Original Articles

BOVINE TUBERCULOSIS

Salman H. Siddiqi and Nasreen Niaz

Abstract

An investigation was made to find out type of mycobacteria involved in the cattle. Suspected lung, liver, mesenteric node specimens of cow and buffaloes were obtained from slaughter house animals, and were cultured by concentration method. Out of 500 specimens 51 yielded growth. All the isolates were identified as *M.bovis* except 8 cultures which were atypical mycobacteria. The results are compared with those obtained from human tuberculosis studies. *M. bovis* was never isolated from pulmonary and extra-pulmonary infection in man. It appears that there is no cross infection between man and cattle as the causative agents isolated from each host are different. Thus a tentative conclusion may be drawn that cattle in general and milk in particular pose no threat to human beings so far tuberculosis is concerned.

Introduction

Tuberculosis is an infectious disease attacking man and cattle equally. The relationship of cattle and human tuberculosis was pointed out as early as 1846 (Marshall 1932; Moore 1913), but it is still to be understood clearly. Generally speaking, it was established that tuberculous infection may occur in two forms in man. Alimentary and lymph gland infection caused by the bovine type and lung infection caused by inhalation of the human type tubercle bacilli. The communicability of bovine infection through raw milk is still a firm belief in medical profession.

In recent years, it has been established that *M. tuberculosis* and *M. bovis* are two separate species and are not just a variety as believed earlier. Moreover, the spread of human tuberculosis, as suggested by Flick in 1925, is said to be through Europe from Mediterranean region, while bovine tuberculosis is said to be of Indian origin as cattle were first domesticated in Asia and later brought to Europe (Francis 1947). The incidence and type of disease, however differs to some extent from country to country depending upon the type of the parasite and living conditions and to some extent breed and genetic make-up of the host.

Tuberculosis Research Centre, Pakistan Medical Research Council, Mayo Hospital, Lahore, Pakistan.

It has been a general observation that tuberculosis of pulmonary and extra-pulmonary sites is quite common in Pakistan (Siddiqi, 1973; Siddiqi et al., 1976). However, practically no information is available and no report has been cited in literature concerning bovine type of infection in human beings.

Considering such a high prevalence of tuberculosis in man, it was felt by many workers in this field that man may be infecting cattle rather cattle giving infection to man. Thus *M. tuberculosis* might have taken place of *M. bovis* in cattle as well.

With these observations in mind, this study was carried out to investigate type of mycobacteria involved in cattle infection and their relationship with human infection. The results are compared with that of human tuberculosis in order to establish existence of cross infection between man and cattle.

Material and Methods

Suspected portions of cow and buffalo lungs, liver and mesenteric nodes having some gross lesion were collected in sterile plastic container from slaughter house through the cooperation of Veterinary Research Institute, Lahore. The specimens were refrigerated till the next morning when they were processed for culture.

For culture, concentration method was followed (Kubica and Dye, 1967; Vestal, 1975). The specimen was first washed with distilled water to remove contamination from outside. A portion of suspected tissue was removed and was macerated in a sterile morter and pestle with some sand. About 10 ml phosphate buffer pH 6.8 was also added. After thorough maceration, the supernatent was carefully decanted in a 50 ml screw cap centrifuge tube. To this approximately equal quantity of 4% sodium hydroxide was added for digestion and decontamination. After mixing well it was allowed to stand for 15 minutes, shaking the tube frequently.

This mixture was then neutralized and then centrifuged at 3000 rpm for 20 minutes in an International Centrifuge. The supernatent fluid was discarded and the sediment was resuspended in about 1-2 ml sterile buffer. This suspension was used for inoculation of two regular Lowenstein Jensen (LJ) medium tube with glycerin and two tubes of LJ medium without glycerin, adding about 3-4 drops in each tube by means of a pasteure pipette.

All the inoculated tubes were incubated at 37°C and were checked weekly for a maximum of 10 weeks. Whenever any growth was observed, it was checked for Acid Fast Bacteria (AFB). All the mycobacterial isolates were subcultured

on LJ medium while slow growing non-chromogenic mycobacteria were also subcultured in special LJ medium with sodium pyruvate. These subcultures were used for identification by colonial morphology, pigment production, rate of growth and biochemical reactions (Vestal 1975).

All the isolated mycobacteria were subjected to the drug sensitivity testing following proportion method (Canetti et al., 1969). Lowenstein Jensen medium without potato starch and with 0.5% sodium pyruvate was used for drug sensitivity test. One medium concentration of the test drug was incorporated in the LJ medium and two different dilutions of the test culture were inoculated for each drug to be tested. Mycobacteria which showed more than 1% growth on drug containing medium as compared with control were taken as resistant in case of Isoniazid, PAS and Rifampicin while in case of Streptomycin, Ethionamide, Ethambutol and Thiacetazone, 10% growth was taken as critical proportion for resistance.

Selective isolates were also inoculated in tuberculin negative rabbits following method of Mitchison (1964) for virulence testing with some modifications. Scoring was done describing extent of involvement of each organ after autopsy of the animal whenever it died. If the animal survived, it was killed after 8 weeks. Direct smears were also made from the infected organs and were stained by Ziehl Neelsen method to confirm the presence of AFB. The specimens were also processed for histopathology.

Results

Results of 530 specimens are reported here. There were 510 specimens from cow and bulls comprising of 90 livers, 415 lungs and 5 mesenteric nodes. Only 20 specimens from buffaloes were obtained having 4 livers, 15 lungs and 1 mesenteric node. A total of 51 specimens were found positive from cows and 5 from buffaloes (Table 1). Further analysis of the type of mycobacteria showed that majority of the isolates were *M. bovis* as judged by the growth, characteristics and biochemical reactions (Table II and III). *M. tuberculosis* was not isolated in any case. However, atypical mycobacteria were isolated in 9 cases belonging to Runyon Group II, III and IV. There were two isolates each of *M. flavescens*, *M. scrofulaceum*, *M. gordonae*, and *M. terrae* complex while there was one isolate of rapid growing *M. smegmatis*.

The drug sensitivity results of *M. bovis* are summarized in Table IV. Most of the organisms were found resistant to Isoniazid. Drug sensitivity of atypical mycobacteria is not included because of doubtful pathogenic nature of some of the organisms.

Table I: AFB Culture Results of Specimens from Slaughter House.

	Total specimens	*Culture Positive*		
		M. bovis	*Atypical*	*Total*
Cow	510	43	8	51 (10%)
Buffalo	20	5	0	5 (25%)
Total:	530	48	8	(56) (10.6%)

Table II: Identification of Mycobacteria isolated from Slaughtered Cattle.

Type of Mycobacteria	*Total No. of Specimens Positive*
M. tuberculosis	0
M. bovis	48
Runyons Group I	0
Runyons Group II	6
Runyons Group III	2
Runyons Group IV	1
Total:	56*

*One specimen yielded two types of growth.

Table III: Cultural Characteristics and Biochemical Reactions of the isolated *M. bovis*

Test	*Result*
Rate of growth	Very slow (slower than *M. tuberculosis*)
Appearance of growth	Dysogonic small colonies
Pigment production	None
Growth in glycerol media	Scanty
Enhancement of growth in sodium pyruvate medium	Good
Niacin	Negative*
Nitrate	Negative
Catalase	Slightly positive
Rabbit virulence	High

*One specimen showed Niacin ±

Table IV: Drug Sensitivity Results of *M. bovis* isolated from Slaughtered Animals

Sensitive to all the drugs	4 (8.3%)
Resistant to Isoniazid	44 (91.7%)
Resistant to Streptomycin	0
Resistant to PAS	0
Resistant to Ethionamide	0
Resistant to Ethambutol	0
Resistant to Thiacetazone	0
Resistant to Rifampicin	0

The virulence results indicated great variation in virulence of *M. bovis* for rabbit, but overall, the test strains were found highly virulent giving rise to generalized tuberculosis involving lungs, liver, spleen and omentum. Kidneys were also found to have large tubercles which is a characteristic of *M. bovis* infection.

Discussion

This study was aimed at the investigation of type of mycobacteria involved in cattle tuberculosis and thus no efforts were made to establish the incidence of tuberculosis in animals slaughtered in this area. There have been many reports concerning incidence of the disease in cattle, ranging from 6.7% to as high as 33% (Ahmad et al., 1967; Qureshi et al., 1966). All these reports are based on tuberculin tests and not on any bacteriological work and thus exact information is not available. However, it is a general belief and personal observation of those involved in this field that tuberculosis is quite common in cattle in this country and thus there is a strong possibility that cattle may be playing an important role in spreading this infection in human beings.

As it is well known that tuberculosis is wide spread in human population in this country, with a large number of open cases coughing out tubercle bacilli, infecting other people and possibly also cattle. Living conditions are such that there is a very close association of cattle and man. As a matter of fact sometimes they live in the same room, closed in together in winters. The gawalas, milkman and meat handlers are continuously in contact with tuberculous cattle while cattle are continuously exposed to human tuberculosis. Thus, the cross infection between the two species becomes quite evident since both species are equally susceptible to *M. tuberculosis* and *M. bovis* (Francis 1947).

Our studies on human tuberculosis indicate that men are infected mainly by *M. tuberculosis* (Siddiqi 1977). Atypical mycobacteria are rare in pulmonary infection. In certain extra-pulmonary infection like tubercular lymphadenitis and urogenital tuberculosis, atypical mycobacterial infection is more frequent (Siddiqi 1974, 1975, 1977). After processing more than 15000 sputa from pulmonary patients and more than 2000 specimens from different extra-pulmonary sites including bone, abdomen, lymph gland and genito-urinary tract, we can safely conclude that there is no K. *bovis* in these infections. *M. bovis* infection in man has been reported from Western countries (Francis 1947, 1958). However, there has been no report in literature indicating *M. bovis* infection in man in this sub-continent. The only case report in literature by Soparker is of dubious nature (Abdussalam 1956). It is, therefore, believed that in this sub-continent *M. bovis* infection in human beings does not exist.

This study on cattle tuberculosis further indicates that there is only *M. bovis* infection in cattle and no human *M. tuberculosis* is infecting cows and fuffaloes. Similar findings have been reported from India (Lall, 1969; Mallick et al., 1942). We can thus, tentatively draw a conclusion that there is no cross infection between man and cattle and thus both the species pose no danger to each other so far tuberculosis is concerned. This, infact, breaks the century old concept of raw milk causing tuberculosis of intestine and lymph nodes.

A possible explanation is that there may be some difference in the degree of virulence of the infecting agent and degree of susceptibility of each species. Heriditary factor of the host may also play an important role in acquiring different types of infection. Absence of *M. tuberculosis* in cattle leads us to believe that cattle in this area have developed resistance to human infection. This study is confined to this area and further studies at different places of the country are needed before establishing this fact.

Cross immunity may be an important factor which may play an important role in making man resistant to bovine infection. Since the exposure rate to tubercle bacilli is very high and 80% of the adult population is tuberculin positive (Roelsgaard et al., 1957; Siddiqi and Stauffer, 1973), it is possible that they become immune to *M. bovis* infection through subclinical infection or primary complex. Geographic variation in the virulence of the prevalent strains has been reported by many workers (Lall 1969; Mitchison 1964; Soparker 1926). It is possible that *M. bovis*, prevalent in this area are of low virulence and thus are not capable of producing disease in man.

Generally under infection is the main source of spread of bovine infection through milk. There have been some reports that there is no udder tuberculosis in this area (Lall 1969; Qureshi et al., 1966) and thus milk is not the source of infection even if it is taken unboiled and unpasturized. A thorough investigation is needed on these lines. Moreover, the possibility of aerogenic and other source of infection from cattle to human should also be considered.

One thing which we should keep in our mind is that with changing time, traditions and living habbits, the trend of tuberculosis infection has also changed. The West has overcome the problem of this infection which has caused sudden slack in the research work and accumulation of further knowledge. The East is still fighting with this disease. We have to go a long way before we can find out answers to many questions and uptodate not only our knowledge but also the medical literature.

Acknowledgement

The authors are grateful to Dr. M.S. Jaffery, Director Veterinary Research Institute, Lahore, for his cooperation. Special thanks to Dr. M.

Zulfiqar, Veterinary Officer who made this study possible by helping in collection of the specimens from slaughter house.

References

Abdussalam, M. (1956) Bovine tuberculosis in Indo-Pakistan. Pakistan J. Health, 6:175.

Ahmad, R., Anwar, M. and Qureshi, A. Incidence of tuberculosis amongst cattle in West Pakistan. Proceedings of Pakistan Science Conference, 1967, pp. 6-41.

Canetti, G., Fox, W., Khomenko, A., Mahler, H.T., Menon, N.K., Mitchison, D.A., Rist, N. and Smelev, N.A. (1969) Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. Bull. Wld. Hlth. Org., 41:21.

Francis, J. Bovine tuberculosis, including a contrast with human tuberculosis. London, Staples, 1947.

Francis, J. Tuberculosis in animals and man; a study in comparative pathology. London, Cassell, 1958.

Kubica, G.P. and Dye, W.E. (1967) Laboratory methods for clinical and public Health Mycobacteriology, U.S. Dept. of Health, Education and Welfare, Public Health Service Publication No. 1547.

Lall, J.M. (1969) Tuberculosis among animals in India. Veterinary Bulletin, 30:385.

Mallick, S.M., Aggarwal, H. and Dua, R.L. (1942) An investigation into the incidence and type of tuberculous infection in cattle at Amritsar with special reference to human infections. Int. Med. Gaz., 77:668.

Marshall, C.J. (1932) Progress in controlling bovine tuberculosis. J. Amer. Vet. Med. Ass., 33:625.

Mitchison, D.A. (1964) The virulence of tubercle bacilli from patients with pulmonary tuberculosis in India and other countries. Bull. Int. Un. Tuberc., 35:287.

Moore, V.A. Bovine tuberculosis and its control. Ithaca, N.Y. Carpenter and Company, 1913.

Qureshi, M.H., Chughtai, A.H. and Bhatti, M.A. (1966) Observations on the incidence of bovine tuberculosis. W. Pakistan J. Agri. Res., 4:168.

Roelsgaard, E., Christensen, H. and Iversen, E. (1957) BCG vaccination programme in Pakistan. Bull. Wld. Hlth. Org., 187:202.

Siddiqi, S.H. Bacteriological study of tuberculosis, final report, University of Maryland, Pakistan Medical Research Center, Lahore, 1973.

Siddiqi, S.H. and Stauffer, J.C. (1973) Tuberculin sensitivity to PPD-S and three atypical antigens in Pakistan. Pakistan J. Med. Res., 12:11.

Siddiqi, S.H., Stauffer, J.C., Ali, M.A. and Ahmad, B. (1974) Tubercular lymphadinitis. A study of mycobacteria isolated from cervical lymph glands. Pakistan J. Med. Res., 13:63.

Siddiqi, S.H., Stauffer, J.C., Ali, M.A. and Middlebrook, G. (1976) Some bacteriologic aspects of the epidemiology of pulmonary and extrapulmonary tuberculosis. Amer. J. Epidemiology, 103:101.

Siddiqi, S.H. (1975) Extra-pulmonary tuberculosis (Editorial). Prog. Med., 4:1.

Siddiqi, S.H. (1977) Progress Report 1976, Pakistan Medical Research Council.

Soparker, M.M. (1926) The relative susceptibility of Indian milk cattle of various breeds to tuberculosis. Ind. J. Med. Res., 13:755.

Vestal, A.L. (1975) Procedures for the isolation and identification of mycobacteria, Centre for Disease Control, Atlanta, Georgia, U.S.A., DHEW Publication No. (C.D.C.) 75-8236.