INT J TUBERC LUNG DIS 5(1):87–91

Isolation of *Mycobacterium bovis* from human cases of cervical adenitis in Tanzania: a cause for concern?

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SUMMARY

SETTING: Pastoralist communities in the Northern and Southern zones of Tanzania.

DESIGN: Observational study.

OBJECTIVES: To determine the involvement of *Mycobacterium bovis* in tuberculosis cases presenting at tuberculosis (TB) clinics in rural areas in these zones.

METHODS: A total of 149 tuberculosis cases identified on the bases of clinical manifestation were sampled. Appropriate specimens were cultured on two Löwenstein Jensen slants with respectively glycerol and pyruvate added. Forty-one isolates were cultured and subjected to biochemical typing.

RESULTS: Overall, 31 (70.5%) of the mycobacterial isolates recovered from all forms of tuberculosis were identified as *M. tuberculosis*, seven (16.0%) were identified as *M. bovis*, and six (13.6%) were other mycobacterial species. There was a significantly higher isolation rate ($P < 0.05$) of *M. bovis* among strains recovered from extra-pulmonary (26.8%) than pulmonary tuberculosis samples (4.3%).

CONCLUSION: Based on these findings, it is imperative that *M. bovis* be considered as a pathogen of concern to people living in rural areas of Tanzania. Further work is required to establish a zoonotic link between cattle and the people in these communities who rear them.

KEY WORDS: *Mycobacterium bovis*; human; Tanzania

TUBERCULOSIS is a disease of worldwide distribution, and most cases are found in the developing countries.[1] In Africa much is known about tuberculosis in the animal population; 33 of the 56 countries have reported *Mycobacterium bovis* in animals;[2] however, few countries have reported infection due to *M. bovis* in humans.[3–5] The appearance of endemic human immunodeficiency virus (HIV) infection has exacerbated the prevalence of tuberculosis in many developing countries.[6] In Tanzania, where the human population is estimated at 30 million, the annual number of new cases of tuberculosis is now approaching 40 000;[7] 17% of these cases are extra-pulmonary tuberculosis,[7] and in the absence of culture facilities there is no indication of whether it is *M. bovis* or other mycobacteria that are involved.[8]

A preliminary survey conducted in Tanzania in early 1992 revealed that most cases of extra-pulmonary tuberculosis were found in regions with a high ratio of cattle to human population.[9] This observation was particularly true of the Arusha region in the north of the country, which has the highest cattle population, and where 30% of all cases of extra-pulmonary tuberculosis in Tanzania occur. As regards the disease in cattle, the Southern Highland zone of Tanzania has recorded a high prevalence of cases of bovine tuberculosis amongst slaughter cattle for many years.[9–11]

Although there is a possibility of the human population acquiring tuberculosis from cattle in Tanzania, the paucity of data on infection caused by *M. bovis* in man resulted in a failure on the part of the medical authorities in the country to recognise *M. bovis* as a potential risk to human health.[12] One relevant study, in which 271 cases of extra-pulmonary tuberculosis diagnosed at Muhimbili Medical Centre, Dar es Salaam, were examined, was able to isolate mycobacteria from 6% of the samples, but could not identify the species involved.[13]

The problem in proving the presence of *M. bovis* in man has many facets, among them the lack of interest amongst medical physicians and microbiologists in requesting typing of strains after primary culture.[14,15] Second is that it is not always possible, due to lack of facilities, to collect specimens from extra-pulmonary organs affected by the disease.[8]

In order to determine the involvement of bovine tuberculosis in tuberculosis morbidity in Tanzania, a

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Article submitted 16 March 2000. Final version accepted 28 September 2000.

study was conducted to identify the mycobacterial species found in tuberculous cases among people involved in livestock keeping.

MATERIALS AND METHODS

Sampling of human cases

A study lasting from March 1993 until December 1996 was conducted in the Arusha region in the North and the Southern Highlands of Tanzania. The sampling frames were identified due to high rates of tuberculosis in man and cattle. There were three hospitals in each of the sampling frames. In the Southern Highlands, two health centres were also included in the study, as the area encompasses more than one region. A few samples came from a referral hospital in Dar es Salaam—these were from patients originating from the study area.

Sputum samples were collected from cases of pulmonary tuberculosis as recommended by the National Tuberculosis and Leprosy Programme (NTLP);[12] in brief, early morning sputum was collected on 3 consecutive days. For cases of extra-pulmonary tuberculosis, biopsies of cervical and mesenteric lymph nodes were collected; this involved minor surgery on enlarged nodes on the neck, and excision of enlarged mesenteric lymph nodes during emergency laparotomies.

Data collected on the patients included age, sex, ethnicity and history of tuberculosis.

Isolation of mycobacteria

All processing of specimens was carried out in a Class I safety cabinet (Medical Air Technology, Manchester, UK). Lymph nodes were taken from containers aseptically using sterilised forceps and placed in sterile containers. Using sterile scalpel blades, samples were further macerated to obtain fine pieces and placed in a stomacher bag containing about 5 ml of distilled water and homogenised for 5 minutes using a Stomacher 80 Lab blender (Seward Laboratory, London, UK). The lymph node homogenate was then put into a universal container and 3% oxalic acid was added to fill the universal for decontamination. Decontamination was carried out for 30 minutes with intermittent shaking, after which the lymph node homogenate was centrifuged at 1500 $\times$ *g* for 20 minutes and the supernatant discarded into a disinfectant. 2% sodium hydroxide (NaOH) was added to neutralise the pH of the samples.

Sputum samples were transferred into a 25 ml universal container and decontaminated using 4% NaOH. Homogenisation was achieved within 15 minutes, after which an indicator, phenol red, was added prior to neutralisation using concentrated hydrochloric acid. Neutralisation was achieved when the suspension colour changed from purple to pink.

After neutralisation, suspensions were centrifuged and the supernatant discarded to leave at least 2 ml of the sediment to be used as inoculum for the cultivation of mycobacteria. 0.1 ml aliquots of the sediments from each sample were spread on the surface of each of the Löwenstein-Jensen slants with glycerol (IUT) or pyruvate (L-J pyruvate). Cultures were incubated aerobically at 37°C for at least 6 weeks, with weekly observation for signs of growth.

Identification of mycobacteria species was done on pure cultures using established protocols.[16,17] Differentiation of *M. tuberculosis* and *M. bovis* from other mycobacterial species was initiated by visual observation of cultures. Isolates which gave eugonic growth on both media were tentatively identified as *M. tuberculosis*, while those which produced eugonic growth on L-J pyruvate were suggestive of *M. bovis*. The following biochemical tests were also carried out as described by Grange and Collins:[16,17] nitratase activity, oxygen preference, susceptibility to pyrazinamide and 2 tiophen-carboxylic acid hydrazide (TCH). For identification of other species of mycobacteria, methods described by Marks were utilised.[18]

Statistical analyses

Statistical analysis was performed using the EPITABLE facility of Epi-Info version 6.0 (Centre for Disease Control, Epidemiology Program Office, Atlanta, GA, USA/World Health Organization, Global Programme on AIDS, Geneva, Switzerland). The χ^2 Yates corrected analysis was used to measure the strength of differences observed in the proportion of isolation rates between the different forms of tuberculosis and the different species of mycobacteria isolated.

RESULTS

Isolation of mycobacteria

A total of 44/149 (29.5%) specimens collected from human cases of tuberculosis were found to be positive for mycobacteria. There was a significant difference ($P < 0.05$ [$\chi^2 = 4.03$; df = 1; $P = 0.045$]) between the isolation rates of mycobacteria from pulmonary and extra-pulmonary tuberculosis. Twenty-one isolates (39.6%) were cultured from 53 cases of extra-pulmonary tuberculosis, while 23 isolates (24.0%) were recovered from 96 cases of pulmonary tuberculosis.

Overall, 31/44 (70.5%) isolates of mycobacteria recovered from specimens from all forms of tuberculosis were classified as *M. tuberculosis*, while seven (16.0%) were identified as *M. bovis*. Of these, six were from cervical lymph nodes while only one was from mesenteric lymph nodes. Six (13.6%) isolates were other mycobacterial species (Table). There was no significant difference in the isolation rate of *M. tuberculosis* from either pulmonary or extra-pulmonary cases of tuberculosis (69.1% vs. 71.4%, $P > 0.05$ [$\chi^2 = 2.81$; df = 1; $P = 0.09$]). In contrast, the isolation rate of *M. bovis* among strains recovered from cases of extra-pulmonary tuberculosis (28.6%) was significantly

Table Distribution of *Mycobacterium* species amongst positive cases of tuberculosis

Mycobacterium spp.	Isolates *n* (%)*	Form of tuberculosis	
		Extra-pulmonary *n* (%)	Pulmonary *n* (%)
M. tuberculosis	31 (70.5)	15 (71.4)	16 (69.1)
M. bovis	7 (15.9)	6 (28.6)	1 (4.3)
Other species	6 (13.6)	0	6 (26.6)
Total isolates	44	21	23

* Proportion of total isolates.

higher ($P < 0.05$ [$\chi^2 = 6.03$; df = 1; $P = 0.014$]) than that from pulmonary tuberculosis (4.3%) (Table). None of the other species of mycobacteria were isolated from cases of extra-pulmonary tuberculosis.

DISCUSSION

In Tanzania, no concise studies have so far been carried out to specifically identify *M. bovis* as a cause of human tuberculosis. As a consequence the NTLP has provisionally regarded this disease agent as being of no significant consequence to human health.[12] The present study, the first of its kind, has demonstrated the involvement of *M. bovis* in tuberculosis morbidity in humans in Tanzania, albeit in few isolates. The results of the present study have also shown that *M. bovis* was more prevalent in cases of extra-pulmonary than pulmonary tuberculosis ($P < 0.05$). This finding concurs with the suggestion made by others that the main manifestation of *M. bovis* infection in man is the involvement of the extra-pulmonary organs.[8] However, recent literature has shown that this agent can also be more common in pulmonary cases in countries where bovine tuberculosis has been or is on the verge of being eradicated.[19] Nevertheless, the recovery of these few isolates of *M. bovis* from cases of extra-pulmonary tuberculosis in the present study has provided the first data on the involvement of *M. bovis* in Tanzania; the magnitude of the problem may be greater than reported, as the method of diagnosis was based mainly on culture of the pathogen. No attempt was made to carry out histopathological examinations, as the main focus was to obtain live organisms for molecular studies.[11] However, despite the lower number of isolates of *M. bovis* recovered in the present study, the observed ratio of *M. bovis* isolates in cases of extra-pulmonary tuberculosis to those in pulmonary tuberculosis (6:1) was similar to that experienced in European countries (5:1) at the start of the bovine tuberculosis eradication programme.[8,20]

Previous analysis of epidemiological data in cases in which *M. bovis* was isolated has revealed important observations such as that *M. bovis* infections are more confined to people with cattle contact, implying that these people might have acquired the infection from animal sources.[11] The present study observed pastoralist groups, particularly the Maasai, whose custom it is to keep younger stock indoors as well as to drink untreated raw milk and blood. These two activities provide an ideal scenario for transmission of infection from cattle to man. Similar observations were made in Europe before the bovine tuberculosis eradication campaigns were instituted.[21] In a much larger study in Tanzainia, *M. bovis* was more common in younger people.[11] This finding is in agreement with what happened in the developed countries in the 1930s to 1940s, where bovine tuberculosis affected children more than other age groups.[22] The situation in industrialised countries has changed due to the control measures instituted by these countries, and the disease is now mainly confined to old people.[23]

NTLP data on the incidence of tuberculosis in Tanzania for the period 1984–1995 have shown a steady increase from 12 092 to 39 847 new cases per year, equivalent to an increase of 230%. During the same period the proportion of extra-pulmonary cases rose from 7.9% to 17%.[7] An increase of this magnitude calls for further studies on the contribution of *M. bovis* to tuberculosis morbidity in Tanzania, where the likelihood of certain communities such as pastoralists contracting the disease from cattle should be considered as high. Tuberculosis in cattle has been demonstrated to be prevalent in a number of foci within the country;[9,11,24] in the Southern Highlands in particular, a high proportion (14%) of cattle react to the single comparative intradermal tuberculin test, and tuberculous lesions are found in up to 26.8% of slaughtered cattle.[11] These cattle serve as potential reservoirs of infection to man in the absence of concrete control measures.

Souring of milk, which is accompanied by a reduction of pH of milk, has been reported to prevent the multiplication of many micro-organisms. However, others have found *M. bovis* to persist in soured milk for up to 14 days.[7,25]

The isolation of *M. bovis* from both man and cattle is not adequate evidence that the infection is transmitted from one population to another. The biochemical tests used to identify this species are not adequately equipped to sub-type strains of *M. bovis* and other members of the tuberculosis complex.[16,17] Conventional typing techniques, such as determination of antibiograms, phage typing or serotyping, have been found not only to be ineffective but are cumbersome to perform.[26] This study therefore calls for the use of the recently developed DNA typing techniques to link the disease in the two populations, as has been done elsewhere.[27]

Acknowledgements

We wish to express our sincere thanks for the support and acceptance of the National Tuberculosis and Leprosy Programme and hospital under this programme to undertake this study in Tanzania. Thanks to the staff of Mycobacterium Laboratories at Sokoine

University of Agriculture, Morogoro, Tanzania and at City Hospital, Edinburgh, UK, who worked tirelessly in perfecting this study. Special thanks to Drs N Haegreves and Mrs J Champion, members of the Edinburgh Veterinary Research Expedition Team 1994, for provision of surgical equipment which assisted in obtaining lymph node biopsies.

The financial and material support from UK Department of International Development (formerly Overseas Development Administration) through the Animal Health Programme is acknowledged. Assistance provided by British Council Office in Dar es Salaam, Tanzania is also highly appreciated.

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RÉSUMÉ

CADRE : Communautés pastorales dans les zones Nord et Sud de la Tanzanie.

SCHÉMA : Etude d'observation.

OBJECTIFS : Déterminer l'implication de *Mycobacterium bovis* dans les cas de tuberculose se présentant dans les polycliniques de tuberculose, dans les régions rurales de ces zones.

MÉTHODES : On a échantillonné 149 cas de tuberculose identifiés sur la base de leurs manifestations cliniques. Les spécimens appropriés ont été mis en culture sur deux tubes de Löwenstein-Jensen additionnés respectivement de glycérol et de pyruvate. Dans 41 isolats, on a pratiqué la culture et un typage biochimique.

RÉSULTATS : Globalement, 31 isolats (70,5%) de mycobactéries prélevés dans toutes les formes de tuberculose ont été identifiés comme *M. tuberculosis*, sept (16,0%) identifiés comme *M. bovis* et six (13,6%) comme d'autres espèces mycobactériennes. Le taux d'isolement de *M. bovis* est significativement plus élevé ($P < 0,05$) dans les souches provenant de tuberculose extra-pulmonaire (26,8%) que de tuberculose pulmonaire (4,3%).

CONCLUSION : Sur la base des observations de cette étude, il est impératif de considérer *M. bovis* comme un pathogène préoccupant pour les personnes vivant dans les zones rurales de Tanzanie. Des études complémentaires s'imposent pour établir des liens entre une zoonose du bétail et les individus de ces communautés qui les élevent.

RESUMEN

MARCO DE REFERENCIA : Comunidades pastorales en las zonas Norte y Sud de Tanzania.
MÉTODO : Estudio de observación.
OBJETIVOS : Determinar la presencia de *Mycobacterium bovis* entre los casos de tuberculosis presentados en los centros de TB de las áreas rurales de estas zonas.
MÉTODOS : Se identificaron 149 casos de tuberculosis en base al estudio clínico y los materiales fueron cultivados en dos medios de Löweinstein Jensen, a los que se le agregaron glicerol y piruvato, respectivamente.
RESULTADOS : Del total, 31 (70,5%) de las cepas de micobacterias recuperadas de todas las formas de tuberculosis se identificaron como *M. tuberculosis*, siete cepas (16,0%) fueron identificadas como *M. bovis*, mientras que seis (13,6%) cepas pertenecían a otras especies de micobacterias. Existió una tasa significativamente más alta de aislamiento ($P < 0,05$) de *M. bovis* entre las cepas recuperadas de tuberculosis extrapulmonares (26,8%) que entre las de tuberculosis pulmonares (4,3%).
CONCLUSIÓN : En base a los hallazgos de este estudio es imperativo considerar a *M. bovis* como un agente patógeno importante entre la gente de áreas rurales de Tanzania y es necesario poner énfasis en establecer relaciones entre la zoonosis en el ganado y las personas de estas comunidades que viven en su proximidad.