ISOLATION OF MYCOBACTERIUM SPECIES FROM RAW MILK OF PASTORAL CATTLE OF THE SOUTHERN HIGHLANDS OF TANZANIA

R.R. KAZWALA1, C.J. DABORN2, L.J.M. KUSILUKA1, S.F.H. JIWA3, J.M. SHARP4 AND D.M. KAMBARAGE1
1Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, PO Box 3021, Morogoro, Tanzania; 2Centre for Tropical Veterinary Medicine, University of Edinburgh, Roslin, Easter Bush, Edinburgh, EH25 9RG, UK; 3Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture, PO Box 3086, Morogoro, Tanzania; 4Moredun Research Institute, Edinburgh, EH17 7JH, UK

ABSTRACT

A study to determine the secretion of Mycobacterium spp. in milk from indigenous cattle was carried out in pastoral cattle reared in the Southern Highlands of Tanzania. The study was aimed at elucidating the dangers associated with milk-borne zoonoses in a society where milk is normally consumed raw. Out of 805 milk samples, 31 (3.9%) were positive for mycobacteria. There was a preponderance of atypical mycobacteria (87%) whereas only two isolates (6.5%) were confirmed as M. bovis. Atypical mycobacteria included: M. terrae (n = 7), M. fortuitum (n = 2), M. flavescent (n = 13), M. gordonae (n = 1) and M. smegmatis (n = 4). Although the number of M. bovis positive samples was low, the habit of pooling milk may still pose great public health dangers to milk consumers in this part of the world. Moreover, isolation of atypical mycobacteria should also be considered to be a danger to human health in countries such as Tanzania, where the number of people with lowered immunity due to HIV infection is on the increase.

INTRODUCTION
The cattle population of Tanzania comprises mainly (98%) indigenous cattle scattered throughout the country, and only 2% exotic dairy and grade (crosses) cattle (Anonymous, 1997). The latter are mostly confined to large government dairy farms and the small scale dairy sector in urban and peri-urban areas. This structure of the dairy industry dictates that much of the milk consumed in Tanzania is from the traditional communally grazed livestock sector.

In Tanzania, bovine tuberculosis is known to be present in a number of foci (Chillaud, 1995). For example, the Southern Highlands, particularly the Usangu Plains, have continuously recorded a high proportion of cattle with tuberculous lesions at slaughter (Kazwala, 1996). In a nationwide survey (Kazwala et al., 1993) it was found that the proportion of lesion-positive cattle in regions excepting the Southern...
Highlands was between 0.1% and 2%, whereas in regions constituting the Usangu Plains (Mbeya and Iringa), the regional average was between 4% and 10%.

In view of inadequate veterinary services offered to rural areas of Tanzania, coupled with the lack of epidemiological data regarding the disease status in the traditional livestock sector (Jiwa, 1997), the rural population continues to be at risk of acquiring milk-borne mycobacterial infections. Cattle-derived tuberculosis in man is attributed to *Mycobacterium bovis* and occasionally *Mycobacterium tuberculosis* (Sinha, 1994) and is mainly transmitted through milk (Sigurdsson, 1945; Kleeberg, 1984). Therefore habits, such as consumption of raw milk and sour milk prepared by fermentation, may predispose people to such infections (Kleeberg, 1984). The purpose of this study was to establish whether mycobacteria species were being secreted in milk and to ascertain the extent of the zoonotic implications related to consumption of raw milk in rural communities.

**MATERIALS AND METHODS**

*Milk sample collection*

Milk samples were collected from 805 pastoral cattle in villages in the Southern Highlands of Tanzania which comprised Iringa and Mbeya administrative regions where other studies have indicated the presence of tuberculosis in cattle at slaughter (Maiseli et al., 1987; Kazwala, 1996). The choice of herds from which milk was collected was based on the cooperation of animal owners.

Milk samples were collected by cattle owners from previously washed udders into 30 ml sterile universal containers and then placed in cool boxes. Samples were later frozen at −20°C at local animal health centres prior to culturing at the Mycobacterium Laboratory at Sokoine University of Agriculture.

*Sample processing and isolation of mycobacteria*

In order to minimize contamination, 10 ml of milk sample was transferred into another sterile universal container for decontamination using 4% sodium hydroxide and then neutralized using concentrated hydrochloric acid. In order to monitor neutralization, 1 or 2 drops of phenol red were added. Neutralization was achieved when the suspension colour changed from purple to pink. Suspensions were then centrifuged at 13,000g and the supernatant discarded to leave at least 2 ml of the sediment to be used as inoculum for the cultivation of mycobacteria species.

Primary isolation of *Mycobacterium* spp. was done on 2 egg media, namely IUT (Jensen, 1955) and Loewenstein-Jensen with added pyruvate (L-J pyruvate) (Stonebrink, 1958). For cultivation, 0.1 ml of the sediments from each sample was spread on the surface of each of the media using a sterile pastette and incubated at 37°C for at least 6 weeks, with weekly observation for signs of growth. Positive cultures with colony morphology similar to that described by Vestal and Kubica (1966) were subcultured onto another set of media (2 slopes of each medium per culture) and incubated for another 3 to 4 weeks for further identification.