دراسة عن الحالة الصحية للألبان فى مدينة أسيوط
« مدى انتشار ميكروب السل البقرى وطريقة حديثة لتصنيفه »

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يشمل هذا البحث فحص ٨٥ عينة لبن مجمعة من محال بيع الألبان وكذلك الباعة الجائلين بمدينة أسيوط لمعرفة مدى انتشار ميكروب السل البقرى فى اللبن ومدى خطورته على الصحة العامة للمستهلكين وصنفت الميكروبات المعزولة باستخدام اختبارات عديدة .

وأثبتت النتائج أن هناك عينة ايجابية .

وبهذا يتعين مدى خطورة اللبن كمصدر لميكروب السل للمستهلكين اذا لم يتعرض البائعين للكشف الدورى كذلك محلات البيع يجب أن تكون مطابقة للمواصفات الصحية المناط عليها بقرارات وزارة الصحة .

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SANITARY CONDITION OF MARKET MILK IN ASSIUT CITY. INCIDENCE OF MYCOBACTERIUM BOVIS AND A MODIFIED SCHEME FOR TYPING

(with one Figure and 7 Tables)

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SUMMARY

The role played by market milk as a vehicle in transmitting Mycobacterium bovis to man was denoted by the bacteriological investigation of 85 samples collected from dairy shops and street venders in Assiut City.

The means of identification and classification used were chiefly thiosemicarbazone sensitivity, sodium salicylate susceptibility, photochromogenicity test, catalase activity at the three different temperatures, Peknice and Asmus — Gorschagen's niacin test, indirect virtanen — Gries test, and nicotinamidase test.

The results obtained from this piece of work revealed that 13 out of 85 milk samples examined (15.3%) were culturaly positive. of these, one (1.2%) was found to be Mycobaterium bovis, 6 (7.06%) scotochromogens, one (1.2%) non-photochromegens and 5 (5.9%) as rapid growers.

INTRODUCTION

The bovine type tubercle bacillus constitutes a major proplem in the epidemiology of tuberculosis among human beings. The localization of the lesions and character of the disease depend greatly upon the manner by which organism gain enterance to the human body.

Regardless of the anatomic situation of the resultant disease, milk and its products are considered the most likely source of infection of bovine tuberculosis in man.

The contamination of milk with tubercle bacilli was proved as early as 1893 by MONTIFUSKO in Naples. In 1907, BOUEGGE in Schleswig-Holstein, proved the presence of tubercle bacilli in 35 out of 258 bulk

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milk samples (13.5%), and in 32 out of 597 individual milk samples (5.2%). MOHLER (1911) showed that out of 73 samples of examined, 3% only contained tubercle bacilli. TONNEY *et al.* (1927) complied data from European cities stating that of 16, 700 samples market milk examined, 8.66% contained tubercle bacilli. ROUSHDY (1948), likewise, succeeded in isolating the bovine tubercle bacilli from two (1.5%) out of 156 samples collected from shops and street venders in Cairo city. HOFMANN*et al.* (1957) could also isolate the tubercle bacilli in 48% of the unboiled milk samples in a town in Oberfals Germany.

Because of the epidemiological importance of bovine tuberculosis and its bearing on the public health, and as milk is one of the most important sources of infection, the authors had made an attempt in the present work to denots an additional evidence on the role played by market milk in transmitting the disease to man.

MATERIAL AND METHODS

Source of samples :

85 milk samples were collected at random from Assiut City, of which 50 were taken from dairy shops and 35 were from street venders. Each sample was collected in a sterile Mac Cartney bottle (One ounce capacity) and laboratory examination was done as soon as possible.

Laboratory investigation :

The laboratory investigation entailed the following :

I — Isolation :

Modified Petroff's method was used for the treatment of samples. Each sample was firstly centrifuged at 3000 r.p.m. for 30 minutes. The resultant sediment as well as the cream layer were mixed thoroughly with an equal volume of 6% hydrochloric acid and incubated at 37°C for 15-30 minutes. The mixture was then centrifuged at 300 r.p.m. for 15-30 minutes and the supernatant fluid was decanted. The deposit was then neutralized with 4% sodium hydroxide solution, using phenol red as indicator. The neutralized sediment was drawn up and down in a sterile Pasteur pipette and was evenly distributed onto four slopes of modified Lowenstein-Jensen medium, two with glycerine while the other without glycerine. The inoculated bottles were then placed in a horizontal position and left overnight at room temperature to assure even distribution of the sediment on the entire surface of the slope media. The cultured slope media were incubated in an upright position for 8

weeks at 37°C. Each cultured medium was examined daily for 7 days and once a week thereafter. The positive cultures were examined microscopically using Ziehl-Neelsen's stain.

II — Identification of the isolated strains :

In this work, a selection of invitro methods for grouping and typing of the isolated strains were carried out. These methods were conveniently grouped under the following headings :

A. Culture screening test

1 — Speed of growth and its character.

2 — Drug sensitivity test .

Thio-semicarbazone sensitivity test as described by MARKS *et al.* (1960) for differentiation of the mammalian type of tubercle bacilli and photochromogens from other anonymous mycobacteria was carried out.

3 — Sodium salicylate susceptibility test :

The technique described by TSUKAMURA (1962) for the differentiation of Mycobacterium tuberculosis from other mycobacteria by sodium salicylate susceptibility test was used.

4 — Photochromogenicity test :

The varying ability of some of the isolated strains to produce pigment as described by TIMPE and RUNYON (1954) and RUNYON (1955) was done.

B. Cytochemical reaction tests :

1 — Catalase activity test :

The catalase activity test at room temperature as described by MIDDLEBROOK (1954) as well as at several temperatures as mentioned by KUBICA *et al.* (1960) were applied.

2 — Niacin test :

PEKNICE and ASMUS GARSCHAGEN's niacin test as described by ASMUS and GARSCHAGEN'S (1953), PEKNICE (1950), BONICKE *et al.* (1958) and JUHLIN (1960) for the differentiation of Mycobacterium tuberculosis other mycobacteria was carried out.

3 — Nitrate reduction test :

Indirect Virtanen-Gries test as described by SULA *et al.* (1963) for differentiation of Mycobacterium tuberculosis var hominis from other mycobacteria was applied.

4 — Nicotinamidase test;

The technique described by MUFTIC (1964) for detecting formamidase as means of differentation of some of isolated Strains, was used.

The scheme used for typing the isolated organisms was according to the following figure.

RESULTS

The results obtained are presented in the following tables.

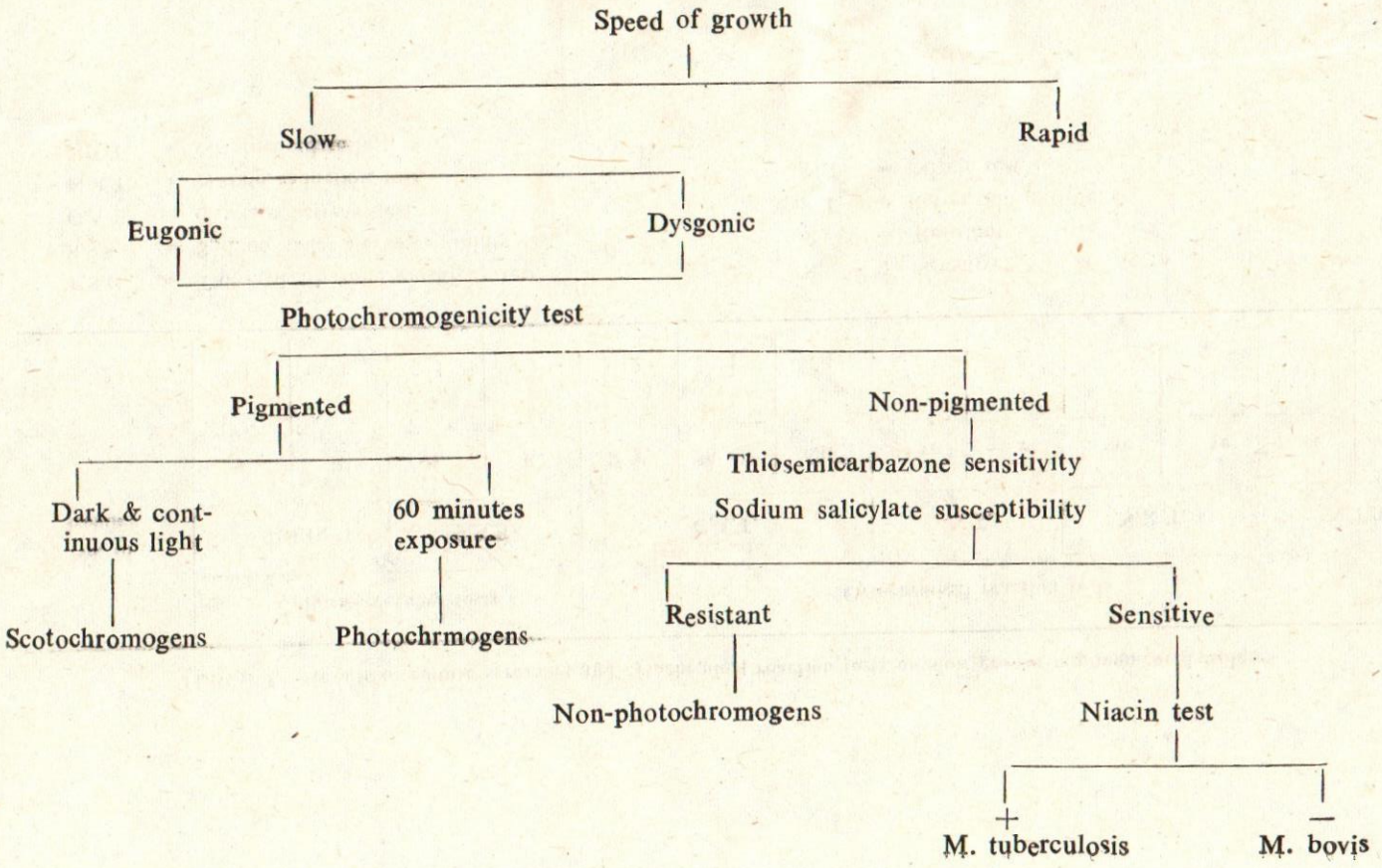
TABLE 1.—**Growth characterestics of the isolates**

No,. of specimens	Isolates		Growth					
			Rapid		Slow			
					Euogenic		Dysgonic	
	No.	%	No.	%	No.	%	No.	%
85	13	15.3	5	5.9	7	8.3	1	1.2

TABLE 2.—**Results of pigment production on the slow grower strains**

No. of Samples	Noof isolates	Pigment+Production		
		Pigmented Strains,		Non Pigmentedstrains
		Dark & cont. light	60 min. exposure	
85	8	6	—	2

The scheme used for typing the isolated organisms



TLBLE 3.—Results of culture screening and cytochemical reaction tests on slow grower-non-pigmented isolates

No. of isolates	Culture screening tests				Cytochemical reaction tests								
	T.S.C.		S.S.S.		C.A.T.			N.T.		N.R.T.		N.T.T.	
	S.	R.	S.	R.	R.T.	56 C°	68C°	+ ve	— ve	+ ve	— ve	+ ve	— ve
2	1	1	1	1	2	1	1	—	2	—	2	—	2

T.S.C. = Thiosemicarbazone sensitivity test.
S.S.S. = Sodium salicylate susceptibility test.
C.A.T. = Catalase activity test.
N.R.T. = Nitrate reduction test.
N.T.T. = Nicotinamidase test.
S. = Sensitive.
R. = Resistant.
R.T. = Room temperature.
N.T. = Niacin test.

TABLE 4.—Schematic representation of the results obtained with culture screening and cytochemical reaction tests in Mycobacterium bovis.

No. of isolates	Growth		Culture Screening tests		Cytochemical reaction tests				
					Catalase		Niacin	Nitrate red.	Nicotinamidase.
	speed	character	Thiosemicarb.	Sod. salicyl	R.T.	56. 68 C			
1	slow	dysgonic	sensitive	sensitive	+	—	—	—	—

TABLE 5.—Schematic representation of the results obtained with culture screening and cytochemical reaction tests in non-photochromogens strains

No. of isolates	Growth		Culture Screening tests		Cytochemical reaction tests				
					Catalse		Niacin	Nitrate red.	Nicotinami-
	speed	character	Thiosemicarb.	Sod. Salicyl.	R.T.	56.58 C			
1	slow	Eugonic	resistant	resistant	+	+	—	—	—

R.T. = Room Temperature.

TABLE 6.—The different types of mycobacteria isolated from market milk

Source of specimen	No. of Samples	isolates		M. tuberculosis				Type of isolates Photochrom.		Anonymous mycobacteria					
				var. hom.		bovis				Schotochrom.		Non-photo.		Rapid grow.	
		No.	%	No.	%	N.	%	No.	%	No.	%	No.	%	No.	%
Dairy shops	50	8	16	—	—	—	—	—	—	6	12	1	2	1	2
Street vendors . .	35	5	14.3	—	—	1	2.9	—	—	—	—	—	—	4	11.4

TABLE 7.—Percentage distribution of M. bovis, Schotochromogens, non-photochromogens and rapid growers isolated from market milk.

No. of specimens	Isolates		Type of isolates							
			M. bovis		Schotochrom.		Non-photochrom.		Rapid growers	
	No.	%	No.	%	No.	%	No.	%	No.	%
85	13	15.3	1	1.2	6	7.06	1	1.2	5	5.9

Var. hom. = var. hominis.

DISCUSSION

Alcid-fast bailli amounting to 13 strains were isolated from 85 milk samples collected from dairy shops and street venders. These strains were subjected to a planed series of procedures for identification.

The speed of growth as well as its character was used as a starting point in the screening procedure. From the 13 isolated strains 5 were found to be rapid growers while the remaining eight were slow growers. One of these slow grower isolates revealed a few, buff, smooth, dysgonic colonies. On subculturing the original growth, the colonies appeared as tiny and translucent and the growth remained dysgonic even after incubation for 6 weeks which is quite unlike the other isolates.

The eight slow growers including the dysgonic one, were subjected to the photochromogenicity test and revealed that 6 strains had the ability of producing pigment in dark as well as in continuous light. These strains were identified according to TIMPE and RUNYON (1954) and RUNYON (1955 as Scotochromogens.

The remaining 2 strains were placed in a special study group. Identification of those strains by using Thio-semicarbazone sensitivity, Sodium salicylate susceptibility and catalase activity at room temperature as well as at 56°C and 68°C revealed that one strain was Non-photochromogens, while the other strain was Mycobacterium tuberculosis var hominis or bovis. Further examination of this strain by its dysgonic growth character as well as Peknice and Asmus-Garschagen's niacin test, indirect Vartenen-Gries test and nicotinamidase test proved to be Mycobacterium bovis.

From the epidemiological point of view, the isolation of Mycobacterium bovis from market is of great hazardous. This contaminated milk may have incapacitating human being especially the children with the mycobacterial affection of the extrapulmonary type. PRICE (1938) in Torento, Canada, had accumulated data during thirteen on 500 tuberculous children and found that 9.6% of the extrapulmonary tuberculosis was due to the bovine type of infection. The youngest child in Price's series was $6^1/_2$ months old and without exception, the children came from a locality that did not provide pasteurization for milk before consumption. Later, VAN ZWANENBERG *et al.* (1956) reported seven cases of tuberculosis of the cervical lymph nodes. Twelve additional cases were found during a tuberculin

survey in the same community. All the cases gave a history of having drunk raw milk from the same dairy. On the other hand, KIMURA *et al.* (1939) attributed that the low rate of infection with the bovine type of the tubercle bacillus in Japan was largely due to the fact that the amount of raw milk consumed by the Japanese is minimal. However, the frequency of the bovine type tuberculous infection in human being closely dependant on the incidence of the disease in cattle, the milk drinking habits of the populace, and the application of heat treatment processes.

The contamination of milk with Mycobacterium bovis frequently occurs either endogenous or exogenous. Data assembled by WILLIAMS *et al.* (1927) stated that dung from tuberculous cattle and even from cattle that were apparently healthy, contained tubercle bacilli virulent for Guinia pigs.

Regarding the mycobacterial contamination of milk, the efficiency of pasteurization in killing these organisms was recorded by PRICE (1938) out of 200 milk samples of pooled raw milk obtained from the pasteurization tanks prior to heating. 52 samples (26%) were containing virulent tubercle bacilli, this was proved by animal inoculation tests. One hundred samples were taken after heating milk at 145 °F for 30 minutes and examined for the presence of tubercle bacilli by animal inoculation, all samples proved negative to tuberculosis.

The Anonymous mycobacteria were isolated from 14% of the examined samples. Such groups were also isolated by CHAPMAN *et al.* (1965). They found representatives of Runyon's groups II, III, and IV in 261 out of 770 samples of raw milk taken from tank trucks. These groups were regarded as of etiological significance in human tuberculous sufferers by many investigators (SELKON *et al.*, 1959; WOLINSKY, 1960; PRATHES *et al.*, 1961; WAYNE, 1962 and ISSA, 1963). However, the question of the origin and normal habitat of these anonymonus mycobacteria, and their relation to soil and animal life are still open. Work dealing with the sources of these organisms in milk as well as the probability of their being food-borne agents are still under investigation.

From the results achieved and according to the local conditions as well as our habits in consuming milk, one may safely concluded that the market raw milk and milk products play a role in infecting man with the bovine type of tubercle bacillus. Therefore, effective measures should be inforced to control and eliminate the disease among farm animals as well as heat treatment of milk should be applied.

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