic material surrounded by a connective tissue capsule (13, 14, 20), characteristically, *Corynebacterium pseudotuberculosis* is a pyogenic organism capable of tissue invasion and production of a filterable exotoxin (8, 9, 13). Contamination of superficial skin wounds (due to shearing, docking and castration) with discharges from ruptured lymph nodes, is considered the usual mode of transmission (5, 17, 20). Heavily encapsulated lesions result when the microorganism becomes established in a regional lymph node (15). Chemotherapeutic treatment is usually of no value at this stage of the disease, because the antimicrobial agent is unable to penetrate the heavily encapsulated lesions (5, 17). Although pseudotuberculosis lesions in the lungs may produce signs of respiratory tract infection, infected sheep may exhibit no specific clinical signs, other than the occasional presence of detectable superficial abscesses to suggest a diagnosis of generalized psuedotuberculosis. frequently, there are no specific clinical signs indicative of pseudotuberculosis in sheep infected with the visceral form of pseudotuberculosis (13, 16, 17, 22). So this investigation was done to clarify, explore this point as well as to detect the infected animal with visceral form of pseudotuberculosis via allergic skin test.

MATERIAL AND METHODS

Experimental animals :

A total of 30 mature sheep, aged from 2 to 7 year old, were obtained from both private and Governmental farms is Sharkia province. The infected sheep exhibited various degrees of superficial lymph nodes abscesses and emaciation. Examination of faecal material indicated that they were free of clinical problems caused by internal parasites.

Bacteriological examination :

A total number of 220 different samples from mature sheep were taken at postmortum inspection at an abattoir. Abscesses and other lymph nodes tissues from the infected sheep were collected in sterile containers. The surface of the tissues were heat sterilized and cultured within 4 hours of collection. the tissue was incised with a sterile scalpel and scissors, and purulent material at the periphery of the abscess

was transferred with a sterile swab to be cultured. Abscess material, incised tissue from different enlarged lymph nodes and caseated nodules from different viscera were examined by culturing on Columbia blood agar base (Difco) supplemented with 5 % defibrinated sheep blood. Duplicate plates were aerobically incubated at 37°C for 24-48 hours, and examined daily for detection of any growth, and were kept 5 days before discarding. Isolates were identified, using standard procedures for isolation, propagation and identification (6, 7, 8). Enlarged lymph nodes and caseated nodules on different organs were examined macroscopically as in Fig. (1, 2).

Skin allergic test:

a) Preparation of test reagents (allergin): Reagents consisting of sonicated bacteria were prepared from *C. pseudotuberculosis* and *C. pyogenes* cultures. Field isolated strains of the organisms were cultured onto 10 % sheep blood agar plates, then incubated aerobically at 37°C for 48 hours, and the bacterial colonies were rubbed by sterile glass rod with sterile saline solution. The bacterial suspension was examined for purity by Gram stained smear. The bacterial cells were collected by centrifugation at 4000 r.p.m. for 15 minutes and washed three times in sterile saline solution. the packed organisms were resuspended in sterile saline solution to adjust the optical density at opacity tube No. 5 on mcFarland's nephelometer (11,19). these cells were sonicated (Dismembrator Sonifier, Dynatech Fisher Scientific, NY, USA) at 70,000 V/m for 20 minutes at 4°C in an icebath. The resultant material was merthiolated (0.1 %) and the extract was routinely stored at 4°C until used.

b) Technique of test: The potential diagnostic value of the skin test was examined in 30 debilitating sheep suffering from emaciation and debilitation. Responses to intradermal inoculation with 0.2 ml of C. pyogenes allergin in the left axillary region was compared with that produced by intradermal inoculation of 0.2 ml of both sterile saline solution and C. pseudotuberculosis allergin in the in left axillary region was compared with that produced by intradermal inoculation of 0.2 ml of both sterile saline solution and *C. pseudotuberculosis* in the right axilla. Such inoculations were done by using sterile tuberculin syringes. Skin test responses were evaluated after 24 and 48 hours post inoculation. The diameter of the area of induration and swelling was measured with calipers and the results were recorded as it was recommended by (19). Susceptibility of the most predominant isolates to different chemotherapeutic agents was tested by the disc diffusion method according to (10).

RESULTS

The results obtained from skin testing with the sonicate preparations for delayed hypersensitivity to both *C. pseudotuberculosis* and A. pyogenes, necropsies and bacteriological examination of abscesses and lymph nodes tissues are summarized in tables (1&2). Internal abscesses were found during necropsy in 80 % (24/30) of emaciated and debilitating animals.

Table (1): Results of intradermal skin tests, necropsies, and bacteriological examinations of abscesses and lymph nodes from mature sheep.

Animal number	Age (Years)	Diameter of skin test response to allergin (cm)				Location of abscess and lymph nodes	Bacteria recovered from animals with and without internal abscesses
		C. ovis		*C. pyogenes*			**a) With internal abscesses**
		24h.	48h.	24h.	48h.		
1	7	-	-	1.9	1.0	Lung Bronchial lymph node	C. pseudotuberculosis, S. epidermidis C. pseudotuberculosis, M. bovis, Bacillus spp.
2	4	-	-	-	-	lung Bronchial lymph node Kidney Prescapular lymph node Greater omentum	C. pseudotuberculosis C. pseudotuberculosis C. pseudotuberculosis, Bacillus spp. P. aeruginosa, Bacillus spp.,C. pseudotuberculosis S. epidermidis M. bovis, S. aureus, S. epidermidis, Bacillus spp.
3	5	1.3	-	1.4	-	Bronchial lymph node	C. pseudotuberculosis
4	3	-	-	1.0	-	Lung	C. pseudotuberculosis
5	6	1.0	-	1.2	-	Trachea	C. pseudotuberculosis, R.equi
6	7	-	0.9	-	-	Bronchial lymph node mammary gland	C. pseudotuberculosis M. bovis, S. epidermidis, Bacillus spp.
7	6	-	-	-	-	Lung, Brochial L. nds and Popliteal L. nds	C. pseudotuberculosis
8	7	1.0	-	1.6	1.1	Lung Bronchial lymph node Liver	M. bovis, Bacillus spp. C. pseudotuberculosis (nothing recovered)
9	6	-	-	-	-	Bronchial lymph node	C. pseudotuberculosis
10	5	1.0	-	1.0	-	Lung	C. pseudotuberculosis
11	5	-	-	-	-	Bronchial lymph node	C. pseudotuberculosis, M. bovis, Bacillus spp.
12	7	0.9	-	1.0	-	Lung, prescapular L. nds.	C. pseudotuberculosis M. bovis
13	7	1.2	-	1.6	-	Lung\ prescapular lymph node	C. pseudotuberculosis C. pseudotuberculosis, M. bovis, Enterbacter spp., Bacillus spp.
14	4	-	-	-	-	Bronchial lymph node	C. pseudotuberculosis, Bacillus spp.
15	6	1.6	-	1.3	-	Bronchial lymph node	C. pseudotuberculosis, Bacillus spp.
16	6	-	-	-	-	Lung	C. pseudotuberculosis, S. epidermidis, S. aureus.
17	7	1.6	-	1.5	-	Lung, Bronchial L. nds	C. pseudotuberculosis
18	6	1.0	-	1.3	-	Bronchial lymph node	C. pseudotuberculosis
19	6	1.4	-	1.8	1.8	Diaphragm mammary gland	M. bovis, S. epidermidis, P. aeruginosa, Bacillus spp. Bacillus spp.
20	3	1.2	0.8	1.3	-	Lung	A. pyogenes
21	2	1.4	-	1.3	-	Lung	M.nonliquefaciens, S. epidermidis, Lactobacillus spp.Bacillus spp.
22	7	-	-	-	-	Bronchial L. nds, mesenteric L.nds	C. pseudotuberculosis, S. epidermidis, Bacillus spp.
23	5	1.1	-	1.0	-	Parotid L. nds, Prescapular L.nds	C. pseudotuberculosis, Bacillus spp.
24	4	1.1	-	-	-	Prescapular L. nds, Mediastinal L. nds.	C. pseudotuberculosis, S. aureus
							a) Without internal abscesses
25	6	1.4	-	1.1	0.9	Lung	**M.nonliquefaciens,M.bovis,P.aeruginosa,S.epidermidis,E. coli.**
26	2	1.0	-	1.0	-	Lung	**M . bovis, S.epidermidis, Bacillus spp.**
27	4	1.0	-	1.3	-	Lung	**Lactobacillus spp.**
28	3	1.3	-	-	-	Lung	**Bacillus spp.**
29	3	-	-	-	-	Lung	**Micrococcus spp.**
30	4	1.0	-	-	-	Lung	**Micrococcus spp.**

- = no skin test response
L.nds = Lymph nodes
A = Actinomyces
M = Moraxella
P. = Pseudomonas
R = Rhodococcus
S = Staphylococcus
C = Corynebacterium

Corynebactrium pseudotuberculosis was isolated from abscesses and other lymphoid tissues from 87.5 % (21/24) of the animals with internal abscesses. *Actinomyces pyogenes* was recovered from one animal only. A dual infection with *C. pseudotuberculosis* and R. equi was recovered from tracheal abscess in one infected sheep. In animals from which *C. pseudotuberculosis* was recovered, the abscesses were observed most frequently in the bronchial lymph node 61.9 % (13/21) and lung parenchyma 42.8 % (9/21). While, they were observed less frequently in popliteal, prescapular, mesenteric, trachea, greater omentum, kidney, mammary gland and liver. in a number of samples to be examined, C. pseudotuberculosis was the only bacterium isolated from abscessed tissue (Table 1). In other instances where more than one isolate was recovered, *C. pseudotuberculosis* was usually the predominant microorganism in mixed infection. In addition to the *Corynebacterium spp.* isolated from abscesses and other lymph node tissues of the infected sheep, other pyogenic bacteria such as Staphylococcus spp. and Pseudomonas spp. were recovered.

Staphylococcus epidermidis, S. aureus and *P.* aeruginosa were recovered in association with *C. pseudotuberculosis* from abscesses of 6, 2 and 1 animals respectively (Table 1). The percentage of *Moraxella spp.* was isolated in association with *C. pseudotuberculosis* 42.9 % (9/21) from of the animals. *Moraxella bovis* was isolated from abscesses in the different lymph nodes, diaphragm, greater omentum and mammary gland. Isolations of *C.*

Table (2): The total isolates of bacterial groups in samples of mature sheep with internal abscesses at post mortum inspection.

Bacterial groups	Total number of Samples (220)	
	No. of isolates	Percentage
Gram Positive Group:		
C. pseudotuberculosis	193	87.7
A. pyogenes	10	4.5
R. equi	9	4.0
S. aureus	28	12.7
S. epidermidis	65	29.5
Bacillus spp.	110	50.0
Total	415	188.6
Gram Negative Group:		
Moraxella bovis	46	20.9
Moraxella nonliquifaciens	9	4.0
P. aeruginosa	18	8.2
Lactobacillus spp.	9	4.0
Enterobacter spp.	9	4.0
Total	91	41.4

Table (3): Results of antibiotic sensitivity test to the most predominant representative bacterial isolates

Chemotherapeutic disc	C. pseudotuberculosis (20)			A. pyogenes (10)			S. aureus (20)		S. epidermidis (10)		M. bovis (20)			P. aeruginosa (15)	
	R	I	S	R	I	S	R	S	R	S	R	I	S	R	S
Ampicillin	13 (65)	2 (10)	5 (25)	6 (60)	1 (10)	3 (30)	12 (60)	8 (40)	6 (60)	4 (40)	19 (95)	1 (5)	0 (-)	15 (100)	0 (-)
Erythromycin	(5) (25)	5 (25	10 (50)	3 (30)	2 (20)	5 (50)	10 (50)	10 (50)	4 (40)	6 (60)	10 (50)	1 (5)	9 (45)	11 (73.3)	4 (26.7)
Tetracycline	10 (50	2 (10)	8 (40)	7 (70)	1 (10)	2 (20)	7 (35)	13 (65)	3 (30)	7 (70)	18 (90)	0 (-)	2 (10)	14 (93.3)	1 (6.7)
Gentamicin	0 (-)	0 (-)	20 (100)	0 (-)	0 (-)	10 (100)	2 (10)	18 (90)	1 (10)	9 (90)	0 (-)	6 (30)	14 (70)	10 (66.7)	5 (33.3)
Streptomycin	13 (65)	1 (5)	6 (30)	7 (70)	0 (-)	3 (30)	14 (70)	6 (30)	7 (70)	3 (30)	12 (60)	4 (20)	4 (20)	7 (46.7)	8 (53.3)
Chloramphenicol	10 (50)	0 (-)	10 (50)	8 (80)	1 (10)	1 (10)	8 (40)	12 (60)	4 (40)	6 (60)	10 (50)	0 (-)	10 (50)	15 (100)	0 (-)
Cephalothin	7 (35)	1 (5)	12 (60)	2 (20)	0 (-)	8 (80)	6 (30)	14 (70)	3 (30)	7 (70)	20 (100)	0 (-)	0 (-)	15 (100)	0 (-)
Nalidixic acid	3 (15)	6 (30)	11 (55)	2 (20)	3 (30)	5 (50)	8 (40)	12 (60	4 (40)	6 (60)	11 (55)	0 (-)	9 (45)	9 (60)	6 (40)
Flumequine	1 (5)	2 (10)	17 (85)	0 (-)	1 (10)	9 (90)	9 (45)	11 (55)	5 (50)	5 (50)	18 (90)	2 (10)	0 (-)	8 (53.3)	7 (46.7)
Trimethorpim Sulfamethoxazole	12 (60)	3 (15)	5 (25)	7 (70)	1 (10)	2 (20)	0 (-)	20 (100)	5 (50)	5 (50)	12 (60	0 (-)	8 (40)	14 (93.3)	1 (6.7)

Figures between parenthesis represent percentage value.
R : Resistant. I: Intermediate. S. Sensitive

Fig. (1): Submandibular enlarged lymph node of sheep infected with pseudotuberculosis.

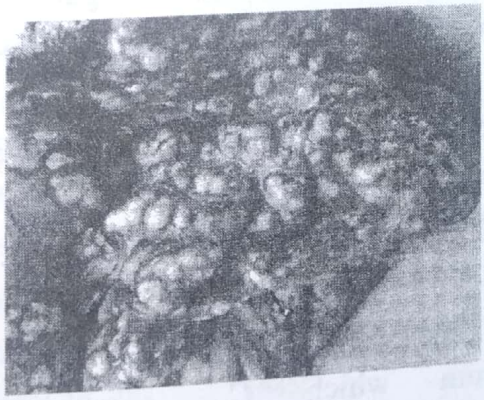

Fig. (2): Visceral form (caseated nodules) of *C. pseudotuberculosis* in lung of infected sheep.

*pseudotuberculosis*were not made from three sheep with internal abscesses. In these animals, the predominantly isolated microorganisms were *M. bovis, M. nonliquifaciens* and *A. pyogenes. Staphylococcus epidermidis* was isolated from two of these three animals. Thus from the 24 animals with internal abscesses, Moraxella spp. was isolated from 9 (37.5 %) and Staphylococcus spp. from 8 (33.3 %). *Staphylococcus epidermidis* and *Moraxella spp.* were isolated in large numbers from ecchymotic lung tissue of two animals without internal abscesses. Bacillus, Lactobacillus and Micrococcus spp. were isolated from abnormal lung tissue of the other four sheep, however, they were isolated in small numbers and were thought to be extraneous contaminants. The results which are listed in Table (2) revealed that the most predominant isolated pathogenic, Gram positive microorganisms were *C. pseudotuberculosis* (87.7%), S. epidermidis (29.5 %) S. aureus (12.7 %) and Gram negative bacteria were *M. bovis* (20.9 %) and *P. aeruginosa* (8.2 %).

The obtained data revealed that the skin allergic test by sonicating *C. pseudotuberculosis* was of limited value in detecting sheep infected with the visceral from of pseudotuberculosis due to *C. pseudotuberculosis*. All three sheep that had internal abscesses from which *C. pseudotuberculosis* was not isolated, responded to both C. pseudotuberculosis and A. pyogenes skin allergic test at 24 hours. Meanwhile 5 of 6 (83.3 %) of sheep without internal abscesses responded to *C. pseudotuberculosis* allergin; 3 of 6 (50 %) responded to the A. pyogenes and 3 of 6 (50 %) responded to both two allergins. The animals with internal abscesses from which *C. pseudotuberculosis* had been isolated (12/21) 57.1 % responded to the A. pyogenes allergin while only 11 (52.4 %) responded to the C. *pseudotuberculosis* allergin and 10 (47.6 %) of these animals were positive for both two allergins. More positive skin test responses were observed at 24 than at 48 hour

DISCUSSION

Results of the present study indicate that the visceral form of caseous lymphadenitis may be an important contributing factor to the development of a chronic debilitating condition of mature and this agrees with that obtained by (12). Internal abscesses were observed in 80 % of necropsied sheep and *C. pseudotuberculosis* was isolated from 87.5 % of the animals with internal abscesses. These agree with results obtained by many authors (19, 23). Several other microorganisms were isolated from abscesses. in most instances, they were isolated in association with *C. pseudotuberculosis*, but occasionally, *C. pseudotuberculosis* was not recovered. Isolation of pyogenic organisms such as *A. pyogenes, R. equi, S. epidermidis, S. aureus* and *P. aeruginosa* from abscesses, either in association or not in association with *C. pseudotuberculosis* was not unexpected. Similarly, isolation of various *Bacillus spp.* which were assumed to be of limited importance, could be expected. However, the isolation of several *Moraxella spp.* from the abscesses and from other abnormal tissues was some what surprising. These attributed to when visceral structures are involved with visceral form, the viability of the animal

may be compromised, especially in cases wher a significant portions of vital organs are involved. The debilitated animal may be more susceptible to other infective agents, development of some metabolic diseases and predation (23). Severe lung lesions diminish respiratory functional capacity, increase susceptibility to systemic disease, and limit the animal's ability to cope with environmental stresses. It has been reported that affected animals do not cope well with stress and when confronted with secondary organisms, the infection often overcomes their anti-infection defenses and death results (16). The visceral caseous lymphadenitis may contribute signifiantly as a cause of debilitation and death of sheep. So that these give an opinion exists about the economic importance of caseous lymphadenitis to the sheep industry (2, 4, 13, 16, 17, 19, 22). Also information about the economic losses resulting from condemnation of infected carcasses and parts of carcasses is available (13, 17). Dissemination of the infection from the respiratory system and superficial lymph nodes to other organs occurs (13, 17). In many animals infected with the visceral form of caseous lymphadenitis there are no specific clinical signs indicative of the disease. identification of generalized caseous lymphadenitis as the cause of emaciation is further complicated, because an accurate diagnostic technology to detect animals with infected abscesses has not been perfected.

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