ISOLATION OF MYCOBACTERIA FROM TUBERCULIN POSITIVE COWS AND BUFFALOES IN KAFR EL-SHIEKH GOVERNORATE

BADIER T. H. SH.

Animal Health Research Institute - Agricultural Research Center, Ministry of Agriculture, Dokki – Giza - Egypt

(Manuscript received 29 July 2003)

Abstract

This study regarded the prevalence of tuberculosis disease among cattle and buffaloes in Kafr El-Shiekh governorate during the period from January 2000 to December 2001. The incidence of tuberculin test reactors among cows and buffaloes using the single intradermal cervical test was 1.12% and 1.34%, respectively. The tuberculin positive cows had macroscopic tuberculosis lesions of bronchial lymph nodes that had visible (V.L.N) and non-visible (N.V.L.N) (69.01) and 30.98 lesions, respectively. In buffaloes, it had (41.03%) and (58.97%), respectively. On the other hand, the isolation rates of *Mycobacterium bovis* were (85.29%) and (71.43%) and atypical mycobacteria (14.7% and 28.56%) form the bronchial lymph nodes of both V.L.N and N.V.L.N from tuberculin positive cows and buffaloes respectively. Generally, the isolated strains of atypical mycobacteria were *mycobacterium intracellulure* and *Mycobacterium kansassi* (2 and 1 strains) respectively from V.L.N but *mycobacterium fortuitum* and *Mycobacterium smegmatis* (3 and 1 strains) respectively were isolated from non visible lymph nodes.

Mycobacterium bovis could be isolated from the milk of 3 (10%) out of 30 tuberculin reacting dairy cattle. However, the examined milk samples of 15 tuberculin reacting dairy buffaloes were negative in culture medium for acid fast bacilli except one which was positive in ratio 6.67%.

INTRODUCTION

Tuberculosis is considered as a major issue for farming economies and an important zoonotic disease in many areas (Waters *et al.* 2000).

Infections may be due to *M. tuberculosis* complex, and non-tuberculosis mycobacteria (Corner 1994). Since 1981, the General Organization of Veterinary

Cervices in Egypt had begun a national project for eradication of tuberculosis in dairy cattle and buffaloes by using the regular obligatory tuberculin testing via 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 to be an effective method for tuberculosis control in farm animals, yet, 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). 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). Also, 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 Kafr El-Shiekh governorate, where large numbers of cattle and buffalo farms are reared, this study was undertaken to determine the incidence of mycobacterial infections of cattle and buffaloes in Kafr El-Shiekh Governorate and to assess the level of awareness with regard to the risk through milk consumption.

MATERIALS AND METHODS

A total of 9278 animals (6361 cattle and 2917 buffaloes) were examined for tuberculosis during the period from January 2000 to December 2001 through the routine campaign of control programs for tuberculosis among animals in Kafr El-Sheikh governorate. All animals were tested by the single intradermal cervical test (SICT) using mammalian purified protein derivative (PPD) tuberculin (Weybridge Standard 2 mg/ml), which was obtained from the Veterinary Serum and Vaccine Research Institue, 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 by O'Reilly (1992).

Bacteriological examination

Sampling

A total of 45 milk samples was aseptically collected from 30 and 15 tuberculin reacting dairy cows and buffaloes, respectively. Also, at the postmortem examination in the abattoir a total of 110 bronchial lymph node specimens was aseptically collected from the tuberculin reacting 71 cows and 39 buffaloes. These specimens were submitted rapidly as possible to the Animal Health Research Institue, provincial lab in Kafr El-Shiekh Governorate, where they were subjected to bacteriological examination according to Neill *et al.* (1992).

Isolation and Identification of Mycobacteria

The collected lymph node specimens were prepared and homogenized in a sterile saline (0.85%). Of the homogenate , 0.1 ml of each specimen was inoculated directly into dorest egg media and incubated at 37°C for 2-3 weeks. Smears were stained by Ziehl Neelsen's stain and examined for the presence of acid-fast bacilli according to Bailey and Scott (1974).

Aseptically collected milk samples were centrifuged at 3000 for 15 minutes, smears were prepared from the cream layer and sediment of each specimen stained by Ziehl Neelsen's stain and examined under the microscope for the presence of acid fast bacilli. The suspected milk samples were cultured on dorest egg media. Identification of mycobacteria was based on the finding of acid fast organisms, the growth of characteristic colonies with pigment production and the appropriate reaction of the organisms to some biochemical tests as Niacin, Nitrate reduction, Catalase, Arylsulfatase, Tween hydrolysis, Nacl and urease (Vestal, 1981).

RESULTS

The incidence of tuberculin reacting cows and buffaloes was summarized in Table 1. The overall incidence of tuberculosis among the examined animals by the single intradermal cervical tuberculin test was 1.19 % (110 out of 9278 animals). The

incidence of tuberculin reacting cows and buffaloes was 1.12 % (71 out of 6361 animal) and 1.34% (39 out of 2917 animal), respectively.

Generalized tuberculosis was recognized in all districts of Kafr El-Sheikh Governorate of tuberculin-reacting cows, and buffaloes in which, calcified, yellowish and firm gritty lymph nodules were seen in the pleura, lungs, liver and spleen. On contrast, there were non-visible lesions (N.V.L.N.) is some of the tuberculin reacting animals.

As shown in Table 2, 49 (69.01%) had (VL), and 22 (30.99%) had (N.V.L.) out of the tuberculin reacting cows while, 16 (41.03%) and 23 (58.97%) of tuberculin reacting buffaloes had V.L.N. and N.V.L.N., respectively.

As recorded in Table 3, it was found that 29 V.L.N. out of 71 of the examined lymph nodes were positive in culture medium for acid fast bacilli. On typing the isolates, 93.10% were *Mycobacterium bovis* (27 strains) and 6.90% were atypical mycobacteria (2 strains). The examined 5 N.V.L.N. were positive in culture medium for acid fast bacilli (2 strain) of *Mycobacterium bovis* (40%), and 3 strains of atypical mycobacteria (60%). On the other hand, the bacteriological examination of lymph nodes of tuberculin reacting buffaloes revealed the isolation of mycobacteria from 5 isolates recovered from V.L.N. These were identified as 4 strains of *Mycobacterium bovis* (80 %) and one strain of the atypical mycobacteria (20 %), while, the 2 isolates obtained from N.V.L.N. were identified as one of *M. bovis* and 1 strain of atypical mycobacteria.

Generally, the isolation rates of *Mycobacterium bovis* and atypical mycobacteria from the respiratory tract lymph nodes of the tuberculin positive animals respectively were 85.29 % and 14.70 % in cows, and 71.43 % and 28.57% in buffaloes respectively. The atypical mycobacteria were identified as *Mycobacterium intracellulure* (2 strains), *Mycobacterium kansassi* (1 strain), *Mycobacterium fortuitum* (3 strains) and *Mycobacterim smegmatis* (1 strains).

The bacteriological examination of milk samples collected from the tuberculin reacting dairy cows and buffaloes revealed that *Mycobacterium bovis* could be isolated from the milk of 3 (10%) out of 30 tuberculin reacting dairy cows and 1 out of 15 buffaloes as shown in Table 4.

Table 1. Incidence of bovine tuberculosis during two years (2000-2001) among cows and buffaloe tested by tuberclin test in Kafr El-Shiekh Governorate.

Type of Animal	Total	Result of tubercline test			
		-		+	
		No	%	No	%
Cattle	6361	6290	98.88	71	1.12
Buffaloe	2917	2878	98.66	39	1.34
Total	9278	9168	98.81	110	1.19

Table 2. Correlation between the results of tuberculin test and the presence of visible or non-visible leasions.

Type of Animal	No. of animals tested	Tuberculin reacting No	Visible leasons (V.L.)		Non-visible leasons (N.V.L.)	
			No	%	No	%
Cattle	6361	71	49	69.01	22	30.98
Buffalloe	2917	39	16	41.02	23	58.97
Total	9278	110	65	59.09	45	40.91

Table 3. *M. bovis* and atypical mycobacteria (a.m.) isolated from respiratory lymph node (rLn) of tuberculin reacting cows and buffaloes.

Animal type	No. of examined lymph nodes	Culture positive for acid fast bacilli				Isolates				Total	
		V.L.N.*		N.V.L.N.**		V.L.N.*		N.V.L.N.**		No.**** type	%
		No***	%	No***	%	No.**** type	%	No.**** type	%		
Cows.	71	29	40.84	5	7.04	27 M.b.	93.10	2 M.b.	40	29 Mb.	85.29
						1 Mai	3.45	3 Mf	60	1 Mai	2.94
						1 Mk	3.45	0	0	1 Mk 3Mf	2.94 8.82
Buffaloes.	39	5	12.82	2	5.31	4 Mb.	80	1 Mb.	50	5 Mb.	71.42
						1 Mai.	20	1Ms	50	1 Mai	14.28
						0	0	0	0	1 Ms	14.28
Grand total	110	34	30.91	7	6.36	31 Mb.	91.18	3 Mb.	42.86	34 Mb.	82.93
						2 Mai	5.88	3 Mf	42.86	2 Mai	4.88
						1Mk	2.94	1 Ms	14.28	3 Mf 1Mk 1Ms	7.32 2.44 2.44

M.ai = Mycobacterium intracellulure.
M.K. = Mycobacterium kansassi
M.F. = Mycobacterium fortuitum
M.S. = Mycobacterium smegmatis
Mb. = *Mycobacterium bovis.*
VLN* = Visible lymph node.
NVLN** = non visible lymph node.
No*** = Number of respiratory lymph node examined.
No**** = Number of serotype of mycobactria isolated.

Table 4. *Mycobacterium bovis* isolated from milk samples of tuberculin test reactions in dairy cows and buffaloes.

Animal species	No. of examined specimens	Culture positive M. bovis	
		No	%
Dairy cattle	30	3	10
Dairy buffaloe	15	1	6.67
Total	45	4	8.98

DISCUSSION

The results of tuberculin reactors among cows and buffaloes by using the single intradermal cervical test were nearly similar with those reported in Egypt by Abou Eisha *et al.* (2002), who indicated that the low incidence of infection in cows and buffaloes could be attributed 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 systems, uncontrolled animal movements, unhygienic local habits, other stress factors due to other diseases and mass vaccination against various diseases (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. The obtained results were in line with those reported by Abou-Eisha *et al.* (2002), who found that 78.9% and 52.1% of tuberculin reacting cattle and buffaloes were diagnosed as tuberculous cases 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 tuberculous at slaughtering. 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. 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 are in agreement with those recorded by Neill `. (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 organisms are entrapped (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* (Scanlon and Quinn 2000).

Regarding the isolation of *Mycobacterium bovis* and atypical mycobacteria from the lymph nodes either visible or non visible lesions (41.13% or 42.86%) and (8.82% or 57.14%) respectively, were cultural positive for acid fast bacilli.

On typing most isolates of the visible lesions, they were found to be *Mycobacterium bovis* (Thoen and Steele 1995). On contrast, atypical mycobacteria were the most frequent isolates from cases of non-visible lesions. These results indicated that the persistence and propagemation of *Mycobacterium bovis* is due, in part, to intensive management by raising the animals in close confinement in large numbers. In cases with non-visible lesions the tuberculin reaction may be attributed to the non-tuberberculous mycobacteria (Bonsu *et al.* 2000).

The atypical mycobacteria were identified as one *Mycobacterium kanassi,* 3 *Mycobacterium fortuitum, M. intracellulure* and 1 *Mycobacterium smegmatis.* These results indicated that *Mycobacterium intracellulare* may affect cows and buffaloes and the lesions in these animals are usually restricted to the lymph nodes.

* Human cases which suffered from tuberculosis in hospital in Kafr Sheikh governorate, in this research no isolation from these human cases.

In the bacteriological examination of milk from the tuberculin reacting dairy cattle and buffaloes, *Mycobacterium bovis* could be isolated form 10% and 6.67% of milk samples, respectively. These findings indicate that dissemination of the disease to the udder is relatively infrequent (Collins and Grang, 1983). However, it was reported that one animal with tuberculous mastitis of the udder amongst a herd of milking cows may contaminate the whole output of the herd and this in turn, will represent a risk of milk borne infections (Kazwala *et al.* 2001).

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

REFERENCES

1. Abdallah J. S. and G. Abdel-Galil. 1972. Studies on the eradication of tuberculosis in a dairy farm J. Egypt Vet. Med. Assa. P 17-21.

2. Abou-Eisha A.M., A. A. EL- Atlar and El-Sheary. 2002. Bovine and atypical mycobacterial infections of cattle and buffaloes in Port Said province, Egypt Assiut Vet. Med. J., 47 (93): 152.

3. Acha P.N. and B. Szyfers. 1989. Zoonoses and Communicable diseases common to man and animals. 2 nd ed. Pan American health organization Washingtton USA pp 74-81.

4. Adawy A.T. 1985. Incidence of tuberculosis in Egyptian buffaloes 1st. world Bufallo congress, Cairo, pp. 806-818.

5. Bailey W. Robert and G. Elvyn Scott. 1974. Diagnostic microbiology. The C.V. Mosby Company, Saintlousis. 4th ed., Bailliere Tindull.

6. Bonsu O.A., E. Laing and B.D. Akanmoti. 2000. Prevalence of tuberculosis in cattle in the Dangme west district of chana public health implications. Acta Tropica, 26 (1): 9-14.

7. Colins Citt. and M. Grange. 1983. The bovine tubercle bacillus a review. J. Appl. Bact., 55: 13-29.

8. Corner L.A. 1994. Postmortem diagnosis of Mycobacterium bovis infection in cattle. Vet. Microbiol., 40: 53-63.

9. El-Sayed A.A. 1986. Studies on some blood and serum constituents in tuberculin negative and tuberculin positive cattle and buffaloes. Thesis, M.V. Fac. Vet. Med. Cairo University.

10. Kazwala R.R., C.J. Daborn, J. M. Sharp, D.M. Kambarage, S.F. Jiwa and N.A. Mbe. 2001. Isolation of Mycobacterium bovis from human cases of cervical adenitis in Tanzania: acuse for concern. Int. J. Tuberc. Lung Dis., 5 (1): 87-91.

11. Neill S.D., J. Hanna, D.P. Mackie and T.G.D. Bryson. (1992): Isolation of Mycobacterium bovis from the respiratory tracts of skin test negative cattle. Vet. Rec., 131: 45-47.

12. O'Reilly L. M. 1992. Specificity and Sensitivity of tuberculin test. Proc. Intern. Conf. Anim. T.B. in Africa and Middle East, Cairo.

13. Pritchard D. G. 1988. Acentury of bovine tuberculosis 1888-1988. Conquest and controversy. J. Comp. Path., 99, 130: 357-399.

14. Refai M. and H. Hammam. 1993. Tuberculosis in farm animals in Egypt. J. Egypt. Vet. Med. Ass °c . 53 (4): 581-607.

15. Scanlon M.P. and P.J. Ouinn. 2000. The survival of Mycobacterium bovis in sterilized cattle slurry and its relevance to the persistence of this pathogen in the environment. Rish Vet. J., 53 (8): 412-415.

16. Thoen C. O. 1992. Pathogenesis of bovine tuberculosis. Proc. Internat. Conf. Anim. Tuberculosis in Africa and Middle East.

17. Thoen C. O. and J. H. Steele. 1995. Mycobacterium bovis infection in animals and humans. 1 st ed. Iowa State University press Ames.

18. Vestal A.L. 1981. Procedures for the isolation and identification of mycobacterium. Department of health and human services, Publication No. [CDC] 81-8230.

19. Waters W.R., M.V. Palmer, B.A. Resch, S.C. Olsen, M.J. Wannemuehler and D.L. Whipple. 2000. Lymphocyte subset proliferative responses of Mycobacterium bovis infected cattle to purified protein derivative. Vet. Immunol. immuno. Pathol., 77 (3/4): 257-273.

عزل ميكروب السل من الأبقار والجاموس الإيجابى لاختبار التيوبركلين بمحافظة كفر الشيخ

طلعت حامد شعيشع بدير

معهد بحوث صحة الحيوان- مركز البحوث الزراعية – وزارة الزراعة – الدقي – جيزة – مصر

أوضحت الدراسة بمحافظة كفر الشيخ فى الفترة من يناير ٢٠٠٠م حتى ديسمبر ٢٠٠١م أن نسبة الإصابة بمرض السل البقرى كانت ١,١٢ % فى الأبقار و ١,٣٤ % فى الجاموس نتيجة استخدام اختبار التيوبركلين الحقلى.

وقد أظهرت نتائج فحص الغدد الليمفاوية للحيوانات الإيجابية لاختبار التيوبركلين والتى ذبحت بالسلخانة أن النسبة فى الأبقار المصابة كانت ٦٩,٠١ % من الإصابة الغير واضحة بالغدد الليمفاوية وأما النسبة فى الجاموس المصاب كانت ٣,٩٩ % وعلى الجانب الآخر أوضحت نتائج فحص الغدد الليمفاوية والتى لم تظهر بها أعراض ظاهرية للإصابة بمرض السل بالرغم من أنها إيجابية اختبار التيوبركلين أن النسبة كانت ٤١,٠٣ % فى الأبقار و ٥٨,٥٧ % للجاموس.

وكانت النسبة المئوية للإصابة بالسل البقرى وغير البقرى المعزول من الغدد الليمفاوية (بها أعراض ظاهرية وغير ظاهرية للسل) المصاحبة للجهاز التنفسى (٨٥,٩% ، ٧١,٤٣ %) بالنسبة للأبقار و (١٤,٧٠% ، ٢٨,٥٦%) بالنسبة للجاموس على التوالى.

وقد تم تصنيف ميكروبات السل الغير بقرية والمعزولة من الغدد الليمفاوية التى بها أعراض ظاهرية ميكروبكستريوم انتراسيليورارى (٢ عترة) و ميكروبكتريوم كنساسى (١ عترة) ومن الغدد الليمفاوية التى ليس بها أعراض ظاهرية تم عزل ميكروب ميكروبكتريوم فورتيوتم (٣ عترة) وميكروبكتريوم سيجماتيس (١ عترة) .