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BOVINE AND ATYPICAL MYCOBACTERIAL INFECTIONS OF CATTLE AND BUFFALOES IN PORT SAID PROVINCE, EGYPT

(With 4 Tables)

By

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إصابة الأبقار والجاموس بالسل البقرى والسل الغير قياسي فى محافظة بورسعيد - بمصر

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فى دراسة على مرض السل فى الأبقار والجاموس بمحافظة بورسعيد، فى الفترة من يناير ٢٠٠٠ حتى ديسمبر ٢٠٠١، تم فحص عدد ٤٧٥٦ من الأبقار و٢٧٦٩ من الجاموس باستخدام اختبار التيوبركلين وكانت نسبة الإصابة بين الأبقار والجاموس هى ٠,٩٦% و ٠,٥% على التوالى. وبفحص هذه الحيوانات الموجبة لاختبار التيوبركلين بعد الذبح تبين أن معظم الإصابة بدرنات السل كانت فى الرئة والغدد الليمفاوية للجهاز التنفسى لهذه الحيوانات. وبالفحص البكتريولوجى لعينات ألبان من الحيوانات الحلوب الموجبة لاختبار التوبركلين، تم عزل ميكوبكتريم بوفس (ميكروب السل البقرى) من عدد ٢ من ٢٦ بقرة (٧,٧%)، بينما كانت عينات ألبان الجاموس التى تم فحصها بكتريولوجيا سلبية. وعلى الجانب الأخر كان معدل عزل ميكوبكتريم بوفس والميكوبكتريا الغير قياسية من الغدد الليمفاوية للأبقار والجاموس الموجبة لاختبار التيوبركلين على التوالى؛ ٦٣,٠٤% و ٨,٧% فى الأبقار و ٤٢,٩% و١٤,٣% فى الجاموس. وعامة أنواع الميكوبكتريا الغير قياسية التى تم عزلها؛ ميكوبكتريم افيام انتراسلولارى، ميكوبكتريم كانساسى، ميكوبكتريم فورتويتم وميكوبكتريم سمجمينتس. وأتضح من هذه الدراسة أن الأبقار والجاموس مازالت مصدرا محتمل لاصابة الإنسان بمرض السل وهذا يتطلب التعاون بين الأطباء البيطريين وهيئات الصحة العامة لمكافحة هذا المرض والتخلص منه.

SUMMARY

In a study on tuberculosis among cattle and buffaloes in the Port Said Province, Egypt, during the period from January, 2000 to December,

2001, the incidence of tuberculin reactors from cattle and buffaloes by using the single intradermal cervical test was 0.96% and 0.5%, respectively. Pulmonary tuberculosis with the involvement of the respiratory tract lymph nodes has been recognized as the most frequent form of tuberculosis in the slaughtered tuberculin-reacting cattle and buffaloes. Of the tuberculin positive cattle; 34 (73.9%) had macroscopic tuberculous lesions (visible lesions) and 12 (26.1%) has non-visible lesions. While, 8 (57.1%) and 6 (42.9%) of tuberculin positive buffaloes had visible lesions and non-visible lesions respectively. *Mycobacterium bovis* could be isolated from the milk of 2 (7.7%) out of 26 tuberculin-reacting dairy cattle. However, the examined milk samples of 8 tuberculin-reacting dairy buffaloes were culturely negative for acid fast bacilli. On the other hand, the isolation rates of *Mycobacterium bovis* and atypical mycobacteria from the respiratory tract lymph nodes of tuberculin positive animals respectively, were 63.04% and 8.7% in cattle, and 42.9% and 14.3% in buffaloes. Generally, the isolated atypical mycobacteria were *Mycobactrium avium-intracellulare*, *Mycobacterium kansassi*, *Mycobacterium fortuitum* and *Mycobacterium smegmatis*. The current research indicates that cattle and buffaloes still acting as a potential reservoir of tuberculosis to man.

Key words: *Bovine, mycobacterial infections.*

INTRODUCTION

In spite of eradication programmes, tuberculosis still be a major issue for farming economies in many countries, as well as an important zoonosis in many areas (Bollo *et al.*, 1998 and Waters *et al.*, 2000). Since 1981, the general organization of veterinary services in Egypt has begun a national project for eradication of tuberculosis in dairy cattle and buffaloes. This national project started in 3 governorates of the Suez Canal area. In 1986, the programme extended to all governorates with regular obligatory tuberculin testing, using the single intradermal cervical tuberculin test, in addition to continuous surveillance for tuberculosis in slaughtered animals in Egyptian abattoirs for identification of tuberculosis foci and tuberculous herds in different locations (Refai and Hammam, 1993). Although test and slaughter policy proved an effective methods for tuberculosis control in farm animals, a significant increase of non-visible reactors has been documented by detailed necropsy reports in the later stages of control programmes when the prevalence of tuberculosis becomes low (Pritchard, 1988). These infections are due both to *M. tuberculosis*

complex and non-tuberculous mycobacteria (Corner *et al.*, 1990 and Corner, 1994). In studies on tuberculosis in Egypt, El-Taweel (1992) reported that in 1989 the incidence of tuberculosis among bovines was 0.04% for cattle and 0.16% for buffaloes. In 1990 the incidence was 0.25% and 0.12% in the order given, while in 1991 it was 1.23% and 0.11% in cattle and buffaloes respectively. Generally in developing countries, especially in Africa where *M. bovis* infection is present in various animal species, there is a substantial lack of knowledge of distribution, epidemiological patterns and zoonotic implication of this important disease. It is shown that the area with high prevalence of bovine tuberculosis may represent a risk to human health (Thoen and Steele, 1995). Atypical mycobacteria attracted the attention of many research workers due to the increasing reports of its role as etiological agents of diseases in man and animals (Acha and Szyfers, 1989). In view of the intensive management system for raising farm animals in El-Kaboty, Port Said Province where large numbers of cattle and buffalo farms are existed. This study was undertaken to determine the incidence of mycobacterial infections of cattle and buffaloes in this area and to assess the level of awareness with regard to the risks through milk consumption.

MATERIAL and METHODS

Animals:

A total of 7525 animals (4756 cattle and 2769 buffaloes) were examined for tuberculosis, during the persod from Jan., 2000 to December, 2001 through the routine compaign of control prgams for tuberculosis among animals in Part Said provine..

Tuberculin test:

The animals were tested by the single intradermal cervical test (SICT) using mammalian purified protein derivative (PPD) tuberculin (Weybridge standard 2mg/ml) which was obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. This test was performed by assistance of the accredited veterinarians of the veterinary services in the investigated area. The application and interpretation of tuberculin test was the same as that previously recommended (O'Reilly, 1992).

Bacteriological examination:

Sampling:

A total of 34 milk samples were aseptically collected from the tuberculin-reacting dairy cattle (26) and buffaloes (8). Also a total of 60 tissue specimens were aseptically collected from the bronchial lymph

nodes of the tuberculin-reacting cattle (34) and buffaloes (16), at the post-mortem examination it the obattoiv in Port Said Province.

These specimens were submitted as rapidly as possible to the laboratory at the Faculty of Veterinary Medicine, Suez Canal University, where they subjected to bacteriological investigation.

Isolation and identification of Mycobacteria:

Aseptically collected milk samples were centrifuged at 3000 xg for 15 minutes and the cream layers and sediments inoculated directly onto solid media; Lowentein Jensen tubes (L-J), Middlebrook 7H-10 and 7H-11 (Sigma-Aldrich, Inc.). Smear was prepared from the cream layer and sediment of each specimen by spreading a drop over an area 1x2 cm of the microscope slide, air dried, heat fixed, stained by Ziehl Neelsen stain and the examined under the microscope for the presence of acid-fast bacilli.

On the other hand, the collected tissue specimens were seared and then cut with sterile instruments. Each tissue specimen was homogenized in a sterile saline (0.85%), using a sterile tissue grinder. Inoculate 0.1 ml of the homogenate of each specimen directly into solid media: L-J tubes, 7H-10 and 7H-11. Smears were made of these homogenates, stained by Ziehl Neelsen stain and examined for the presence of acid-fast bacilli.

The inoculated 7H-10 and 7H-11 plates were placed in individuals Co_2-permeable polyethylene bags and all the inoculated media incubated for 4 to 8 weeks at 37 ^{0}C under 10% co_2.

Identification of mycobacteria was based on the finding of acid-fast organisms, the growth of characteristic colonies, pigment production and the appropriate reaction of the organism to some biochemical tests as Niacin, Nitrate reduction, Catalase, Arylsulfatase, Tween hydrolysis, Nacl and urease (Vestal, 1981).

RESULTS

The incidence of tuberculin-reacting cattle and buffaloes was summarized in Table 1. The overall incidence of tuberculosis among the examined animals by the single intradermal cervical tuberculin test was 0.8% (60 out of 7525). The incidence of tuberculin-reacting cattle and buffaloes was 0.96% (46 out of 4756) and 0.5% (14 out of 2769) respectively.

Regarding the meat inspection of slaughtered tuberculin-reacting cattle and buffaloes, pulmonary tuberculosis with the involvement of the respiratory tract lymph nodes has been recognized as the most frequent

form of tuberculosis in these animals. Generalized tuberculosis was recognized in only three cases of tuberculin-reacting cattle in which, calcified, yellowish and firm gritty nodules were seen in the pleura, lungs, liver and spleen. . In contrast, there were non-visible lesions (N.V.L.) in some of the tuberculin- reacting animals.

Table 1: Incidence of tuberculosis among cattle and buffaloes based on tuberculin testing using Single Intradermal Cervical Tuberculin Test (SICTT).

Animal species	Total No. examined	Positive	
		No.	%
Cattle	4756	46	0.96
Buffaloes	2769	14	0.5
Total	7525	60	0.8

In Table 2 out of the tuberculin-reacting cattle; 34 (78.9%) had visible lesions (VL) and 12 (26.1%) had non-visible lesions (NVL). While, 8 (57.1%) and 6(42.9%) of tuberculin-reacting buffaloes had VL and NVL respectively.

Table 2: Correlation between tuberculin reactions and results of meat inspections among the examined cattle and buffaloes.

Animal species	No. of tuberculin reactors	Meat inspection	
		Visible lesions(V.L) No. (%)	Non-visible lesions (N.V.L.) No. (%)
Cattle	46	34(78.9)	12(26.1)
Buffaloes	14	8(57.1)	6(42.9)
Total	60	42(70)	18(30)

In bacteriological examination of milk samples collected from the tuberculin-reacting dairy cattle and buffaloes for mycobacteria, *Mycobacterium bovis* could be isolated from the milk of 2 (7.7%) out of 26 tuberculin-reacting dairy cattle. However, the examined milk samples of 8 tuberculin-reacting dairy buffaloes were culturely negative for acid fast bacilli (Table 3).

Table 3: *Mycobacterium bovis* isolated from milk samples of tuberculin-reacting dairy cattle and buffaloes.

Animal species	No. of examined specimens	Culture positive for acid fast bacilli No(%)	Type of isolates
Dairy cattle	26	2 (7.7)	*Mycobacterium bovis* (2 strains)
Dairy buffaloes	8	0 (0.0)	-ve

In Table 4 it was found that 30 (88.2%) out of 34 V.L. of the examined cattle were culturely positive for acid fast bacilli. On typing the isolates 93.3% were Mycobacterium bovis (28 strains) and 6.6% were atypical mycobacteria (2 strains). While, of the examined N.V.L. of cattle; 3 (25%) were culturely positive for acid fast bacilli (one strain of *Mycobacterium bovis* (33.3%) and 2 strains of atypical mycobacteria (66.7%).

Table 4 *Mycobacterium bovis* (M.b.) and *atypical mycobacteria* (a.m.) isolated from tissue specimens of tuberculin-reacting cattle and buffaloes.

Animal species/ Type of specimens	No.of examined specimen V.L.	N.V.L.	Culture positive for acid fast bacilli V.L. No(%)	N.V.L. No(%)	Isolates V.L No. type(%)	N.V.L. No. type(%)	Total No. type(%)
Cattle L. node	12		30(88.2)	3(25)	28 M.b.(93.3) 1 M.a.i.(3.3) 1 M.k. (3.3)	1 M.b.(33.3) 2 M.f.(66.7)	29 M.b.(63.04) 1 M.a.i.(2.2) 1 M.k. (2.2) 2 M.f.(4.4)
Buffaloes L. node	6		7(87.5)	1(16.7)	6 M.b.(85.7) 1 M.a.i.(14.3)	1 M.s.(100)	6 M.b.(42.9) 1 M.a.i.(7.1) 1 M.s. (7.1)
Grand total	42	18	37(88.1)	4(22.2)	34 M.b.(91.9) 3 a.m.(8.1)	1 M.b.(25) 3 a.m.(75)	35 M.b.(58.3) 6 a.m. (10)

M.a.i. = *Mycobacterium avium intracellulare* M.f. = *Mycobacterium fortuitum*
M.k. = *Mycobactrium kansassi* M.s. = *Mycobacterium smegmatis*

On the other hand, the bacteriological examination of lymph nodes of tuberculin reacting buffaloes for mycobacteria; 7(87.5%) and one (16.7%) of V.L. and N.V.L. were culturely positive for acid fast bacilli. Of the culture positive V.L.; 6 strains of *Mycobacterium bovis* (85.7%) and one strain of the atypical mycobacteria (14.3%) could be identified, while, only one strain of atypical mycobacteria was identified from the N.V.L. positive culture.

Generally, the isolation rates of *Mycobacterium bovis* and atypical mycobacteria from the respiratory tract lymph nodes of the tuberculin positive animals respectively were 63.04% and 8.7% in cattle, and 42.9% and 14.3% in buffaloes. The atypical mycobacteria isolated

from the examined lymph nodes of tuberculin-reacting cattle and buffaloes were *Mycobacterium avium-intracellularae* (two strains), *Mycobacterium kansassi* (one strain), *Mycobacterium fortuitum* (two strains) and *Mycobacterium smegmatis* (one strain).

DISCUSSION

The results of tuberculin reactors among cattle and buffaloes by using the single intradermal cervical test, were nearly similar with those reported in Egypt by Adawy (1986) and El-Taweel (1992) who reported that tuberculin reactor rates were ranged from 0.04% to 2.2% in cattle and 0.12% to 3.3% in buffaloes. While, it was significantly low as compared with those reported by Adawy (1982) who found that 43.4% of cattle and 63% of buffaloes were had positive tuberculin reactions in Military farm in Giza.

These results indicate that the low incidence of the infection in cattle and buffaloes could be is attributable to the repeated removal of tuberculin-sensitive animals. The tuberculous infection rates are governed by many factors such as herd size, density, different breeding and management system, uncontrolled animal movements, unhygienic local habits and stress factors due to other diseases and mass vaccination against various diseases (Abou-Eisha and El-Sheary, 1995 and Thoen and Steele, 1995).

Many authors are concerned with the reliability of tuberculin test. In the correlation between tuberculin reactions and results of meat inspection, our results were in line with those reported by El-Sayed (1986) who found that 78.9% and 52.1% of tuberculin-reacting cattle and buffaloes were tuberculous at slaughter respectively. However, Abdalla and Abdel-Galil (1972) stated that all tuberculin positive cattle in a dairy farm in Assiut were tuberculous at postmortem examination. Adawy (1985) reported that 91% and 92.3% of tuberculin-reacting buffaloes and cattle respectively were found to the tuberculous at slaughter. The obtained data suggest that a number of these positives can be attributed to other factors as sensitization by atypical mycobacteria or other unrelated infections (Bachvarova *et al.*, 1999). Tuberculin test is less sensitive in buffaloes than in cattle (El-Sayed, 1986).

In the present study, pulmonary tuberculosis with the involvement of the bronchial and mediastinal lymph nodes has been recognized as the most frequent form of tuberculosis in cattle and buffaloes. These results in agreement with those recorded by Neill *et al.* (1992). Study evidence indicates that the most tuberculous cattle and

buffaloes are infected by the respiratory route and that many animals have lesions in their lungs. Lymph nodes have been found to be more commonly infected than other tissues because tissue fluids in an animal eventually pass through the lymph nodes where the organism is entrapped (Collins and Grange, 1983; Neill *et al.*, 1992 and Thoen, 1992). Moreover, these results emphasized the importance of occupational exposures to aerosols containing infectious material, with the result that disease is more frequently pulmonary (Fanning, and Edwards, 1991; De-Kantor, 1992 and Scanlon and Quinn, 2000).

In the bacteriological examination of milk from the tuberculin-reacting dairy cattle and buffaloes, *Mycobacterium bovis* could be isolated from 7.7% of milk samples of tuberculin positive dairy cattle. While, the buffalo milk samples were culturely negative for acid fast bacilli. These findings indicate that dissemination of the disease to the udder is relatively infrequent (Collins and Grange, 1983). However, it was reported that one animal with tuberculus mastits of the udder amongst a herd of milking cows will contaminate the whole output of the herd and this in turn, will represent a risk of milk-borne infections (Gallagher and Jenkins, 1998 and Kazwala *et al.*, 2001).

Regarding the isolation of *Mycobacterium bovis* and atypical mycobacteria from the lymph nodes of the tuberculin positive cattle and buffaloes, a high percentage of lymph node with visible lesions were culturely positive for acid fast bacilli (88.1%). While most of the non-visible lesions were culturely negative for acid fast bacilli.

On typing, most isolates of the visible lesions were found to be *Mycobacterium bovis* (Zidan, 1971, Osman, 1974 and Thoen and Steele, 1995). In contrast, atypical mycobacteria were the most frequent isolates of non-visible lesions. These results indicate that the persistence and propagation of *Mycobacterium bovis* is due in part to the intensive management by raising the animals in close confinement in large numbers. The infection in non-visible lesions of reactors may be attributed to the nontuberculous mycobacteria (Thoen and Steele, 1995; Bollo *et al.*, 1998 and Bonsu *et al.*, 2000).

In respect to the isolated atypical mycobacteria from the examined lymph nodes, in the present study, *Mycobacterium avium-intracellulare* isolated from the visible lesions of tuberculin-reacting cattle and buffaloes. *Mycobacterium kansassi* was isolated from visible lesion of tuberculin-reacting cattle. While, *Mycobacterium fortuitum* and *Mycobacterium smegmatis* were isolated from non-visible lesions of tuberculin-reacting cattle and buffaloes respectively. These results

indicate that *Mycobacterium avium intracellulare* and *Mycobacterium kansassi* may affect cattle and buffaloes and the lesions in these animals are usually restricted to the lymph nodes (Acha and Szyfres, 1989 and Thoen and williams, 1994). The rarity of systemic atypical mycobacteriosis, despite the ubiquitous distribution of these organisms, is a reflection of the low pathogenicity of the causative organisms in clinically normal animals. However, the opportunistic nature of atypical mycobacteria allows them to cause infection when normal defenses are disrupted (Grooters *et al.*, 1995).

In conclusion, most tuberculous cattle and buffaloes were infected by the respiratory route and many animals have lesions in their lungs. Several of these open cases are potentially capable of spreading infection with *Mycobacterium bovis*. Public health significance of animal infected with atypical mycobacteria is difficult to assess, because these organisms can exist as saprophytes. Nevertheless, it may be prudent for owners to be careful when handling animal infected with atypical mycobacteria. There is no doubt that this situation indicates that cattle and buffaloes still acting as a potential reservoir of tuberculosis to man. This fact stresses the need for close surveillance and control by veterinarians as well as by public health authorities.

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