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CASEATION IN LYMPH NODES OF SLAUGHTERED CATTLE WITH A SPECIAL REFERENCE TO BOVINE TUBERCULOSIS

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SUMMARY

Two hundred samples of caseated lymph nodes were collected aseptically from 167 cattle during routine meat inspection at Omdurman central abattoir.The samples were taken on suspecious of Bovine tuberculosis and were subjected to bacteriological investigations. The age of cattle , sites and locations of the caseated lymph nodes were determined during postmortem exmination .

Mycobacterium bovis was isolated from 11 (5.5 %) of the caseated lymph nodes .

The frequency distribution and locations of isolates of M.bovis includes 6 from the bronchial lymph nodes, 2 from the mediastinal lymph nodes and 3 from the retropharyngeal lymph nodes. With regard to the age of cattle; all isolates of M.bovis were from caseated lymph nodes of old cattle. Other Mycobacterium species involved in caseation of lymph nodes includes 18 (9%) M.farcinogenes, 3 (1.5 %) M.avium, 2 (1 %) M.fortuitum and 2 (1%) M.pheli.Isolation of Mycobacterium species is important in differential diagnosis of Bovine tuberculosis which is a significant zoonotic disease that can transmitted to humans by contamination of meat from infected animals and through aerosols .The role of meat inspection in control of Bovine tuberculosis is discussed .

INTRODUCTION

Caseation of the lymph nodes of cattle is encountered in many abattoirs in Sudan during the routine postmortem inspection. Such caseated lymph nodes lead to suspicious of Bovine tuberculosis, and may be judged as tuberculosis without identifying the causative agents. Caseous affections of the lymph nodes of cattle therefore must be thoroughly and properly investigated before making a final judgement as tuberculosis.

The objectives of the present study is to identify Mycobacterium bovis and other Mycobacterium species involved in the caseated lymph nodes and to discuss recovered results from the standpoint of the public health significance of M.bovis and the role of meat inspection in control of Bovine tuberculosis

MATERIALS AND METHODS

1- Postmortem examination of cattle:

A total of 200 samples of caseated lymph nodes were aseptically collected from 167 out of 3175 cattle during postmortem examination at Omdurman Central Abattior. Sites and positions of the caseated lymph nodes were determined. The samples were taken following the method described by Lepovetsky et al (1953).

Age of cattle was estimated according to the Sudanese meat inspection law (1974). The slaughtered cattle were divided into 2 age groups young cattle (under 2 years) and old cattle (over 2 years)

2- Bacteriological investigations:

2.1- Microscopial examination

Direct smear is made and left to dry from the caseated material taken from the periphery of the node.The film is fixed by heat to become ready for staining with the Ziehl Neelsen's stain.

2.2- Isolation on Löwenstein Jensen medium

Aseptic removal of the node was made by repeated searing of the fat capsules using a red hot spatula. The node was dipped in 95% ethyl alcohol then held in a flame until the alcohol was burned off. This procedure was repeated twice.

The caseated lymph node is incised by a sterile scalpel blade and the caseated material is exposed.

Primary culture On Löwenstein Jensen medium is made by inoculation of 4-5 grams of the caseated material in 10 ml of 4% NaoH within a sterile conical-capped tube. The mixture is shaken and allowed to stand at 37°C for 30 minutes. The mixture is centrifuged at 3000 r.p.m. for 30 minutes.

The supernatant fluid is poured off then 10 ml of sterile distilled water is added and mixed with the sediment. Again centrifugation is made and the supernatant is poured off. The sediment is inoculated on duplicate of screw capped bottles containing slopes of Löwenstein Jensen medium. The inoculated medium bottles were incubated at 37°C for 8 weeks.

The cultural and biochemical characters used in identification of acid fast bacteria used in current study were those detailed by Runyon et al (1974).

RESULTS

1-Postmortem Findings :

The allocations and numbers of the 200 caseated lymph nodes from the various meat inspection sites in the two age groups are shown in table (1)

2- Bacteriological findings:

2-1. Detection of Acid fast bacteria by the direct smear stained with Ziehl Neelsen's:

Twenty four caseated lymph nodes revealed acid fast bacteria out of 200 caseated L.n examined by direct smears stained with Ziehl Neelsen's stain (table 2) .

Table (1): Allocation and numbers of caseated L.Ns in the tow age groups of the slaughtered cattle.

The Age group	Caseate L.NS in the site / Numberof cattle affected	Head			Carcass						Pluck		Mesentery	Total of caseated L.NS
		SubMaxillary	InternalRetro pharyngeal	Parotid	Prescapular	Axillary	Precrural	External irguinal	Internal Irguinal	Internal iliac	Medi-stinal	Bronchial	Mesenteric	
Group (1) The young cattle	12	6 (40%)	1 (6.6%)	3 (20%)	5 (33.33%)	0 (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	15
Group (2) The old cattle	155	37 (20%)	31 (16.75%)	25 (13.5%)	34 (18.37)	1 (0.5%)	10 (5.4%)	17 (9.18%)	4 (2.16%)	1 (0.5)	11 (5.9)	13 (7.02%)	1 (0.5%)	185
Total	167	43	32	28	39	1	10	17	4	1	11	13	1	200

With regard to the age groups, acid fast bacteria were detected in two (13.33%) of 15 caseated lymph nodes in young cattle. Whereas acid fast bacteria were detected in 22 (11.89%) of 185 lymph nodes of the old cattle.

2-2. Isolation of acid fast bacteria by cultural method on Löweinstein Jensen medium

Thirty-six were isolated on Löweinstein Jensen medium from the 200 examined caseated lymph nodes.With regard to the age groups, two (13.33%) acid fact bacteria were isolated from 15 caseated lymph nodes in the young cattle (group1). Where as 34 (18.38%) acid fast bacteria were isolated from 185 caseated lymph nodes in old cattle (group 2).

Acid fast bacteria were obtained by the direct smear method and the cultural method on Löweinstein Jensen medium from 21 caseated lymph nodes.

Table (2) show comparison of the results of detecting and isolation of acid fast bacteria by the two methods.

Table (2) Comparison of the direct smear and cultural methods in detection site of acid fast bacteria.

Caseated lymph nodes	Number of caseated lymph nodes	Acid –fast bacteria by direct smear (microscopically)	Isolation on L. J. (culture media)
Submaxillary	43 (21.5%)	4 (2%)	6 (3%)
Internal retrophryngeal	32 (16%)	4 (2%)	5 (3.5%)
Parotid	28 (14%)	0 (0.0%)	4 (2%)
Prescapular	39 (19.5%)	4 (2%)	4 (2%)
External inguinal	17 (8.5%)	0 (0.0%)	2 (1%)
Precrural	10 (5%)	1 (0.5%)	3 (1.5%)
Internal inguinal	4 (2%)	1(0.5%)	0 (0.0%)
Axillary	1 (0.5%)	1(0.5%)	1 (0.5%)
Internal iliac	1 (0.5%)	1(0.5%)	1(0.5%)
Bronchial	13 (6.5%)	3 (1.5%)	7 (3.5%)
Mediastinal	11 (5.5%)	5 (2.5%)	3 (1.5%)
Mesentric	1 (0.5%)	0 (0.0%)	0 (0.0%)
Total	200	24 (12%)	36 (18%)

Regarding the prevalence of isolated Mycobacteria from examined caseated L.n which proved to be tuberculous (36 L.n). which proved to be tuberculous (36 L.n). M. bovis was detected in 11(30.56%),

Table (3) the frequency of isolation of the species of Mycobacterium species from the various caseated lymph nodes

n = 36

Isolated bacteria / *Lymph nodes*	*Mycobacterium. bovis*	*Mycobacterium. farinogenes*	*Mycobacterium avium*	*Mycobacterium fortuitum*	*Mycobacterium phlei*
Submaxillary	0 (0.0%)	3 (16.6%)	1 (33.3%)	1(50%)	1(50%)
Retropharyngeal	3 (27.27%)	1(5.5%)	1 (33.3%)	0	0
Parotid	0 (0.0%)	4 (22.2%)	0	0	0
Prescapular	0 (0.0%)	4 (22.2%)	0	0	0
Ex-inguinal	0 (0.0%)	1(5.5%)	0	0	1(50%)
Precrural	0 (0.0%)	1(5.5%)	1 (33.3%)	1(50%)	0
Axillary	0 (0.0%)	1(5.5%)	0	0	0
Int.iliac	0 (0.0%)	1(5.5%)	0	0	0
Bronchial	6 (54.54%)	1(5.5%)	0	0	0
Mediastinal	2 (18.18%)	1(5.5%)	0	0	0
Total	11 (30.56%)	18 (50%)	3 (8.33%)	2 (5.56%)	2 (5.56%)

DISCUSSION

Caseation was recorded in 12 different positions of lymph nodes of old cattle, compared with 4 different positions in young cattle .This show a wider exposure potential to infection of old cattle than the young ones.

The high incidence of caseation of the lymph nodes of the heads 103 (55.69 %)out of 200 examined L.n. indicated that they are more exposed to infection (table 1).

Acid fast bacteria were detected in 12% of the examined samples by the direct smear method. Meanwhile using the cultural method on Löweinstein Jensen medium it was possible to isolate acid fast bacteria from 18% of the same samples. This recorded indicated that negative results of acid fast bacteria by the direct smear

method could be positive by the cultural method. Such findings was in conformity with the comparative achieved results by Murphy et al (1938).

In the present study three of the samples showed acid fast bacteria with the direct smear however not on Löweinstein Jensen medium. This could be attributed to either death of the acid fast bacteria during decontamination process or due to the growth of inhibtor acid fast bacteria.

Acid fast bacteria were isolated from 10 (41.67%) of 24 caseated lymph nodes in 13 plucks, 11 (15.28%) of 72 caseated lymph nodes in 61 carcasses, and from 15 (14.56%) of 103 caseated lymph nodes in 93 bovine heads. The caseated lymph nodes of the plucks represented the highest percentage of isolation in comparison to other sites. Such findings suggested that the main route of entrance of the acid fast bacteria that cause caseation of lymph nodes in cattle is the respiratory tract.

Mycobacterium bovis was isolated from 11 (5.5%) of the caseated lymph nodes. The frequency distribution of *M.bovis* was 6 from the bronchial, 3 from the retropharyngeal and 2 from the mediastinal lymph nodes. The thoracic and pulmonary lymph nodes were the most commonly affected sites as stated by several authors, (Keller 1941, Lepper and Pearson 1973, Pritchard et al 1975, Cheneau and Blancu 1976, Mcilroy 1986, Olivera et al 1986 and Whipple et al 1996). Hagan and Bruner (1961) stated that the lymph nodes of the bronchial, mediastinal and retropharyngeal exhibited lesions of tuberculosis more than the lungs and that the mesentric lymph node is less often involved.

Alhaji (1976) stated that cattle were much more susceptable to tuberculosis infection by the aerogenous route and that 80-90% of all tuberculous cattle were infected by the aerogenous route.

Isolation of *M.bovis* from the caseated lymph nodes of the bronchial, retropharyngeal and mediastinal was in conformity with the above references.

In the present study *M.bovis* was not isolated from any of the caseated lymph nodes of the carcass site. This indicated that M.bovis infection rarely occurred through the skin route. Thewes (1939) reported 1% involvement of lymph nodes of the musculature in generalized bovine tuberculosis.

The incidence of bovine tuberculosis was found to be 4.77% of the slaughtered cattle with caseated lymph nodes in Omdurman central abattoir. With regard to age, all strains of *M.bovis* isolated, were from the caseated lymph nodes of the old cattle. Such finding was in agreement with Francis (1958) who reported that the incidence of bo-

vine tuberculosis in meat inspection plants in Europe in beef cattle to be zero at birth and increased steadily to 35% at the age of 7 years.

Pritchard (1988) stated that Mycobacterium species other than *M.bovis* may be isolated from the bovine tissue since members of this genus are widely distributed in the environment. Some of these species occasionally produce lesions in cattle, whereas the presence of others may be incidental. Pallaske (1935) mentioned that the type of bacilli present in different tuberculous organs of cattle can't be determined by macroscopic examination of the lesion. Barton (1972) stated that histopathological examined differentiate bovine tuberculosis from other infections of Mycobacterium species. These statements showed that the caseation produced by these Mycobacterium species may false tuberculosis and that the bacteriological examination is necessary to determine the etiological agents.

Mycobacterium farcinogenes was isolated from 18 (9%) of the caseated lymph nodes. The frequency distribution of *M.farcinogenes* was in 3 submaxillary, 4 parotid, 4 prescapular, and one in each of the retropharngeal, the external inguinal, the bronchial, the mediastinal, the precural, the axillary and the internal iliac lymph nodes.

M.farcinogeres was also considered when isolated from caseation of the bronchial and the mediastinal lymph nodes. Awad (1958) noticed the close resembelance of the pulmonery form of bovine farcy and tuberculosis.

Isolation of *M.farcinogenes* in the present study is in conformity with the reports of several authors, (Awad and Mustafa 1974, Salih et al 1978, and Hamid et al 1991).

In the present study three strains of Mycobacterium avium, two strains of *M.fortuitum* and two strains of *M.pheli* were isolated from the caseated lymph nodes.

Runyon et al, (1974) stated that *M.avium* is less frequently to be found in lesions of the lymph nodes of cattle, whereas *M.fortuitum* was isolated from the lymph glands of cattle and that *M.pheli* was isolated from hay and grasses and that these organisms are present in soil. In the present study isolation of *M.avium* , *M.fortuitum* and *M.pheli* was attributed to ingestion and or to contamination of wounds with soil.

It is concluded that a bacteriological investigation is of value as supplementary test to assist judgment of caseated lymph nodes suspected to be Bovine tuberculosis.

M.bovis is classified as a risk group 3 pathogen and can infect humans so constitutes a public health problem .In this respect meat inspection

provides safe wholesome meat to the Public and protect the community from infection with *M.bovis*. In control and eradication programs of Bovine tuberculosis, the routine postmortem meat inspection is one of the main elements used. As a result of eradication of Bovine tuberculosis from cattle, the chances of human infection by *M.bovis* have been reduced in most countries.

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