MICROBIAL ISOLATES FROM MASTITIC UDDERS FOLLOWING CHEMOTHERAPY FAILURE

Sanga, A. A., Kambarage, D. M., Kazwala, R.R., Kusiluka, L.J.M., Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania.

Mastitis is one the most important diseases which cause immense economic losses to the dairy industry world-wide (Hungerford, 1975; Harbans and Singh, 1982). Losses are attributed to reduced milk production, discarding of milk, treatment costs and occasionally fatalities (Kinabo and Assey, 1983; Blood *et al.*, 1989; Radostits *et al.*, 1995).

In Tanzania, the prevalence of mastitis in large scale dairy farms has been shown to be high (Kinabo and Assey, 1983; Said, 1987; Mbwambo, 1988; Mshana, 1989). Annual incidences of the clinical and sub-clinical forms of the disease have been observed to vary from 2.3-3% and 40-70% respectively. Comparable incidence rates of the disease have also been observed in the small scale dairy sector in Tanzania (Shekimweri, 1992).

The number of clinical cases of mastitis which are unresponsive to chemotherapy are on the increase in the country (Kambarage, personal observation) leading to speculations that either there is involvement of micro-organisms other than the conventional aerobic bacteria or drug resistance probably attributed to improper usage of anti-microbial.

In routine diagnosis of mastitis carried out by a number of laboratories in the country, most samples are cultured aerobically as it is assumed that aetiological agents are often aerobic bacteria (viz. *Staphylococcus* spp.; *Streptococcus* spp.; *E. coli*; etc.). Therefore, no efforts are made to search for micro-organisms which are either anaerobic or require specialised growth conditions such as fungi, yeast and *Mycobacterium* spp. These micro-organisms may be responsible for some of the non-responsive mastitis.

Therefore, in order to determine the range and antibiogram of micro-organisms responsible for non-responsive mastitis, a total of 42 milk samples were collected from dairy animals in Morogoro and Dar es Salaam. Samples were collected after washing the udders with soap and water, and discarding the first few streams of milk. Samples were then transported in cool boxes to the laboratory for cultures for aerobic bacteria, fungi and yeast. A portion of each sample was kept frozen until cultured for *Mycobacterium* species.

A portion of each sample was aseptically inoculated on blood, nutrient and McConkey agar media, incubated aerobically at 37°C and examined for bacterial growth at either 24 and/or 48 hr. Characterisation of bacterial isolates was based on gram staining and biochemical tests. Bacterial isolates were further inoculated on nutrient agar containing anti-microbial paper discs (Mast Laboratories Ltd, Merseyside, UK) and incubated as above for 24 hr. Resistant bacterial isolates were identified on the basis of growth in the presence of drugs (Emmanuel, 1980; Carter *et al.*, 1991; Watt *et al.*, 1993).

Fungi and yeasts were isolated by inoculating a loopful of milk samples on Saboroud's Dextrose Agar and incubated at room temperature for 24 to 48 hr. Identification was based on gross and microscopic appearances of colonies. The latter was carried out after staining

with Giemsa. An additional portion of each sample was decontaminated using 4% sodium hydroxide and then neutralised using 2% potassium dihydrogen orthophosphate (BDH Laboratory Supply, England) in the presence of phenol red (Fissons Scientific Equipment, UK). The samples were then inoculated on Lowesten-Jensen medium containing pyruvate (L-J Pyruvate) and that with glycerol for isolation of *Mycobacterium* spp. The media were then incubated aerobically at 37°C and examined for presence of growth every week for a maximum of 12 weeks.

The results showed that out of 42 samples of milk collected, 72% (32) were positive for bacteria, fungi or yeasts. Of the positive samples, 85% had various bacteria; 27% showed yeast growth and 3% were positive for fungi. Overall, the isolation rates for bacteria, yeast and fungi were 64%, 17% and 2.5%, respectively (Table 1). Culture negativity (28%) of the samples observed in this study has also been reported by others (Radostits *et al.*, 1995) and may be attributed to the presence of antimicrobials in milk samples as no concise treatment history (especially as regards the drug type and last date of treatment) was made available for each case. However, it is also possible that the udders were infected by micro-organisms other than those investigated in the course of this study. Such micro-organisms may include viruses, *Mycoplasma* spp and anaerobic bacteria which have also been implicated in mastitis (Radostits *et al.*, 1995).

The most prevalent bacterial isolates were *Streptococcus* species other than *Streptococcus agalactiae* (24%); *Staphylococcus* species (17%); *Actinomyces pyogenes* (14%) and *Mycobacterium* species (10%). The preponderance of *Streptococcus* and *Staphylococcus* species even in udders unresponsive to chemotherapy compares well with the observation in other studies carried out in Tanzania (Shekimweri, 1992; Kinabo and Assey, 1983; Mahlau and Hyera, 1984; Mbise *et al.*, 1983; Msanga *et al.*, 1989; Kambarage *et al.*, in press).

Four isolates from milk were identified as *Mycobacterium* species. These included *M. bovis* (1), *M. flavescens* (1) and *M. terrae* (2). The latter two are environmental micro-organisms of little public health importance in man. Of major public health significance is the isolation of *M. bovis*, albeit from one animal. Although the isolation rate of *M. bovis* is rather low as also observed in other studies (Appuswamy *et al.*, 1980; Kazwala 1996) but in contrast to the high isolation rate from mastitic animals elsewhere (Kastandi *et al.*, 1989), pooling of milk often leads to contamination of bulk milk (Kleeberg, 1984). Contamination of bulk milk by one animal may lead to infections in a significant number of milk consumers.

Much of the milk in Tanzania is consumed raw and this is mainly attributed to lack of enforcement of legislatory regulations pertaining to pasteurisation of milk and low public awareness. Consumption of unpasteurised milk greatly posses a great risk of infections to milk consumers. The public health implications of *M. bovis* in milk is high in the wake of the current HIV/AIDS pandemic which has a devastating effect on human life and economies of the affected countries.

Most of the gram positive bacterial isolates were resistant to cloxacillin, penicillin G; weakly sensitive to cephalospan (<5%), ampicillin (10%) and lincomycin (25%) and moderately (50-60%) sensitive to erythromycin, novobiocin , gentamicin and tetracycline. *Streptococcus* species were mainly sensitive to tetracyclines (83%),

moderately to gentamicin and novobian (50%) and weakly or resistant to other drugs. *Staphlococcus* species were sensitive to erythromycin and novobiocin (83%) and weakly or resistant to other antimicrobials. *Actinomyces pyogenes* were sensitive to tetracyclines (83%) and weakly or resistant to other drugs. Gram negative bacterial isolates showed weak (20%) and moderate (60%) sensitivity to tetracyclines and gentamicin respectively.

Table 1: Isolation of different micro-organism from non-responsive mastitis

Type of Isolates	Samples	%
Staphylococcus aureus	3	7.1
Staphylococcus epidermides	3	7.1
Streptococcus spp.	1	2.4
Streptococcus spp. other than *Streptococcus agalactiae*	10	23.8
Actinomyces pyogenes	6	14.3
Micrococcus spp.	2	4.8
ß-Hemolytic *E. coli*	1	2.4
Proteus millabiris	1	2.4
Other gram negative Bacilli	3	7.1
Mycobacterium bovis	1	2.4
M. flavescens	1	2.4
M. terrae	2	4.8
Asperlillus spp.	1	2.4
Candida albicans	7	16.6
Total	42	100

In summary, the variable sensitivity to drugs including low sensitivity to penicillin G which is the main ingredient in intra-mammary infusions available in the country, calls for the need to carry out anti-microbial sensitivity profiles where chemotherapy is ineffective. The isolation of *Candida* spp., *Aspergillus* spp. and *Mycobacterium* spp. is indicative of the spectrum of micro-organisms which may be involved in causing mastitis and thus calls for the need of field veterinarians to include fungal, yeast and mycobacterial infections in the differential diagnosis of bacterial mastitis. Therefore, tuberculin testing of animals and mandatory pasteurisation of milk are becoming essential if the lives of Tanzanian people have to be saved. Definitely, more studies are required to assess the magnitude of fungal and yeast infections, drug resistance and the public health implications of *M. bovis* in milk.

REFERENCES

Appuswamy, S., Batish, V.K., Parkash, O. and Ranganathan, B. 1980. Prevalence of mycobacteria in raw milk sampled in Karnal, India. Journal of Food Protection, 43: 778-781.

Blood, D.C. and Radostits, O.M. (1989). Veterinary Medicine: A Textbook of Diseases of horses, sheep, goats and pigs. Bailliere Tindall, London.

Harbans, S. and More, E. (1982). A Textbook of Livestock and Poultry Production. Prantice Hall, India. pp 221.

Hungerford, T.G. 1975. A Textbook of Diseases of Livestock. 8th edition. MacGraw-Hill Book Company. Sydney. pp 224-225.

Kambarage, D.M., Mtambo, M.M.A., Kimera, S.I. and Muhairwa, A.P. Microbial isolates from milk samples of animals with clinical mastitis in a large dairy farm in Tanzania. Bulletin of Animal Health and Production in Africa. In press.

Kastandi, J.C., Al-Samarrae, S.A.G. and Kamalapur, P.N. 1989. Tubercular mastitis in cows in Irag. Indian Veterinary Journal, 66: 1078-1080.

Kazwala, R.R. 1996. Molecular Epidemiology of Bovine Tuberculosis in Tanzania. PhD Thesis. The University of Edinburgh. pp 315.

Kinabo L.D.B. and Assey, R.J. 1983. Bovine mastitis in selected dairy farms in Morogoro District in Tanzania. Beitrage Tropic Landwirtch Veterinarmed, 21: 65-71.

Kleeberg, H.H. 1984. Human tuberculosis of bovine origin in relation to public health. Revue Scientifique et Technique, Office International des Epizooties, 3: 11-76.

Mahiau, E.A. and Hyera, J.M.K. 1984. Mastitis in dairy cattle in Tanzania.

Bulletin of Animal Health and Production in Africa, 32: 39.

Mbise, A.M., Nyange, J.F.C., Otaru, M.M., Mbasha, E.M.S. 1983. Isolation and drug sensitivity of bacteria from bovine milk samples with clinical mastitis at Arusha Veterinary Investigation Centre between 1975-82. Bulletin of Animal Health and Production in Africa, 33: 31.

Mbwambo, K.E. 1988. A study of occurrence of mastitis in dairy herds in Morogoro. Special Project. Department of Animal Science and Production, Sokoine University of Agriculture, Morogoro.

Msanga, J.F., Tungaraza, R., Olumu, L.M.U. and Muhairwa, A. 1989. Bovine mastitis bacterial isolates from subclinical and clinical mastitis in the Lake zone of Tanzania. Tanzania Veterinary Bulletin, 9: 13-17.

Mshana, Y. 1989. A study of effect of hypochlorite in control of subclinical mastitis. Special Project. Department of Animal Science and Production, Sokoine University of Agriculture, Morogoro.

Said, R. 1987. A study of problems associated with hand and machine milking in large scale dairy farms in Tanzania. Special Project. Department of Animal Science and Production, Sokoine University of Agriculture, Morogoro.

Shekimweri, T.M. 1992. A study of incidence of clinical and subclinical mastitis in small holder dairy farms in Morogoro. MSc Dissertation. Sokoine University of Agriculture, Morogoro. pp 69.