Epidemiological Studies on Bovine Mastitis in Smallholder Dairy Herds in the Dar es Salaam Region, Tanzania

F. M. Kivaria

Utrecht, 2006

Kivaria, Fredrick Mathias
Epidemiological Studies on Bovine Mastitis in Smallholder Dairy Herds in the Dar es Salaam Region, Tanzania
2006

Dissertation Utrecht University, Faculty of Veterinary Medicine
- with summary in English and Dutch

ISBN : 9987- 475-01-9

Layout : Tina Kulewa
Cover : Fredrick Kivaria
Print : DTP, Dar es Salaam, Tanzania

Epidemiological Studies on Bovine Mastitis in Smallholder Dairy Herds in the Dar es Salaam Region, Tanzania

Epidemiologische studies over mastitis bij runderen op kleine familiebedrijven in de Dar es Salaam region van Tanzania

(met een samenvatting in het Engels en Nederlands)

proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. W. H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 16 november 2006 des middags te 2:30 uur

door

Fredrick Mathias Kivaria

geboren op 16 march 1968 te Kilimanjaro, Tanzania

Promoters: Prof. Dr. J. P. T. M. Noordhuizen

Prof. Dr. J. A. P. Heesterbeek

This research was financially supported by the International Foundation for Science (IFS), Stockholm, Sweden, through a grant (NO B /3211) to Fredrick M. Kivaria, the Royal Dutch Academy for Science (KNAW) and the Tanzanian Government.

To Joshua & Margaret

Contents

1

Chapter

General introduction

F. M. Kivaria

Population Studies
Animal Diseases Research Institute
P. O. Box, 9254, Dar es Salaam, Tanzania
fredkiv@yahoo.com

Dairy industry in Tanzania

Historically speaking the dairy industry in Tanzania begins in 1921(Sumberg, 1997), when the colonial government introduced the first nucleus herds of Ayrshire and Holstein cattle, and established the Temeke dairy farm, located on the grounds of Animal Diseases Research Institute, in Dar es Salaam. Since before independence in 196l and with increasing emphasis, the government of Tanzania has tried to encourage more domestic milk production to achieve self-sufficiency. During the 1970s large-scale milk production on farms run by a parastatal organisation (Dairy Farming Company/DAFCO), the use of relatively intensive production systems was encouraged. Another state enterprise, Tanzania Dairies Ltd. (TDL), was set up to collect, process, and market dairy products. The outcomes were disappointing: DAFCO farms were often poorly managed and ran at high costs; whilst TDL built large, "modern' processing plants close to the consumers which produced good quality pasteurised products, but at relatively high cost. This in turn made it difficult for TDL to offer attractive prices to local farmers for their milk, and so capacity needs could only be maintained met by using imported skimmed milk powder (SMP). When SMP imports declined in the 1980s, owing largely to national economic problems and the loss of ability to import, the plants found themselves operating substantially below capacity, with correspondingly high overheads per litre of milk processed. As DAFCO's problems became clear, a new approach was adopted in 1983 (MLD, 1983), switching attention to the promotion of smallholder dairying. It was decided to supply improved cattle, animal feeds, veterinary medicines, extension services, etc. to smallholders, and to improve price incentives to them.

As in the rest of the developing world, increased domestic dairy production by smallholders has the potential in Tanzania to generate income and employment on a wide scale, and thus to improve the welfare of human populations on an economically sustainable basis (Winrock international, 1992). In urban areas, dairy farming typically has been part of the adjustment of household economic activities, especially when families have been faced with rising costs of living. Under such conditions, dairy farming may be one of the few agricultural activities that can provide adequate and stable income to maintain the economic viability of urban dwellers.

The smallholder dairy production system in Tanzania began to emerge in 1983 (Mdoe and Wiggins, 1997). At that time high and mid-

ranking civil servants started keeping a number of grade cattle in their residential compounds in the low and medium density urban areas. Their aim was to contribute to the milk market supply after the parastatal and cooperative farms collapsed. The sector is predominantly a back-yard production system where pigs, sheep and goats, and different poultry species are kept along with dairy cattle. Such intensification (of animals) requires adequate management and increased resources per animal. Moreover, the intensification of dairying, especially under tropical conditions, presents new disease problems. Mastitis is an important example.

Bovine mastitis

Literally bovine mastitis means an inflammatory response of the bovine mammary gland tissue to noxious agents; the agents can be either infectious or non-infectious by nature. Most frequently the aetiology is infectious by nature, organisms as diverse as bacteria, mycoplasma, yeasts and algae have been implicated as causes of the disease (Watts, 1988; Quinn *et al*., 2000; Radostits *et al*., 2000). Mastitis could also be the result of sterile inflammation due to chemical, physical or mechanical trauma. Bovine mastitis can be accompanied by visible signs, such as changes in the milk and swollen udder. When signs are discernable with the naked eye, infection has caused clinical mastitis. When no signs are visible, udder pathogen presence has resulted in subclinical mastitis, and laboratory techniques such as measurement of somatic cell count (SCC) and microbiological culture are needed to detect inflammation and infection causes. Compared with subclinical mastitis, clinical mastitis is much less costly, is of short duration, tends to be an individual cow problem, and is detected without special tests. Bovine mastitis reduces milk yield, increases culling rates, and brings with it treatment costs and the occasional death from severe infections. In addition, some udder pathogens affect food safety because they produce toxins that cause food poisoning, as in the case of *Staphylococcus aureus* (Rosec *et al*., 1997), or because the pathogens, e.g. *Mycobacterium tuberculosis* (Weinhaupl *et al*., 2000; Shirima *et al*., 2003), Verotoxin producing *Escherichia coli* (Roberts, 1990; Cullor, 1997; Stephan and Kuhn, 1999) are zoonotic agents.

Mastitis is a management-related disease the prevention and control of which depends, among other factors, on the type of management employed. With the right management there is a reduction of mastitis

and vice versa. As with most infectious diseases, mastitis risk factors depend on three components: exposure to udder pathogens, cow defence mechanisms, and environmental and management factors (Suriyasathaporn *et al*., 2000). In Tanzania, mastitis is also a common problem in smallholder dairy animals. It has been shown that the annual incidence risk of clinical mastitis ranges between 1.5 and 3.2 cases per 100 cows (Kinabo and Assey, 1983), whereas the prevalence of the subclinical form ranges between 60 and 80% (Shekimweri, 1992; Karimuribo, 2002). A thorough examination of laboratory records at ADRI ranked bovine mastitis number fourth after the three major cattle tick-borne diseases (namely anaplasmosis, babesiosis and theileriosis) **as** the leading animal health constraint among smallholder dairy cattle in the coastal regions.

However, compared to other diseases mastitis is ranked low in priorities by the national veterinary authority, and consequently mastitis has received little attention in Tanzania. Extension efforts have therefore been focused on the treatment of clinical cases rather than tackling the disease from the control point of view. The classic ten-point mastitis control programme (Radostatis *et al*., 2000; Radostatis, 2001) was developed for machine milked herds, the plan may thus not be appropriate for the 100% hand milked smallholder dairy herds. To this end, a study of 125 randomly selected urban and peri-urban smallholder dairy herds in the Dar es Salaam region, Tanzania was initiated. The general objective of the study was to identify and quantify potential risk factors for mastitis as a guide for initiation of low cost and appropriate control strategies for the urban and peri-urban smallholder dairy herds.

Aim and design of the study

Information on risk factors associated with mastitis is therefore of great importance as this is essential for the design and implementation of an appropriate control programme. When designing a sector and herd-specific mastitis control programme, intramammary infections (IMI) as detected by the California mastitis test (CMT) and microbiology cultures, the distribution of risk indicators in time and space has to be taken into account. The scope of this study is to contribute to the knowledge necessary for the design of herd-specific udder health programmes given the resource constraints of a Tanzanian smallholder dairyman. More specifically the aims of this study were:

- To estimate prevalence figures of clinical and subclinical mastitis, as well as the putative causative micro-organisms in small holder dairy

farms in Tanzania
- To evaluate different cow factors, and herd management practices related to (presence or absence of) intramammary infections.
- To study risk factors for intramammary infections at herd level
- To study risk indicators associated with good or poor quality of milk (as determined by the presence of drug residuals and acaricides, potential hazards of public health) from both smallholder dairy herds and milk-selling premises in the Dar es Salaam region, Tanzania.

The dynamics of mastitis at herd level was observed in a longitudinal prospective dynamic cohort study in smallholder dairy herds. The longitudinal design allowed for the observation of the time order of events and hence for identification of possible cause-effect relationships (dynamics as well as interrelations of different risk indicators). The design also allowed for measurement of duration of infections and therefore quantification of the magnitude of economic damage (if resources will allow). Herds with at least $2 \leq n \leq 18$ lactating cows were eligible for the study. In addition, the owner must be willing to participate in the study. Study duration was set at 18 months. Since dynamics in an individual herd can be very different from the average dynamics in herds with similar management, proportional probability sampling was initially used to select herds from the study population to include representative herds from the major three husbandry systems (zero, semi and extensive grazing) practiced in the study area. To strike a balance between the need for frequent sampling and the need to include multiple herds, and within the logistic constraints of the project, a two weekly sampling interval was chosen.

Three procedures were followed in the investigation: clinical, farm and data inspection all according to pre-set field protocols (Brand *et al.*, 2001). Clinical inspection, this focused on udder health of all cows, observations on udder quality, teat shape, teat lesions and teat end callosity scoring. Cows treated for mastitis, body condition score of dry and lactating cows, percent of blind and soiled quarters, and percent of cows with oedematous quarters. Management of mastitis cases, cows that should be culled due to mastitis. This procedure also involves clinical and bacteriologic evaluation of cows. Cows will be inspected for general cleanliness, milk leaking, presence of flies (and biting insects tabanids and ticks) on cows and whether hair is clipped from udders. Farm inspection, this procedure consisted of a thorough inspection of management practices and of environmental conditions to detect risk

factors that influence the multifactorial background of mastitis. The following management categories were investigated: Milking practices, farm environment and management practices related to mastitis treatment and control procedures. Management practices are often interrelated; therefore categorisation is often subjective. Nevertheless, we divide the risk factors into 9 categories. 1: General management included size of the property, number of people working on the farm, herd size, breed, other farming activities, manure management and record keeping. 2: Housing conditions of lactating and dry cows included type of housing, floor, ventilation, grazing / feeding, barn size and bedding materials. 3: Cleaning procedures included cleaning of the barn, cleaning of the cubicles, bedding replacement and cleaning, disinfection procedures and frequency. 4: Hygiene of cubicles and cows included collecting information on the cleanliness of cubicles/barns, bedding, and cows every 30 days at a farm visit. The percentage of cows with dirty udders, thighs and dirty anal regions were estimated. A mean parameter for each category will be calculated for each farm. Observations were done separately for lactating and dry cows. 5: Feeds and feeding of lactating and dry cows included minerals for lactating and dry cows, methods of concentrate feeding and source of water. 6: Management of dry cows and cows before and during calving included presence of maternity or disease pens, bedding in calving area, cleaning and disinfection in calving area, milking in calving area, drying off procedure, dry cow treatment, teat disinfection in dry cows, mastitis checks in the dry period, length of the dry period and seasonal calving pattern. 7: Milking procedures included frequency of milking, number of milkers, wet or dry udder preparation, use of milking salve, pre and post dipping and management of mastitic cows, and a milking technique observation protocol focusing on time-intervals between handlings during milking. 8: Production data included milk production data for 18 months of the study, average pedigree / herd index. 9: Disease and disease prevention included estimate of cows with leaking milk, cows with udder lesion, wounds, cows sleeping on dirty floors and in alleys, use of herd health programmes, use of preventive measures such as vaccinations, treatments, clipping of the udder and dry cow therapy. The data inspection this procedure is directed at assessing the accuracy, validity and quality of data collected by the farmer and at getting insight into the current mastitis status.

Microbiological investigation

Quarter milk samples from all lactating cows for microbiological investigation, were collected at 14 days intervals from July 2003 to March 2005. Throughout the study period, additional quarter milk samples from all lactating cows were collected by the livestock field officer (LFO) at calving, purchase (entry into the milking herd), dry off or culling (exit from the milking herd), and when clinical mastitis is observed. LFO used standardised forms for recording of the relevant (CMT results, clinical mastitis) events. All milk samples were used for microbiological culture according to IDF (1981) and/or NMC (1987) standards. Additionally isolates from sub/clinical cases were deep frozen for detailed studies (antimicrobial susceptibility and molecular characterisation). Bulk milk samples were collected (once) from milking, storage and transporting containers at the study herds and milk collection centres and selling points for evaluation of milk quality as defined by total bacterial counts and drug residuals tests. Microbiological quality of water from the study herd and milk handling points was also evaluated (samples of water used for dairy activities at farm level, and for washing of dairy utensils at the selling points were bacteriologically evaluated). During the study period the farmers were interviewed on relevant aspects of mastitis control and prevention, using a standardised questionnaire. Questionnaires are commonly used in epidemiological investigations to collect information on disease occurrence, associated risk factors, and opinions, and they have been used successfully in studies on mastitis (Schukken *et al*., 1989, Brand *et al*., 2001). The questionnaires were conducted during the first herd visits, and a follow up questionnaire focusing more on the udder health was conducted during the subsequent visits.

Outline of the thesis

In this thesis the research conducted on the aforementioned aspects is presented. In Chapter 2 and Chapter 3 the distribution of mastitis pathogens, is studied in order to detect changes in pathogen profiles. This information is crucial both for adjusting control programmes and for providing an advisory service. One of the major tasks of veterinary epidemiology is the identification and quantification of risk factors for livestock diseases and production problems. Information on risk factors of mastitis is of great importance as this is required for the design and implementation of appropriate prevention and control strategies.

Chapters 4, 5, and 6 give an in-depth coverage of the risk factors that are likely to influence the epidemiology of bovine mastitis in smallholder dairy herds in the Dar es Salaam region. California Mastitis test (CMT) is currently the only available cow-side test which can be economically used for the diagnosis of subclinical mastitis in smallholder dairy herds. However, its utility in low-producing smallholder dairy cows is not well established. This is addressed in Chapter 7. One of the study goals was to follow up the microbiological quality of milk from the herd to the market, thus factors which influence the microbiological quality of milk marketed by the smallholder in the Dar es Salaam region are discussed in chapter 8. In order to understand the dairy sub-sector and to identify constraints to, and opportunities for improving smallholder dairying's contribution to poverty alleviation and to increased food security in the study area, an in-depth study of the smallholder dairy herds is presented in Chapter 9.

References

A.Brand, J.P.T.M.Noordhuizen and Y.H.Schukken., 2001. Herd Health and Production Management In Dairy Practice, 3rd edn (Wageningen Pers Publ. Wageningen, The Netherlands), 351-415

Cullor, J. S., 1997. Risks and prevention of contamination of dairy products. Scientific and Technical Reviews (OIE) **16**: 472-481

International Dairy Federation 1981. Bulletin No. 132. Laboratory Methods for Use in Mastitis Work. IDF, Brussels, Belgium.

Karimuribo, E. D., 2002. Epidemiological studies of mastitis in smallholder dairy farms in Tanzania. PhD Thesis. University of Reading, UK.

Kinabo, L. D. B., and R. J. Assey., 1983. Bovine mastitis in selected dairy farms in Morogoro district, Tanzania. Beitrge Trop. Landwirtsch. Veterinarmed. **21:** 65-71.

Mdoe. N. and Wiggns. S., 1997. Returns to smallholder dairying in the Kilimanjaro region, Tanzania. *Agricultural economics*. **17**: 75-87

MLD., 1983, the livestock policy of Tanzania. Ministry of Livestock Development. Government Printer, Dar es Salaam

National Mastitis Council, Inc. 1987. Microbiological Procedures for the Diagnosis of Bovine Udder Infections.

Quinn, P.J., Carter, M. E., Markey, B. K. & Carter, G.R., 2000. Clinical veterinary microbiology. Mosby-year book Europe limited, London. pp. 120-121

Quinn, P.J., Carter, M. E., Markey, B. K. and Carter, G.R. 2000. Clinical veterinary microbiology. Mosby-year book Europe limited, London. pp. 120-121

Radostits, O. M., 2001. Herd health: food animal production medicine, 3rd edition. W. B Sounders Company, Philadelphia, PA, pp. 397-433.

Radostits. O.M., Gay. C. C., Blood.D.C. and Hinchcliff. K. W., 2000. Veterinary Medicine; A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edition. W. B. Saunders Company. London. pp. 603-660

Roberts, D., 1990. Sources of infection: Food. Lancet, **336**: 859-861

Rosec, J. P., Guiraud, J. P., Dalet, C., and Richard, N., 1997. Enterotoxin Production by staphylococci isolated from foods in France. International Journal of Food Microbiology. **35**: 213-221

Schukken, Y. H., Grommers, F. J., van de Geer, D. and Brand, A., 1989. Incidence of clinical mastitis on farms with low somatic cell counts

in bulk milk. Veterinary Record, **125**: 60-63

Shekimweri, M. T., 1992. Mastitis: Incidence, Predisposing Factors and the Strategy of Control in Smallholder Dairy Farms in Morogoro. MSc Dissertation. Department of Animal Science, Sokoine University of Agriculture, Morogoro-Tanzania.

Shirima, G. M., R. R. Kazwala, and D. M. Kambarage., 2003. Prevalence of bovine tuberculosis in different farming systems in the eastern zone on Tanzania. Preventive Veterinary Medicine. **57**: 167-172.

Stephan, R. and Kuhn, K., 1999. Prevalence of Verotoxin-producing Escherichia coli (VTEC) in bovine coli mastitis and their anitiobitic resistance patterns. Zentralb. Veterinarmed. (B) **46**: 423-427

Sumberg J., 1997. Policy, milk and the Dar es Salaam peri-urban zone: a new future for an old development theme? *Land use policy*, **14** (**4**): 277-293

Suriyasathaporn, W., Schukken, Y. H., Nielen, M. and Brand, A., 2000. Low somatic cell count: a risk factor for subsequent clinical mastitis in a dairy herd. Journal of Dairy Science. **83**: 1248-1255

Watts, J.L. (1988). Etiological agents of bovine mastitis. Veterinary microbiology, **16**: 41-66

Weinhaupl, I., K. C. Schopf, D. Khaschabi, A. M. Kapaga, and H. M. Msami., 2000. Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es Salaam region and in zebu cattle in Lugoba area, Tanzania. Tropical Animal Health and Production. **32**: 147-154.

Winrock International., 1992. Assessment of Animal agriculture in sub-sahara Africa. Winrock International, Morrilton, AR.

2

Chapter

A Retrospective Study on the Aetiology and Temporal Distribution of Bovine Clinical Mastitis in Smallholder Dairy Herds in Dar es Salaam Region, Tanzania

F.M. Kivaria [a,*], J.P.T.M. Noordhuizen [b]

[a]Population Studies, Animal Diseases Research Institute P.O. Box 9254 Dar es Salaam, Tanzania
[b]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

* Corresponding author: Tel: +255 222 863 104; fax: + 255 222 864 369;
E-mail address: fredkiv@yahoo.com (F.M. Kivaria)

The Veterinary Journal (2006) doi:10.1016/j.tvjl.2006.01.008

Abstract

A 31-year record-based retrospective study was carried out to determine the aetiology and temporal distribution of bovine clinical mastitis in smallholder dairy herds in the Dar es Salaam region of Tanzania, for the period November 1971-December 2002. Laboratory information on 1964 quarter samples from 1365 cows in 281 smallholder dairy herds were retrieved, compiled and studied. 88% of the quarter samples were culture-positive. The predominant mastitis pathogens were *Staphylococcus aureus* (25.7%), *Streptococcus agalactiae* (15.4%), *Klebsiella pneumoniae* (14.3%) and *Escherichia coli* (14.1%). Other isolates included *Pseudomonas aeruginosa* (7.5%), *Streptococcus dysgalactiae* (5.2%) and *Streptococcus uberis* (4.2%). Contagious mastitis pathogens were isolated from 45.6% of the culture-positive samples, whereas environmental and miscellaneous pathogens were isolated from 48.2% and 5.7% of the culture-positive samples; 30% of the miscellaneous mastitis pathogens were *Candida* species.

The results demonstrate a steady increase in clinical *Candida albicans* mastitis. The prevalence of *Candida albicans* has increased from ≤1% in 1971 to ≥17.0% in November 2002. Contrarily, despite some fluctuations, the prevalence of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* and *Klebsiella pneumoniae* have remained over the years above 10%. The possible risk factors for these observations are discussed. It is concluded that udder health in smallholder dairy herds in Tanzania is poor.

Keywords: Contagious mastitis; *Candida albicans*; environmental mastitis; Tanzania.

Introduction

Bovine mastitis is the inflammatory response of the udder to noxious agents that can be either infectious or non-infectious. Most frequently the aetiology is infectious and organisms as diverse as bacteria, mycoplasma, yeasts and algae have been implicated as causes of the disease (Watts, 1988; Smith and Hogan, 2001). Mastitis agents are categorised as either 'contagious' or 'environmental' (Smith and Hogan, 1993). Contagious pathogens are intramammary infections transmitted from cow to cow with the principal transmission occurring during milking (Bramley and Dodd, 1984; Smith and Hogan, 1993).

Environmental pathogens are ubiquitous, opportunistic invaders of the mammary gland; their primary reservoir is the environment and not the infected udder (Schukken *et al*., 2005). Such categorisation reflects the basic epidemiology of mastitis pathogens in dairy herds and the practices likely to be effective in the control of particular pathogens within a herd. However, there is an increasing body of evidence to suggest that this classification may not be as clear cut as previously thought (Bradley, 2002; Zadoks, 2003). The epidemiology of mastitis pathogens is better represented by a sliding scale where the balance of contagious and environmental transmission shifts gradually, than by a species based dichotomy (Zadoks, 2003).

The prevalence/incidence of clinical bovine mastitis in Tanzania is not accurately known and although there have been some recent studies on mastitis, research has been very limited and there are no recent data available on the dynamics of mastitis pathogens. The aim of the present study was to document the aetiology and temporal distribution of individual clinical mastitis pathogens from cows in smallholder dairy herds in the Dar es Salaam region of Tanzania, based upon the routine culturing of bovine milk samples over a 31-year period.

Materials and methods

Study herds and Source of data

The Central Veterinary Laboratory, now known as the Animal Diseases Research Institute (ADRI), was established in 1961, by which time Tanzania had very few privately owned dairy herds. The majority (≥80%) of the herds that were present were owned by para-State organisations and a small number of private farmers (<20%). The herd owners and their agents submitted samples either to the zonally-located

veterinary investigation centres or to ADRI. However, by early 1980s the number of smallholder dairy herds within and around the Dar es Salaam region increased substantially (Kivaria *et al*., unpublished data) and most milk samples were submitted to ADRI.

Records of all milk samples submitted to ADRI by veterinarians, livestock field officers and farmers from the para-state, private commercial and smallholder dairy herds within and around the Dar es Salaam region between November 1971 and December 2002 were retrieved, compiled and analysed. All samples were exclusively from clinical mastitis cases, and to qualify for this study a sample had to have complete information on herd identification (ID), cow ID, quarter ID, date of submission and the culture results. A mastitic-sample was defined as a quarter milk sample taken and submitted by the farmer[1] upon recognition of signs of clinical mastitis before treatment, and from which a known udder pathogen was positively cultured. A recurrent mastitic sample was one from the same quarter of the same cow, with an interval of >14 days between cases, and caused by the same[2] microbial isolate. Samples submitted within 14 days from the same herd with the same cow and quarter ID were considered to be duplicates and were excluded from this study. A sample with more than two microbial isolates was considered to be contaminated and was also excluded.

Sampling procedures and Microbiological methods

Anecdotally, all quarter milk samples were collected according to the following standard procedure. Prior to sampling, teat ends were swabbed with 70% ethyl alcohol; the initial milk stripped from each quarter was discarded and the next 5-15 mL were collected in a sterile, pre-labelled tube[3]. Separate samples were taken from each quarter and chilled to 4 °C until delivery to ADRI.

Microbiological culturing was carried out according to standard protocols (Cruickshank, 1965; Buchanan and Gibbons, 1974; IDF, 1981; NMC, 1987, Hogan *et al*, 1999). Briefly, a sterile, aluminium bacteriological loop was used to spread approximately 0.01- 0.03 mL of each milk sample into blood agar and MacConkey agar (Oxoid). The plates were incubated at 37°C, and examined after 24 and 48 h. When

[1] The term 'farmer' denotes anybody who could follow the standard sampling procedures.
[2] Same microbial isolates' refers to the same microbial species and not to the same microbial strain.
[3] A case is reported at ADRI, ADRI technician will visit the herd to collect the samples, the containers are pre-labelled for herd/cow/quarter/date of collection. The containers are sterilised at ADRI.

slow growing or unusual bacteria were suspected, longer incubation periods or an environment of 10% Co_2 were used. If growth did not appear within seven days, plates were considered negative. Gram stain and culture characteristics (colony morphology, pigmentation, aroma and haemolysis) were used for presumptive identification for all isolates. For the isolation of fungi and yeasts, 0.03 mL of the suspected fungal mastitic sample was inoculated onto Sabouraud's dextrose agar plates, which were incubated at 37ºC, aerobically, for up to five days. The microbes were identified to species level using growth characteristics, morphology and other standard microbiological procedures (Davis *et al.*, 1973).

All staphylococci with β-haemolytic pattern, a positive coagulase reaction in rabbit plasma and a positive CAMP[4] reaction were presumed to be *Staphylococcus aureus*. Starting in 1996, specific tests were carried out as recommended by National Mastitis Council/NMC (1987) and/or International Dairy Federation/IDF (1981). Coagulase-negative staphylococci (CNS) were identified to the genus level. However, up to 1996, whenever logistics permitted, CNS were identified to species level. This followed the findings by Kapaga *et al.*, (1995) who suggested that CNS could be important causes of clinical mastitis in the smallholder dairy herds. Streptococci were identified by haemolytic patterns, CAMP reaction and hydrolysis of aesculin on aesculin blood agar (sheep blood agar with 0.05% and 0.01% ferric citrate).

Coliform bacteria and other Gram-negative bacilli were identified using culture characteristics on MacConkey agar, growth in triple sugar iron and urease, catalase, oxidase and indole production. Corynebacteria and *Actinomyces pyogenes* were identified using culture characteristics on blood agar, motility and catalase and urease production.

Results

A total of 3429 quarter samples were retrieved and investigated in this study. Mean number of samples per year was $107 \pm 72.2_{Sd}$, varying from 23 to 321 samples per year. Median was 98 samples. Distribution of samples per year is presented in Table 1. 69% (2366/3429) of the samples had complete information and were therefore further studied. Twenty five percent (857/3429) of the samples had incomplete information and 6% (206/3429) were duplicate samples which were therefore excluded from

[4] CAMP-reaction, a microbiological cultural phenomenon called after the originators Christie, Atkins and Muench-Petersen. The phenomenon is used to presumptively diagnose the presence of Streptococcus agalactiae and Staphylococcus aureus in mastititc cow's milk.

further analysis. Of the 2366 samples, (2200/2366; 93%) were culture positive, whereas 7% (166/2366) were culture negative. Eighty three percent (1964/2366) of samples came from the smallholder dairy herds, while 9% (213/2366) and 8% (189/2366) of the samples came from the para-State and private commercial dairy herds, respectively.

Table 1. Number of samples per year and results of microbiologically culture-positive clinical mastitis samples as percentage of 1732 clinical mastitis samples submitted at the Animal Diseases Research Institute for the period December 1971 – November 2002, in smallholder dairy herds in Dar es Salaam region, Tanzania

Year	Total number of samples retrieved	Number of samples from smallholder dairy herds	Udder pathogens from smallholder herds	Number of isolates	Percent in culture positive samples
1971	96	76	*Staphylococcus aureus*	445	26
1972	110	82	*Streptococcus agalactiae*	267	15
1973	102	73	*Klebsiella pneumoniae*	248	14
1974	118	99	*Escherichia coli*	244	14
1975	230	77	*Pseudomonas aeruginosa*	130	7
1976	317	103	*Streptococcus dysgalactiae*	90	5
1977	321	97	*Streptococcus uberis*	73	4
1978	209	99	*Enterobacter aerogenes*	44	3
1979	110	46	*Candida species*	36	2
1980	126	71	*Arcanobacter pyogenes*	35	2
1981	98	63	*Staphylococcus epidermidis*	32	2
1982	92	66	*Bacillus cereus*	29	2
1983	78	57	*Streptococcus pyogenes*	12	1
1984	66	53	*Corynebacterium bovis*	10	1
1985	74	64	*Serratia marcescens*	7	0.4
1986	81	61	*Trichophyton species*	5	0.3
1987	23	20	*Proteus mirabilis*	5	0.3
1988	49	38	*Streptococcus lactis*	3	0.2
1989	52	43	*Clostridium perfringens*	3	0.2
1990	98	51	*Citrobacter diversus*	2	0.1
1991	63	33	*Yersinia pseudotuberculosis*	2	0.1
1992	89	67	*Acinetobacter lwoffii*	2	0.1
1993	114	56	*Brucella abortus*	2	0.1
1994	107	52	*Pasteurella multocida -A*	2	0.1
1995	108	68	*Nocardia asteroids*	2	0.1
1996	101	60	*Mycobacterium bovis*	2	0.1
1997	23	16			
1998	153	93			
1999	117	87			
2000	41	34			
2001	34	30			
2002	29	29			
Total	**3429**	**1964**		**1732**	**100**

(Source: Animal Diseases Research Institute, Data base – December 1971-to- November 2002)

Microbial isolates were obtained in 100% of the 213 samples from the para-State herds; the major isolates were *Staphylococcus aureus* (50 %), *Streptococcus agalactiae* (27%), *Escherichia coli* (21%), and *Candida albicans* (2%). Twenty nine percent (54/189) of the samples from the private commercial herds were culture negative, whereas 71% (135/189) of the samples were culture positive with the major isolates being *Staphylococcus aureus* (33%), *Escherichia coli* (22%), *Streptococcus dysgalactiae* (10%), and *Klebsiella pneumoniae* (6%). Since the focus of this study is on the smallholder dairy herds, samples from the para-State and private herds were excluded from further analysis.

Of the 1964 quarter milk samples from the smallholder dairy herds, 1041 (53%) came from 814 dairy cows in 139 herds in the Kinondoni district[5], which is currently estimated to have 509 herds with 3,525 dairy cows (MOAC, 2001). Twenty-nine per cent (569/1964) of the samples came from 365 cows in 111 smallholder dairy herds in Temeke district, currently estimated to have 603 smallholder dairy herds with 4,509 dairy cows (MOAC, 2001).

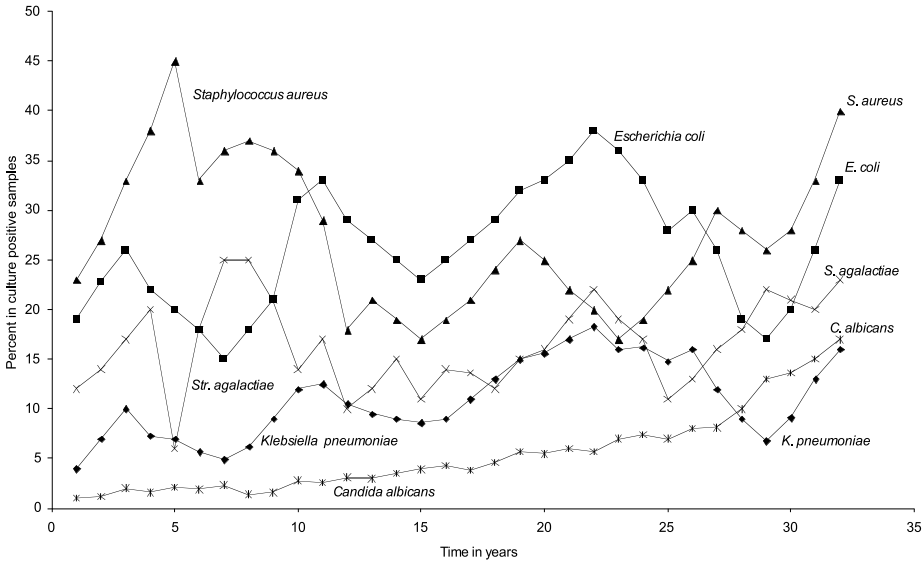
Eighteen per cent (354/1964) of the samples came from 186 cows in 31 smallholder dairy herds in the Ilala district, currently estimated to have 196 smallholder dairy herds with 615 dairy cows (MOAC, 2001); 236 (12%) of the 1964 quarter milk samples from the smallholder dairy herds were negative, while 1732 (88%) were culture-positive.

The most prevalent bovine-mastitis pathogens were *Staphylococcus aureus* (25.7%); *Streptococcus agalactiae* (15.4%); *Klebsiella pneumoniae* (14.3%); *Escherichia coli* (14.1%). Other isolates included *Pseudomonas aeruginosa* (7.5%), *Streptococcus dysgalactiae* (5.2%), and *Streptococcus uberis* (4.2%). Culture results for the samples originating from the smallholder dairy herds are shown in Table 1. Mixed cultures were isolated from 9 (0.52%) of the culture positive samples and were excluded from this study. Contagious pathogens were isolated from 790 (45.6%) of the culture-positive samples, whereas environmental and miscellaneous pathogens were isolated from 835 (48.2%), and 98 (5.7%) of the culture-positive samples, respectively. Thirty per cent of the miscellaneous pathogens were *Candida* species and included *Candida albicans* (33%); *Candida guillermondi* (29%); *Candida tropicalis* (19%); and *Candida pelliculosis* (19%). On the other hand, *Trichophyton verrucosum*, constituted 7% of the miscellaneous positive samples.

[5] The Dar es Salaam region has three districts, namely, Kinondoni, Ilala and Temeke. Of an estimated 12,000 heads of dairy animals, 55%, 40% and 5% are in Temeke, Kinondoni and Ilala district, respectively. This cattle population represent 3% of the national dairy animals (MOAC, 2001).

Figure 1 shows the pattern of isolation of selected micro-organism species over 31 years (for readability, the respective dots have been connected to show trends). In Figure 1, it can be deduced that there is a steady increase in clinical *Candida albicans* mastitis. The prevalence of *Candida albicans* has increased from ≤1% in 1971 to ≥17.0% in November 2002. Contrarily, despite some fluctuations, the prevalence of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* and *Klebsiella pneumoniae* have remained over the years above 10%. Since cows could have multiple infections in one quarter and/or multiple quarters, the proportional prevalence of microbial isolates were also calculated for cow cases. In total, 1365[6] cows in 281[7] smallholder dairy herds were involved. A single quarter was affected in 28% (383/1365), two quarters in 40% (546/1365), three quarters in 23% (314/1365) and four quarters in 9% (122/1365) of the affected cows. The distribution of the affected quarters was 34% fore-right, 33% rear-right, 19% fore-left, and 14% rear-left. Most cases were associated with environmental pathogens 73% (574/786), Yeast and fungal species 20% (157/786) and contagious pathogens 7% (55/786).

Figure 1. Observed prevalence of isolation of *S. aureus, S. agelactiae*. *E. coli, K. pneumoniae* and *C. albicans* in culture positive sample for the period December 1971 to November 2002, in smallholder dairy herds in Dar es salaam region, Tanzania



[6] (814 + 365 + 186) cows, in Kinondoni, Temeke and Ilala districts, respectively
[7] (139 + 111 + 31) herds, in Kinondoni, Temeke and Ilala districts, respectively

Discussion

In this study we used data which were already available in a diagnostic laboratory for epidemiological studies on clinical mastitis. The use of such data is inexpensive and (if every case of clinical mastitis is assumed to have been recorded) is probably more representative of the target population than experimental data. Unfortunately, the assumption that every case is reported is rarely valid. The main problems associated with the ADRI database system include under-reporting, estimated to be 90% (Kivaria and Kapaga, 2002), and in some cases not all the necessary epidemiological data are recorded.

Under-reporting occurs for a number of reasons, such as lack of farmers' awareness, or because of the costs associated with sampling and laboratory charges. Consequently, the routine analyses of laboratory data are of marginal value. However, despite these inadequacies, our samples are most likely random representative of the clinical mastitis cases in the study period and area. The results are in good agreement with random mastitis survey results from smallholder and commercial dairy herds in the Dar es Salaam region (Kapaga *et al*., 1995), Iringa and Tanga regions (Phiri *et al*., 1998; Karimuribo *et al*., 2003), and Coast and Morogoro regions (Mdegela *et al*., 2004). Our results are also comparable with those obtained from the commercial (Workineh *et al*., 2002), and smallholder (Omore *et al*., 1996) dairy herds in Kenya and Ethiopia, respectively. The samples were and are still taken by the livestock field officers, exclusively from cows with clinical mastitis, and are still submitted to ADRI for analysis by standard procedures. It is therefore logical to assume that the above standard sampling and laboratory methodologies were followed.

The most common organisms isolated here were *S. aureus*, *S. agalactiae, K. pneumoniae*, and *E. coli*. These pathogens represent two distinct epidemiological and clinical entities of bovine mastitis (Bradley, 2002). *S. aureus* and *S. agalactiae* are the two major proto-typical contagious mastitis pathogens (Fox and Gay, 1993). In contrast, *K. pneumoniae* and *E. coli* are environmental pathogens that are in most cases responsible for acute clinical mastitis in the same cow in the same lactation (Dopfer *et al*., 1999; Bradley, 2002). These observations could be due to the fact that there are no established mastitis control practices that are employed by the smallholder producers, but instead mastitis control relies heavily on drug use. Moreover, the unhygienic housing and milking practices observed among the smallholder dairy herds increase both exposure and infection pressure to cows, with the subsequent high infection levels.

Contagious udder pathogens are involved in both clinical and subclinical mastitis (Bradley, 2002). Poor clinical mastitis treatment procedures or not responding to treatment usually leads to a subclinical mastitis (Radostits *et al*., 2000), with recurrent clinical flare ups. Environmental udder pathogens are commonly associated with acute clinical mastitis, often with signs of systemic disease. Coliform mastitis is known to repeatedly occur in the same cow in the same lactation (Dopfer *et al*., 1999). Both different types of recurrences may be a reason for sample submission to a diagnostic laboratory.

In the present study, environmental pathogens were found to be more common than contagious pathogens (Table 1). This may not be surprising if it is a reflection of the likely presence of pathogens more likely to be contaminants. However, another interpretation could be that this is a reflection of the acute and recurrent (Dopfer *et al*., 1999) nature of environmental clinical mastitis. This form of mastitis may lead to treatment failures (Blowey and Edmundson, 2000). Therefore, cows with recurrent clinical mastitis are more likely to be selected for culture so the percentage of environmental pathogens will probably be higher than in a random survey. Only nine samples were classified as contaminated, but this does not necessarily reflect the quality of sampling and sample handling and could be due to missing information or recording bias where the technicians tend to record more negative and pure isolates while overlooking the mixed cultures.

Miscellaneous bovine-mastitis pathogens such as yeasts and fungal species are associated with unhygienic udder preparation, contamination, unsanitary intramammary infusion practices, and indiscriminate use of antibiotics, particularly tetracyclines (Radostits *et al*., 2000). Despite the fact that our observations are not population based, the steady increase (Fig. 1) in the prevalence of *C. albicans* seems to be real. A recent longitudinal study involving 317 lactating cows from 87 smallholder dairy herds (Kivaria unpublished data), demonstrated that the incidence rate of clinical *C. albicans* mastitis was 124.3 clinical mastitis episodes per 100 cow-years at risk. In that study the quality of housing and milking hygiene featured strongly in the results, though these and other factors identified need further investigation. At the start of culturing, coagulase-negative staphylococci (CNS) were not considered to be important in clinical mastitis. This is probably the reason that CNS do not feature in our present results.

The observed fluctuations over time in the prevalence of the microbial isolates (Fig. 1) could be attributed to drops in the laboratory submission of samples. Initially samples were submitted by the extension staffs and farmers, and laboratory work was conducted free of charge. During the late 1980s the government introduced laboratory fees which were accompanied with a general decline in the number of specimen submission. However, the mid 1990s saw an increased level of specimen submission. The increased level of specimen submission could be due to increased problems of mastitis. Since the classic five-point mastitis control plan is not practised in Tanzania, the fluctuations are due to changes in specimen submission, probably being influenced by the nature of the mastitis problems, and not due to any interventions. Cyclical changes in risk indicators, such as housing conditions, nutrition and water availability could also account for the fluctuations.

Despite the observed fluctuations (Fig 1), the prevalence of *S. aureus, S. agalactiae, E. coli* and *K. pneumoniae* have almost always remained over the years above 10%. This implies the existence of maintenance mechanisms for these and other udder pathogens, suboptimal udder hygiene, housing conditions and lack of mastitis control skills by the smallholder farmers. An alternative argument is the development of an intramammary reservoir from which udder pathogens are released periodically. This argument is probably supported by the previous (Kivaria *et al*., 2004) observations that 77% and 5% of 182 lactating cows in 62 smallholder dairy herds from the same study area had fibrotic (palpable mass) and atrophic quarters, respectively. A single quarter was affected in only 28% of the 1365 cows while multiple infections were observed in 72% of the cows. This observation supports the previous observations that udder health, milking practices and housing conditions are generally poor among the smallholder dairy herds (Kivaria *et al*., 2004; 2005). Poor udder health, suboptimal milking and housing hygiene facilitate the rapid within-herd spread and maintenance of intramammary infections. Although public health hazards attributed to mastitis pathogens are not addressed in this paper, the isolation of *Nocardia asteroids*, *Mycobacterium bovis* and other potentially zoonotic agents, is of great public health concern, particular in Tanzania where 86% of milk consumers prefer raw milk (Kivaria, unpublished data).

It is hard to make a conclusion, based on this work, on the improvement or deterioration in udder health in the Tanzanian smallholder dairy herds. Certainly, the current observed poor and unhygienic housing, milking and feeding practices; coupled with poor extension services, lack of mastitis awareness (85% of the producers are aware of the clinical mastitis, but they are not aware of how does mastitis comes about) among the producers, indiscriminate use of antibiotics and the non implementation of mastitis control programme would suggest that, if anything deteriorated udder health rather than improved during the period under study.

References

Blowey, R. and P. Edmondson. 2000. Mastitis control in dairy herds, Farming press books, Ipswich, UK.

Bradley, A.J. 2002. Bovine mastitis: An evolving disease. The Veterinary Journal **164**: 116-128

Bramley, A. J. and Dodd, F. H. 1984. Reviews of the progress of dairy science: Mastitis control progress and prospects. Journal of Dairy Research **51**: 481-512.

Buchanan, R. E. and Gibbons, N.E. 1974. Bergey's Manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co. Baltimore, USA.

Cruickshank, R. 1965. Medical microbiology, Livingstone, London

Davis, B. P. O., Dulbecco, R., Eisen, H. N., Ginsberg, H. S., Wood, W. F. 1973. Microbiology 2nd ed., Harper and Row Publication, London.

Dopfer, D., Barkema, H. W., Lam, T. J. G. M., Schukken, Y. H., and Gaastra, W 1999. Recurrent clinical mastitis caused by Escherichia coli in dairy cows**.** Journal of Dairy Science. **82:** 80-85

Fox, L. K. and Gay, J. M. 1993. Contagious mastitis. Veterinary Clinic of North America, Food Animal Practices. **9**: 475-487

Hogan. S. J., Gonzalez R. N., Harmon, J. R., Nickerson, S.C., Oliver, S. P., Pankey,.J. W., Smith, L. K. 1999. Laboratory Handbook on Bovine Mastitis. Published by National Mastitis Council, Inc., W D Hoard, Fort Atkinson, USA.

International Dairy Federation 1981. Bulletin No. 132. Laboratory Methods for Use in Mastitis Work. IDF, Brussels, Belgium. pp 27

International Dairy Federation 1987. Bulletin No. 221. Bovine mastitis, definitions and guidelines for diagnosis. Brussels, Belgium. pp 3-16

Kapaga, A.M., Weinhaupl.I., Baumann, M.P.O. 1995. Risk indicators and Mastitis Prevalence in Dairy Cattle in The Region of Dar es Salaam- Tanzania. Livestock Production and Diseases. Proceedings of the 8th Conference, Institute of Tropical Veterinary Medicine. Berlin. Germany

Karimuribo, E., Fitzpatrick, J.L.; Bell, C.E.; Swai, E.S.; Kambarage, D.M.; Ogden, N.H., French, N.P. 2003. Mastitis in smallholder dairy farms in Tanzania: From risk to intervention and knowledge transfer. In: Reid, S.W.J. and Menzies, F.D. (Editors). Proceedings of Society for Veterinary Epidemiology and Preventive Medicine held at Warwick, UK, 31st March – 2nd April, 2003: pp 83-94.

Kivaria F.M. and Kapaga A.M. 2002. Review of current problems and shortcomings in the Tanzanian animal health information system with suggestions on improvement, Onderstepoort Journal of Veterinary Research, **69**: 305-314

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M. 2004. Risk Indicators Associated with Subclinical Mastitis in Small-holder Dairy Cows in Tanzania. Tropical Animal Health and Production, **36**: 581- 592

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M. 2005. Risk Indicators Associated with *Staphylococcus aureus* Subclinical Mastitis in Small-holder Dairy Cows in Tanzania. In: H. Hogeveen (editor). Mastitis in Dairy production; current knowledge and future solutions. Wageningen academic publishers, Wageningen, The Netherlands.

Mdegela, R. H., L. J. M. Kusiluka., A. M. Kapaga., E. D. Karimuribo., F. M. Turuka., A. Bundala., F. Kivaria., B. Kabula., A. Manjurana., T. Loken., D. M. Kambarage., 2004. Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in eastern Tanzania. Journal of Veterinary Medicine B. **51:** 123-128.

Ministry of Agriculture and Cooperatives (MOAC), 2001. District integrated agricultural survey 1998/99, survey results-Dar es Salaam report.

National Mastitis Council, Inc (1987). Microbiological Procedures for the Diagnosis of Bovine Udder Infections. National Mastitis Council, Inc., W D Hoard, Fort Atkinson, USA. pp 34

Omore. A.O., J.J. Mcdermott., S.M. Arimi., M.N. Kyule., D. Ouma., 1996. A longitudinal study of milk somatic cell counts and bacterial culture from cows on smallholder dairy farms in Kiambu district,

Kenya. Preventive Veterinary Medicine. **29**: 77-89

Phiri, E. C. J. H., Pereka, A. E., Mgasa, M. N., Larsen. T. 1998. Clinical mastitis and bacteria isolates in dairy cows at ASAS dairy farm Iringa, Tanzania. Tanzania Veterinary Journal, **18**: 173-179

Quinn, P.J., Carter, M. E., Markey, B. K. and Carter, G.R. (2000) Clinical veterinary microbiology. Mosby-year book Europe limited, London. pp. 120-121

Radostits. O.M., Gay. C. C., Blood.D.C., Hinchcliff. K. W. 2000. Veterinary Medicine; A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th ed. W. B. Saunders. London. pp. 603-660

Smith, K. L. and Hogan, J. S. 1993. Environmental mastitis. Veterinary Clinic of North America, Food Animal Practices. **9**: 489-498

Smith, K. L. and J. S. Hogan. 2001. The world of mastitis. Pages 1-12 in Proceedings of 2nd International symposium on mastitis and milk quality, September 13-15, Vancouver, BC, Canada.

Watts, J.L. (1988). Etiological agents of bovine mastitis. Veterinary Microbiology, **16**: 41-66

Workineh, S., Bayleyegn, M., Mekonnen, H., Potgieter. L. N. D. 2002. Prevalence and aetiology of mastitis in cows from two major Ethiopian dairies. Tropical Animal Health and Production, **34**: 19-25

Zadoks, R. N. 2003. Contagious and Environmental pathogens: from dichotomy to sliding scale. International Dairy Federation IDF, Mastitis newsletter No 25, Brussels, Belgium. pp 16-17

3

Chapter

Prevalence and Antimicrobial Susceptibility of Bacteria Isolated from Milk Samples of Smallholder Dairy Cows in Tanzania

Kivaria. F.M.,[1]* Noordhuizen. J.P.T.M.,[2] Kapaga. A.M.[1]
[1]Animal Diseases Research Institute, P.O.Box 9254,
Dar es Salaam, Tanzania

[2]Department of Farm Animal Health, Faculty of Veterinary Medicine,
Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

*Corresponding author at: Fax: + 255 222 119 001;
E-mail address: fredkiv@yahoo.com;

This chapter has been accepted for publication in the journal Research in Veterinary Science

Abstract

A cross-sectional study involving 69 smallholder dairy herds and 230 dairy cows in Tanzania was designed with the aim of elucidating the prevalence and cause of bovine intramammary infections. Secretions from 878 quarters from lactating cows, and from pre-calving dry cows and in-calf heifers were bacteriologically examined. Sensitivity testing of pathogens towards seven commonly used antibiotics was conducted. California Mastitis tests (CMT) were carried out on 762 quarters from 191 lactating cows. The observed prevalence of clinical mastitis was 4.5 cases per 100 cows, whereas the prevalence of sub-clinical mastitis, defined by positive (≥ +1) CMT score or bacteriologically positive culture was 90.3% and 90.7% respectively. The most important micro-organisms detected from quarter samples were *Staphylococcus aureus* (48%), *Streptococcus agalactiae* (17.6%), *Candida albicans* (7.2%), *Streptococcus pyogenes* (7.2%), *Escherichia coli* (4.1%), *Arcanobacterium pyogenes* (3.6%) and *Pseudomonas aeruginosa* (3.0%). A higher prevalence of antibiotic resistance was observed for the commonly used antimicrobials than for the newly introduced antimicrobials, and this difference was statistically significant ($\chi^2 = 225$; $P = 0.00$). It is concluded that intensive farmer education programmes are necessary to improve udder health in the smallholder dairy sector in Tanzania.

Keywords; Antibiotic susceptibility; Bovine mastitis; Smallholder dairy cattle; Tanzania

Introduction

Keeping dairy cattle in Tanzania is a relatively new activity. Animals are reared under different management and milking regimen conditions, and there is little knowledge about dairying among farmers. There is insufficient information locally about bovine udder health, including the aetiology of intramammary infections (IMI), antimicrobial susceptibility patterns of the isolated microorganisms, and the use of indirect methods for diagnosis of subclinical mastitis. Few studies on mastitis have been carried out in smallholder dairy herds in Tanzania (Kapaga *et al*., 1995; Karimuribo *et al*., 2003). These studies showed point prevalence of clinical mastitis of 12, and 19 cases per 100 lactating cows, in Dar es Salaam, and Iringa regions respectively. While the point prevalence of subclinical mastitis, defined by a CMT positive sample was 70 and 80 cases per 100 lactating cows, for the two regions respectively. Analysis of laboratory records at the Animal Diseases Research Institute (ADRI) in Dar es Salaam demonstrate that the major causes of clinical mastitis are Coliforms (20%), *Staphylococcus* spp (21%), *Streptococcus* spp (15%) and Mycotic infections (8%). Although not quantified, the records also indicate an increasing incidence of antimicrobial resistance amongst the commonly isolated bacteria. Furthermore, little information regarding susceptibility of bacteriological isolates in Tanzania exists, thus hindering the choice of appropriate antibiotics for veterinary use.

The objective of this study was to establish the prevalence of the etiological causes of udder infections in smallholder dairy herds, and to assess their susceptibility patterns to antibiotics commonly administered on farm.

Materials and methods

Sampling technique

This cross-sectional study was carried out between June and September 2002. The target population was all smallholder (with $1 \leq n \geq 15$ animals) dairy herds in the Dar es Salaam region, while the sampling unit was the udder quarter. Initially a total of 65 dairy herds were randomly selected from a sampling frame of 300 herds. The sample size was based on a preceding study (Kivaria *et al*., 2004) concerning subclinical mastitis. A maximum of two herds were visited per day at milking time and milk samples were inoculated on the same day. Cows

with clinical mastitis were those with clinical signs of mammary gland inflammation and from whom mammary secretions mastitis pathogen was detected by culture; cows with subclinical mastitis were those with apparently normal udder secretions but with at least one positive CMT-quarter (regardless of the culture status).

Sampling and processing

CMT was performed on all lactating quarters without evidence of clinical mastitis. In order to get insight into the udder microbiological status of the animals joining the lactating pool, pre-calving dry cows and in-calf heifers were included in this study. Samples for microbiological culturing were taken from each quarter of all lactating and pre-calving dry cows and in-calf heifers in their last trimester as described by the International Dairy Federation (1981) and Hogan *et al*., (1999). Milk samples were stored on ice and transported to the laboratory within six hours of the collection. Microbiological culturing and identification of the micro-organisms to species level was performed according to standard procedures described by Hogan *et al*., (1999). In addition, *Arcanobacterium pyogenes* and *Bacillus* spp. were identified based on colony appearance on blood agar, and the results of the catalase test and Gram stain (Quinn *et al*., 2000). When slow growing or unusual bacteria were suspected, longer incubation periods or on incubator environment of 10% Co_2 were used.

Antimicrobial susceptibility testing

The owners of dairy cows sampled were asked to provide details of antimicrobials used to treat dry cows or cases of clinical mastitis, this information was the only factor considered for selecting the antimicrobials for sensitivity testing. The commonly on-farm used antibiotics against which bacterial isolates were tested included: Gentamicin, Kanamycin, Streptomycin, Neomycin, Tetracycline, Penicillin, and Bacitracin. The cultures were tested for antimicrobial susceptibility by the Kirb-Bauer disc diffusion method (Quinn *et al*., 2000), using the Oxoid-antibiotic disks. Quality controls were run using *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Cultures were classified as sensitive, intermediate and resistant on the basis of the diameter of the zone of inhibition (Quinn *et al*., 2000).

Data analysis

The point prevalence of sub/clinical mastitis was calculated as the number of sub/clinical cow-cases with at least one quarter affected per 100 cows. The quarter-sub/clinical mastitis prevalence was defined as the number of quarters infected per 100 quarters. Data on antimicrobial susceptibility was compared by Chi-square test for independence.

Results

In some animals milk samples could not be collected from all four quarters. A total of 878 quarters from 198 lactating cows, 25 pre-calving dry cows and 7 pre-calving in-calf heifers were bacteriologically evaluated, while CMT was carried out on 762 quarters from 191 lactating cows. A total of 10 clinical cow cases (7 from lactating and 3 from dry cows) were observed.

The overall prevalence of clinical mastitis was 4.5 cow-cases per 100 cows, while a prevalence of 12 and 3.5 cases per 100 cows was observed in pre-calving dry and lactating cows respectively. The quarter prevalence was 1.4 quarter-cases per 100 quarters; 4% of investigated quarters were blind. The clinical cow-cases were observed in 8.7% of the investigated herds. At herd level, prevalence of clinical cases varied from 12 to 50 cases per 100 cows. The cow-level prevalence of subclinical mastitis defined by either CMT positive (≥ +1) sample or microbiologically positive culture was 90% and 91% respectively. On a quarter basis 84.6% of all quarter samples tested were CMT-positive (≥ +1).

Microorganisms isolated from quarter milk samples are displayed in Table 1. 96% of all isolates were associated with subclinical mastitis. The most important pathogens were *Staphylococcus aureus* (48%), *Streptococcus agalactiae* (17.6%), *Candida albicans* (7.2%), *Streptococcus pyogenes* (7.2%), *Escherichia coli* (4.1%), *Arcanobacterium pyogenes* (3.6%) and *Pseudomonas aeruginosa* (3.0%); 58.7% (515/878) of the samples were negative on culture. 56% of the CMT positive quarters were negative on culture; the relationship between CMT scores and selected isolates is shown in Table 2. The susceptibilities of bacterial isolates against the commonly used antimicrobial agents are presented in Table 3.

Table 1: Udder pathogens isolated from 363 quarter milk samples from 198 lactating smallholder dairy cows with subclinical mastitis in the Dar es Salaam region, Tanzania.

Microorganisms	Lactating cows		Pre-calving dry cows		Pre-calving in-calf heifers		Overall	
	Number of Quarters	Frequency* (%)	Number of Quarters	Frequency (%)	Number of Quarters	Frequency (%)	Number of Quarters	Frequency (%)
Staphylococcus aureus	151	41.6	18	5.0	5	1.4	174	47.9
Streptococcus agalactiae	58	16.0	3	0.8	3	0.8	64	17.6
Candida albicans	23	6.3	3	0.8	0	0.0	26	7.2
Streptococcus pyogenes	21	5.8	5	1.4	0	0.0	26	7.2
Escherichia coli	5	1.4	10	2.7	0	0.0	15	4.1
Arcanobacterium pyogenes	11	3.0	2	0.6	0	0.0	13	3.6
Pseudomonas aeruginosa	4	1.1	6	1.6	0	0.0	10	2.7
Proteus mirabilis	5	1.4	3	0.8	0	0.0	8	2.2
Bacillus subtilis	6	1.6	0	0.0	1	0.3	7	1.9
Klebsiella pneumoniae	5	1.4	0	0.0	1	0.3	6	1.7
Staphylococcus saprophyticus	3	0.8	2	0.6	0	0.0	5	1.4
Proteus vulgaris	3	0.8	0	0.0	0	0.0	3	0.8
S. aureus & S. pyogenes	3	0.8	0	0.0	0	0.0	3	0.8
Streptococcus faecalis	0	0.0	0	0.0	1	0.3	1	0.3
Candida albicans and S aureus	1	0.3	0	0.0	0	0.0	1	0.3
S. aureus and S. saprophyticus	1	0.3	0	0.0	0	0.0	1	0.3

* Computed as (151/363)* 100 = 41.6

Table 2: The relationship between California mastitis test (CMT) results and isolated udder pathogens from 601 quarter milk samples of 150 smallholder dairy cows with subclinical mastitis in the Dar es Salaam region, Tanzania.

	CMT score			
Culture				
	0	**1**	**2**	**3**
Negative	86	133	43	64
Staphylococcus aureus	5	8	26	111
Streptococcus agalactiae	4	5	13	36
Candida albicans	5	1	7	10
Arcanobacterium pyogenes	9	1	1	0
Streptococcus pyogenes	0	4	5	12
Klebsiella pneumoniae	0	0	1	3
Escherichia coli	0	0	1	3
Pseudomonas aeruginosa	0	0	0	4

Discussion

In this study, microorganisms responsible for bovine intramammary infections were found to be prevalent in smallholder dairy cows. Kapaga *et al*., (1995), Omore *et al*., (1996) and Karimuribo *et al*., (2003), have recorded similar observations. In this study, *Staphylococcus aureus* was observed to be the predominant cause of bovine-udder infections. This is consistent with other studies carried out on medium ($n \leq 50$), and/or large scale dairy herds ($n \geq 300$ dairy cattle), and smallholder farms; *Streptococcus* species and enteric bacteria being other important pathogens (Kinabo and Assey, 1983; Phiri *et al*., 1998; Kapaga *et al*., 1995, and Karimuribo *et al*., 2003).

The predominance of contagious mastitis pathogens is supported by the high prevalence of positive CMT-quarter scores for the same milk samples (Table 2). Environmental factors such as unhygienic housing, warm and humid weather, and the general lack of farm cleanliness and sanitation, may account for the observed high prevalence of environmental pathogens. On the other hand, deficient milking procedures, poor hygiene and treatment practices could contribute to a high prevalence of contagious pathogens.

The observed overall prevalence of clinical mastitis is relatively low as compared to that quoted in the developed countries (Leigh, 1999, Bradley, 2002). This observation is explained by the fact that smallholder

Table 3: Antimicrobial susceptibility of the commonly isolated udder pathogens from 181 quarter milk samples from 198 lactating smallholder dairy cows with subclinical mastitis in the Dar es Salaam region, Tanzania.

Isolate	Gentamicin (10 µg)			Kanamycin (30 µg)			Streptomycin (10 µg)			Neomycin (30 µg)			Tetracycline (30 µg)			Penicillin (10 µg)			Bacitracin (10 µg)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S. aureus	88	5	3	77	16	3	21	69	6	67	29	-	48	32	16	47	42	6	51	44	1
(n = 96)	(92)	(5)	(3)	80	(17)	(3)	(22)	(72)	(6)	(70)	(30)	-	(50)	(33)	**(17)**	(50)	(44)	(6)	(53)	(46)	(1)
S. agalactiae (n = 36)	31	4	1	25	10	1	3	29	4	19	17	-	16	9	11	11	22	3	11	22	3
	(86)	(11)	(3)	(69)	(28)	(3)	(8)	(81)	**(11)**	(47)	(53)	-	(44)	(25)	**(31)**	(31)	(61)	(8)	(31)	(61)	(8)
S. pyogenes ***(n = 18)***	17	-	1	16	1	1	5	10	3	8	10	-	4	7	7	8	9	1	7	9	2
	(94)	-	(6)	(89)	(6)	(5)	(28)	(55)	**(17)**	(44)	(56)	-	(22)	(39)	**(39)**	(44)	(50)	(6)	(39)	(50)	**(11)**
E. coli (n = 11)	8	3	-	8	3	-	1	6	4	8	3	-	3	3	5	3	1	7	6	-	5
	(73)	(27)	-	(73)	(27)	-	(9)	(55)	**(36)**	(73)	(27)	-	(27)	(27)	**(46)**	(27)	(9)	**(64)**	(55)	-	**(45)**
Klebsiela pneumoniae (n = 9)	7	2	-	6	3	-	2	1	5	4	4	1	2	5	2	3	1	5	1	3	5
	(78)	(22)	-	(67)	(33)	-	(22)	(11)	**(56)**	(44)	(44)	**(12)**	(22)	(56)	**(22)**	(33)	(11)	**(56)**	(11)	(33)	**(56)**
P. aeruginosa (n = 8)	8	-	-	6	2	-	-	6	2	5	3	-	-	5	3	-	1	7	2	-	6
	(100)	-	-	(75)	(25)	-	-	(75)	**(25)**	(63)	(37)	-	-	(63)	**(37)**	-	(13)	**(87)**	(25)	-	**(75)**
A. pyogenes (n = 3)	3	-	-	3	-	-	-	-	3	3	-	-	1	2	-	1	2	-	3	-	-
	(100)	-	-	(100)	-	-	-	-	**(100)**	(100)	-	-	(33)	(67)	-	(33)	(67)	-	(100)	-	-

S = sensitive; I = intermediate sensitive; R = resistant: Figures in parentheses are the relative frequency for the respective variables

farmers were found to be keen on treatment of clinical mastitis[8], and that 94% of antibiotics used on farm were used for mastitis (Kivaria *et al*., "in press, Outlook on Agriculture"). 56% of the CMT positive quarters were negative on culture, possibly related to hand milking techniques resulting in microscopic trauma of the teat epithelium that may become infected (Mulei, 1999), and consequently leading to elevated somatic cell count, and/ or intracellular bacteria that were unavailable for isolation (Hogan *et al*., 1999). The isolation of *Staphylococcus aureus* and *Streptococcus agalactiae* and other bacteria from CMT-negative quarters may be explained by the possibility that the isolated bacteria may have originated from micro-abscesses or from the teat canal, respectively (Hogan *et al*., 1999, Quinn *et al*., 2000). The isolation of high numbers of *Arcanobacterium pyogenes* from CMT-negative samples (Table 2) could probably be due to the inability of the minor pathogens to elicit a strong cellular response in the udder, or increased resistance of the colonised quarter to invasion protection by major-pathogens (Schukken *et al*., 1991).

Little published local information on antimicrobial susceptibility of pathogens responsible for bovine intramammary infections in Tanzania is available. A study by Mbise *et al*., (1983), indicated a high prevalence of bacterial resistance to ampicillin, penicillin, streptomycin and tetracycline. Antimicrobial resistance is influenced by the different practices of administering antimicrobial products (dosage, timing, frequency, type and frequent change of types) (Aarestrup, 1999). Our data on the susceptibility to commonly used antimicrobials support this. For example, penicillin-streptomycin combination, and tetracycline are heavily used for the treatment of bacterial infections across all animal species in Tanzania (personal observations), while bacitracin is used in many of the intramammary formulations. This is reflected in the higher prevalence of resistance to streptomycin, penicillin, tetracycline and bacitracin. Similarly, gentamicin (as injectable), kanamycin and neomycin (both as intramammary formulations), are expensive and quite new in the Tanzanian livestock industry and therefore less frequently used, and this difference is reflected in a lower prevalence of resistance. The difference in susceptibility between the "older" and the "newer" products was statistically significant ($x^2 = 225$; $P \leq 0.01$). The former may also be related to the long period of inappropriate use of antimicrobial

[8] Smallholder farmers are generally aware of the presence of clinical mastitis, and they respond quite promptly up on positive diagnosis. But the producers do not know how does mastitis comes about and therefore they do not know how to prevent and control.

products, since in Tanzania these are dispensed without a prescription. Furthermore, under-dosing or incomplete treatment of animals is not uncommon. On the other hand, in The Netherlands for example, the prevalence of antibiotic resistance has been noted to decline over the last 50 years, with a decline in antibiotic use (Sol, 2002). Two factors: effective dry cow therapy and strict and efficient culling as practiced in the Netherlands, were cited as being the major reasons for the declining antibiotic resistance (Sol, 2002). These two factors do not apply in Tanzania.

The general lack of knowledge on dairy cattle husbandry by the smallholder producers, and the paucity of livestock extension services, may be associated with a relative lack of awareness by the smallholder producers of the importance of the principles of mastitis control. Furthermore, the inappropriate use of antibiotics by the smallholder producers increases the risk of resistant bacteria in herds. These resistant bacteria do not respond well to the antibiotics used, and therefore the mastitis pathogens in such herds develop into a chronic problem. It is recommended that intensive farmer education programmes are needed to improve udder health in smallholder dairy herds in Tanzania.

References

Aarestrup, F. M. 1999, Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. Journal of Antimicrobial Agents. **12**: 279-285.

Bradley A.J. 2002. Bovine mastitis: An evolving disease. The Veterinary Journal, **164**: 116-128.

Hogan. S. J., Gonzalez R. N., Harmon, J. R., Nickerson, S.C., Oliver, S. P., Pankey,.J. W. and Smith, L. K. 1999. Laboratory Handbook on Bovine Mastitis. Published by National Mastitis Council, Inc., W D Hoard, Fort Atkinson, USA.

International Dairy Federation 1981. Bulletin No. 132. Laboratory Methods for Use in Mastitis Work. IDF, Brussels, Belgium.

Kapaga.A.M., Weinhaupl.I. and Baumann, M.P.O. 1995. Risk Factors and Mastitis Prevalence in Dairy Cattle in The Region of Dar Er Salaam- Tanzania. Livestock Production & Diseases. Proceedings of The 8th Conference, Institute of Tropical Veterinary Medicine. Berlin.Germany.

Karimuribo, E., Fitzpatrick, J.L.; Bell, C.E.; Swai, E.S.; Kambarage, D.M.; Ogden, N.H. & French, N.P. 2003. Mastitis in smallholder dairy farms in Tanzania: From risk to intervention and knowledge transfer. In: Reid, S.W.J. & Menzies, F.D. (Editors). Proceedings of Society for Veterinary Epidemiology and Preventive Medicine held at Warwick, UK, 31st March-2nd, April, 2003: pp 83-94.

Kinabo. L.D.B. and Assey. R.J. 1983. Bovine mastitis in selected dairy farms in Morogoro district in Tanzania. Beitrage trop. Landwirtsch. Veterinarmed. **21** (**1**): 65-71

Kivaria, F.M. J.P.T. Noordhuizen., A.M. Kapaga (2006). Prospects and constraints of small holder dairy husbandry in Dar es Salaam region, Tanzania *Outlook on Agriculture* 35 (3) 209-215

Leigh J. A., 1999. *Streptococcus uberis*: A permanent barrier to the control of bovine mastitis? The Veterinary Journal, **157:** 225-238.

Mbise, A.N., Nyange, J.F.C., Otaru., M.M., and Mbasha, E.M.S. 1983. Isolation and drug sensitivity of bacteria from milk samples with clinical mastitis at Arusha veterinary investigation centre between 1975 and 1982. Bulletin of Animal Health and Production, **33**: 31-33

Mulei C.M. 1999. Teat lesions and their relationship to intramammary

infections on small-scale dairy farms in Kiambu district in Kenya. Journal of the south African Veterinary Association. **70**: (**4**) 156-157

Omore. A.O., J.J. McDermott., S.M. Arimi., M.N. kyule., and D. Ouma., 1996. A longitudinal study of milk somatic cell counts and bacterial culture from cows on smallholder dairy farms in Kiambu district, Kenya. Preventive Veterinary Medicine. **29**: 77-89

Phiri, E. C. J. H., Pereka, A. E., Mgasa, M. N. and Larsen. T., 1998. Clinical mastitis and bacteria isolates in dairy cows at ASAS dairy farm Iringa, Tanzania. Tanzania Veterinary Journal, **18** (**3**): 173-179

Quinn, P.J., Carter, M. E., Markey, B. K. and Carter, G.R. 2000. Clinical veterinary microbiology. London, Mosby-year book Europe limited, pp. 120-121

Schukken, Y. H., Grommers, F. J., Van de Geer, D., Erb, H. N., Brand, A. 1991. Risk factors for clinical mastitis in herds with a low bulk milk somatic cell count. 2. Risk factors for *Escherichia coli* and *Staphylococcus aureus*. Journal of Dairy Science, **74**: 826-32

Sol, J. 2002. Cure of *Staphylococcus aureus* mastitis in Dutch dairy cows. PhD thesis; Utrecht University, The Netherlands.

4

Chapter

Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania

Kivaria F.M.[1]* Noordhuizen. J.P.T.M.[2] Kapaga. A.M.[1]

[1]Population Studies, Animal Diseases Research Institute, P.O.Box 9254; Dar es Salaam, Tanzania

[2]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

*Corresponding author at: Fax: + 255 222 119 001:
E-mail address: fredkiv@yahoo.com

Tropical Animal Health and Production (2004) 36(6): 581-592
doi: 10.1023/B:TROP.0000040935.87175.bb

Abstract

Smallholder dairy farmers in Tanzania appear to be unaware of the subclinical mastitis situation in their cows. A cross-sectional study was carried out between June and September 2002 on smallholder dairy herds in the Dar es Salaam region. The study objectives were to establish the prevalence of subclinical mastitis and related risk indicators, and to assess their contribution to the occurrence of subclinical mastitis. Three field procedures based on the principles of herd health and production management were followed: clinical, farm and farm-records inspection. The California mastitis test (CMT) was carried out on quarter milk samples to determine the prevalence of subclinical mastitis. A total of 182 lactating cows from 62 herds were investigated. Clinical inspection indicated that 3.8% of the lactating cows had clinical mastitis. Subclinical mastitis was detected in 90.3% of lactating cows screened. Farm inspection revealed that, water scarcity, barn size, residual suckling, single udder-towel and dairy-labourers as most substantial ($P < 0.05$) risk indicators. Although most of the risk indicators studied were not found to be statistically significantly associated with the occurrence of subclinical mastitis possibly due to sample size and the presence of confounders, the epidemiological need to address such risk indicators cannot be over-emphasized.

Keywords: Clinical mastitis, Subclinical mastitis, Smallholder dairy cattle, Risk indicators, Tanzania
Abbreviations: ADRI, Animal Diseases Research Institute; CMT, California mastitis test; NMC, National Mastitis Council, SCC, somatic cell count.

Introduction

The keeping of dairy animals is a popular activity in many urban and peri-urban areas of Tanzania (Kapaga *et al*., 1995). Most of these dairymen have little knowledge of dairy husbandry and the management practices are therefore of sub optimal standards. Laboratory records indicate that mastitis is an important problem in the Tanzanian dairy sector. Because the dairy industry in Tanzania is still in its infancy, it is possible that the risk factors of the disease may be greatly influenced by management practices and poor feeding among others. The dairy herds range in size from 1 to over 250 head, grade cattle are predominantly held in small herds averaging nine head, and overall, 64% of herds have less than five dairy cattle. The distribution of dairy cattle amongst the herds is such that 50% of the herds contain only 18.7% of all the animals, while the 20 largest herds (approximately 2% of all herds) contain 25% of all the animals.

Studies conducted in large-scale dairy farms ($n \geq 250$ dairy-cattle) in Tanzania show that mastitis is common (Kinabo and Assay, 1983; Phiri *et al*., 1998). Little work has been done in the smallholder dairy sector. One of the studies in Dar es Salaam region, reported the point prevalence of clinical mastitis was 27 and 70 cases per 100 cows for clinical and subclinical mastitis respectively (Kapaga *et al*., 1995).To date however, information on the occurrence of subclinical mastitis remains sparse while information on the major risk indicators for mastitis in smallholder dairy cows in the country is lacking.

The present study was therefore designed to (1) establish the prevalence of subclinical mastitis, (2) determine the risk indicators for subclinical mastitis in smallholder dairy cows and (3) to assess their relationship to the occurrence of subclinical mastitis in lactating smallholder dairy cows.

Materials and methods

Study design

This cross-sectional study was conducted between June and September 2002 and it was designed to investigate animals on urban / peri-urban based smallholder dairy herds in the Dar es Salaam region, Tanzania. All urban / peri-urban dairy herds formed the population from which study herds were randomly selected. To qualify for inclusion in the study, a smallholder herd had to have an average herd size of $1 \leq n \leq 15$, all

lactating cows in selected herds were eligible for the study. The following information was used in computing the sample size required, a priori prevalence (P) of subclinical mastitis of 70% based on results from previous study in the same study area (Kapaga *et al*., 1995). There are a total of ('N') 383 herds, listed in the proposed study area; 150 herds were eligible and the average herd size was five dairy cattle. A total of 233 herds had more than 15 cattle / or had no lactating cows, were therefore excluded from the study. The sample size needed was determined as $n = (Z^2_{\alpha(2)} * PQ) / L^2$, (Martin *et al*., 1987); where $Z_{\alpha(2)} = 1.96$; P = the disease risk; and Q =1-P = disease free risk; L = the desired absolute precision level, this was set at 5%. $n = 1.96^2*0.30*0.70 / 0{,}05^2 = 322.69$ cows, the number of herds to be sampled was given as: 322.69/5 = 64.54.Thus, 65 dairy cattle clusters were randomly selected; the ultimate sample size depended on the number of eligible animals in the selected herds. The epidemiological unit of concern was defined as the individual cow that is likely to have subclinical mastitis at the defined prevalence level that the study is attempting to detect, i.e. 70% if it is present in the study area.

Field survey

Three procedures were followed in this cross sectional study: clinical, farm and data inspection during one herd visit, all according to a locally adapted pre-set field observational protocol (Brand *et al*., 2001). A summary of activities conducted during the farm visits are presented in Table I. The activities were investigated and evaluated according to the scoring system developed by Brand *et al*., (2001).

Screening for sub clinical mastitis

CMT was applied as a cow-side test, evaluated as described by Hogan *et al*., (1999). Based on the thickness of the gel formed by CMT reagent-milk mixture, test results were scored as 0 (negative / trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive). Positive CMT-cows were defined as having at least one CMT-positive quarter. In this study, milk samples with test results of negative / trace were assessed as having originated from cows free of subclinical mastitis while CMT results of ≥ +1 were classified as evidence of subclinical mastitis.

Table I: Summary of the variables studied

CLINICAL INSPECTION

- Udder lesion, blind teats, estimates of cows leaking milk, udders with increased fibrotic tissue (palpable lumps in the gland), and evidence of clinical mastitis. CMT evaluation of lactating cows

FARM-RECORD INSPECTION

- Record keeping, the purpose of this procedure was to use the available farm records as standard for comparison with farmers' information and with own observations.

FARM INSPECTION

- **General management**
  Herd size, number of the people working on the herd, breed, other farming activities, manure management, experience in dairying, calf feeding methods, grazing system, beddings, daily milk yield per cow for the previous 12 months, and daily milk yield per cow.
- **Housing of lactating and dry cows**
  Type of housing, floor type (concrete or rammed mud), ventilation, barn size, feeding area, separate calf units
- **Cleaning procedures**
  Cleaning of the barn, bedding replacement, disinfection procedures, frequency of cleaning and disinfection
- **Hygiene of barns and cows**
  Cleanliness of barn, beddings and cows, presence of too many flies and biting insects, the percentage of cows with dirty thighs, dirty udders, and dirty perineum, and cows sleeping on dirty floor.
- **Feeding of lactating and dry cows**
  Minerals for lactating and dry cows, methods of concentrate feeding, water sources
- **Management of dry cows**
  Dry off procedure, dry cow therapy, teat disinfection in dry cows, mastitis checks in the dry period, and length of the dry period.
- **Management of cows before and during calving**
  Presence of calving pen, isolation pen, beddings in calving pen, cleaning and disinfection of calving pen.
- **Milking procedures**
  Udder preparation (whole udder versus teat wash), pre and post teat dipping, use of milking salve, management of mastitic cows, and milking technique (striping versus five finger method), use of soap for hand washing.
- **Diseases and disease prevention**
  Method of treatment of clinical mastitis, duration of treatment, number of antibiotic treatments per clinical case, provision of fresh feed after milking, clipping hairs of cow.

Questionnaire execution

A pre-tested structured questionnaire with the primary objective of elucidating the multifactorial background of subclinical mastitis was conducted in an interactive manner at every farm visited. Two herds were visited per day by the study team of three people, at milking time. In this way the research team had ample time to conduct the questionnaire, as well as the data, clinical and farm inspection.

Data analysis

Occurrence or non-occurrence of subclinical mastitis at cow level as defined by CMT-score was the dependent variable in our study. Prior to statistical analyses, data files were screened for unlikely observations, proper coding, missing and out of range data. Fifteen out of 191 observations were omitted for these reasons, while errors in data entry were corrected. Variables at the cow level included dirty thighs, parity, length of the dry period, milk yield, days in milk (DIM), and body condition score. Variables defined at the quarter level were presence or absence of a palpable mass in the gland, udder lesions, soiled udders, and blind teats. The analyses did not consider the quarter position. Descriptive statistics of herd, cow, and quarter factors were estimated using SPSS version 11.5 (SPSS Inc, 2002).

In this study, clustering of observations was expected to occur within cows, and within herds. However, due to the small herd sizes, multi-level analysis was not feasible, as computational limitations or non-convergence would arise. Since subclinical mastitis risk indicators may depend on herd management factors and on exposure to infected cows/udders, and cow-level variables are highly correlated with quarter-level variables, analyses were run with herd effect and cow effects forced into the different model-building steps, as fixed effects. Analyses were run using logistic regression procedure in SPSS as follows. Univariate analysis using 2X2 tables was employed to screen the variables; all variables whose univariable test had a *P*-value ≤ 0.25 were selected for the next step. The selected variables were all subjected to one multivariable logistic-regression model, and significant associations were determined at a *P*-value of 0.10 by the forward-Walds' selection option in SPSS. Selected variables were offered for selection in the final model, variables were removed by backward elimination. Only variables significant at a *P*-value ≤ 0.05 in the likelihood ratio test were retained in the final model. The goodness of fit of the final model was assessed by the Hosmer-Lemeshow (Hosmer and Lemeshow, 1989) statistic option in SPSS.

Results

The study animals were mostly crosses of Zebu and exotic dairy cattle. A total of 191 lactating cows from 62 herds were investigated, the average herd size being 12 ± 7 cattle. However, in some cows CMT samples could not be collected from all four quarters. The mean parity was 2.8 ± 1.6; the average daily milk yield per cow at 120 days post calving

was 10 ± 8, and 6 ± 4 litres at the time of visit, this resulted into an overall daily mean of 8.5 ± 5 litres per cow, over duration of lactation of 450 ± 90days. The results from farm inspection are summarised in Table II. The results of the final multivariable-logistic model are displayed in Table III. In our model the Hosmer-Lemeshow statistic had a value close to one, indicating good model fit and suggesting appropriate model selection.

Clinical inspection

Clinical inspection of 182 (nine cows were excluded) lactating cows revealed that 86 % had dirty thighs, 53.8% had soiled udders, 30% had various forms of udder lesions, 77% had increased udder fibrosis (palpable lumps in the gland) and five percent had blind and atrophic quarters. Other observed lesions were, open wounds (52%); extra teats (21%); insect bites (17%); miscellaneous lesions, such as teat erosions, teat fistula and blocked teat canal (5%). Clinical evaluation further revealed that 3.8% of the lactating cows had clinical mastitis. A total of 698 (15 cows and 6 quarters were not sampled) clinically healthy quarters from 176 cows were CMT tested. Herd based prevalence of subclinical mastitis was 100% (that is in every farm there was at least one cow testing CMT positive in at least one quarter). 90.3% of cows were CMT positive, the prevalence ranged from 33.3% to 100% between herds. CMT results of 0, +1, +2, and +3 were observed in 15.5%, 21.3%, 20.8%, and 42.4% of quarter milk samples, respectively. Overall, 0, +1, +2, and +3 CMT scores were observed in 10%, 14%, 35%, and 41% of lactating cows, respectively.

Farm-records inspection

Fifty seven dairy farmers (92%) kept herd records, but the available data were incomplete. Missing event dates and misclassifications of cow's status were some of the observed deficits of the few available farm records. The available information indicated a recorded annual prevalence of 62.4 cases of clinical mastitis per 100 lactating cows, during the previous 12 months. Subclinical mastitis was never detected so was never recorded before by either the farmer or by the visiting livestock extension officer. Only 5% of dairymen were aware of the presence of subclinical mastitis. Long calving intervals, up to 20 ± 2 months appeared to be characteristic of the surveyed herds. Purchasing of new animals was common; these cows are not screened for mastitis or any other disease prior to entry into the herd.

Table II: Distribution of the farm variables among 62 smallholder dairy herds in Dar es Salaam region, Tanzania

Variable studied		Number of herds	Percentage
General management			
Herd size			
	$1 \leq n \leq 5$	22	35
	$6 \leq n \leq 10$	26	42
	$n \geq 11$	14	23
Number of labourer			
	$n \leq 2$	59	95
	$n > 2$	3	5
Calf feeding			
	Residual suckling	41	66
	Bucket feeding	21	34
Experience in dairying			
	0-5 years	21	34
	6-10 years	20	32
	11-15 years	13	21
	≥ 16 years	8	13
Grazing			
	Indoors	46	74
	Outdoors	16	26
Manure disposal			
	Good	18	29
	Poor	44	71
Record keeping			
	Poor	15	24
	Good	47	76
Housing			
Barn size			
	Adequate	57	92
	Not adequate	5	8
Floor type			
	Concrete	47	76
	Earth	15	24
Beddings			
	Yes	6	10
	No	56	90
Sleeping area for cows			
	Same as feeding	54	87
	Separate area	8	13
Animals tethered while in house			
	Yes	39	63
	No	23	37
Cleaning procedures			
Sanitary practices			
	Good	2	3
	Poor	60	97
Presence of too many flies			
	Yes	46	74
	No	16	26
Floors disinfected periodically			
	Yes	10	16
	No	52	84

Table II continued

Variable	Number of herds	Percentage
Feeding		
Water source		
Tap	39	63
Bore well / pond	23	37
Occurrence of water scarcity		
Frequent	28	45
Rare	34	55
Cow management		
Dry cow therapy		
Yes	3	5
No	59	95
Length of dry cow period		
60 days	26	42
< 60 days	20	32
> 60 days	16	26
Milking procedures		
Cows restrained for milking		
Yes	34	55
No	28	45
Hand washing		
With soap	11	18
Without soap	51	82
Screening for mastitis		
Yes	18	29
No	44	71
Milking techniques		
Five finger squeezing	6	10
Stripping	56	90
Udder preparation		
Wash only the teats	8	13
Wash the whole udder	54	87
Udder cloth		
Single towel	54	87
Separate towel	8	13
Type of teat lubricant		
Commercial milking salve	30	48
Cooking oil/petroleum jelly	32	52
Feed after milking		
Yes	40	65
No	22	35
Mastitic cows milked last		
Yes	57	92
No	5	8
Clinical cases cultured		
Yes	3	5
No	59	95

(Source: Survey data June - September 2002)

Discussion

In Tanzania, the factors responsible for low milk production in the dairy industry are not fully known. It is well established that high somatic cell counts (SCC) reflect intramammary infections and have negative effects on milk quality and milk production with associated loss (Dutta *et al*., 1995; Omore *et al*., 1996). Culture results were not available in this study due to logistic reasons. For developing treatment and prevention strategies those culture results would have been necessary. The observed cow-prevalence of subclinical mastitis in this study is relatively higher than the 40% and 70% reported by Kinabo and Assey (1983), and Kapaga *et al*., (1995) respectively. Workineh *et al*., (2002) in Ethiopia, reported a cow-prevalence of 38.2%, and in Kenya a cow-prevalence range of 43% to 80% on smallholder dairy herds was reported by Omore *et al*., (1999). The high prevalence (90.3%) of subclinical mastitis and low overall prevalence (3.8%) of clinical mastitis observed agrees with reported observations that subclinical mastitis is more prevalent than clinical mastitis on most farms (Schukken *et al* 1995). Similar observations have been reported in Tanzania by Kapaga *et al*., (1995).

Table III

Final logistic regression model explaining relationship between occurrence of subclinical mastitis and risk indicators for 176 lactating dairy cows on 62 smallholder dairy farms in Dar es Salaam, Tanzania (Hosmer-Lemeshow statistic = $X^2 = 1.283$ on 7 df, $P = 0.989$).

Variable	ß	$SE_ß$	*P*	OR
Intercept	1.862	1.123	0.098	- -
Scarcity of water (rare)	2.380	0.780	0.002	10.802
Adequate barn size (yes)	2. 320	1.013	0.022	10.17
Residual suckling (yes)	-1.748	0.674	0.009	0.174
Single udder towel (yes)	1.818	0.797	0.023	6.162
Number of dairy labourers (≤ 2)	-2.190	0.848	0.010	0.112

Clinical examination of the animals and the subsequent farm inspections were the basis for this study. Furthermore, the results of the questionnaire were used together with the results of inspection of farm records. Interpretation of our data depended directly on the validity of the information given by the farmers. Such information is more or less

subjective and might therefore be biased. However, there was no other feasible method to describe farmers' practices that might influence the prevalence of subclinical mastitis. Validation of the farmers' information was thus, limited to plausibility verification. Odds ratios describing the strength of association for statistically significant relationships between farm variables and CMT-cow scores were provided in the results (Table III). However, between herds correlation might be present, based on socio-economic conditions, likely also the within-herd correlation. Therefore, risk indicators found should be interpreted with care. The probability of subclinical mastitis for an individual cow will depend not only on the risk indicators, but also on the herd in which the cow lives (Atwill *et al*., 1995).

The results found in this study could be due to a number of reasons. A stripping type of hand-milking is employed in 90% of the 62 herds. Such a technique probably causes microscopic trauma of the teat epithelium, and may have resulted in an elevated SCC. Furthermore, none of the farmers employ CMT, a test whereby estimates of the somatic cell counts at herd, cow or quarter level, can be made and therefore provides a screen for subclinical mastitis. Dairy farmers' practice of fore-milk squirting on the hand, floor or into a teacup does not detect subclinical mastitis. The farmers were therefore unaware of the existence of subclinical mastitis in their lactating cows and no efforts were made to treat or control infections as is recommended (Murin *et al* 1998; NMC, 2003). Application of the CMT would assist in improving this situation.

Associated with the increased risk of subclinical mastitis is the use of contaminated water for dairying activities (Schukken *et al*., 1988, 1991). In the present study, scarcity of water was significantly ($P = 0.002$) associated with subclinical mastitis. Abundance of water (e.g. for udder washing) may lead to dirty, wet udders at milking time. The water supply to dairy farms is generally of poor quality. It was also found that water contamination often occurred in the storage containers used, thus cows might have experienced increased exposure to dirt. This suggests that it is the (microbiological) quality, rather than the amount of water, which is associated with subclinical mastitis.

Subclinical mastitis was significantly ($P = 0.022$) more prevalent on farms with adequate barn size compared to those farms with inadequate barn sizes. This finding could be explained, in part, by the fact that, in 63 % of the study herds; animals were tie-stalled, and restricted to smaller areas which were very wet and dirty. The exposure of teats to such a dirty

environment, and teat lesions resulting from various causes, probably resulted in the increased intramammary infections with subsequent elevated SCC. In addition, the multifactorial nature of subclinical mastitis coupled with the failure to control a number of these factors may explain the observations.

Sixty six percent of the farmers allowed calves to suckle after milking. This practice is good in that poor and irregular milking by the milker can be corrected by the calf. However, calves may be efficient transmitters of udder infections between cows.

Overall, poor hygiene may result in increased exposure and transmission of mastitis pathogens during milking. Thus, the observation that 87 % of dairymen in the study area only use a single towel for udder preparation, coupled with the use of contaminated water, and the general poor udder-hygiene practices, may explain the statistically significant ($P = 0.023$) association between use of udder-towel and the occurrence of subclinical mastitis. Dairy-labourers are also involved in other farming activities, this together with their lack of knowledge on dairy husbandry result in poor hygiene and inadequate udder preparation, and consequently leading to mastitis.

Only five (13 %) out of 40 risk indicators studied were found to be statistically significantly ($P < 0.05$) associated with subclinical mastitis. This could be due to the strong similarity of all herds; as there was not much difference in their management and husbandry standards. The small herd sizes would make clustering difficult to detect even if farm factors are important risk indicators for subclinical mastitis. Consistent with the lack of clustering by farm, failure to eliminate completely the effects of confounders may also be responsible for the lack of statistical significance since this is a cross-sectional study rather than a quantitative longitudinal study where these factors could be better controlled, as has been done for studying post-milking teat disinfection (Lam *et al*., 1997).

Although few significant associations were found, epidemiologically it is worth considering all the respective risk indicators at smallholder herd level. It is essential that a quantitative longitudinal study be conducted followed by intervention studies in order to determine the importance and roles of these risk indicators in subclinical mastitis. The risk indicators determined in such a study may be transferred into an extension and training programme for smallholders. Such programmes should emphasize awareness of subclinical mastitis via CMT, knowledge on intervention options, and data collection.

References

A.Brand, J.P.T.M.Noordhuizen and Y.H.Schukken 2001. Herd Health and Production Management In Dairy Practice, 3rd edn (Wageningen Pers Publ. Wageningen, The Netherlands), 351-415

Atwill, E. R., Mohammed, H.O., Scarlett, J.M., McCulloch, C.E., 1995. Extending the interpretation and utility of mixed effects logistic regression models. Preventive Veterinary Medicine. **24:** 187-201

Dutta. G.N., R.K. Saxena, and J. Buragohan. 1995. Economic implications of treatment of lactating cows for subclinical mastitis, Indian Veterinary Journal. **72:** 420-422.

Hogan. S. J., Gonzalez R. N., Harmon, J. R., Nickerson, S.C., Oliver, S. P., Pankey,.J. W. and Smith, L. K. 1999. Laboratory Handbook on Bovine Mastitis. Published by National Mastitis Council, Inc., W D Hoard, Fort Atkinson, USA.

Hosmer D. W. and Lemeshow. S. 1989. Applied logistic regression (Wiley-Interscience publication, New York), 83-134.

Kapaga.A.M., Weinhaupl.I. and Baumann, M.P.O. 1995. Risk indicators and Mastitis Prevalence in Dairy Cattle in The Region of Dar Er Salaam- Tanzania. Livestock Production & Diseases. Prceedings of The 8th Conference, Institute of Tropical Veterinary Medicine. Berlin.Germany

Kinabo. L.D.B. and Assey. R.J. 1983. Bovine mastitis in selected dairy farms in Morogoro district in Tanzania, Beitrage trop. Landwirtsch. Veterinarmed. **21:** 65-71

Lam, T.J.G.M., J.H.van Vliet, Y.H. Schukken, F.J. Grommers, A. van Velden-Russcher, H.W. Barkema, and A. Brand. 1997. The effect of discontinuation of post milking teat disinfection in low somatic cell count herds. I. Incidence of clinical mastitis. Veterinary Quarterly **19:** 41-47

Martin, S.W., Meek, A.H. and Willeberg, P., 1987. Veterinary Epidemiology. Principles and Methods. Iowa State University Press, Ames, p.32.

Murin. D. F., R.D. Shanks, and G. C. McCoy., 1998. Comparison of antibiotic administration in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows with clinical mastitis. Journal of American Veterinary Medical Association, **213:** 676-684.

National Mastitis Council. 2003, Information and resources. http://www.nmconline.org/info.htm

Omore, A. O., McDermott, J. J., Arimi, S. M., Kyule, M. N., 1999. Impact of mastitis control measures on milk production and mastitis indicators in smallholder dairy farms in Kiambu district, Kenya. Tropical Animal Health and Production, **31:** 347-361

Omore. A.O., J.J. McDermott., S.M. Arimi., M.N. kyule., and D. Ouma., 1996. A longitudinal study of milk somatic cell counts and bacterial culture from cows on smallholder dairy farms in Kiambu district, Kenya. Preventive Veterinary Medicine. **29**: 77-89

Phiri, E. C. J. H., Pereka, A. E., Mgasa, M. N. and Larsen. T., 1998. Clinical mastitis and bacteria isolates in dairy cows at ASAS dairy farm Iringa, Tanzania. Tanzania Veterinary Journal, **18**: 173-179

Schukken. Y.H., F.J. Grommers., D. van de Greer., H.N. Erb, and A.Brand. 1991. Risk factor for clinical mastitis in herds with a low bulk milk somatic cell count. 2. Risk factor for *Escherichia coli* and *Staphylococcus aureus*. Journal of Dairy Science, **24:** 826-832

Schukken. Y.H., H.N. Erb, P. M. Sears, and R.D. Smith. 1988. Ecologic study of the risk indicators for environmental mastitis in cows. American Journal of Veterinary Research, **49:** 766-769.

Schukken. Y.H., T. J. Lam., M. Nielen., H.Hogeveen., H. W. Barkema., and F. J. Grommers. 1995. Subclinical mastitis on dairy farms in the Netherlands: epidemiological developments. Tijdschrift van Diergeneeskunde (in Dutch). **120:** 208-213

SPSS for windows, release 11.5.0, SPSS Inc, 2002. http://www.spss.com

Workineh, S., Bayleyegn, M., Mekonnen, H. and Potgieter. L. N. D. 2002. Prevalence and aetiology of mastitis in cows from two major Ethiopian dairies. Tropical Animal Health and Production, **34:** 19-25

5

Chapter

Risk Factors Associated With Incidence Rate of Clinical Mastitis in Smallholder Dairy Cows in Dar es Salaam Region, Tanzania

Kivaria F.M. [a],*. J. P. T. M. Noordhuizen [b], Msami, H. M [a].

[a] Population Studies, Animal Diseases Research Institute, P.O. Box 9254; Dar es Salaam, Tanzania.

[b] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands.

* Corresponding author: Tel: +255 222 863 104; fax: + 255 222 864 369.
E-mail address: fredkiv@yahoo.com (F.M. Kivaria)

The Veterinary Journal (2006) doi: 10.1016/j.tvjl.2006.01.009

Abstract

Smallholder dairy herds around the Dar es Salaam region, Tanzania, supply 86% of raw milk consumed by the city dwellers. Previous studies indicated that clinical mastitis is also an important disease affecting the smallholder dairy cows in Tanzania. An 18-month questionnaire-based longitudinal field-study was conducted between July 2003 and March 2005 to elucidate risk factors associated with the incidence rate of clinical mastitis among the smallholder dairy cows in Dar es Salaam region, Tanzania. A total of 6057 quarter-level observations from 317 lactating cows on 87 randomly selected smallholder dairy herds were analysed at the quarter and cow level using logistic and Poisson regression models, respectively.

At the cow level, the average incidence rate of clinical mastitis was 38.4 cases per 100 quarter-years at risk. Whereas, at the cow level the incidence rate was 43.3 clinical mastitis cases per 100 cow-years at risk. The incidence rate of clinical mastitis was significantly ($P \leq 0.001$) associated with cow factors (body condition score, parity, stage of lactation, and udder consistency), housing (floor type) conditions and milking (cow and udder preparation) practices. It is concluded that the extrapolation of the classic ten-point mastitis control plan into the smallholder dairy herds should be undertaken cautiously. An integrated approach in dairy extension should focus more on creation of mastitis awareness among the smallholder producers and on improvement of animal nutrition and reproduction indices, factors that may also have a direct impact on milk yield.

Keywords: Clinical mastitis; Risk factors; Smallholder dairy herds; Tanzania

Introduction

The smallholder dairy industry around the Dar es Salaam region of Tanzania supplies 86% (APA & HPI[9], 2000) of the raw milk consumed by the city of Dar es Salaam. The industry has expanded rapidly in recent years and the sector employs many primary school leavers and provides a regular source of cash for farmers. Animals are reared under different management and milking conditions, and there is little knowledge about dairying among farmers. The intensification of dairy production, especially under the hot and humid conditions, presents new disease problems and bovine mastitis is an important example of this. Previous studies in Tanzania (Kapaga *et al*., 1995, Mdegela *et al*., 2004; Kivaria *et al*., 2004) indicated that clinical mastitis is an important disease among the smallholder dairy herds.

Compared with other diseases, mastitis is ranked low in priorities by the national veterinary authority and consequently has received little attention in Tanzania. Extension efforts have therefore been focused on the treatment of clinical cases rather than tackling the disease from the control point of view. A classic ten-point mastitis control programme (Radostits *et al*., 2000; Radostits, 2001) was developed for machine milked herds and may not be appropriate for smallholder dairy herds in Africa, which are almost all hand milked. Information on risk factors associated with mastitis is of major importance in designing and implementing appropriate control programmes.

The aim of the present study was to elucidate the risk factors associated with the incidence rate of clinical mastitis longitudinally in the urban and peri-urban based smallholder dairy herds as an initial stage in the design of appropriate preventive strategies for the smallholder dairy industry in Tanzania.

Materials and methods

Study population, selection of herds and sample size

An 18-month longitudinal study was planned to investigate animals on urban/peri-urban based smallholder dairy herds in the Dar es Salaam region of Tanzania. All urban/peri-urban dairy herds formed the population from which study herds were randomly selected. In order to obtain an arguably representative sample of herds, a smallholder dairy

[9] APA & HPI - Austrian Development Cooperation and Heiffer project international

herd was defined as one with herd size of $1 \leq n \leq 50$. All lactating cows in selected herds were eligible for the study.

The study herds and field procedures have been described in an earlier paper (Kivaria *et al*., 2004). In short, the smallholder herds were subsistence backyard herds with 2-30 30 cattle producing ≤ 5000 kg of milk per year. The producers were known to have other enterprises (e.g. poultry, pigs and vegetables) and used family labour. Milk is sold to obtain a continuous cash flow to support the families. The herds are predominantly (≥ 70%) Friesian-Boran (40%) or Jersey-Boran (30%) crosses, the level of exotic blood is first filial (13%), second filial (35%) and third filial (52%).

A total of 87 herds were randomly selected using computer generated random numbers, from 383 smallholder herds listed in the study area. This provided 22.7% of the target population. All lactating cows in selected herds that calved up to a month before commencement of the study were eligible for investigation, and followed up to 305 days post partum; 36 visits were scheduled for each herd at an interval of 14 days between July 2003 and March 2005. The potential power of this study could not be estimated a priori, since little information concerning prevalence estimates and information on the distribution of management practices existed. The sample size of 87 herds was therefore based on logistical considerations.

Case definition and risk factors survey

Clinical mastitis was diagnosed at the quarter level, based on visible and palpable signs (hard and swollen quarter, kick up on udder touching, watery secretions, clots in milk and blood tinged secretions), on clinical examination by a trained staff. At each herd visit, farm management and individual cow data were collected using a standardised questionnaire and protocols (the questionnaire and protocols were previously used in the prevalence study (Kivaria *et al*., 2004), this was assumed to be a pre-testing, and the questionnaire and the protocols were amended to be more focused on the risk factors) and in order to avoid inter-interviewer variations, one person permanently administered the questionnaire. Initially the questionnaire covered a broad range of topics including past health, reproduction, production performance, disease control practices, housing and nutrition, and herd demographics (herd size, number of heifers, female and male calves, lactating and dry cows, and bulls), and herd dynamics (purchases and culling) Individual animal data included: breed, sex, parity, and disease history, disease control measures

administered at an individual, animal, production and reproduction performance. The questionnaire was administered on the first visit to the herd. During subsequent visits a shorter follow-up survey was conducted, in which health, production and management events occurring in the past two weeks were recorded. In the event of clinical mastitis episodes occurring between the visits, the farmers were requested to call one of the project staff who would then attended and recorded the case. A thorough clinical examination of the udder and specific information on the udder health (cases of mastitis and treatment history, udder hygiene, drying-off practices) were the main focus during the subsequent visits. A summary of the field activities and risk factors studied is presented in Table 1.

Statistical analyses

Initially, descriptive statistics, frequency distribution, histograms and scatter, plots, and Kolmogorov – Smirnov test statistic procedures in SPSS version 11.5.0 (SPSS Inc., 2002) were used to explore the data set for errors, outliers and normality. As a result of this procedure, the final data set available for statistical analyses included 87 herds, 317 lactating cows and 6057 quarter-observations. Parametric measures of central tendency and dispersion were used to investigate continuous variables and frequency distributions to investigate categorical variables. At the quarter level, only one clinical mastitis episode per quarter was included in the analysis. Therefore, a logistic regression model that always included herd as a fixed effect was built to illustrate the magnitude of association between the investigated risk factors and clinical mastitis episodes.

The logistic model selection process involved three steps. In the first step, Yates' corrected x^2-test or Fisher's exact test for discrete data were used to identify and select the risk factors; only those variables with significant associations ($P \leq 0.25$) with the outcome variable were selected. In the binary logistic regression procedure of SPSS, all variables selected in the first step were included, and significant associations were determined at a *P*-value of 0.10 by the forward-Walds' selection option in SPSS (Hosmer and Lemeshow, 2000). Selected variables were offered for selection in the final model, variables were removed by backward elimination. Only variables significant at a *P*-value ≤ 0.05 in the likelihood ratio test[10] were retained in the final model. The goodness of fit of the final model was assessed by Hosmer-Lemeshow (Hosmer and

[10] The Wald statistic and the likelihood ratio test essentially do the same thing, but the latter is more powerful than the former, their consecutive use here was meant to be less restrictive in variable screening stage and stricter in the final model.

Table 1. Summary of field procedures and the variables studied

CLINICAL INSPECTION

- Udder lesion, blind teats, estimates of cows leaking milk, udders with increased, fibrotic tissue (palpable lumps in the gland), and evidence of clinical mastitis.

FARM-RECORD INSPECTION

- Record keeping, the purpose of this procedure was to use the available farm records as standard for comparison with farmers' information and with own observations.

FARM INSPECTION

- **General management**
  Herd size, number of the people working on the herd, breed, parity, body condition scores, other farming activities, manure management, experience in dairying, calf feeding methods, grazing system, beddings, daily milk yield per cow for the previous 12 months, current daily milk yield per cow, calving date

- **Housing of lactating and dry cows**
  Type of housing, floor type (concrete or rammed mud), lighting and ventilation, barn size, feeding area, separate calf units, separate milking area, separate dry cow units

- **Cleaning procedures**
  Cleaning of the barn, bedding replacement, disinfection procedures, frequency of cleaning and disinfection

- **Hygiene of barns and cows**
  Cleanliness of barn, bedding and cows, presence of too many flies and biting insects, the percentage of cows with dirty thighs, dirty udders/teats, and dirty perineum, cows sleeping on dirty floor, magnitude and direction of the slope

- **Feeding of lactating and dry cows**
  Minerals for lactating and dry cows, methods of concentrate feeding, water availability and sources

- **Management of dry cows**
  Dry off procedure, dry cow therapy, teat disinfection in dry cows, mastitis checks in the dry period, length of the dry period.

- **Management of cows before and during calving**
  Presence of calving pen, isolation pen, bedding in calving pen, cleaning and disinfection of calving pen.

- **Milking procedures**
  Udder preparation (whole udder versus teat wash), pre and post teat dipping, use of milking salve, management of mastitic cows, milking technique (stripping versus five finger method), use of soap for hand washing, milking order, udder towel (single versus shared udder towel)
- **Diseases and disease prevention**
  Method of treatment of clinical mastitis, duration of treatment, number of antibiotic treatments per clinical case, drug types (intramammary-tubes or indictable), provision of fresh feed after milking, clipping of cow.

Lemeshow, 2000) statistic option in SPSS. At the cow level, repeated episodes in the same cow were taken into account, with the exception of those episodes recorded at shorter intervals than the lag time of ≤ 14-days. The total number of clinical mastitis episodes was therefore the outcome of interest.

A main effects Poisson model was fitted using the general log-linear procedures in SPSS, to evaluate the magnitude of association between clinical mastitis and management related risk factors. A bivariate analysis for screening of observations was conducted in the Poisson regression model that always included herd as a fixed effect. Finally, all independent variables with $P \leq 0.20$ for at least one level in the bivariate analysis were submitted to a multivariate mixed model, based on maximum likelihood estimation equations. The goodness-of-fit of the final model was assessed by the deviance x^2 statistic (Kleinbaum *et al*., 1998). Milk yield was not included in the analyses because of the focus on identifying the management practices and cow factors (other than the level of production) associated with the incidence rate of clinical mastitis rather than the association between clinical mastitis and milk yield. In the last two steps, two-way biologically meaningful interactions were tested as described by Kleinbaum *et al*., (1998). The inclusions of interactions in the final multivariable models resulted in a poor fit, and were therefore omitted from further analysis. The risk factors for use in the logistic and Poisson regression models are given in Table 2.

At quarter level the incidence rate (IR) was expressed as the number of clinical mastitis episodes per 100 quarter- years at risk. The observation period for each quarter was from the calving date, until the occurrence of the first clinical mastitis episode. At cow level the incidence rate was expressed as the number of clinical mastitis episodes per 100 cow-years at risk. The observation period for each cow was from the calving date until 305 days postpartum, minus 14 days after each clinical mastitis episode. The International Dairy Federation (1997) proposed a lag time of 8 days. However, based on the following assumptions; a 7-day therapy regimen followed by a 4-days withdrawal period will allow the farmer to sell milk from the treated cow on day 12. A new treatment from day 13 onwards would indicate a new episode, we decided to use a lag time of 14 days in this study period. Multivariate adjustment of odds ratios for confounding analyses were done as described by Kleinbaum *et al*., (1998). Calculations of clinical mastitis therefore included the total number of episodes and separate calculations were performed per herd size, parity, body condition score, and days in milk (DIM) categories.

Table 2. Variables used in the final logistic and Poisson regression models for the clinical mastitis risk factors

Variable	Levels	Code
Dry cow therapy	2	Yes = 1; No = 0
Grazing system	2	Out doors = 1, Zero = 0
Are animals tethered while in house	2	Yes = 1, No = 0
Bedding	2	Yes = 1, No = 0
Water availability	2	Yes = 1, No = 0
Udder washing	2	Teats only = 1, the whole udder = 0
Udder towel	3	Individual = 1, no towel = 2, common = 0
Mastitic cows milked last	2	Yes = 1, No = 0
Provision of feed after milking	2	yes = 1, No = 0
Proper records[11]	2	Yes = 1, No = 0
Milking	2	All quarters = 1, leave some teats = 0
Body condition score	3	Fair = 1, Good = 2, poor = 0
Parity	3	1 – 4 = 0, ≥ 5 – 8 = 1, > 8 = 2
Herd size	4	1 – 10 = 0, ≥ 11 – 20 = 1, ≥ 21 – 30 = 2, ≥ 31 = 3
Type of labourers	3	Family = 0, Hired = 1, both = 2
Barn floor type	2	Concrete = 1, rammed soil = 0
Dirty barn floor	2	Yes =1, No = 0
Milking salve	2	Yes = 1, No = 0
Dry off method	2	Gradual = 1, Abrupt = 0
Cow preparation	3	calf = 0, concentrate = 1, massage = 2
Hand preparation	2	With soap = 1, Without soap = 0
Calf feeding system	3	Bucket = 0, Residual suckling = 1, some quarters left = 2
Sleeping area for cows	2	Separate = 1, Same as feeding area = 0
Restraining for milking	2	Back legs = 0, Around the neck = 1
Soiled quarters/teats	2	Yes = 1, No = 0
Udder consistency	2	normal = 1, fibrotic = 0
Soiled animal	2	Yes = 1, No = 0
Teat lesions	2	Yes = 1, No = 0

[11] Proper records referrers to udder health records with date of clinical episode, cow Id, quarter Id, type of medication used.

Results

Descriptive statistics and Multivariate regression models

A total of 317 lactating grade cows on 87 smallholder dairy herds were examined at least ten times over the 18-months study period. Of the 1268 quarters investigated 10 and 51% were blind and soiled, respectively. Blind quarters were excluded from the multivariate analyses. Of the investigated quarters 54% had a hard (fibrotic) consistency. The mean herd size was 24 ± 10Sd[12]. The mean daily milk yield per cow in all the study herds was 8.4 ± 3.4Sd litres, while the mean parity was 3 ± 2Sd. The distribution of categorical variables is summarised in Table 3.

Table 3. Distribution of categorical herd level variables among 87 smallholder dairy herds in and around Dar es Salaam region, Tanzania

Variable	Percentage
Grazing system – (zero/outdoors)	53/47
Calf feeding (bucket/residual/some quarters left)	53/44/3
Floor type (concrete/rammed soil)	90/10
Dirty barn floor (Yes/No)	51/49
Beddings (Yes/No)	5/95
Dung disposal (piled up near the barn/pile faraway)	59/41
Animal tethered while in house (Yes/No)	37/63
Sleeping area for cows (same as feeding area /separate)	57/43
Water availability (frequent/rare)	88/12
Water source (tap/bore-well)	78/22
Type of labour (family/hired/both)	57/43/0
Hand preparation (with soap/without soap)	33/67
Udder towel (no towel/individual/common)	46/2/52
Udder washing (teats only/whole udder)	3/97
Milk let down (introduce calf/concentrate/massage)	12/85/3
Milking salve (Yes/No)	69/31
Type of milking (all quarters/leave some quarters)	78/22
Restraining for milking (back legs/around the neck)	73/27
Mastitic cows milked (last/between)	2/98
Feed immediately after milking (Yes/No)	43/57
Record keeping (Yes/No)	63/37
Dry off method (gradual/abrupt)	94/6
Dry cow therapy (Yes/No)	4/96

[12] Sd = standard deviation

Over the study period, 937 new clinical mastitis episodes were recorded at the quarter level. This figure represented an incidence rate of 38.4 per 100 quarter-years at risk. Stratum incidence rate is summarised in Table 4. The total number of clinical mastitis episodes at the cow level was 1472[13], representing an incidence rate of 43.3 per 100 cow-years at risk over the study period.

Table 4. Summary results of stratum - specific incidence rate and multivariate analysis of associations between clinical mastitis and potential risk factors

Variable	Variable distribution (%)	Cow-years at risk	Number of CM episodes	Incidence rate (IR)	β	SE_β	Wald χ^2	P	e^β	95% CI for e^β	
Herd size										Lower	Upper
1 – 10	46.30	1144.38	318	0.278	0.512	0.452	1.283	0.257	1.669	0.688	4.047
11 – 20	30.00	693.21	283	0.408	0.971	0.476	4.161	0.041	2.640	1.039	6.712
21 - 30	8.10	217.07	96	0.442	1.442	0.553	6.800	0.009	4.229	1.431	12.502
≥ 31	15.60	387.69	240	0.619	1.863	0.549	11.515	0.001	6.442	2.197	18.900
Parity											
1 – 4	85.20	2154.35	771	0.358	0.251	0.133	3.562	0.059	1.285	0.990	1.668
5 – 8	13.80	274.98	156	0.567	1.313	0.280	21.989	< 0.001	3.717	2.147	6.435
≥ 9	1.00	13.03	10	0.767	1.681	0.077	476.600	< 0.001	5.370	4.619	6.246
Body condition score											
Poor	36.70	823.34	355	0.431	1.931	0.699	7.632	0.006	6.895	1.752	27.141
Fair	43.50	1084.62	403	0.372	0.782	0.332	5.548	0.019	2.186	1.140	4.190
Good	19.80	534.39	179	0.335	-0.291	0.122	5.689	0.017	0.748	0.589	0.949
Days in milk											
≤ 120 days	41.40	412.42	350	0.849	1.843	0.455	16.407	< 0.001	6.314	2.589	15.407
≥ 121 - ≤ 210 days	32.40	901.84	322	0.357	0.644	0.221	8.492	0.004	1.904	1.235	2.936
≥ 211 days	26.20	1128.09	265	0.235	0.227	0.633	0.129	0.720	1.255	0.363	4.339

In these analyses, the Hosmer-Lemeshow - x^2 statistic was 8.422 on 8 df, P = 0.395, while for the Poisson regression model, the deviance x^2_{LR}– statistic was 613.537 on 5168 df, P = 1.000, indicating good model fit and suggesting appropriate model selection. The results of the final multivariate logistic and Poisson regression models are summarised in Tables 5 and 6, respectively.

Discussion

Longitudinal studies with repeated measurement of intramammary status are necessary to identify factors associated with the risk of clinical intramammary infection. Identification of these factors will improve understanding of how smallholder dairy herds in Tanzania might control clinical intramammary infections. The study presented here was specifically designed to identify risk factors for the incidence rate of clinical mastitis episodes in smallholder dairy herds. Unknown or poor case definition combined with reporting or submission biases are

[13] Includes those cases occurred within two weeks on the same quarters.

Table 5. Final logistic regression model explaining the occurrence of first episodes of clinical mastitis for 1141 quarters from 317 lactating cows on 87 smallholder dairy herds in Dar es Salaam region, Tanzania

Variable	β	SE_β	$Wald_{\chi^2}$	*P*	e^β	95 % CI for e^β	
						Lower	Upper
Constant	- 0.302	0.343	0.775	0.379	-	-	-
Dry cow therapy (Yes)	-1.202	0.202	35.408	0.000	0.301	0.202	0.447
Grazing system (out doors)	-0.656	0.098	44.808	0.000	0.519	0.429	0.628
Animals tethered(Yes)	0.219	0.078	7.883	0.005	1.245	1.068	1.452
Water availability (Yes)	-0.603	0.103	34.274	0.000	0.547	0.447	0.670
Udder washing (teats only)	0.791	0.203	15.183	0.000	2.205	1.482	3.282
Udder towel							
Udder towel (individual)	-0.038	0.080	0.226	0.635	0.963	0.823	1.126
Udder towel (N0 towel)	-0.642	0.257	6.240	0.012	0.526	0.318	0.870
Mastitic cow milked (last)	-2.927	0.304	92.704	0.000	0.054	0.030	0.097
Feed after milking (Yes)	-0.730	0.094	60.310	0.000	0.482	0.401	0.579
Records (Yes)	-0.591	0.076	60.471	0.000	0.554	0.477	0.642
Milking (All quarters)	0.762	0.084	82.291	0.000	2.143	1.817	2.529
Body condition score							
BCS (Fair)	-0.278	0.067	17.216	0.000	0.757	0.665	0.863
BCS (Good)	-0.175	0.086	4.141	0.042	0.840	0.709	0.994
Parity							
Parity (5-8)	0.393	0.086	20.883	0.000	1.482	1.253	1.753
Parity (>8)	1.031	0.282	13.366	0.000	2.804	1.612	4.878
Herd size							
Herd size (11-20)	0.755	0.084	80.786	0.000	2.128	1.805	2.509
Herd size (21-30)	-0.183	0.143	1.638	0.201	0.833	0.630	1.101
Herd size (≥31)	0.297	0.145	4.195	0.241	1.345	1.013	1.787
Labourer (hired)	0.277	0.101	7.522	0.006	1.320	1.083	1.608
Floor type (concrete)	1.300	0.115	127.788	0.000	3.669	2.927	4.599
Milking salve (Yes)	0.421	0.075	31.509	0.000	1.523	1.315	1.765
Dry off method (Gradual)	0.760	0.153	24.674	0.000	2.139	1.584	2.888
Cow preparation							
Cow preparation (concentrate)	-0.494	0.111	19.806	0.000	0.610	0.491	0.759
Cow preparation (massage)	-0.442	0.212	4.347	0.037	0.643	0.424	0.975
Soiled teats (Yes)	0.447	0.060	55.502	0.000	1.563	1.391	1.757
Udder consistency (fibrotic)	0.196	0.059	11.036	0.000	1.217	1.085	1.365
Teat lesions (Yes)	0.683	0.126	29.383	0.000	1.980	1.547	2.534

common problems affecting field studies. Having an investigator live on each of the study farms and observe every animal each day can eliminate these problems. The design and execution of the study enabled us to minimise such inaccuracies. We believe that the two-weekly frequency of sampling visits was useful to monitor changes in herd management, to detect when clinical mastitis cases occurred.

The incidence rates we found in this study are considerably high compared to the previous reported incidence rate for a similar smallholder dairy farming systems in the southern highlands of Tanzania (Karimuribo *et al.*, 2003), and Kiambu district in Kenya (Omore *et al.*,

Table 6. Final Poisson regression model for the incidence rate of clinical mastitis for 317 lactating cows on 87 smallholder dairy herds in Dar es Salaam region, Tanzania

Variable	ß	$SE_ß$	*Z*	*P*	$e^ß$	95 % CI for $e^ß$	
						Lower	Upper
Constant	6.8910	0.3925	-17.56	0.000	-	-	-
Dry cow therapy (No)	1.1242	0.1985	5.66	0.000	3.077	2.086	4.542
Udder towel (Common)	0.8369	0.1058	7.91	0.000	2.309	1.877	2.841
Udder towel (Individual)	-2.1664	0.4220	-5.13	0.000	0.115	0.050	0.262
Udder washing (whole udder)	0.7208	0.1085	6.64	0.000	2.056	1.662	2.543
Hands preparation (without soap)	0.1266	0.0952	1.33	0.184	1.135	0.942	1.368
Milking salve (No)	-0.2224	0.9220	-2.41	0.016	0.801	0.131	4.878
Milking (leave some teats for calf)	-0.1420	0.0468	-3.03	0.002	0.868	0.792	0.951
Restraining for milking (rope around neck)	0.1937	0.0373	5.19	0.000	1.214	1.128	1.306
Sleeping area (same as feeding area)	0.2314	0.0571	4.05	0.000	1.260	1.127	1.410
Dirty barn floor (Yes)	0.8671	0.0953	9.10	0.000	2.380	1.974	2.869
Calf feeding (bucket)	0.9247	0.2381	3.88	0.000	2.521	1.581	4.020
Calf feeding (residual suckling)	1.0573	0.2383	4.44	0.000	2.878	1.796	4.571
Udder consistency (fibrotic)	0.6478	0.2011	3.22	0.001	1.911	1.289	2.835
Teat lesions (Yes)	0.3535	0.0499	7.08	0.000	1.424	1.291	1.570

1996), respectively. However, similar or even higher estimates have been reported in the literature (Sviland and Waage, 2002). This difference could most probably be attributed to the differences in the distribution of mastitis risk factors. Moreover, such differences may be attributed to the differences in case definition, laboratory techniques, study design, climate, the level of management and animal studied.

The increased incidence rate of clinical mastitis episodes (Table 4) with herd size, parity, body condition score, and days in milk conform to previous reports (Barkema *et al*., 1998; Sviland and Waage, 2002; Mungube *et al*., 2004). The apparent effect of herd size might be a result of differences in the distribution of risk factors. The observed risk factors (Tables 5 and 6) presented in this study are in agreement with the results described by many other studies (Radostits *et al*., 2000; Radostits, 2001; Zadoks *et al*., 2001; Kivaria *et al*., 2005). Although the risk factors were statistically significant, they are not necessarily causally related, and they should be interpreted in light of the causal criteria that have been proposed to transpose an observed association between a risk factor and a disease into a causal relation (Thrusfield, 1995; Dohoo *et al*., 2003).

As a result of the paucity of dairy extension services in Tanzania, the smallholder dairy sector is characterised by two major features; poorly planned dairy infrastructures and the lack of dairy knowledge and skills among the producers. Consequently, dairy cows are housed under conditions of sub optimal hygiene. Sub-optimal housing hygiene has been associated with high incidences of clinical mastitis (Radostits *et al*., 2000;

Radostits, 2001). In an unpublished study, we established that 80% of the smallholder producers in the same study area are aware of the existence of clinical mastitis. But 90% did not know what the causal factors were, and therefore they did not know how to prevent the disease. The lack of knowledge leads to failures to implement standard preventive hygiene, which has proven to be effective in temperate and tropical countries (Brown *et al*., 1998; Radostits *et al*., 2000).

Although microbiological investigations were not the objective of this investigation, from previous studies in the same area (Kapaga *et al*., 1995; Mdegela *et al*., 2004, Kivaria *et al*., 2005) and unpublished laboratory data, it is apparent that environmental pathogens such as *Escherichia coli*, as well as the contagious pathogens such as *Staphylococcus aureus,* play an important role in causing clinical mastitis in the study herds. The observed shortcomings in management practices and milking hygiene encourage a rapid within-herd multiplication, spread and maintenance of both environmental and contagious mastitis pathogens, a situation that translates itself into the observed high risk of clinical mastitis.

These observations, coupled with the observed poor nutrition and long calving intervals (Kanuya *et al*., 2000) imply that the extrapolation of the classic ten-point mastitis control plan into smallholder dairy setting has to be undertaken cautiously. Through an integrated approach, dairy extension efforts should focus more on creation of both clinical and subclinical mastitis awareness among the smallholder producers and on improvement of animal nutrition and reproduction indices factors that may also have a direct impact on milk yield.

References

APA and HPI. 2000. National Dairy Development Conference. Proceedings of the 3rd conference, Kibaha education centre, Kibaha, Coast region. June 27th -29th 2000.

Barkema, H. W., Y. H. Schukken., T. G. M. Lam., D. T. Galligan., M. L. Beiboer., A. Brand. 1997. Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. Journal of Dairy Science. **80**: 1592-1599.

Brown, D. F., D. V. Ardaya., Ribera, H.C., A. M. Cuellar., P. J. Kerby. 1998. Mastitis control programme in the developing dairy industry of tropical lowland Bolivia. Tropical Animal Health and Production, **30**: 3-11.

Dohoo, I., Martin, W., H. Stryhn., 2003. Veterinary Epidemiologic Research. AVC Inc, Charlottetown, Prince Edward Island, Canada.

Fang, W., Jiang, C., Liu, H., 1993. Epidemiologic aspects of bovine mastitis and its control in several dairy herds in southern China. Preventive Veterinary Medicine **15**: 169-180.

Hosmer D. W. and Lemeshow. S., 2000. Applied Logistic Regression. New York. Wiley, John and Sons, Inc. 392 pp.

International Dairy Federation, 1997. Recommendations for presentation of mastitis-related data. Bulletin No 321, Brussels, Belgium.

Kanuya N. L., Kessy B. M., Bittegeko S. B. P., Mdoe N. S. Y., Aboud A. A. O., 2000. Suboptimal reproductive performance of dairy cattle kept in smallholder herds in a rural highland area of northern Tanzania. Preventive Veterinary Medicine **45:** 183-192

Kapaga, A.M., Weinhaupl, I. and Baumann, M.P.O. 1995. Risk indicators and Mastitis Prevalence in Dairy Cattle in The Region of Dar Es Salaam- Tanzania. Livestock Production and Diseases. Proceedings of the 8th Conference of the Institute of Tropical Veterinary Medicine. Berlin. Germany

Karimuribo, E., Fitzpatrick, J.L.; Bell, C.E.; Swai, E.S.; Kambarage, D.M.; Ogden, N.H. and French, N.P. 2003. Mastitis in smallholder dairy farms in Tanzania: From risk to intervention and knowledge transfer. In: Reid, S.W.J. and Menzies, F.D. (Editors). Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine held at Warwick, UK, 31st March – 2nd April, 2003: pp 83-94.

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M., 2004. Risk

Indicators Associated with Subclinical Mastitis in Smallholder Dairy Cows in Tanzania. Tropical Animal Health and Production, **36**: 581- 592

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M., 2005. Risk Indicators Associated with Staphylococcus aureus subclinical mastitis in smallholder dairy cows in Tanzania. In H. Hogeveen (editor). Mastitis in Dairy Production – Current Knowledge and Future Solutions. Wageningen academic publishers, The Netherlands.

Kleinbaum D. G., Kupper L. L., Muller K. E., Nizam A., 1998. Applied Regression Analysis and other Multivariable Methods. London: Duxbury press. pp 186-211

McDermott, J. J., and Y. H. Schukken., 1994. A review of methods used to adjust for cluster effects in explanatory epidemiological studies of animal populations. Preventive Veterinary Medicine. **18**: 155-173.

Mdegela, R. H., L. J. M. Kusiluka., A. M. Kapaga., E. D. Karimuribo., F. M. Turuka., A. Bundala., F. Kivaria., B. Kabula., A. Manjurano., T. Loken., D. M. Kambarage., 2004. Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in eastern Tanzania. Journal of Veterinary Medicine B. **51:** 123-128.

Mungube, E. O., Tehagen, B. A., T. Kassa., F. Regessa., M. N. Kyule., M. Greiner., Baumann, M. P. O., 2004. Risk factors for dairy cow mastitis in central highlands of Ethiopia. Tropical Animal Health and Production, **36:** 463-472.

Omore. A.O., J.J. McDermott., S.M. Arimi., M.N. Kyule., and D. Ouma., 1996. A longitudinal study of milk somatic cell counts and bacterial culture from cows on smallholder dairy farms in Kiambu district, Kenya. Preventive Veterinary Medicine. **29**: 77-89

Radostits, O. M., 2001. Herd Health: Food Animal Production Medicine, 3rd edition. Philadelphia, W. B. Saunders.

Radostits. O.M., Gay. C. C., Blood, D.C. and Hinchcliff. K. W., 2000. Veterinary Medicine; A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edition. pp. 603-660. W. B. Saunders. London.

Rothman, K.J. and Greenland, S., 1998. Modern Epidemiology, 2nd ed. Philadelphia: Lippincott, Raven.

SPSS for windows, release 11.5.0, SPSS Inc, 2002. http://www.spss.com

Sviland S., Waage S., 2002. Clinical bovine mastitis in Norway. Preventive Veterinary Medicine **54:** 65-78

Thrusfield, M., 1995.Veterinary Epidemiology, 2nd Edition. London: Black Well Science. pp 220-237

Zadoks R. N., H. G. Allore., H. W. Barkema., O. C. Sampimon, Wallenberg. G. J., Y. T. Grohn., Y. H. Schukken., 2001. Cow and quarter level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. Journal of Dairy Science. **84**: 2649-2663.

6

Chapter

Management Practices Associated With Pathogen Specific Incidence Rates of Clinical Mastitis in Smallholder Dairy Cows in Dar es Salaam Region, Tanzania

Kivaria F.M [a],*, J. P. T. M. Noordhuizen [b]., Msami, H. M[a].

[a] Population Studies, Animal Diseases Research Institute, P.O. Box 9254; Dar es Salaam, Tanzania.

[b] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands.

* Corresponding author: Tel: +255 222 863 104; fax: + 255 222 864 369.
E-mail address: fredkiv@yahoo.com (F.M. Kivaria)

This chapter has been accepted for publication in the journal Tropical Animal Health and Production

Abstract

Management related risk factors for the pathogen specific incidence rate of clinical *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*), *Candida albicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) mastitis were longitudinally studied with sampling of all lactating cows at an interval of 14 days between July 2003 and March 2005, in 317 lactating cows from 87 smallholder dairy herds in the Dar es Salaam region, Tanzania. Quarter milk samples were used for bacteriology. Standardised questionnaires were used to obtain information on the potential management related risk factors for clinical mastitis. Four multivariate Poisson regression models with clustering at herd and cow levels were fitted to determine the association between risk factors and clinical mastitis.

6057 quarter milk samples were investigated. The study pathogens were isolated from 42% of the 6057 quarter milk samples. *S. aureus* was isolated from 23%, *S. agalactiae* from 8%, *E. coli* from 7%, and *C. albicans* from 4% of investigated quarter milk samples. The final multivariate Poisson regression models indicated that, the incidence rate for clinical *S. aureus* and *S. agalactiae* mastitis was significantly ($P < 0.05$) related to residual calf suckling, lack of dry cow therapy, and hired herd labourers, , respectively. Whereas, the incidence rate of clinical *C. albicans* and *E. coli* mastitis was significantly ($P < 0.05$) associated with gradual drying off, and dirt barn floors, respectively. Failure to use soap for hand preparation, leaving some teats for calf to suckle, use of udder towels, and water shortage were significantly associated ($P < 0.05$) with clinical mastitis caused by the four study pathogens. In view of the current results we can conclude that, udder hygiene among the smallholder dairy herds is generally poor.

Keywords: Clinical mastitis, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, Risk factors, Tanzania

Introduction

Tanzania is an emerging dairy country with an estimated 400,000 head of specialised dairy cattle (MOAF, 2002). Of these dairy animals 90% and 10% are owned by smallholder and commercial dairy producers, respectively (MOA, 2002). There are about 1749 herds with approximately 11571 head of improved dairy cattle in and around the Dar es Salaam region (MOAF, 2001). These herds supply 86% of raw milk consumed by the city dwellers. However, most of these farmers have little knowledge of dairy husbandry, and thus herd management practices and dairying hygiene are sub-optimal. Furthermore the sector's productivity is limited by a number of diseases and socio-economic constraints, mastitis occurring in clinical and subclinical forms is an important constraint (Kivaria *et al*., 2004; Mdegela *et al*., 2004).

The prevalence of clinical mastitis in the urban/peri-urban based smallholder dairy herds was estimated to be ≤ 19% (Kapaga *et al*., 1995). This figure was reported as a single disease. However, the epidemiology of clinical mastitis caused by different pathogens is different (Schukken *et al*., 1991). Risk factors for environmental pathogens such as *Escherichia coli* do not necessarily apply to contagious pathogens such as *Staphylococcus aureus* (Schukken *et al*., 1991). Retrospective analysis of 1732 culture-positive clinical bovine mastitis specimens submitted to the Animal Diseases Research Institute between December 1971 and November 2002 indicated the major microbial isolates to be *Staphylococcus aureus* (25.7%), *Streptococcus agalactiae* (15.4%), *Escherichia coli* (14.1%) and *Candida albicans* (2.1%). This study was designed to identify the management risk factors associated with the incidence rate of these most frequently isolated clinical mastitis pathogens.

Materials and methods

Herds, animals and data collection

The investigation was planned as a longitudinal observational study, milk samples were collected from 317-lactating cows at different stages of lactation on 87 smallholder dairy herds located within and around the Dar es Salaam region. The 87 herds were a subset of 125 randomly selected herds which were recruited in the studies of the epidemiology of bovine mastitis in smallholder dairy herds in the Dar es Salaam region. Herds were selected that had at least two lactating and/or dry animals,

participated in the previous studies (Kivaria *et al*, 2004; Mdegela e*t al*, 2004), had a history of at least one of the target clinical mastitis pathogens, and had cross bred cows. 36 visits were scheduled for each herd at an interval of 14 days between July 2003 and March 2005.

Management practices studied were categorised into (1) risk indicators associated with exposure to pathogens (housing, hygienic condition of barns, grazing management and milking procedures), (2) risk indicators associated with cure of existing intramammary infections (treatment of clinical mastitis cases, and dry cow therapy). Clinical mastitis was diagnosed at the quarter level, based on visible and palpable signs (hard and swollen quarter, kick up on udder touching, watery secretions, clots in milk and blood tinged secretions), on clinical examination by a trained staff. At each herd visit, farm management and individual cow data were collected using a standardised questionnaire and protocols, and in order to avoid inter-interviewer variations, one person permanently administered the questionnaire. Initially the questionnaire covered a broad range of topics including past health, reproduction, production performance, disease control practices, housing and nutrition, and herd demographics (herd size, number of heifers, female and male calves, lactating and dry cows, and bulls), and herd dynamics (purchases and culling). Individual animal data included: breed, sex, parity, and disease history, disease control measures administered at an individual, animal, production and reproduction performance. The questionnaire was administered on the first visit to the herd. During the subsequent visits a shorter follow-up survey was conducted, in which health, production and management events occurring in the past two weeks were recorded. In the event of clinical mastitis episodes occurring between the visits, the farmers were requested to call one of the project staff who would then attended and recorded the case. A thorough clinical examination of the udder and specific information on the udder health (cases of mastitis and treatment history, udder hygiene, drying-off practices) were the main focus during the subsequent visits. A summary of management activities studied is described in Table 1.

Screening for clinical mastitis and microbiological examination of milk samples

All lactating cows in a given herd were subjected to a thorough clinical examination of the udder. Cows that had swollen udder/quarters, showed pain upon touching of the udder/quarter produced abnormal

(flakes, clots, watery or blood tinged) udder/quarter secretions were judged to have clinical mastitis. Milk samples for microbiological culturing were collected from all clinically affected quarters. Each teat was wiped to remove gross contamination, washed with soap soaked cotton gauze, and wiped dry. Each teat was then scrubbed with 70% ethyl-alcohol and allowed to dry; the teat ends were then scrubbed again with a cotton wool swab soaked in 70% ethyl-alcohol before the sample was collected. Samples were collected before antimicrobial drugs were given, 10-20 mL of milk were squirted into sterile universal containers held as nearly as possible horizontal. The sample containers were identified by herd, cow, quarter and sample date. The samples were transported to the laboratory ice-cooled and then transferred to a freezer at -20^{o}C.

About a week after collection, 10µL from each sample was inoculated and spread with a loop on 5% sheep blood agar, Edward's agar, and Sabouraud dextrose agar. 100 µL of samples was also inoculated on to MacConkey agar to enhance the detection of Coliform organisms (Smith *et al*., 1985). The plates were incubated at 37ºC, aerobically and read after 24 and 48 h. Sabouraud dextrose agar plates were incubated at 37^{o}C, aerobically, for up to five days. The microbes were identified to species level by standard microbiological procedures described by Quinn *et al*., (2000). The aetiology of the mastitis was established on the basis of the isolation of an organism in pure growth, or as the pre-dominant growth or as the only recognised mastitis pathogen. A sample was classified a mixed growth if two known mastitis pathogens were isolated, and it was labelled contaminated if more than two organisms were isolated. A mastitic sample was the one taken from a lactating quarter with positive clinical signs and from which one of the four study pathogens was isolated.

Statistical analyses

Occurrence or non-occurrence of clinical mastitis cases was the dependent variable in our study. Prior to statistical analyses, data files were screened for unlikely observations, proper coding, missing and out of range data. No observations were excluded for this reason. The final data set available for statistical analyses at the herd, cow and quarter levels included 87 herds, 317 lactating cows and 6057 quarter-observations. Descriptive procedures for continuous data included calculations of frequencies; categorised data were summarised as

contingency tables using SPSS version 11.5 (SPSS Inc, 2002).

In the present study, clustering of repeated observations over time was expected to occur at the quarter level, quarters were clustered within cows and cows were clustered within herds. However, due to the small number of cases relative to the total number of the observations, multi-level analysis was not feasible, as computational limitations or non-convergence would arise. Using variance-components procedure in SPSS, it was determined that cow level accounted for more variability than the quarter level. Therefore, analyses were run with herd as fixed effect and cow as a repeated effect, accounting for correlation at herd level and cow level, but not at quarter level. Analyses were run with the log-linear procedure of SPSS using a regression model with log link and Poisson distributed error.

The modelling procedures involved three steps. In the first step, all single explanatory variables were screened in a bivariate Poisson regression model that always included herd as a fixed effect. Only variables with a *P*-value of ≤ 0.25 in the likelihood ratio test were considered for further analysis. Clinical mastitis incidence data is a 'person-time rate' and use of Poisson regression is conventional for analysis of person-time rates (Dohoo *et al*., 2003). In the second step, a backward elimination selection procedure of variables was performed; statistical significance was assumed at a *P*-value of ≤ 0.10 by the likelihood ratio test. In the final step, all risk factors with $P \leq 0.10$ for at least one level in the bivariate analysis were submitted to a multivariate Poisson regression mixed model with a log link, a backward selection of variables was performed. Only variables significant at a *P*-value of 0.05 in the likelihood ratio test were retained in the final model. In the last two steps, two way biologically meaningful interactions were tested as described by Kleinbaum *et al*., (1998). However, their inclusions in the final multivariable models resulted into poor model fit, and were therefore omitted from further analysis. The goodness of fit of the final model was assessed by the ratio of the Pearson Chi square statistic to the remaining degrees of freedom (McDermott *et al*., 1994).

Observations on cows or quarters within a herd are not independent. The within-herd correlation of measures must be considered in study design or data analysis to avoid invalid statistical inferences (McDermott and Schukken, 1994). When a common within herd correlation cannot be assumed, mixed models with fixed effects for herd and additional random effects for subgroups within herds (cows) are needed (McDermott *et al*., 1994). In the present study, within herd correlation was not the subject of

interest, but rather an effect that had to be corrected for to estimate the effect of cow and quarter level variables correctly. This correction was done as suggested by McDermott *et al.*, 1994.

The cow incidence rate (IR_{CM}) of clinical mastitis was computed as described by Thrusfield (2005), and expressed as the number of quarter cases per 100 cow days at risk. Intervals between clinical mastitis episodes in the same quarter had to be ≥ 14 days for a case to be included in the analysis. Cow-days at risk were calculated as the total number of days during the study that a cow was present in the herd, starting 7 days after parturition minus 14 days after each case of clinical mastitis episode.

Results

Descriptive statistics and clinical inspection

Eighty-seven herds with 317 lactating cows were used to estimate the number of cow-days at risk. Mean herd size of the 87 herds evaluated in this study was $11 \pm 6_{Sd}$ lactating cows, varying from 1 to 26. Median herd size was 10 cows. Mean daily production at the start of the study was 8.6 $\pm$ 4_{Sd} litres for the 87 herds. Other descriptive results are summarised in Table 1. At the quarter level 937 new clinical mastitis episodes were recorded over the study period, while at the cow level, the total number of clinical mastitis episodes was 547. The total cow-days at risk was 425667, the mean was $148 \pm 77_{Sd}$ cow days at risk, the mode and median were 228 and 153 cow-days at risk, respectively. First-single cases were observed in 122 quarters (122-cows), first-multiple cases were observed in 197 quarters (71-cows), recurrent single cases were observed in 217 quarters (217-cows), and recurrent multiple cases were observed in 401 quarters (134-cows). In 62% of the affected quarters, more than one case occurred during the same lactation. Recurrent episodes of clinical mastitis from which the same pathogen was isolated from the same quarter during the same lactation were found in 468 quarters. *Staphylococcus aureus* and *Candida albicans* were involved in 19% and 13% of these cases, respectively.

Animals calving for the first time had the lowest IR_{CM}, 11 quarter cases per 100 cow-days at risk. The IR_{CM} increased with parity, cows that had calved ≥ 2-times had the highest IR_{CM}, 38 quarter cases per 100 cow-days at risk. All herds had cases of clinical mastitis during the study period, and in five herds all lactating cows had clinical mastitis at one

Table 1. Frequency distribution of management risk factors and parameter estimates with standard error from the final multivariate Poisson regression models for prediction of clinical *Staphylococcus aureus, Streptococcus agalactiae, Candida albicans* and *Escherichia coli* mastitis in 317 lactating cows from 87 smallholder dairy herds in Dar es Salaam region, Tanzania

variable	Frequency (%)	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Candida albicans*	*Escherichia coli*
		± SE	± SE	± SE	± SE
Constant		-6.22 ± 0.38***	-6.03 ± 0.55***	5.71 ± 0.75***	4.97 ± 0.44***
Calf feeding					
Bucket	53	Reference	Reference	Reference	Reference
Residual suckling	47	1.22 ± 0.11***	0.68 ± 0.18***	0.31 ± 0.85*	0.06 ± 0.32*
Cows tethered while in house					
No	63	Reference	Reference	Reference	Reference
Yes	37	0.58 ± 1.85*	0.64 ± 0.21**	0.43 ± 1.21*	1.46 ± 1.40*
Dry cow therapy					
Applied	4	Reference	Reference	Reference	Reference
Not applied	96	1.34 ± 0.22***	1.25 ± 0.36***	2.01 ± 1.90*	0.01 ± 0.32*
Drying off procedure					
Abrupt	4	Reference	Reference	Reference	Reference
Gradual	96	1.31 ± 1.14*	1.11 ± 1.30*	2.19 ± 0.92**	0.71 ± 0.25***
Floor type					
Rammed soil	10	-0.82 ± 0.17***	-0.64 ± 0.38*	0.18 ± 0.64*	0.88 ± 0.32***
Concrete	90	Reference	Reference	Reference	Reference
Floor cleanness					
Not clean	49	0.06 ± 0.32*	0.48 ± 0.81*	1.43 ± 0.53***	0.60 ± 0.20***
Clean	51	Reference	Reference	Reference	Reference
Grazing management					
Indoors	53	0.81 ± 0.90*	0.22 ± 1.02*	1.69 ± 0.90*	0.922 ± 0.30***
Outdoors	47	Reference	Reference	Reference	Reference

Table 1 continued

variable	Frequency (%)	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Candida albicans*	*Escherichia coli*
		± SE	± SE	± SE	± SE
Hand preparations					
No soap used	33	$0.44 \pm 0.12^{***}$	$1.11 \pm 0.35^{***}$	$1.75 \pm 0.55^{***}$	$0.89 \pm 0.26^{***}$
Soap used	67	Reference	Reference	Reference	Reference
Labourers					
Family	57	Reference	Reference	Reference	Reference
Hired	43	$0.54 \pm 0.18^{***}$	$0.65 \pm 0.20^{***}$	$0.08 \pm 0.16^{*}$	$0.57 \pm 0.19^{***}$
Mastitic cow milked					
Last	2	Reference	Reference	Reference	Reference
In between	98	$0.96 \pm 0.58^{*}$	$0.50 \pm 0.16^{***}$	$0.53 \pm 0.70^{*}$	$0.03 \pm 0.42^{*}$
Milking procedures					
Leave some teats	22	$0.79 \pm 0.15^{***}$	$0.85 \pm 0.28^{***}$	$1.94 \pm 0.58^{***}$	$0.58 \pm 0.21^{***}$
All teats	78	Reference	Reference	Reference	Reference
Provision of feed after milking					
No feed provided	57	$0.07 \pm 0.06^{*}$	$0.05 \pm 0.31^{*}$	$0.63 \pm 0.61^{*}$	$0.63 \pm 0.25^{**}$
Feed provided	43	Reference	Reference	Reference	Reference
Sleeping area					
Same as feeding area	57	$0.11 \pm 0.32^{*}$	$0.01 \pm 0.11^{*}$	$2.70 \pm 0.81^{***}$	$1.42 \pm 0.33^{***}$
Separate from feeding area	43	Reference	Reference	Reference	Reference
Udder washing					
Whole udder	93	$0.54 \pm 0.12^{***}$	$2.02 \pm 0.91^{**}$	$0.73 \pm 0.14^{***}$	$0.23 \pm 0.36^{*}$
Teats only	7	Reference	Reference	Reference	Reference

Table 1 continued

variable	Frequency (%)	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Candida albicans*	*Escherichia coli*
		± SE	± SE	± SE	± SE
Udder towel					
Shared towel	52	$1.25 \pm 0.30^{***}$	$0.96 \pm 0.20^{***}$	$0.73 \pm 0.35^{**}$	$0.61 \pm 0.30^{**}$
Individual towel	2	$-2.02 \pm 0.43^{***}$	$-1.85 \pm 0.74^{***}$	$1.40 \pm 0.52^{***}$	$- 0.24 \pm 0.50^{*}$
No towel used	46	Reference	Reference	Reference	Reference
Water availability					
Adequate	88	Reference	Reference	Reference	Reference
Not adequate	12	$1.10 \pm 0.36^{***}$	$2.03 \pm 0.32^{***}$	$0.99 \pm 0.32^{***}$	$0.68 \pm 0.06^{***}$
Water source					
Bore-well	22	Reference	Reference	Reference	Reference
Tap	78	$1.10 \pm 0.63^{*}$	$1.61 \pm 0.48^{***}$	$1.99 \pm 0.96^{**}$	$0.89 \pm 0.26^{***}$
Model fit		$^{2} = 0.91$/DF	$^{2} = 0.87$/DF	$^{2} = 0.73$/DF	$^{2} = 0.80$/DF

Data source; Field survey July 2003-to- March 2005

* $P > 0.05$; ** $P \leq 0.05$; *** $P \leq 0.01$

point in time. Distribution of the IR per lactation period was such that 32%, 35% and 33% of cases occurred during 0-100 days, 101-200 days, and 201-305 days in milk, respectively.

Of the 937 clinical cases, 36 % had flakes/clots with inflammation of the udder, 41% had watery secretion with udder inflammation, 15% had blood tinged secretions without udder inflammation, and 8% had pus with a swollen quarter. 26% of the observed clinical cases occurred in animals that calved for the first time, 14.5% of the clinical cases occurred in cows producing > 15 L, 31.4 % of clinical cases occurred in fresh cows (within 14 days post calving), and 51.5 % of the cases occurred in combination (i.e. in all these categories). Ten per cent of the 1268 quarters (from the 317 study animals) had open wounds, 17% had insect bites, and 5% had miscellaneous lesions, such as teat erosions, teat fistula and blocked teat canal. 5% of the investigated quarters were blind. Extra teats were observed in 21% of the 317 cows.

Microbiological investigation and risk factors

The final data set presented for statistical analysis had 6057 quarter observations with complete risk factors and microbial observations. 30%, and 28% of the 6057 quarter-milk samples yielded no microbial growth, and other microbial isolates which were not of interest in this study. These included *Streptococcus pyogenes* (9%), *Enterococcus faecalis* (6%), *Klebsiella pneumoniae* (6%), *Staphylococcus epidermidis* (4%), and *Candida tropicalis* (3%). Study-udder pathogens were isolated from 42% of the 6057 quarter milk samples. The frequency of microbial isolates was *Staphylococcus aureus* (23%), *Streptococcus agalactiae* (8%), *Escherichia coli* (7%), and *Candida albicans* (4%). There was a good correlation between clinical examination and culture results (*Kappa*-statistic = 52%). The IR_{CM} was 0.78, and 0.73 cases per 100 cow-days at risk for *Staphylococcus aureus* and *Streptococcus agalactiae* positive quarters, respectively. While, the IR_{CM} was 0.83 and 1.24 cases per 100 cow-days at risk for *Escherichia coli* and *Candida albicans* positive quarters, respectively.

The variables in the final model associated ($P \leq 0.05$) with exposure to both *Staphylococcus aureus* and *Streptococcus agalactiae* clinical mastitis were calf feeding, hand and udder preparation, use of udder towels, milking procedures, dry cow therapy, labourers, water source, floor type and in house tethering of cows. Variables associated ($P < 0.05$) with exposure to both *Escherichia coli* and *Candida albicans* clinical

mastitis were grazing, sleeping area, floor cleanness, udder and hand preparation, water source and availability, labourers, post milk feeding and milking procedures. Corrected parameter estimates for the pathogen specific risk factors are summarised in Table 1. The Pearson Chi square statistic was 0.91 per degree of freedom, indicating good model fit.

Discussion

The recruitment into this study was 100% and all 87 randomly selected herds cooperated. Assuming that there was no unknown bias, the frequent two-weekly visits and the free-of-charge diagnosis, laboratory culture and treatment of clinical cases, and the random study design that took into consideration the cluster effects, probably improved the data quality and gave accurate estimates of influences on clinical mastitis for smallholder dairy herds in Tanzania. The 937 clinical mastitis episodes observed in this study is quite high, as compared to the observations made by Omore *et al*., (1996), and Karimuribo *et al*., (2003). In this study there was no distinction between mild, severe and chronic mastitis, also the two-weekly intervals of visit probably arose great interest and keenness among the producers and herd attendants, thus it was possible for a mild case to be detected and reported. 21% of the 937 cases were fresh-cases affecting more than one quarter. Whereas, 66% (618) of cases were recurrent-cases (occurring after ≥ 14days), 65% (401) of 618 case occurred in more than one quarter. All these may explain why we observed such a high number of cases.

Observations made in this study (table 1) are no doubt all pointing to one general factor, and that is shortcomings in husbandry. Superficial washing of the udder, in some cases without any disinfectants, the use of same udder cloths for several cows and poor hygiene of the milkers were prevalent in all 87-study herds (Table 1). Such factors are well described (Radostits *et al*., 2000) as being important risk factors for bovine mastitis. Moreover, unhygienic milking procedures, wet and muddy barn-floors, and persistent teat lesions, the absence of pre-and-post milking teat dips, and the failure to use dry period therapy, lack of proper treatment for clinical cases, and the absence of culling (chronically infected cows are not culled, in expectation that the particular cow will one day calve a female calf) that should have served to remove an infectious reservoir from the herd may be risk factors for the persistent risk of clinical mastitis throughout the lactation. Barkema *et al*., (1999) reported a significant correlation between the incidence rate of clinical mastitis and sub-optimal management practices, such as housing, udder hygiene,

feeding and milking practices. This might well be applicable to the study herds.

The most important sources of *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae* (*S. agalactiae*) within the dairy herds are infected udders/quarters, and infected teat lesions (Mulei, 1999). So these pathogens are spread during milking, and in the smallholder settings, transmission occurs via udder towels, milkers' hands, or free-running suckling calves. Therefore, good control of *S.aureus* and *S. agalactiae* mastitis can be achieved by good milking time hygiene combined with dry period therapy and culling. However, environmental sources may also help in the spread of *S. aureus* and *S. agalactiae* udder infections (Larsen *et al*., 2000). So, poor housing hygiene, contaminated water, and floors may provide an additional source of infection (Roberson, 1999). Thus the generally deficient management and milking hygiene, and the high risk of teat lesions observed in this study will favour the persistence of *S. aureus* and *S. agalactiae* mastitis. The primary reservoir of *Escherichia coli* (*E. coli*) is the cow's environment (Radostits *et al*., 2000); this type of mastitis is therefore common among housed cows especially where sanitation is sub-optimal. In 53% of the study herds (Table 1), cows were zero grazed. Majority of these zero grazed units have poor drainage, muddy floor, and small unit area per cow. Such a situation coupled with hot and humid temperatures of Dar es Salaam increases *E. coli* concentration in the cows' environment with subsequent increase in new *E. coli* mastitis. In addition, poorly designed structures subjects the animals to unnecessary stressful conditions which then increase the risk of infection of the cows, probably as a result of decreased phagocytic activities of the leukocytes. It is also important to note that, *E. coli* mastitis is also not uncommon among the cows reared outdoors, especially those who get access to ponds (Schukken *et al*., 2005). The isolation percentage of *S. agalactiae* and *E. coli* was low compared to isolation percentage of *S. aureus*; it is possible that freezing of milk samples prior to culture may have reduced the isolation rate (Schukken *et al*., 1989; Sol *et al*., 2002).

Candida albicans is an opportunistic udder pathogen (Radostits *et al*., 2000), its isolation from milk samples may imply udder infections that could be due to use of contaminated water to wash the udder/teats; sustained use of antibiotics or introduction of infections by use of contaminated intra-mammary infusion gadgets; factors which are commonly practiced by farmers in the study area. Teat lesions may also be a risk factor for *Candida albicans* udder infections. The isolation of fungal infections calls for the need to consider them in the differential

diagnosis of mastitis and in reviewing the treatment protocols in cases of treatment failures, which are occasionally encountered. 30% of the 6057 quarter milk samples yielded no bacterial growth. It is possible that failing to isolate an udder pathogen from clinically positive quarter could be due to incorrect treatment history. This is not uncommon especial in the study herds where some farmers try to treat their cows before calling a veterinarian. Another possible explanation is that culture negative result could be due to false positive results from clinical udder inspection.

Although 88% of the study herds (Table 1) reported to have enough water for dairying, we observed that there is a general shortage of water in the study area. Farmers collect and store water in open drums which are kept adjacent to the cow sheds, and water becomes grossly contaminated with cow dung, maize bran and dust. Thus the water used for dairying activities is generally of poor quality, and may explain the observed association between water attributes (source and availability) with clinical mastitis. When studying relatively large data sets, the possibility of finding associations due to chance alone increases, and many variables might be highly correlated. We suspect that the association between gradual dry off and *Candida albicans* clinical mastitis (Table 1) was the result of this process. In view of the current results we can conclude that, udder hygiene among the smallholder dairy herds is generally poor. An overall better hygiene and sanitation would decrease the exposure and transmission of udder pathogens.

References

Barkema, H. W., Schukken, Y. H., T. J. Boiboer., Bededictus, G., and A. Brand., 1999. Management practices associated with the incidence rate of clinical mastitis. Journal of Dairy Science. **82:** 1643-1654

Dohoo, I., Martin, W., H. Stryhn., 2003. Veterinary epidemiologic research. AVC Inc, Charlottetown, Prince Edward island, Canada.

Kapaga. A.M., Weinhaupl. I. and Baumann, M.P.O. 1995. Risk Factors and Mastitis Prevalence in Dairy Cattle in the Region of Dar Er Salaam- Tanzania. Livestock Production & Diseases. Proceedings of The 8th Conference, Institute of Tropical Veterinary Medicine. Berlin, Germany.

Karimuribo, E., Fitzpatrick, J.L.; Bell, C.E.; Swai, E.S.; Kambarage, D.M.; Ogden, N.H. & French, N.P. 2003. Mastitis in smallholder dairy farms in Tanzania: From risk to intervention and knowledge

transfer. In: Reid, S.W.J. & Menzies, F.D. (Editors). Proceedings of Society for Veterinary Epidemiology and Preventive Medicine held at Warwick, UK, 31st March – 2nd April, 2003: pp 83-94.

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M., 2004. Risk Indicators Associated with Subclinical Mastitis in Smallholder Dairy Cows in Tanzania. Tropical Animal Health and Production. **36**: 581- 592

Kleinbaum D. G., Kupper L. L., Muller K. E., Nizam A., 1998. Applied Regression Analysis and other Multivariable Methods. London: Duxbury press. pp 186-211

Larsen, H. D., Sloth, K. H., Elsberg, C., Enevoldsen, C., Pedersen, L. H., Eriksen, N. H. R., Aerestrup, F. M., Jensen, N. E., 2000. The dynamics of Staphylococcus aureus intramammary infection in nine Danish dairy herds. Veterinary Microbiology. **71:** 89-101.

McDermott, J. J., and Y. H. Schukken., 1994. A review of methods used to adjust for cluster effects in explanatory epidemiological studies of animal populations. Preventive Veterinary Medicine. **18:** 155-173.

McDermott, J. J., Y. H. Schukken, M. M. Shoukri. 1994. Study design and analytic methods for data collected from clusters of animals. Preventive Veterinary Medicine, **18**: 175-191

Mdegela, R. H., L. J. M. Kusiluka., A. M. Kapaga., E. D. Karimuribo., F. M. Turuka., A. Bundala., F. Kivaria., B. Kabula., A. Manjurano., T. Loken., D. M. Kambarage., 2004. Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in eastern Tanzania. Journal of Veterinary Medicine B. **51**: 123-128.

Ministry of Agriculture and Food Security (MOAF), 2001. District integrated agricultural survey 1998/99. Survey results, Dar es Salaam and Pwani report

Ministry of Agriculture and Food Security, 2002. Basic data-agriculture sector 1994/95 – 2000/2001

Mulei C.M. 1999. Teat lesions and their relationship to intramammary infections on small-scale dairy farms in Kiambu district in Kenya. Journal of the south African Veterinary Association. **70** (**4**) 156-157

Omore. A.O., J.J. McDermott., S.M. Arimi., M.N. Kyule., and D. Ouma., 1996. A longitudinal study of milk somatic cell counts and bacterial culture from cows on smallholder dairy farms in Kiambu district, Kenya. Preventive Veterinary Medicine. **29**: 77-89

Quinn, P.J., Carter, M. E., Markey, B. K. and Carter, G.R. 2000. Clinical veterinary microbiology. London, Mosby International -year book

Europe limited, pp 628.

Radostits. O.M., Gay. C. C., Blood, D.C. and Hinchcliff. K. W. 2000. Veterinary Medicine. A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edition. W. B. Saunders Company, London. pp. 603-660

Roberson, J. R., 1999. Epidemiology of Staphylococcus aureus on dairy farms. In: Proceedings of the 38th Annual meeting of the National Mastitis Council, 14-17 February, Arlington, VA, p. 38.

Schukken, Y. H., F. J. Grommers, D. van de Geer, H. N. Erb, A. Brand., 1991. Risk factors for clinical mastitis in herds with low bulk milk somatic cell count. 2. Risk factors for *Escherichia coli* and *Staphylococcus aureus*. Journal of Dairy Science. **74**: 826-832

Schukken, Y. H., Grommers, F. J., Smit, J. A., Vandegeer, D., Brand, A. 1989. Effect of freezing on bacteriologic culturing of mastitis milk samples. Journal of Dairy Science. **72**: 1900-1906.

Schukken, Y. H., L. L. Tikofsky, R. N. Zadoks. 2005. Environmental control for mastitis prevention, milk quality and safety. In H. Hogeveen (editor), Mastitis in dairy production: current knowledge and future solutions. Wageningen Academic Publishers, Wageningen.

Smith, K. L., Todhunter, D. A., Schoenberger, P. S. 1985. Environmental pathogens and intramammary infections during the dry period. Journal of Dairy Science. **68:** 402-417

Sol, J., O. C. Sampimon, E. Hartman, and H. W. Barkema. (2002). Effect of preculture freezing and incubation on bacteriological isolation from subclinical mastitis samples. Veterinary Microbiology. **85**: 241-249

SPSS for windows, release 11.5.0, SPSS Inc, 2002. http://www.spss.com

7

Chapter

Interpretation of California Mastitis Test Scores Using *Staphylococcus aureus* Culture Results for Screening of Subclinical Mastitis in Low Yielding Smallholder Dairy Cows in the Dar Es Salaam Region of Tanzania

F. M. Kivaria[1]* J. P. T. M. Noordhuizen[2]., M. Nielen[2]

[1]Population Studies, Animal Diseases Research Institute, P.O.Box 9254, Dar es Salaam, Tanzania

[2]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

*Correspondence: Tel: +255 222 863 104; Fax: + 255 222 864 369;
E-mail address: fredkiv@yahoo.com

This chapter has been accepted for publication in the journal Preventive Veterinary Medicine

Abstract

Screening of subclinical mastitis under field conditions is done using the California mastitis test (CMT). CMT score of ≥ +1 corresponding to ≥ 500,000 somatic cells mL^{-1} is commonly used as threshold of subclinical mastitis in temperate countries. However, given the very high background level of somatic cells in low-yielding dairy cows, this threshold may not apply to low-yielding dairy cows. The current study was undertaken to investigate the clinical utility of CMT for screening of *Staphylococcus aureus* subclinical mastitis in low-yielding smallholder dairy cows in Tanzania. There were 1151 of quarter samples CMT tested, of these 914-quarter milk samples from cows with a lactation period post partum of between ≥ 14 days-to- ≤ 305 days were screened for subclinical mastitis by the CMT as well as microbiological culture of single and three consecutive samples as a gold standard. Using a CMT score of ≥ +1 would classify 79.3% of the 914 quarter-samples as positive. 94.6% of the samples in which *Staphylococcus aureus* was isolated had CMT scores ≥ +2; this would classify 56.7% of the 914-quarter-samples as positive. For the single sample, this cut-off had sensitivity, specificity and likelihood ratio for *Staphylococcus aureus* of 98.0%, 70.0%, and 5.65, respectively. Results from three consecutive quarter-milk samples had sensitivity, specificity, and likelihood ratio of 93.0%, 76.0% and 9.60, respectively. This analysis gave a kappa value of 0.62 and 0.66 for the single and three consecutive quarter-milk samples, respectively. Based on these results and practical considerations, it is concluded that CMT score of ≥ +2 is the best cut-off to reliably identify *Staphylococcus aureus* intramammary infections in low-yielding dairy cows in Tanzania.

Keywords: California mastitis test; Subclinical mastitis; *Staphylococcus aureus*; Smallholder; Tanzania

Introduction

Eighty five percent of the urban and peri-urban smallholder dairy farmers in Tanzania are generally aware of clinical mastitis (Kivaria *et al*., "in press") because of the signs exhibited by the cow. Unfortunately, these farmers are not aware that the apparently healthy cow can harbour "hidden" mastitis, which creates tremendous loss in milk production (Omore *et al*., 1999; Bradley, 2002). Mastitis caused by *Staphylococcus aureus* (*S. aureus*) is often subclinical and is typically manifest as an elevation of the somatic cell count (SCC) of the milk from the affected quarter (Radostits *et al*., 2000; Bradley 2002), thus, cows with *S. aureus* intramammary infections (IMI) are not all readily identified. Because management of the disease is focused primarily on preventing spread of *S. aureus* among cows rather than treatment of individual animals, tests that reliably identify cows with *S. aureus* IMI are needed.

Somatic cell count has been accepted as the best index to use to both evaluate milk quality and predict udder infection in the cow (Poutrel and Rainard, 1982). Under field conditions, determination of SCC in cow's milk is usually performed by the California Mastitis Test (CMT). CMT score of ≥ +1, corresponding to ≥ 500,000 cells ml^{-1} is considered an indication of udder infection in cattle (IDF 1979; Quinn *et al*., 2000; Radostits *et al*., 2000). However, the SCC of low-yielding cows is innately (SCC declines as milk yield increases) high regardless of infection status (Omore *et al*., 1996). The usefulness of CMT for screening of subclinical mastitis (and therefore predicting udder bacteriological status) in low-yielding smallholder dairy cows is therefore doubtful. When classifying milk samples from low yielding cows, as either mastitic or non-mastitic, a higher threshold that will detect most of infected quarters and yet give an acceptable number of type II errors should, therefore, be chosen. The purpose of the present study was to explore the appropriateness of CMT for screening, and designing protocols for controlling of subclinical mastitis in low-yielding smallholder dairy cows in Dar es Salaam region, Tanzania.

Materials and methods

Herds and animals

Milk samples were collected from lactating cows with parity from 1 to 7 at different stages of lactation on 95 smallholder dairy herds located within and around the Dar es Salaam region, between October 2003 and

February 2004; the study herds were visited every 14 days. Study population and herd characteristics have been previously described (Kivaria *et al*., 2004). In short, the smallholder herds were subsistence backyard herds with 2-30 cattle producing ≤ 5,000 kg of milk per year. The producers were known to have other enterprises (e.g. poultry, pigs and vegetables) and used family labour. Milk is sold to obtain a continuous cash flow to support the families. The herds are predominantly (≥ 70%) Friesian-Boran (40%) or Jersey-Boran (30%) crosses, the level of exotic blood is first filial (13%), second filial (35%) and third filial (52%).

Sample collection

Initially each cow was examined clinically; particular attention was given to the condition of the udder. All animals selected for this investigation were clinically healthy and showed no mammary disorder. Each teat was disinfected with cotton soaked in 70% ethyl-alcohol, particular attention was given to the teat tip. Sampling was carried out after morning milking, 10-20 ml of milk were squirted into sterile universal containers. The samples were transported to the laboratory ice-cooled and then transferred to a freezer at -20ºC. About a week after collection, the samples were analysed using standard methods described by the International Dairy Federation (1981).

California mastitis test

CMT was applied as a cow-side test after quarter-milk sampling. The results were read and evaluated according to the manufacturer's instructions (Kruuse, Denmark). Scores represented four categories: negative or trace (0); weak positive (+1); distinct positive (+2) and, strong positive (+3). In this study, milk samples with test results of negative / trace were assessed as having originated from quarters free of subclinical mastitis while CMT results of ≥ +1 were classified as evidence of subclinical mastitis.

Bacteriology

Standard procedures for the isolation and identification of *S. aureus* IMI as described by IDF (1981), Hogan *et al*., (1999), and Quinn *et al*., (2000), were used for the isolation and identification of *S. aureus*. Any quarter milk sample with ≥ 1 coagulase-positive colony was classified as

culture positive for *S. aureus* (Hogan *et al*., 1999; Quinn *et al*., 2000). Other microbial isolates were identified by means of standard procedures (Hogan *et al*., 1999; Quinn *et al*., 2000) to the species level.

Data analysis

Data from a given cow were included only if CMT and culture results performed on three consecutive samples were available. Data from individual samples collected from cows that had been lactating ≤ 14 days were excluded (false positive reactions occur frequently in cows that have been fresh less than ten days). Milk samples from cows with blind quarters were also excluded, because infection status of the non-lactating quarters could not be determined. Sensitivity and specificity of CMT in relation to the bacteriological infection status of the quarter were calculated in a series of 2X2 tables at different CMT scores. Likelihood ratios and posterior probabilities were used to determine the clinical utility of the different CMT scores performed on single and three consecutive milk samples. Sensitivity, specificity and likelihood ratios were computed as described by Smith (1995) and Dohoo *et al*., (2003). The kappa statistic (Smith, 1995; Dohoo *et al*., 2003) was used to quantify the level of potential agreement beyond chance exhibited by the CMT scores and the culture results. Test of significance of binomial proportions (Snedecor and Cochran, 1989) was employed to compare other relevant proportions of CMT scores, the type I error was set at 0.05 for all statistical analyses. 95% confidence intervals for proportions were computed as described by (Dohoo *et al*., 2003). The prevalence of IMI caused by pathogens other than *S. aureus* was computed based on microbiological culture of the single quarter milk samples collected from cows from which *S. aureus* was never isolated. An unadjusted prevalence odds ratio, using culture negative quarters as the reference group, was calculated to determine the risk of a positive CMT score for cows with intramammary infection caused by other microbial isolates.

Results

Descriptive statistics

A total of 1,700 quarter-samples from 425 cows in 95 urban and peri-urban smallholder dairy herds were investigated, the average milk yield was 8.4 litres/cow/day (median 8; range = 22, and SE = 0.09). Out of the 1700 quarter-samples investigated 35 (2.1%) were blind, whereas,

34 (2.1%) out of 1,665 quarter-samples had clinical mastitis (clinical mastitis samples will not score positive due to the destruction of leucocytes by toxin from the infecting organisms). Further 100 quarter-samples were excluded because results from three consecutive samples were discordant, 20 quarter-samples were excluded because CMT or culture results were not available and 360 quarter-samples were excluded because they were collected from cows with ≤ 14 days lactation, so the final data set available for CMT evaluation included 1151 quarter samples. Using a CMT cut-off of ≥ +1, 80.6% of the 1151 quarter-samples were judged as being positive for subclinical mastitis. 19.4% of the 1151 quarter-samples showed negative CMT reaction. CMT results of +1, +2 and +3, were predominant, accounting for 20.5%, 22.6% and 37.5% of the screened samples, respectively.

Microbiological findings

Milk samples for bacteriology were available from 1,590 quarters. Of the 1,590-quarter milk samples that were bacteriologically examined, 28 (1.8%) were contaminated, 684 (43.0%) were culture positive, whereas 892 (56%) quarter milk samples yielded no growth of bacteria. *Staphylococcus aureus* was isolated in 26.6% of the culture positive-quarter milk samples. Other microbial isolates included; coagulase negative staphylococci (38.2%), environmental streptococci (14.0%), *Streptococcus agalactiae* (8.2%), coliforms (4.1%), yeast /fungal species (1.6%), *Arcanobacterium pyogenes* (1.6%), *Corynebacterium bovis* (1.3%) and other miscellaneous bacteria (4.4%).

Evaluation of the CMT scores

914-quarter milk samples had complete CMT and culture results; these were used for the ultimate evaluation of clinical utility of the CMT. For the single quarter –samples, CMT score of ≥ +1 would classify 79.3% (CI_{95}: 0.77-0.82) of the 914-quarter milk samples as positive and 40.4% (CI_{95}: 0.37-0.44) of these quarter milk samples would be classified as positive for *S. aureus* IMI. For the three consecutive samples, CMT score of ≥ +1 would classify 78.8% (CI_{95}: 0.76-0.81) of the 914-quarter samples as positive and 44.8% (CI_{95}: 0.42-0.48) of these quarter milk samples would be classified as positive for *S. aureus* IMI. In both cases, *Staphylococcus aureus* positive IMI status agreed more ($Z > 10$; $P \leq 0.01$) often for quarters with high CMT-scores than for quarters with low scores. In the present study, 95.0% (CI_{95}: 0.94-0.96) of the single samples

and 92.0% (CI_{95}: 0.90-0.94) of the three consecutive samples in which *S. aureus* were isolated had CMT scores higher than +1, respectively. This would classify 56.7% (CI_{95}: 0.56-0.58) of the single 914-quarter milk samples as CMT positive, 39.4% (CI_{95}: 0.36-0.43) of the 914-samples would be classified as both CMT and *S. aureus* IMI positive. Likewise, the CMT score ≥ +2 would classify 54.7% (CI_{95}: 0.52-0.58) of the three consecutive 914-quarter milk samples as CMT positive and 41.5% (CI_{95}: 0.38-0.45) as both CMT and *S. aureus* IMI positive. This evaluation yielded a kappa value of 0.62 and 0.66 for the single and three consecutive visits, respectively. The ability of CMT scores to predict *S. aureus* infected quarters of low yielding smallholder dairy cows for the single and three consecutive samples are shown in Table I and Table II, respectively.

Table I. Ability of CMT scores in predicting *Staphylococcus aureus* infected quarters of low producing smallholder dairy cows; single visit

CMT scores	*S. aureus* Positive	Negative culture	Sensitivity[1]	Specificity[2]	False negative[3]	False positive[4]	Sum	Likelihood ratio	Posterior odds	Posterior probability
0	10	179	1.00	0.00	0	197	197	0.08	0.06	0.05
1	9	198	0.97	0.34	10	356	366	0.06	0.05	0.04
2	160	40	0.98	0.70	9	158	167	5.65	4.00	0.80
3	200	118	0.58	0.78	160	118	278	2.39	1.69	0.63
Totals	379	535		[14] kappa = 0.62						

[1] Sensitivity = N° CMT (+)/culture (+) ≥ cutoff e.g. (379-10)/379 = 0.97
Total *S. aureus* culture positive

[2] Specificity = No CMT (+)/culture (-) ≥ cutoff e.g. (535-(118+40+198))/535 = 0.34
Total *S. aureus* culture negative

[3] Number of false negative diagnosis at each cutoff = (379)*(1-sensitivity)

[4] Number of false positive diagnosis at each cutoff = (535)*(1-specificity)

[14] Kappa was computed using the software Winepiscope 2.0. (http://www.clive.ed.ac.uk/winepiscope/) last accessed on 18/01/06

Samples from quarters without *S. aureus* intramammary infection but with intramammary infections caused by other pathogens were no more likely to have positive CMT-scores than were samples from uninfected cows (unadjusted prevalence odds = 0.65; CI_{95}: 0.42-1.00). None of the individual pathogens had a high prevalence odds ratio.

Table II. Ability of CMT scores in predicting *Staphylococcus aureus* infected quarters of low producing smallholder dairy cows; three consecutive visits[15]

CMT scores	*S. aureus* Positive	Negative culture	Sensitivity	Specificity	False negative	False positive	Sum	Likelihood ratio[5]	Posterior odds[6]	Posterior probability[7]
0	5	189	1.00	0.00	0	197	197	0.03	0.03	0.03
1	30	190	0.99	0.38	5	311	316	0.19	0.16	0.14
2	159	20	0.93	0.76	30	121	151	9.60	7.90	0.89
3	220	101	0.62	0.80	159	101	260	2.63	2.18	0.69
Totals	414	500		kappa = 0.66						

[5]Likelihood ratio = Culture (+) ≥ cut-off/total culture (+) e.g. (159/414)/(20/500) = 9.60
Culture (-) cut-off/total culture (-)

[6]Posterior odds at each cut-off (e.g. CMT = 2) were computed in two steps as follows:

- Pre-test odds = 414/(414+500) = 0.45
- Post-test odds = (0.45/(1-0.45))*9.60 = 7.87

[7]Posterior probability at each cut-off = post-test odds/(1+post-test odds) = 7.87/(1+7.87) = 0.89

[15]Three consecutive samples were taken at 14 days interval on the same herd from the quarters of the same cows

Discussion

In the present study, good association between CMT scores and *S. aureus* intramammary infection (IMI) was observed. This agrees with other findings, which indicated that IMI is the most important variable affecting SCC (Radostits *et al.*, 2000). A threshold of 500,000 cells ml^{-1} has been accepted as an indication of IMI (IDF, 1979; EU, 1992). This upper limit was set considering that SCC is indicative for herd mastitis control, hygienic milk production conditions and suitability for use in manufacturing. However, there is no general agreement about what can be considered as a normal SCC for an infected quarter or cow (Harmon, 2001). Likewise, there is a lack of comprehensive studies that can set up criteria to define what is a normal SCC for low yielding dairy cows, taking into account differences in breed, geographical area, husbandry and management conditions and type of milking.

Sensitivity and specificity are intrinsic values of a test and are useful for comparing one diagnostic test with another. However, they cannot be used to determine the probability that a given test result reflects the true disease status of the animal (Greiner and Gardner, 2000). Sensitivity and specificity can thus, be calculated only if there is an objective, independent means of determining whether the disease is truly present. Likelihood ratios, however, when combined with prior probability of the

disease, can be used to predict the likelihood that an animal has the disease when a positive test result is obtained or the probability that an animal does not have the disease when a negative test is obtained (Smith, 1995; Dohoo *et al*., 2003). The likelihood ratio of 5.65 (Table I) indicates that positive results for quarters in herds with prior probability of *S. aureus* IMI of 0.415 would mean at least a 0.80 posterior probability of having a *S. aureus* IMI. This improved to 0.89, when three consecutive samples were considered. Likelihood ratios for CMT score of ≤ +1 were only 0.06 and 0.19 for single and three consecutive samples, indeterminant figures indicating that results of the test did not provide any information on *S. aureus* IMI status that would change the pre-test probability of disease. Although a small number of cows may be misclassified based on the results of a single culture (the proportion of *S. aureus* IMI detected by single culture did not differ significantly (Z = 0.86; P = 0.39) from the proportion detected by three consecutive samples), one must consider the effects of this against the cost of performing multiple microbiological cultures. In this evaluation, there was a "substantial (kappa > 0.60) agreement between the CMT scores and *S. aureus* culture results for single (kappa = 0.62) and three (kappa = 0.66) consecutive samples. Thus, *S. aureus* subclinical IMI in low yielding dairy cows can reasonably be detected by combining CMT scores of ≥ +2 and culture results.

The fact that, in the present study, 94.6 % (CI_{95}: 0.93-0.96) of the samples in which *S. aureus* were isolated had CMT scores > +1, permits one to consider these values as indicators for the presence of *S. aureus* IMI, in low yielding dairy cows. Accordingly, it can be said that CMT score of +1 corresponding to 500,000 somatic cells mls^{-1} is less than one-tenth as likely to come from infected quarters as from non-infected quarters. Conversely, it can be predicted that SCC higher than 800,000 cells ml^{-1} or a CMT score of ≥ +2 is at least five times as likely to come from infected quarters as from uninfected quarters. The likelihood was almost doubled when three consecutive samples were taken into consideration (Table II). These results increase the value of the CMT as a screening tool for subclinical mastitis in low-yielding quarters at herd level.

High proportions of false positives were found in the present survey. This suggests that CMT and microbiological screening, although regarded as the most reliable indicators of on-going IMI, may fail to diagnose subclinical IMI. However, this may be desirable, since failure to

detect the disease screened for, vitiates the principle objective (early detection of asymptomatic disease) of a screening test. On the other hand, positive scores may not be accompanied by the isolation of the aetiological agent because: (1) Microorganisms may be discharged in an intermittent way and in small amounts (Sears *et al*., 1990), (2) IMI-related pathogens are not detectable using the conventional microbiological tests, (3) SCC is a non-specific measure of inflammation, and an elevated SCC can be associated with IMI caused by any one of many organisms. Therefore, determining SCC alone is not reliable for screening *S. aureus* IMI, (4) Intracellular bacteria that were unavailable for isolation and antibiotic treatment prior to sample collection, (5) Another problem with using the results of microbiological culture of a single sample as a gold standard for screening IMI is the need to decide whether a positive culture result truly represents an established IMI, is a transient event, or is the result of contamination, (6) However what is more likely is that the false positives reflect the inherent physiological high SCC in milk of the low-yielding cows. Conversely, the isolation of the mastitis related pathogens might not be accompanied by an increase in SCC, because in many cases the infection process either has a silent debut and progresses slowly without expressing an acute stage or has an acute phase of very short duration (Dopfer *et al*., 1999).

Results of our study indicate that CMT is useful for screening *S. aureus* IMI in low yielding smallholder dairy cows. However, when working with a herd that has *S. aureus* IMI, a practitioner should consider several factors in choosing the diagnostic cut-off to use. First, herd management goals must be evaluated. If eradication of *S. aureus* IMI from the herd is the goal, then re-testing of potentially false positives (microbiological culture of consecutive samples) is the most effective option. If the management goal is to control *S. aureus* IMI within the herd, and procedures to maintain good milking management and hygienic conditions are in effect or are being implemented, a less rigorous testing strategy should be effective. Therefore, the proportion of false-negative CMT results should have little impact on the control programme. False-positive test results are more of a concern, particularly if costly management decisions, such as lactational therapy or culling are made based on these CMT-results.

Decision to use a given cut-off must also include an evaluation of the amount and kind of information gained from test results. The SCC is a non-specific measure of inflammation, and determining SCC provides little information regarding the cause of high counts. Likewise,

microbiological culture is a relatively non-specific diagnostic test that will provide information on a wide variety of pathogens that can cause IMI. Based on results of this study and practical considerations, it appears that CMT score of ≥ +2 is the best cut-off to reliably identify cows with *S. aureus* IMI in low-yielding smallholder dairy cows in Tanzania.

References

Bradley, A. J., 2002. Bovine mastitis: An evolving disease. The Veterinary Journal, **164**: 116-128

Dohoo I., Martin W., Stryhn H, 2003. Veterinary epidemiologic research. AVC Inc, Charlottetown, Prince Edward Isaland, Canada. pp. 107-109

Dopfer, D., Barkema, H. W., Lam, T. G. M., Schukken, Y. H., Gaastra, W., 1999. Recurrent clinical mastitis caused by Escherichia coli in dry cows. Journal of Dairy Science, **82**: 80- 85.

EU, 1992: European Union Directive, 92/46. Official Journal of the European Communities, **L268**

Greiner M., Gardner I. A., 2000. Epidemiologic issues in the validation of veterinary diagnostic tests. Preventive Veterinary Medicine. **45** (**2**): 3-22

Harmon, R. J., 2001: Somatic cell counts: a primer. 40th Annual Meeting Proceedings, pp 3-9. National Mastitis Council Inc., Madison, WI.

Hogan. S. J., Gonzalez R. N., Harmon, J. R., Nickerson, S.C., Oliver, S. P., Pankey,.J. W. and Smith, L. K. 1999. Laboratory Handbook on Bovine Mastitis. Published by National Mastitis Council, Inc., W D Hoard, Fort Atkinson, USA.

International Dairy Federation 1981. Bulletin No. 132. Laboratory Methods for Use in Mastitis Work. IDF, Brussels, Belgium.

International Dairy Federation, 1979. Bulletin No. 114. Somatic cells in milk, their significance and recommended methods for counting. IDF, Brussels, Belgium.

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M., 2004. Risk Indicators Associated with Subclinical Mastitis in Smallholder Dairy Cows in Tanzania. Tropical Animal Health and Production. 36: 581- 592

Omore. A.O., J.J. McDermott., S.M. Arimi., M.N. kyule., and D. Ouma., 1996. A longitudinal study of milk somatic cell counts and bacterial

culture from cows on smallholder dairy farms in Kiambu district, Kenya. Preventive Veterinary Medicine, **29:** 77-89.

Poutrel, B. and Rainard, P., 1982. Predicting the probability of quarter infection (by major pathogens) from somatic cell concentration. American Journal of Veterinary Research, **43:** 1296

Quinn, P.J., Carter, M. E., Markey, B. K. and Carter, G.R. 2000. Clinical veterinary microbiology. London, Mosby-year book Europe limited, pp. 120-121

Radostits. O.M., Gay. C. C., Blood.D.C. and Hinchcliff. K. W. 2000. Radostitis.O.M. 1989. Veterinary Medicine; A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 97th Edition. W. B. Saunders. London. pp. 603-660

Sears, P. M., Smith, B. S., English, P. B., 1990. Shedding patterns of *Staphylococcus aureus* from bovine intramammary infections. Journal of Dairy Science, **73**: 2785- 2789.

Smith, R. D., 1995. Veterinary epidemiology: A problem oriented approach. 2nd ed. CRC Press. London. pp 31 – 69.

Snedecor G. W. & Cochran, G. W., 1989. Statistical Methods, 8th Edition. Iowa: Iowa state university press/AMES

8

Chapter

Evaluation of the Hygienic Quality and Associated Public Health Hazards of Raw Milk Marketed by Smallholder Dairy Producers in the Dar es Salaam Region, Tanzania

F. M. Kivaria[1]*, J. P. T. M. Noordhuizen[2]., A. M. Kapaga[1].

[1]Population Studies, Animal Diseases Research Institute, P.O.Box 9254, Dar es Salaam, Tanzania

[2]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

Tropical Animal Health and Production 38 (2006): 185-194
doi: 10.1007/$_s$ 11250-006-4339-y

Abstract

A cross-sectional study was conducted to determine three parameters of the quality of the raw milk marketed by milk selling points (MSPs) in the Dar es Salaam region. Total bacterial count (TBC) was used as an indicator of the microbial quality of the milk, antimicrobial residues were determined, and the California mastitis test (CMT) was used to screen for milk somatic cells as an indication of the mastitis level in the cows that provided the milk. Moreover, a water sample at each MSP was taken for bacteriological culturing. Finally, a questionnaire survey was conducted with the milk sellers at the MSPs to identify risk factors for poor milk hygiene. A total of 128 milk samples and corresponding water samples were collected from randomly selected milk selling points in the Dar es Salaam region. The mean TBC was 8.2×10^6 cfu $ml^{-1} \pm 1.9 \times 10^6$ cfu ml^{-1}, and major bacterial isolates from the milk samples were *Escherichia coli* (6.3%), *Bacillus cereus* (6.3%), *Staphylococcus aureus* (6.3%) and *Streptococcus agalactiae* (6.3%), *Enterobacter aerogenes* (5.6%) and *Enterococcus faecalis* (4.7%). In most cases, the organisms identified in milk corresponded to those isolated from the corresponding water samples. 79.0 % of milk samples were positive to the CMT and 7.0% were positive for antimicrobial residues. TBC was normalised by log transformation, and the possible predictors of TBC were identified by fitting two linear regression models. In a random effect model, water microbial quality, frequency of cleaning the milk containers, frequency of milk supply, milk storage time and the type of containers, and mixing of fresh and previous milk were significantly ($P < 0.05$) associated with the mean LogTBC. Whereas, in the fixed effect model, in addition to these indicators, water shortage, water source and the refrigerator condition were significantly ($P \leq 0.01$) associated with LogTBC. It was concluded that the milk sold in the Dar es Salaam region is of poor quality and is of public health significance.

Keywords: Antimicrobial residues; CMT; Microbiological quality; Public health hazards; TBC

Abbreviations: CMT, California mastitis test; MSP, milk selling point; TBC, total bacteria count

Introduction

Being a nutritional, balanced foodstuff, milk is a well-known medium that favours the growth of several microorganisms. Milk from a sub/clinically mastitic cow commonly contains the etiological agents, while milk from non-mastitic cows is known to be often contaminated from extraneous dirt or unclean processing water. The health hazards posed by milk-borne zoonotic diseases such as brucellosis, tuberculosis and mastitis-related enterotoxaemia are well-documented (Charles *et al*., 1999; Weinhaupl *et al*., 2000; Shirima *et al*., 2003). Unpublished data at the Animal Diseases Research Institute indicate the prevalence of both bovine brucellosis and tuberculosis to be ≤ 1.0%. However, through consultations with officials of the Muhimbili hospital, a referral hospital in the study area, it was established that 20.0% of 524 human tuberculosis cases reported during 1999 were caused by *Mycobacterium bovis*, the prevalence of human brucellosis for the same year was estimated to ≤ 1.0%.

Smallholder dairy producers supply about 90% of the milk consumed in the Dar es Salaam region. A remarkable feature of this sector is the fact that 74% of all milk is marketed as raw milk through informal channels (Kivaria *et al*., "in press"). Mullins (1993) surveyed 79 households in the Dar es Salaam region: 51% reported predominantly consuming raw milk, while from a survey of 120 households, in Dar es Salaam, Kurwijila *et al*., (1995) estimated that 80 % predominantly consumed raw milk. This practice is of great concern, particularly with regard to the risk for both contracting zoonoses, and other food-borne diseases. Risks of milk-borne zoonoses posed by the informal market are amplified by poor handling procedures on the market, the lack of quality standards and the fact that most consumers prefer raw milk. This situation is further compounded by the fact that many smallholder farmers blindly treat their mastitic cows with little or no consideration of antimicrobial withdrawal periods (Kivaria *et al*., "in press").

Hazard Analysis and Critical Control Points (HACCP) and good farming practice codes have been recognized throughout the world (Noordhuizen and Frankena, 1999), and are becoming increasingly relevant in developing countries as a sometimes compulsory tool to ensure food safety (Ropkins and Beck, 2000). However, the concept is relatively new in the Tanzanian food industry. In Tanzania, information is presently unavailable on the quality of milk to which the consumers are exposed. It therefore becomes necessary to assess the quality of milk for wholesomeness and investigate solutions in order to rectify these

potential problem areas by identifying factors that influence milk quality. The objective of the present study was to determine the hygienic quality and possible sources of contamination associated with milk in randomly selected areas of the Dar es Salaam region.

Materials and methods

Study design and collection of samples

Since we could not obtain the official list of all the MSP from the Ilala, Kinondoni and Temeke districts of the Dar es Salaam region. We initially drove around the city taking inventory of the MSP and their locations/streets. This exercise yielded 90 MSP from 25 streets in the Ilala district, 118 MSP from 31 streets in the Kinondoni district, and 92 MSP from 13 streets in the Temeke district. Since the MSP tend to be clustered in a given street, depending on the population that is passing along that street, streets were first selected followed by the MSP. Simple random sampling was used to select the streets/areas to be sampled. Systematic random sampling (the first MSP was selected at random, and then every second MSP was picked as we drove along a given street/area) was employed in the selection of the MSP along the streets. 128 MSP out of a population of 300 MSP were involved in the study; 20 mL of milk in duplicate was collected from each MSP. Milk samples were aseptically collected by a sterile syringe from each milk container into sterile universal containers. In addition, using separate and a sterile syringe, 10 mL of water was also collected from the respective MSP for microbial assessment. These samples were then ice cooled to restrict microbial multiplication and transported to the laboratory within two hours of collection. The potential power of this study could not be estimated before-hand, since little information concerning milk quality, and no information on the risk of drug residues exists. The sample size of 128 milk samples was therefore based on logistical considerations.

Conducting of a questionnaire

We prepared a comprehensive questionnaire inquiring about the source of milk, the frequency of milk supply, milk storage, type of milk storage containers, the frequency of the cleaning of the storage containers, the method of milk treatment, and water source, shortage[16] and storage, and whether the refrigerator[17] is working or not. We also enquired on the use

[16] MSP receiving water supply of less than 3 days per week was categorised as experiencing water shortage

[17] This point addresses the question whether the refrigerator was on or off (power failures or defective) at the time of visit

of soap or detergent for washing of the utensils, and whether boiled water is used for rinsing the utensils. The questionnaire was conducted by interviewing the person selling the milk by the person collecting the samples. Water was an issue of interest because of the hypothesised contamination of milk using water at the MSPs. The study was conducted over two months, from August to October 2003, and included the MSPs under study.

Screening of milk for somatic cells

The California Mastitis Test (CMT) was used to screen milk for somatic cells as an indication of mastitis. The CMT was evaluated as described by Hogan *et al*., (1999). Based on the thickness of the gel formed by the CMT reagent-milk mixture, test results were scored as 0 (negative / trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive). In this study, milk samples with test results of negative / trace and weak positive (+1) were combined and reclassified as having originated from cows free of subclinical mastitis while CMT results of distinct positive (+2) and strong positive (+3) were as having subclinical mastitis.

Isolation, identification and enumeration of microorganisms

Milk contamination level was determined by the total bacterial count (TBC) method for each sample of milk. Serial ten-fold dilutions (10^{-1} to 10^{-8}) of the milk sample were made using sterile Ringer's solution. An inoculum of 1 mL of each dilution was mixed thoroughly with melted plate count agar (Merck, Darmstadt, Germany); two plates were inoculated with each dilution. The agar was allowed to set and then incubated at 37^{o}C for 24 hrs. Plates that yielded between 30 and 300 colonies per plate were read. Colonies were counted visually using a mechanical counter and the final figure was the average of the two plates inoculated with the selected dilution (the 10^{-5} dilution was found to be most suitable for counting). This figure was multiplied by the dilution factor to get the TBC. A standard plate count of $\leq 1 \times 10^{5}$ colony-forming units (cfu) per mL has been globally adopted for good quality raw milk (Cousins and Bramley 1985). Therefore, a final TBC of less than 100,000 cfu ml^{-1}, was considered good; milk with a TBC greater than 100,000 cfu mL^{-1} indicated gross contamination.

Microbiological culturing and identification of the microorganisms to species level was performed according to standard procedures described by Hogan *et al*., (1999). Dilutions of milk prepared for the TBC were initially plated on blood agar plates, in addition, 0.1 ml of water samples were plated. (microbiological procedures that were used for culturing and enumeration of isolates from the milk samples were also applied to water samples.) Up to five different colonies representing the most predominant types, were randomly picked from the countable plates. After Gram staining, identification of the individual pathogens involved sub-culturing of the presumptive colonies into the respective selective agar media. All inoculated plates were incubated at 37^{o}C for 24 hrs. When slow growing or unusual bacteria were suspected, longer incubation periods and/or an environment of 10 % Co_2 were used (Quinn *et al*., 2000). To enumerate the spores of spore-forming organisms, the samples were exposed to 80^{o}C for 10 minutes to destroy vegetative cells and transferred to the plate count agar plates. Definitive identification of the bacterial isolates to the species level was based on key differential biochemical tests described by Hogan *et al*., (1999), and Quinn *et al*., (2000); all colonies were counted and expressed as cfu of a given pathogen per ml of milk. Sabouraud dextrose agar (Oxoid, CM 41) was used for the detection and enumeration of yeasts and moulds. If three or more microbial species were obtained from a sub-culture, the sample was considered to be contaminated.

Detection of antibacterial residues in milk

A modification of the sensitive organism procedure for the detection of antibiotics and other inhibitory substance in raw milk was used as described by the International Dairy Federation (IDF, 1991). 7.5 ml of fresh yogurt and 0.3 ml of 0.5 % methylen blue were added to 50 mL of fresh UHT-milk. The mixture was incubated at 40^{o}C for 12 hrs. 10 mL of the milk sample were incubated at 85^{o}C for 5 min, and 2 mL of yogurt-methylen mixture was then added to the incubated milk sample, the mixture was then incubated at 43^{o}C for 100 min. The test organism *Streptococcus thermophilus* is sensitive to inhibitory substances such as penicillin, and will detect penicillin at a level of 0.02 IU mL^{-1} (IDF, 1991). Blue colour indicated a positive milk sample containing more than 0.03 IU mL^{-1} of the inhibitory substance, while white colour indicated a negative milk sample. A positive control was made by serial dilution of

a sterile Penicillin G, 2.4 X 10^6 I.U. (Flamingo pharmaceuticals Ltd, India), to give a final solution of 0.03 IU mL^{-1}. Imported UHT-milk was used as a negative control. As a confirmatory procedure, positive milk samples were further subjected to Delvotest® SP (DSM, The Netherlands)[18].

Data analysis

Initially, descriptive statistics, frequency distribution, histograms and scatter plots procedures in SPSS version 11.5.0 (SPSS Inc., 2002) were used to explore the data set for errors and outliers; queries were cross checked with the raw data. Four observations were incorrectly recorded, and these were corrected. The procedures were also used to summarise the variables and confidence intervals (CI_{95}) of proportions were calculated. Preliminary investigation of the data indicated that the dependent variable (TBC) was not normally distributed (Shapiro-Wilks $W = 0.368$, $P(W) < 0.001$), this variable was normalised by a base-10 log transformation (Log). Normality was investigated by visual inspection of the histograms and the Kolmogorov – Smirnov test statistic. Next, bivariate analysis for screening of independent variables was done in linear regression model. Finally, all independent variables with $P \leq 0.25$ were submitted to a multivariate linear regression model as described by (Kleinbaum *et al.*, 1998). Clustering of observations was expected to occur at the street level, multivariate mixed regression models were therefore used to correct for the possible clustering of MSP within streets. Variance components and compound symmetry covariance structures were considered and the variance with structure with the lowest Akaike's information criteria was included in the model when significant. The fit of the model was based on the explained variance (represented by the adjusted R^2) and checked by plotting the standardised predicted values against the standardised residuals. In the final analyses, the type I error was set at the two-tailed $P = 0.05$.

[18] Delvotest is the DSM Food Specialties microbial inhibition assay for the screening of antibacterial substances in milk and milk products. This broad spectrum test has become the gold standard around the world. Delvotest® has been developed for a variety of applications ranging from a single test for testing the milk of an individual cow at the farmhouse to large scale routine analysis in milk quality payment and milk control laboratories. For more information see http://www.dsm.com/en_US/html/dfs/dairy-products-tests-delvotest.htm

Results

Descriptive statistics and CMT results

128 milk selling points were investigated. About 2828 litres of milk are collected and sold daily by the 128 MSPs (a single MSP may receive milk supplies from up to five smallholder producers). It was observed that MSPs on average receive milk every other day, whereas the milk containers were cleaned after every two days, and milk could remain stored for up to seven days. Other descriptive results are summarised in Table I. 78.9% (CI_{95}: 0.72-0.86) of the 128 milk samples screened were

Table I. Descriptive statistics of the milk quality parameters of the 128 milk selling points investigated in the Dar es Salaam region, Tanzania.

			Total bacteria count (cfu/ml)				
Variable	N	%	Minimum	Mean	S.D.	Median	Maximum
Milk source							
SHP[1]	110	86	0.00	$8.08X10^6$	$22.88X10^6$	$1.48X10^6$	$20.80X10^6$
MCC[2]	18	14	0.00	$9.19X10^6$	$15.70X10^6$	$0.98X10^6$	$61.00X10^6$
Milk storage containers							
Plastic	123	96	0.00	$8.28X10^6$	$22.31X10^6$	$1.45X10^6$	$208.00X10^6$
Metal	5	4	$0.12X10^6$	$7.19X10^6$	$11.67X10^6$	$1.42X10^6$	$27.40X10^6$
Plastic type							
Jerry can	86	70	0.00	$8.51X10^6$	$25.06X10^6$	$1.41X10^6$	$208.00X10^6$
Bucket	37	30	0.00	$7.77X10^6$	$15.74X10^6$	$1.47X10^6$	$100.00X10^6$
Water shortage							
Yes	83	65	0.00	$9.25X10^6$	$24.43X10^6$	$2.20X10^6$	$208.00X10^6$
No	45	35	0.00	$6.38X10^6$	$16.59X10^6$	$0.98X10^6$	$100.00X10^6$
Water source							
Tap	37	29	0.00	$3.40X10^6$	$6.72X10^6$	$0.53X10^6$	$27.40X10^6$
Vendors	83	65	0.00	$9.25X10^6$	$24.40X10^6$	$2.20X10^6$	$208.00X10^6$
Bore-well	8	6	$0.16X10^6$	$20.14X10^6$	$35.13X10^6$	$1.90X10^6$	$100.00X10^6$
CMT scores							
0	17	13	0.00	$8.48X10^6$	$9.74X10^6$	$2.76X10^6$	$28.10X10^6$
+	10	8	0.00	$18.94X10^6$	$19.37X10^6$	$13.50X10^6$	$61.00X10^6$
++	43	34	0.00	$6.47X10^6$	$16.42X10^6$	$1.45X10^6$	$100.00X10^6$
+++	58	45	0.00	$7.63X10^6$	$27.71X10^6$	$0.58X10^6$	$208.00X10^6$
Use of boiled water							
Yes	12	9	$0.26X10^6$	$5.64X10^6$	$10.77X10^6$	$1.44X10^6$	$29.80X10^6$
No	116	91	0.00	$8.51X10^6$	$22.82X10^6$	$1.44X10^6$	$208.00X10^6$
Cleaning time							
immediately	34	27	0.00	$6.33X10^6$	$9.30X10^6$	$1.44X10^6$	$29.80X10^6$
before use	94	73	0.00	$8.93X10^6$	$25.00X10^6$	$1.44X10^6$	$208.00X10^6$
Street activities							
High	24	19	0.00	$18.92X10^6$	$45.50X10^6$	$2.38X10^6$	$208.00X10^6$
Low	104	81	0.00	$5.77X10^6$	$10.00X10^6$	$1.04X10^6$	$61.00X10^6$
Refrigerator							
functioning	78	61	0.00	$10.64X10^6$	$27.30X10^6$	$0.99X10^6$	$208X10^6$
defective	50	39	0.00	$4048X10^6$	$7.44X10^6$	$1.64X10^6$	$29.8X10^6$
Mixing of milk							
Yes	106	83	$0.01X10^6$	$9.66X10^6$	$23.88X10^6$	$1.73X10^6$	$208X10^6$
No	22	17	0.00	$1.40X10^6$	$2.83X10^6$	$0.43X10^6$	$13.30X10^6$
Overall	128	100	0.00	$8.24X10^6$	$21.97X10^6$	$1.44X10^6$	$208.00X10^6$

1: SHP – small-holder producers
2: MCC – milk collection centers

CMT-positive, with reactions of +2 or higher; 21.1% (CI_{95}: 0.14-0.28) of the 128 samples showed negative CMT reaction, with reactions of 0 (17), and +1 (10). CMT results of +2 and +3, were predominant, accounting for 33.6% (CI_{95}: 0.25-0.42) and 45.3% (CI_{95}: 0.37-0.54) of the screened samples.

Antibacterial substance in milk, microbiological quality of milk and factors influencing total bacterial count

Nine (7.0%, CI_{95}: 0.03-0.11) out of 128 milk samples tested positive for the presence of antibacterial residues. Different types of microorganisms were isolated in 121 (94.5 %, CI_{95}: 0.91-0.98) out of the 128 milk samples. The microbial isolates are summarised in Table II. Both the Kolmogorov-Smirnov statistic ($Z = 1.361$, $P = 0.173$) and the histogram indicated that the LogTBC was normally distributed. Thus logTBC was used in the linear regression. The final linear regression model with and without random street effect for LogTBC is presented in Table III. When the null model for LogTBC was investigated, the random effect for street with a variance components covariance structure had a strong effect (= 0.52, $P < 0.001$), indicating that a larger proportion of the variability in TBC occurred at the street level. The residual plots showed a random distribution of the standardised residuals around zero.

Table II. Microbial isolates from 128 milk samples and corresponding water samples from milk selling points in the Dar es Salaam region, Tanzania

	Milk samples			Water samples[19]	
Isolates	Number of samples	Percent isolation[20]	Mean cfu/ml ($X10^6$)[21]	Number of samples	Percent isolation
Microbial contaminants[22]	72	56.3	8.65 ± 1.6	18	14.0
Escherichia coli	8	6.3	4.73 ± 3.6	30	23.4
Staphylococcus aureus	8	6.3	6.10 ± 1.4	0	-
Streptococcus agalactiae	8	6.3	10.27 ± 2.5	0	-
Bacillus cereus	8	6.3	11.17 ± 3.0	2	1.6
Enterobacter aerogenes	7	5.5	9.00 ± 1.5	8	6.3
Enterococcus faecalis	6	4.7	2.77 ± 2.4	17	13.3
Actinomyces spp	2	1.6	0.18 ± 1.2	0	-
Serratia marcescens	1	0.8	2.56 ± 0.0	0	-
Yeast spp	1	0.8	0.90 ± 0.0	1	0.8
Citrobacter diversus	0	-	-	5	3.9
Negative	7	5.5	-	47	36.7

[19] Total bacterial counts were not determined for water samples
[20] Percent isolation computed as (number of samples with a given isolate/the total number of samples)*100
[21] Mean cfu ml^{-1} ± standard error of the mean
[22] More than two microbial species were isolated from a given sample

Discussion

It was of clinical significance to note that 79.0% of all milk samples tested were CMT-positive, and therefore considered to have come from sub/clinically mastitic cows. This finding strongly suggests that a considerable proportion of the dairy cows supplying milk to the MSP may be mastitic. It must, however, be realised that since pooled milk samples were studied, the findings do not directly reflect the status of individual cows or herds. Kivaria *et al.,* (2004) had earlier found that 90.0% of 182 lactating cows in the same study area were CMT-positive, while 4.0 % of the studied cows had clinical mastitis.

Table III Random and fixed effects linear regression models for factors that influenced $\log_{10}$ of the total bacteria count among the 128 milk selling points investigated in the Dar es Salaam region, Tanzania

	Random effect model			Fixed effect model		
Variable	ß	$SE_ß$	*P*	ß	$SE_ß$	*P*
Constant	9.322	0.152	< 0.001			
Water shortage	-	-	-	0.461	0.110	< 0.001
Water source	-	-	-	0.324	0.124	0.010
Water microbial quality	0.366	0.131	0.006	0.832	0.212	< 0.001
Refrigerator	-	-	-	0.211	0.011	< 0.001
Cleaning frequency of milk container	0.687	0.273	0.013	0.731	0.321	0.024
Milk supply frequency	0.411	0.110	< 0.001	0.624	0.273	0.024
Milk storage time	0.983	0.221	< 0.001	0.861	0.341	0.013
Milk storage containments	0.961	0.136	< 0.001	0.733	0.282	0.010
Mixing fresh with previous milk	0.822	0.222	< 0.001	0.625	0.143	< 0.001
		$R^2 = 0.61$			$R^2 = 0.43$	

The public health concerns of bovine mastitis relate to the occurrence of both different mastitis pathogens and antibiotic residues in milk for human consumption. *Staphylococcus aureus* is probably the most important species in this respect. In case of consumption of raw milk there can be a real hazard, the main threat being the fact that about 10.0 % of mastitis staphylococci are known to be producers of enterotoxins (Heeschen *et al*., 1985). This toxin may be produced when *S. aureus* counts exceed 10^5-cfu mL^{-1} (Whiting *et al*., 1996). Therefore, in view of the fact that 6.10 X 10^6 of *S. aureus* counts mL^{-1} were observed in 6.0 % of the milk samples, coupled with the heat stability of staphylococcal enterotoxins, this must be regarded as a public health hazard.

Within the bovine mastitis *Escherichia coli* species, a specific verocytotoxigenic strain may cause haemorrhagic colitis (John *et al*., 2001; Leclerc *et al*., 2002), the most important one is the enterohemorrhagic type *E. coli* 0157: H7 (Leclerc *et al*., 2002).

Escherichia coli 0157: H7 is an emerging cause of food-borne illness and is now considered as an important human pathogen (Pennington, 1997). Transmission of the coliform organisms potentially including *E. coli* 0157: H7 occurs through the ingestion of raw milk (Quinn *et al*., 2000). The mean *E. coli* count of 4.73 X 10^6 mL^{-1}, observed in this study is therefore a source of concern since in the presence of a verocytotoxigenic *E. coli* enough toxins may be produced to cause illness to consumers. It is worth mentioning that heating destroys verocytotoxins (Quinn *et al*., 2000), although the possibility of failures during heating cannot be ruled out. The fact that a majority of the Dar es Salaam population consumes raw milk will increase the risk of milk-borne *E. coli* poisoning.

Bacillus cereus produces two distinct forms of food poisoning, the diarrhoeal syndrome caused by heat stable enterotoxin and emetic syndrome involving a very heat-stable enterotoxin (Quinn *et al*., 2000), counts of *B. cereus* in the range of 10^5 to 10^9 $mL^{-1}$are common in contaminated milk (Quinn *et al*., 2000). The fact that 6.3% of milk samples from MSPs had a mean count as high as 1.12 X 10^7 mL^{-1} is of public health significance. As regular inhabitants of the intestine, enterococci may serve as indicators for faecal or soil contamination and implies a risk that other enteric pathogens may be present in the milk. Enterococci are therefore of particular importance in food and public health microbiology. *Enterococcus faecalis* has been suspected as a causative agent of food-borne illness (Stiles, 1989). *Enterococcus faecalis* can cause outbreaks of gastroenteritis, the infective dose has been estimated at 10^9-10^{10} organisms (Charles *et al*., 1999), and raw milk has been incriminated. 5.5% and 36.7% of the milk and water samples investigated were 'negative' (Table II) that is they had no microbial growth. This can be attributed to the presence of antimicrobial agents in milk, and boiling of the milk/water before storage, or the addition of 0.75% sodium hypochlorite to the cleaning water.

Water source, water shortage and water microbiological quality were significantly ($P < 0.05$) associated with milk TBC (Table III). Dar es Salaam region is currently experiencing water shortages due to a number of reasons. Moreover, it has been observed that water contamination often occurred in the storage containers used (Kivaria *et al*., 2004); therefore, most MSPs had no potable water. The presence of *E. coli* and other enteric bacteria in water samples (Table II), strongly suggests faecal contamination of the water at many of the MSPs. If such water gains access to milk or is used for rinsing the equipment and containers, microorganisms present in the water will contaminate the milk. Table I

supports this hypothesis, the table shows that milk quality is affected by extraneous factors; especially those associated with water quality.

The use of soap and good quality water for cleaning the equipment could be expected to remove milk remains, including microorganisms, thereby affecting the microbial quality of the milk. In this study, observations showed that all the MSPs were using soap for cleaning. It was however evident that it was very difficulty to properly clean the narrow mouthed jerry cans. Indeed, a critical observation of the jerry cans revealed that most of them had milky-residues at the corners. It was further indicated that only 9.0% of the MSPs (Table I) use boiled water to rinse the containers. In addition, it was revealed that 73.0% (TableI) of these containers are cleaned prior to use (not immediately after use). In such a situation, microorganisms can rapidly build up in milky-residues in milk storage containers, and if cans are still moist the microbes multiply in milk quite rapidly when lids are put on. Most of the plastic containers used in the MSPs were scratched, which could hinder satisfactory cleaning. Furthermore, it has been found that the spores of *B. cereus* adhere to surfaces better than vegetative cells (Peng *et al*., 2001). The jerry cans can thus be a source of *B. cereus* endospores and other types of thermophilic bacteria in milk. It is therefore not surprising that the milk storage containers played a significant role in the contamination of milk.

The linear regression results (Table III) strongly suggest that milk-handling practices at the MSPs significantly influenced the microbial quality of marketed milk. It is pertinent to mention that 39.0% of the MSPs visited had either defective refrigerator and/or had experienced power failures. It was also observed that the fresh milk supply was mixed with the previous milk. It was of great concern to note that 19.0% of the visited MSPs were located adjacent to a garage, shops and other non-dairy activities. Thus, the general hygiene practices and handling practices resulted in contamination of the milk, Table I supports this hypothesis. The possibility of microbial multiplication in milk between milkings at the farms (given high environmental temperatures), and transportation to the MSPs, coupled with the fact that milk is transported using public transport and bicycles, cannot be ignored.

The presence of antibiotic residues in milk is of public and economic concern, because of the risk of impaired health in persons who consume milk from treated cows (IDF, 1996) and the interference of manufacturing of dairy products by the antibiotics present in milk (IDF, 1996). The detection of antibiotic residues in 7.0% of the market milk samples reflects the abusive use of the antimicrobials by the smallholder

producers. When administered in accordance with the approved labelling, the prevalence of violative drug residues in milk would be less than 1.0% (Sundlof, 1994). Residue violation greater than 1.0% generally indicates that the antimicrobial has been used in some manner that was inconsistent with the labelling. The consumption of sub-therapeutic levels of antimicrobials in the milk can result in the development of antimicrobial-resistant enteric bacteria (Aarestrup, 1999), which may cause disease that is consequently difficult to treat (Mateu and Martin 2001). Furthermore, enteric bacteria (e.g. *E. coli*) are an important reservoir of resistance genes (Mateu and Martin 2001) and the most important likely source of contamination of milk (Table II). It is therefore logical to assume that they are also the most important link between animal and human antimicrobial resistance. Our results indicate that milk contains high levels of bacteria and antimicrobial contaminants and has the potential to be hazardous to the Dar es Salaam population. A majority of the bacteria identified and listed in Table II are potential pathogens (Warburton, 1993; Warburton and Austin, 1997) that can cause severe problems in susceptible populations, particularly the young, elderly, pregnant women, and immunocompromised, who may consume such products to improve their health. High counts of enteric organisms reflect the probable source of the contamination via water or soil, where faecal-oral transmission appears to be of great epidemiological significance. Personal hygiene therefore constitutes a significant preventive measure alongside the use of potable water. Thorough cleaning and disinfection of milk containers is essential after and even before each use. Other essential basic hygiene measures that deserve proper attention are; sufficient separation of "milky-activities" to avoid cross-contamination, pest control as well as temperatures of treatment and storage sites. Those who are familiar with the prevailing conditions in the MSPs are aware of the fact that in Tanzanian practice this requirement is far from being fulfilled. Since compliance with these basic hygienic requirements is not guaranteed it does not make sense to initiate the planning of HACCP concepts, but rather that the HACCP concept should be seen as part of an efficient total hygiene concept in MSPs. This reiterates the need for the adoption of "good farming practices", which ultimately may end up as a foundational practices allowing implementation of on-farm HACCP programmes. To this end, the study team recommends the following suggestions for improvement:

1) Each MSP should ensure that they have enough potable water. The microbial quality of water at the MSPs could be improved by boiling the water or addition of chlorine. (2) In order to reduce contamination of

the milk, utensils used for milking should be rinsed, cleaned using detergent and disinfected immediately after use. Boiled water can be used for rinsing the utensils after washing with detergents and thereafter dried on clean cobweb-free rails. (3) Wide mouthed aluminium containers should be used for milk storage, as these are easy to clean and to maintain. (4) Frequent education of MSP personnel on the various aspects of milk hygiene and handling techniques will have a positive impact on the quality standards of milk sold by the MSP. This could be achieved by teaching and training programmes, using the participatory approach method. Smallholder producers should be educated on the importance of proper antimicrobial use in dairy cows. (5) The general hygiene at milking is known to affect the number of micro-organisms in the milk (IDF, 1990). Therefore, measures to improve the hygiene of the production should be instituted at the farm level. (6) Random samples should be taken at regular intervals from individuals MSPs and analysed in a well-equipped quality control laboratory. To provide better safeguards to the consumer, consumers should be informed about the hazards associated with current raw milk selling practice. (7) Smallholder farmers are not paid according to the quality of their milk. However, the study team is of the opinion that the introduction of a grading system based on the microbiological quality of the milk would be an incentive to improve the quality of the milk delivered to the MSPs and the public at large.

References

Aarestrup, F. M., 1999. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. International Journal of Antimicrobial Agents. **12**: 279-285.

Charles M.A.P. Franz , Wilhelm H. Holzapfel , Michael E. Stiles., 1999. Enterococci at the crossroads of food safety? International Journal of Food Microbiology. **47**: 1-24.

Cousins, C. M., and Bramley, J. A., 1985. The microbiology of raw milk. In dairy microbiology, volume 1, Robinson, R. K., (Ed). Elsevier Applied Science Publication, London, p 119-163

Heeschen, W., G. Suhren., G. Hahn., 1985. Mastitis-significance for processing of milk and public health aspects. Paper presented at the IDF seminar “progress in the control of bovine mastitis”, 21-24 May 1985, Kiel, F. R. Germany

Hogan. S. J., Gonzalez R. N., Harmon, J. R., Nickerson, S.C., Oliver, S.

P., Pankey,.J. W. and Smith, L. K., 1999. Laboratory Handbook on Bovine Mastitis. Published by National Mastitis Council, Inc., W D Hoard, Fort Atkinson, USA.

International Dairy Federation., 1991. Bulletin No 258. Detection and confirmation of inhibitors in milk and milk products 2nd edition. Brussels.

International Dairy Federation., 1996. Mastitis Newsletter No. 144. Brussels.

John E. Coia, Yvonne Johnston, Nicholas J. Steers, Mary F. Hanson., 2001. A survey of the prevalence of *Escherichia coli* O157 in raw meats, raw cow's milk and raw-milk cheeses in south-east Scotland. International Journal of Food Microbiology **66**: 63-69

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M. ("In press"- Outlook on Agriculture). Prospects and constraints of smallholder dairy husbandry in Dar es Salaam region, Tanzania.

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M., 2004. Risk Indicators Associated with Subclinical Mastitis in Smallholder Dairy Cows in Tanzania. Tropical Animal Health and Production, **36** (**6**): 581- 592

Kleinbaum D. G., Kupper L. L., Muller K. E., Nizam A., 1998. Applied regression analysis and other multivariable methods. London: Duxbury press. pp 186-211

Kurwijila R. L., Mdoe N., Nyange D. N., Auerbock R. M. and Malya H. N., 1995. Assessment of fresh milk and milk products and consumption in Dar es Salaam. Report to the Austro project association. Dar es Salaam: Austro project association.

Leclerc, V., B. Dufour., B. Lombard, F. Gauchard, B. Garin-Bastuji, G. Salvat, A. Brisabois, M. Poumeyrol, M-L. De Buyser, N. Gnanou-Besse, C. Lahellec., 2002. Pathogens in meat and milk products: surveillance and impact on human health in France. Livestock Production Science. **76:** 195-202

Mateu, E. and Martin, M., 2001. Why is anti-microbial resistance a veterinary problem as well? Journal of Veterinary medicine B **48**: 569-581

Mullins G. R., 1993. Market policy and market development: a comparison of dairy product consumption in Mombasa, Kenya and Dar es Salaam, Tanzania. Dairy development policy and Implementation: Sharing experiences experiences between Africa and Asia, Harare. FAO, Rome, 12 July 1993.

Noordhuizen J. P. T. M. and Frankena K., 1999. Epidemiology and quality assurance: applications at farm level. Preventive Veterinary

Medicine. **39:** 93-110

Peng, J.S., Tsai, W.C., Chou, C.C., 2001. Surface characteristics of Bacillus cereus and its adhesion to stainless steel. International Journal of Food Microbiology. **65:** 105-111.

Pennington, H., 1997. The Pennington Group: Report on the circumstances leading to the 1996 outbreak of infection with *E. coli* O157 in Central Scotland, the implications for food safety and the lessons to be learned. The Stationary Office, Edinburgh.

Quinn, P.J., Carter, M. E., Markey, B. K. and Carter, G.R., 2000. Clinical veterinary microbiology. London, Mosby-year book Europe limited, pp

Ropkins, K., Beck, A.J., 2000. Evaluation of worldwide approaches to the use of HACCP to control food safety. Trends in Food Science & Technology. **11**, 10- 21.

Shirima, G. M., R. R. Kazwala, D. M. Kambarage., 2003. Prevalence of bovine tuberculosis in cattle in different farming systems in the eastern zone of Tanzania. Preventive Veterinary Medicine **57**: 167-172.

SPSS for windows, release 11.5.0, SPSS Inc, 2002. http://www.spss.com

Stiles, M.E., 1989. Less recognized or presumptive foodborne pathogenic bacteria. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens, Marcel Dekker Inc, New York, pp. 674–735.

Sundlof, S. F., 1994. Antimicrobial drug residues in food-producing animals. In: Prescot J. F., Baggot, J. D. (Eds.), Antimicrobial therapy in veterinary medicine, 2nd edn. Iowa State University Press, Ames. pp. 569-584

Warburton, D.W., 1993. A review of the microbiological quality of bottled water sold in Canada. Part 2. The need for more stringent standards and regulations. Can. J. Microbiol. **38**: 158-168.

Warburton, D.W., Austin, J.W., 1997. Bottled water. In: Microproducts biology of Food. London. Chapman and Hall.

Weinhaupl, I., Schpf, K. C., Khaschabi, D., Kapaga, A. M., Msami, H. M., 2000. Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es Salaam region and in Zebu cattle in Lugoba area, Tanzania. Tropical Animal Health and Production. **32** (**3**): 147-154.

Whiting, R.C., Sackitey, S., Calderone, S., Morely, K., Philips, J.G., 1996. Model for the survival of *Staphylococcus aureus* in non-growth environments. International Journal of Food Microbiology. **31:** 231-243.

9

Chapter

Prospects and Constraints of Smallholder Dairy Husbandry in Dar es Salaam Region, Tanzania

Kivaria F.M.[1]* Noordhuizen. J.P.T.M.[2] Kapaga. A.M[1]
[1]Population Studies, Animal Diseases Research Institute, P.O.Box 9254; Dar es Salaam, Tanzania

[2]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

*Corresponding author at: Fax: + 255 222 119 001:
E-mail address: fredkiv@yahoo.com

Outlook on Agriculture (2006) 35(3) 209-215

Abstract

An in depth field survey was conducted on 125 randomly selected urban and peri-urban based smallholder dairy herds in Dar es Salaam region, Tanzania. The primary objective was to define the sector in terms of its productivity, constraints and sector's relevance to the urban dwellers. Information on such variables as animal management, feeding, housing, prophylaxis and disease history was obtained by means of a standardised interview-questionnaire and by cross-sectional inspection of the available farm records. Results show that dairying has led to improvements in the dairyman's general welfare. 78 % of the respondents indicated that dairying had increased their ability to meet medical and school expenses for their children, while, 6 % of respondents have used dairy income to invest on more capital intensive off-farm activities. It was also learnt that the sector has opened up a number of supporting businesses, creating employment for the people engaged in it. However, the sector's potentials are limited by a number of shortcomings and specific constraints, which among many others, include poor nutrition, poor animal health and diseases, finance, transport, skilled labour, processing facilities, storage, marketing and the delivery of advisory and health services. It is indicated that the system would have future if these technical, and socio-economic constraints are removed through research and technology transfer with appropriate policy support.

Keywords: Animal health; Breeding practices; Mastitis; Smallholder; Tanzania

Introduction

The historical background of the dairy industry in Tanzania begins in 1921(Sumberg, 1997), when the colonial government introduced the first nucleus herds of Ayrshire and Holstein cattle, and established the Temeke dairy farm, located on the grounds of Animal Diseases Research Institute, in Dar es Salaam. After independence dairy development in Tanzania was primarily based on parastatal or state farms, it was then urged that the management skills needed for successful dairying could be developed under a parastatal structure. However, the problems of mismanagement, political interference (e.g. concerning milk prices), low productivity and poor economic performance experienced by these parastatal farms led to a subsequent collapse (Mdoe and Wiggns, 1997). The Tanzanian dairy industry has thus undergone considerable changes since the 1960s. The smallholder dairy production system in Tanzania begun to emerge in 1983 (Mdoe and Wiggins, 1997). At that time high and mid-ranking civil servants started keeping a number of grade cattle in their residential compounds in the low and medium density urban areas. Their aim was to contribute to the market milk supply after the parastatals and cooperative farms collapsed. The sector is predominantly a back-yard production system where pigs, sheep and goats, and different poultry species are kept along with dairy cattle. The household income is mainly derived from formal employment and other off-farm activities; owners produce milk as an additional source of regular and stable income (Msanga *et al*., 2000). The size and structure of the herds vary from purely subsistence herds (n = 1) to large-scale commercial herds (n ≥100). The system is characterized by high input costs particularly for feeds, drugs and veterinary services, and at the output side high milk prices due to high and stable demand. Farmers perceive this system as an important activity that provides a regular income to the household, employment for the household and hired labour, a valuable source of human nutrition (French *et al*., 2001) and is considered to be fore runner of further development (Gitau *et al*., 1994; Schaik *et al*., 1996). Despite its importance, the sector is characterized by low production; the average milk production per cow is estimated at 6.5 litres/day at lactation duration of 18 ± 2 months (Msanga *et al*., 2000).

An important step in evaluating potential development options is to identify the major constraints and opportunities for increasing the productivity in the smallholder dairy sector. To this end, a study of randomly selected smallholder dairy farms in Dar es Salaam region,

Tanzania, was conducted as an input to the ongoing study on the epidemiology of bovine mastitis in smallholder dairy cattle in Tanzania. The objective of this study was to explore the prospects and constraints of the smallholder dairy sector, and to suggest possible solutions for the identified constraints.

Methodology

An interview-questionnaire based cross-sectional study was carried out in urban and peri-urban areas of Dar es Salaam region, between June and November 2002. A total of 125 households were involved in this study. A single interviewer using an interactive and structured questionnaire collected information on producer's socio-demographic data, knowledge and animal husbandry, production (milk; calves), handling and marketing and other variables. The questionnaire was structured to maximise the number of closed (categorical) questions, to ease execution, minimise variations and improve precision. Variations because of administrator bias were minimised by the use of one person to conduct the interviews. Furthermore extra information on farm practices was obtained by personal observation. To get an insight into the current industry performance the available farm records were inspected for accuracy, consistence and quality (the purpose of this procedure was to investigate the utility value of the available farm records and whether they can be used to support some decision e.g. culling or treatment, and if the records can be used to improve the quality of management decision). Also the records were used as a standard for comparison of farmers' information. A total of 62 milk vendors who were found at the farm were also questioned on the amount of milk they are buying, selling prices, transport and handling of milk, and whether the milk is boiled before being sold to the end consumer. Clinical examination (pregnancy diagnosis) of animals was carried out, counts of 'standard ticks' according to Norval *et al*., (1992) were made on each animal, whole blood in plain vacutainers and mammary secretions (68 herds) from each quarter were collected. The potential power of this study could not be estimated a priori, since little information concerning prevalence estimates and no information on the distribution of management practices existed. The sample size of 125 herds was therefore based on logistical considerations.

Data analysis

A descriptive account of the state of nature of smallholder production systems was statistically described by means and standard deviations, and frequency distributions of the variables. Where appropriate, epidemiological indices such as crude odds ratios were computed following univariate analysis (Martin *et al*., 1987).

Results

Producer's socio-demography

125 households with 946 people were visited in the period between June and November 2002. The average household size was 8.5 ± 2.5 persons. Males headed 90 % of the households, whereas females headed only 10% of the households. Many female headed households originated from widowhood, divorce or separation, and not married.

Animals and husbandry practices

The total number of dairy cattle kept was 977; mean herd size was 8.5 ± 6.4. The average observed herd structure was; lactating cows (35%); dry cows (6%); in-calf heifer (5%); non-pregnant heifers (12%); breeding bulls (5%); fattening bulls (3%); male calves (14%) and female calves (20%). 74%, 6% and 20% of visited households practiced zero grazing, semi intensive grazing and extensive grazing respectively. The other types of farm animals kept by the respondents were: chickens (12,572), goats (718), pigs (706), ducks (352) and sheep (78). The premises where farm animals were kept were either individually owned plots (95%) or rented premises (5%). The land distribution is skewed with a few holdings (2%) with over 10 acres of land, 33% of the holdings were less than one acre in size. The mean land size was 3.2 ± 0.4 with a range of 0.3-13 acres. Hired labour is used intensively in 97% of households, and there is a wide range of activities that hired labour undertakes. They include cleaning of animal sheds and general sanitation (92%), tick control practices (75%), cutting and carrying the fodder (85%), feeding animals (80%), milking (82%) and to some extent selling of milk (2%). Members of the household assume a supervisory role in most of these activities, and they are instrumental with regard to purchasing of farm inputs and selling activities.

Housing, feeding practices and feed resources

Animal sheds were categorized based on space per animal, barn size, type of roofing material, general cleanness (abundance of many flies, manure disposal, floor scraping and hosing), and the mode of construction, as good, satisfactory and poor. 30%, 61% and 9% of animal sheds were judged as good, satisfactory and poor respectively. 21% of households visited had a separate calf unit, 79% kept calves in the same shed as adult animals. However, farmers indicated a rather high degree of intervention in managing newborn calves, 84% of owners indicated that feeding of first colostrum was within two hours after birth and 16% stated that it was prior to five hours after birth. Most dairy animals are supplemented with seasonal crop residues and by-products. Some of the major forage species fed to dairy animals were *Hyperrhenia rufa*, *Pennisetum purpureum*, *Panicum maximum*, *Cynodon spp*, *Tripsacum laxum*, and *Desmodium spp*. The distance to the forage sources varied depending on the season (shorter during the rainy season) and means of transport, the distance ranged from less than a kilometre in rainy seasons to over 20 km during the dry spell. 83% of owners use maize bran as a major supplementary feed; variable amounts of concentrates are usually fed to cows at milking time, with many farmers feeding about four kilograms per day throughout the 18 months lactation period. 4.9% of the sampled households practiced bucket calf-feeding, while the rest allowed the calf to suckle from the dam either before (3.3%) or after (1.6%) milking. 57.3% of owners leave at least one quarter for the calf to suckle, whereas 42.7% of owners are not consistent in calf feeding practices. 62%, 36% and 2% of households obtained water for dairy activities from tap water, bore-well and running streams, respectively.

Reproduction practices

General performance indicators for the research area are given in Table 1. 59.7% of owners in this survey rely solely on artificial insemination (AI), which is provided by private veterinarians and livestock field officers (LFO). But, availability of AI is unreliable and costly. Prices paid are between US $ 5.0 and 8.0 per insemination. As a result 40.3% of owners own breeding bulls and charge neighbours for services. The herd boys detect oestrus which is done by brief (≤ 10 minutes) observations at milking and or cleaning times in many zero grazed households. Heifers served by natural mating were 6 times more (odds ratio = 6.3) likely to become pregnant than heifers served by artificial insemination, and the

Table 1. Performance indicators of dairy animals in the smallholder dairy cattle production system in Dar es Salaam region, Tanzania

Parameter	Indicator
Overall annual mortality %	30
Calf annual mortality %	25
Adult annual mortality %	5
Annual calving rate %	35
Calving interval (months)	20 ± 4
Annual off take %	3
Annual migration out %	1.5%
Average age of heifers at first service	24 ± 4 months
Average age of heifers at first calving	33 ± 4 months
Annual migration in %	1.5%
Average number of calving-during cows' on farm life span	5
Culling age	9 years
Number of lactations	5
Age at weaning	3 months
Pre-weaning mortality %	10
Lactation length (days)	450

(Source: survey data June-November 2002)

differences in likelihood of pregnancy was statistically significant (χ^2 = 15.2; $P \leq 0.01$). Likewise, naturally served multi-parous cows were 2 times more likely to become pregnant than their counterparts who were served by AI. The likelihood of pregnancy in naturally bred cows was statistically significant higher (χ^2 = 7.6; P = 0.01) than in artificially bred cows. There is no calving seasonality observed, however, the available farm records indicated that about 70% of calving occurs during and towards the end of wet season.

Animal health services

96.4% of owners have direct access to animal health services. LFO, private veterinarians and state veterinarians provide 76%, 21% and 3% of these services respectively. 45.6% of households keep records on animal health and other parameters, while 54.4% do not keep records. The records are sketchy and not consistent (e.g incorrectly recorded event dates and misclassifications of animals' status). Calving dates, date of service and dates for the next trypanosomiasis prophylactic treatment and de-worming and animal health problems are the major contents of farm records. 11.7% of the records contain information on daily milk yields. Farm records showed that 89% of owners with farm records

routinely de-wormed their calves and 80% practice routine prophylactic treatment against trypanosomiasis, 41% of cattle is vaccinated against theileriosis. 0.1% and 3% of owners have vaccinated against foot-and-mouth disease (FMD) and contagious bovine pleuropneomonia (CBPP) respectively. Despite the fact that only 5% of visited herds reported that they did not practice tick control and only 3% stated that they did not treat their calves, 26.2% of the 977 cattle have never been treated for ticks, while 18.7 % had been last treated more than 9 months prior to sampling. Of the remaining animals examined 44.9% had been treated for ticks within three weeks prior to sampling; 30.6% had been treated within four days. A total of 405 ticks were collected and their distribution was *Rhipicephalus appendiculatus* (92.3%), *Boophilus microplus* (6.7%), *Rhipicephalus pravus* (0.4%), *Amblyoma variegatum* (0.4%) and *Rhipicephalus evertsi* (0.2%).

Major animal diseases and conditions recorded in farm records were anaplasmosis (21.80%), infertility (20%), retained placenta (18.4%), theileriosis (13.2 %), clinical-mastitis (10%), abortions (10%), trypanosomiasis (4%) and milk fever (2.6%), in that order of importance. Anaplasmosis and theileriosis were the major causes of cattle mortality recorded; cattle were about 3 times more likely to die due to anaplasmosis, than theileriosis. The difference in risk of cattle mortality due to anaplasmosis and theileriosis was statistically significant (χ^2 = 22.5; $P = 0.00$). It was observed that calves (≤ 6 months of age) are about 5 times (OR = 4.9) more likely than adult cattle to die due to tick borne diseases (TBD). The risk of death was statistically significantly higher in calves ($\chi^2 = 40$; $P \leq 0.01$), than in adult cattle. It was also observed that female calves were about three times (OR = 2.7) more likely to die than male calves, and the risk of mortality was statistically significantly higher ($\chi^2 = 6.9$; $P = 0.01$) in female calves than in male calves.

Milking practices, milk production, handling and marketing

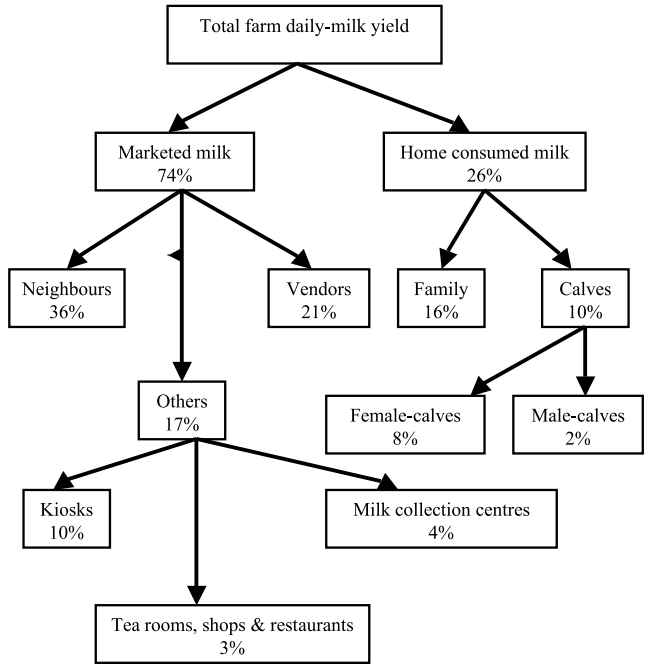
Hand milking is practiced by 100% of the household surveyed, on average a given household will have 2 ± 1 milkers; teat stripping is practiced by 90.3% of the households, whereas 9.7% of the households practice five-finger squeezing type of milking. 100% of households reported to wash hands prior to milking operations, however, 57% reported to wash hands without soap or other disinfectant. Teat lubrication is a common practice by owners, 49% and 48% of owners use commercial milking salve and cooking oil as teat lubricant respectively. The commonly practiced stimulus for milk let down is to give maize bran,

followed by calf suckling, then wash the udder with either warm (74%) or cold (13%) water. 66% of milkers use a single piece of cloth for udder drying for all in herd lactating cows, whereas 30% reported to use bare hands to dry the udder and 4% do not dry the udder. Fore milking is practiced in 70% of households, 24% of these squirt milk on palms of hands to check for milk abnormalities, whereas 27% and 20% squirt on the floor and teacup respectively, 29% do not practice fore milking. Fore milking is regularly done by 39 % of households, while 61% check up on suspicion.

The average daily milk yield per cow at the time of farm visit was 8 ± 4 litres, 86%, 9% and 5% of owners store milk in plastic, metal containers and a combination of the two, respectively. 36% of owners boil milk before consuming or selling it, whereas, 64% sell or consume un-boiled milk. 5% of the owners admitted to have at least in one occasion added water and or wheat flour to milk. Generally milk for home consumption and that marketed by retailers is consumed in the form of naturally fermented milk or informally processed products such as soured milk, warm milk and cooled milk. As can be seen from Figure 1, the predominant market channels for smallholder produced milk, is through direct sales from owners to consumers. Sales are made directly to neighbours who collect it from the farm or the farmer delivers milk to the consumer. Some farmers sell extra milk to shops, hotels, and tearooms. Most of the evening milk is sold in this way or consumed at home while the morning milk is sold mostly to vendors and consumers.

It is estimated from the survey that about 59% (n = 62) of milk vendors use bicycles, foot or public transport to move around farms to collect and deliver fresh or cooled milk. Some of the milk retailers are themselves owners who supplement their supplies by buying milk from other owners, the most common channel for this flow are hotels and tearooms. The amount handled by each small retailer/vendor mostly range from 10 to 150 litres per day. Milk kiosks and tearooms have mushroomed in major cities like Dar es Salaam, in such places milk is boiled and cooled before sale and part of it soured and sold as fermented milk. Almost all marketed milk is sold through contractual arrangements in terms of time of delivery, price and timing of payment to attract customers.

Figure 1. Major milk outlet channels from 125 smallholder dairy herds in Dar es Salaam region and estimation of percentage of milk flow through each channel.



(Source: survey data June-November 2002)

Mastitis awareness

Farmers were asked whether they had ever seen udder diseases in lactating cows. 80 % of farmers were aware of clinical mastitis in lactating cows. 83.7% of the farmers were also aware that mastitis not only reduces the quantity of milk but also its quality. Lack of awareness of subclinical mastitis was apparent among the owners, only 5% of the owners interviewed were aware of the presence of subclinical mastitis. Of the 90 farms that had stated to have experienced mastitis during 2001, 30 (33.3%) did not treat the mastitic cases. 68% of the farmers who did not treat the observed cases, rightly described the clinical signs in their cows but were not aware that it was mastitis. It was evident from farm records that 94% of antibiotics used on farm were used for mastitis. Farm records indicate that mastitis risk was highest at around calving; culling due to

udder health was practiced in 12% of the study herds.

Relevance of smallholder dairy production

Farmers were asked whether dairying had any impact on household consumption of animal proteins, most of the respondents indicated that, dairying had improved the nutritional quality of food eaten by members of the households. The benefits in terms of improved nutritional status also seemed to have extended to non-livestock keeping households, which purchase the surplus milk. Furthermore, the additional and reliable income from sale of milk has increased the purchasing power of the households enabling them to purchase other animal proteins such as beef-meat and poultry products. The use of manure for gardening leads dairy keeping families to have a more assured food security. In addition, these families are producing surplus vegetables for sale, making them economically more independent than families without cattle. The above economic gains from dairying have led to improvements in the farmers' general welfare.

A number of houses for example have been renovated. In addition, several dairy farmers were acquiring various domestic appliances (bicycles, radios and TV sets, sewing machines) and farm implements (rubber-boots, wheel barrows etc) to ease their workload and generally improve their livelihood. All these aspects, although they may appear small, are generally contributing positively towards dairy farmers' economic situation. 22% of households state that dairying has provided steady employment to a member of the family, with labourers hired by the majority (97%) of the dairy farmers. However, the dairy sector creates substantial paid employment opportunities for young men, but not women. 78% of the households indicated that dairying had increased their ability to meet medical expenses and to meet education expenses by getting money for school fees, uniforms, and transport to and from school, largely at primary and secondary school levels, paying development levy (4%) and hiring labour. 6% of the respondents have used dairy income to invest on more capital intensive off-farm activities, such as shops.

Dairying in Dar es Salaam region has made a significant economic contribution to the individual households and to the labourers who are employed by smallholder dairying. Smallholder dairying has opened up additional businesses to support it; the veterinary trade is one of the very fast growing businesses in Dar es Salaam, absorbing a considerable proportion of veterinarians and LFO. Other businesses, which are expanding fairly fast in response to the expanding dairy industry, are the

inputs stockists, like cattle feeds (e.g. pre-mixes, maize bran, seed cakes and molasses), selling of pastures/forage along the road sides, the milk collection centres, processing and marketing business which though still in their infancy, are creating some employment for the people engaged in it. Furthermore, 2 % of the households use cow dung to produce biogas thereby cutting significantly household electricity bills (personal observations).

Specific Constraints

As pointed out earlier livestock diseases, particularly vector borne diseases and mastitis, are major bottlenecks for the optimal performance of smallholder dairy cattle in Tanzania. In this survey the farmers identified a number of specific constraints (as opposed to text book constraints), which were grouped into four classes as summarised in Table 2.

Discussion

The average household size of 8.4 people is rather high, and could be one of the reasons of keeping animals in towns; other reasons could be meagre household income and the availability of ready markets for the products delivered. The number of secondary business supported by the smallholder sector and the fact that 97% of the households depended on hired labour, mostly youths, was a good pointer to the contribution of the smallholder dairy sector to job creation. The average herd size (8.5) per household was found to be rather high in relation to the space and the difficulties of feed acquisition. The relatively low number of households practising semi-intensive and extensive grazing reflects the dwindling of grazing lands as more land is used for buildings.

Owners are keen on routine de-worming, prophylactic treatment and seeking of veterinary services when necessary. However, the recorded high calf mortality and infertility are of great concern. This finding also was reported in similar studies of smallholder dairy farms in Kenya, and Zimbabwe (Gitau *et al* 1999; Maloo *et al*., 2001, French *et al*., 2001). Animal sheds are in general poorly constructed. Moreover, most owners paid little attention to the importance of having properly constructed calf sheds. Thus, calves were easily prone to pneumonia, diarrhoea, parasitic diseases and TBD, mortality, kicking and trampling by mature animals. Furthermore, it is generally accepted that among the TBD, theileriosis is the number one cause of cattle morbidity (Norval *et al*., 1992), the

Table 2. Specific constraints of smallholder dairy cattle production system, as cited by the smallholder dairy farmers in Dar es Salaam region, Tanzania (Source: survey data June-November 2002)

Environmental	Management	Health and reproduction	Extension
• Inadequate, seasonal and irregular supply of forage	• Wasteful feeding practices by not chopping the folder.	• High prevalence of vector borne diseases a. Tick borne disease b. Trypanosomiasis c. Endo-parasites	• Inadequate and weak training programmes for smallholder owners and animal attendants.
• Water shortages (seasonal and poorly developed water resources). • High costs of inputs (drugs, concentrates, water and electricity). • Inadequate supply of appropriate inputs. • Low milk prices • Poor services e.g. electricity. • Lowland availability. • Lack of coordinated dairy development efforts. • Lack of credit and livestock insurance facilities. • Poor marketing infrastructures	• Lack of knowledge on forage conservation. • Inadequate breeding practices. • Lack of bull selection for size, type and ease of calving. • Lack of proper and well-kept production and health records. • Poor housing conditions (overcrowding, poor cleanliness). • Poor calf rearing practices resulting in poor quality heifers. • Poor management of dry cows. • Unbalanced cattle rations. • Poor milking hygiene and inadequate preparation for milking. • Poor management and unskilled labour leading to sub-optimal use of farm resources e.g. feeds. • Low level of technology application at production, processing and marketing.	• Insufficient vector control (irregular and erratic application of ectoparasiticides). • Use of unregistered and expired drugs. • High calf mortality • Infertility • Retained placenta • Abortions • Production diseases particularly subclinical mastitis	• Production of poor quality milk, observation of withdrawal period after antibiotic treatment of mastitis is a problem. • Farmers do not know about the costs of production because majority of them do not keep records. • Farmers unaware of subclinical mastitis • Lack of pre-defined production indices; farmers do not have any specific production goals or disease prevalence levels that they wish to attain. • Lack of extension service

observation that 41% of dairy cattle were vaccinated against theileriosis, and the presence of the tick vector for anaplasmosis (*Boophilus microplus*) might explain our observation that anaplasmosis was the leading cause of cattle mortality.

The long calving intervals reported here are in agreement with data reported by Abdalla *et al*., (1999); the major causes of infertility among the animals were difficulties in heat detection and the unreliability of AI/ bull services, poor semen handling and semen-deposition techniques. Alejandrino *et al*., (1999), indicated that poor breeding management, availability of a bull of proven fertility and good quality semen for A.I, and nutritional stress particularly during critical periods of the cow's reproductive life are the important human factors influencing smallholder dairy cattle productivity. Mastitis has been listed by Walshe *et al*., (1991) as an important constraint to production on smallholder dairy farms in sub-Sahara Africa. In our study (Kivaria *et al*., 2004), it was evident that mastitis, particularly subclinical mastitis is not perceived and unknown to most farmers and that it is one of the largest, unnoticed constraints in smallholder dairy production, probably causing high losses per affected cow.

Our results heavily depended on the information given by the farmers, thus our results were subjected to such errors as recall bias, misinterpretation of farmers' information and reporting/recording bias. However, the use of closed questions and single person to give the interview, and data inspection was meant to minimize these errors. Further more, the 45.6% of farmers with records was assumed to be a random subset of the study population, our study design and assumptions seemed to be valid as our results are comparable with similar published studies conducted elsewhere.

Another remarkable feature of the smallholder sector is the fact that 74% of all milk is marketed as raw milk through the informal channels (Fig 1). Milk collection centres (MCC), handle only 4% of the marketed milk. In contrast, the Kenya Cooperative Creameries (KCC) handles 90% of all marketed (Staal and Shapiro, 1994) milk in Kenya, which, is also the major buyer at the farm level. In Tanzania, the lowest average milk prices are paid by the MCC, followed by sales to kiosks, restaurants and vendors. Individuals pay the highest average prices for raw milk. Staal *et al*., (1997), have reported similar observations in Kenya and Ethiopia. Higher prices obtained from sales to individuals, probably attract most smallholder producers. However, this practice is of great concern, particularly with regard to the risk for both contracting zoonoses such as

brucellosis and tuberculosis, and other food safety failures. Risks of milk borne zoonoses posed by the informal market, the handling procedures on the market, and the handling of milk by consumers are further complicated by the lack of quality standards, longer distance from farm to selling point and delay in the transportation chain (Gran *et al*., 2002).

For attaining reasonable levels of animal health and productivity, feed and other inputs must be purchased when required. Cash income from milk sales should be sufficient for feed and other low cost inputs. However, there are often other demands on income, such as school fees and hospital bills, which take priority over inputs for the dairy enterprise. In addition, income and expenditure are not even throughout the year. During the dry season income from milk sales is low and expenditure on forage high. In dry seasons forage becomes scarce and expensive. If the smallholder does not have savings or credit facilities s/he will be forced into emergency sales of livestock and consequent loss of capital and future income.

From Table 2 it can be concluded that, enterprises' performance is likely to improve if the following would become readily available: reliable, affordable and effective animal health services; adoptable strategies to alleviate under-nutrition of dairy animals and reduce the seasonal variation in feeding availability; a reliable and affordable AI service; a local supply of dairy cattle compounded feed; improved infrastructure for milk collection, processing and distribution; and credit and savings facilities for smallholder owners, and appropriate extension packages.

References

Abdalla, A. L., Louvandini, H., Bueno, I. C. S., Vitt., D. M. S. S., Mierelles, C. F., and Gennari, S. M. 1999. Constraints to milk production in grazing dairy cows in Brazil and management strategies for improving their productivity. Preventive Veterinary Medicine **38:** 217-230

Alejandrino, A. L., Asaad. C. O., Malabayabas, B., De Vera, A. C., Herrera, M. S., Deocaris, C. C., Ignacio, L. M. and Palo. L. P. 1999. Constraints on dairy cattle productivity at smallholder level in the Philippines. Preventive Veterinary medicine **38:** 167-178

French. N. P., Tyrer. J. and Hirst. W. M. 2001. Smallholder dairy farming in the Chikwaka communal land, Zimbabwe: birth, death and demographic trends. Preventive Veterinary Medicine **48:** 101-112

Gitau, G. K., O'Callaghan, C. J. McDermott, J. J., Omore, A. O., Odima,

P. A., Mulei, C. M. and Kilungo, J. K. 1994. Description of smallholder dairy farms in Kiambu district, Kenya. Preventive Veterinary medicine **21** (**2**): 155-166

Gitau, G. K., Perry, B. D., and McDermott, J. J. 1999. The incidence, calf morbidity and mortality due to Theileria parva infections in smallholder dairy farms in Murang'a, Kenya. Preventive Veterinary medicine **39:** 65-79

Gran, H. M., Mutukumira, A. N., Wetlessen, A., and Narvhus, J. A. 2002. Smallholder dairy processing in Zimbabwe: the production of fermented milk products with particular emphasis on sanitation and microbiological quality. Food Control **13:** 161-168

Maloo, S. H., Rowlands, G. J., Thorpe, W., Gettinby, G. and Perry, B. D. 2001. A longitudinal study of disease incidence and case-fatality risks on smallholder dairy farms in coastal Kenya. Preventive Veterinary medicine **52:** 17-29

Martin, S.W., Meek, A.H. and Willeberg, P., 1987. Veterinary Epidemiology. Principles and Methods. Iowa State University Press, Ames, p.32.

Mdoe. N. and Wiggns. S. 1997. Returns to smallholder dairying in the Kilimanjaro region, Tanzania. Agricultural economics 17: 75-87

Msanga, Y. N., Bryant, M. J., Rutam, I. B., Minja, F. N., and Zylstra, L. 2000. Effect of environmental factors and of the proportion of Holstein blood on the milk yield and lactation length of crossbred dairy cattle on smallholder farms in north-east Tanzania. Tropical animal Health and Production, **32:** 23-31

Norval, R.A.I., Perry, B.D. And Young, A.S., 1992. The Epidemiology of Theileriosis in Africa. Academic Press, London.

Schaik, G. van.,, Perry, B. D., Mukhebi, A. W., Gitau. G. K., and Dijkhuizen. A.A. 1996. An economic study of smallholder dairy farms in Murang'a district, Kenya. Preventive Veterinary Medicine **29:** 21-36

Staal, S. J. and Shapiro, B. I. 1994, The effects of recent price liberalization

10

Chapter

General discussion

F. M. Kivaria

Population Studies, Animal Diseases Research Institute,
P. O. Box 9254, Dar es Salaam, Tanzania
fredkiv@yahoo.com

Introduction

The objective of the current research was to contribute to knowledge necessary to design herd specific mastitis control programmes in the urban based smallholder dairy herds. To this end, retrospective (Chapter 2), and prevalence (Chapter 3) studies were carried out to establish the occurrence of endemic udder pathogens among dairy herds in the Dar es Salaam region. The antimicrobial susceptibility patterns of the major microbial isolates were also studied (Chapter 3). Host, environmental and management factors that influence the risk of intramammary infections (IMI) were examined too (Chapters 4-6). Utility of the California Mastitis test (CMT) as a diagnostic tool for subclinical mastitis in low yielding cows was evaluated (Chapter 7). The public health hazards posed by the milk produced by the smallholder were explored (Chapter 8), and finally the constraints to, and the prospects of the smallholder dairy sub-sector were examined in Chapter 9.

Relevance of urban Smallholder dairying in Tanzania

Recently the number of milking cows has increased substantially in the Dar es Salaam region due to an increasing demand for fresh milk in this densely populated urban area. It is estimated that there are 1,765 smallholder dairy herds with 8,233 improved dairy animals in and around the Dar es Salaam region (MOAF, 2006). Urban and peri-urban smallholder dairying is viewed as an agricultural activity that provides a regular and stable income to the household (Our unpublished data shows that in many cases dairy production contributes as much as 60% to the total income of the household), employment for the household and hired labour, a valuable source of human nutrition and is considered to be a forerunner of further development (Walshe *et al.*, 1991; Winrock International, 1992). There are many potential constraints to smallholder dairy farming including difficulties in providing adequate feed and water, lack of dairying skills, and problems with marketing and poor animal health services (National Livestock Policy, 1997).

Bovine mastitis

Mastitis is an inflammation of the udder that affects a high proportion of dairy cows throughout the world. Clinical and subclinical mastitis are the two major forms of the disease. Clinical mastitis results in alterations in

milk composition and appearance, decreased milk production, elevated body temperature, and swelling, redness, or heat in infected mammary quarters. It is readily apparent and easily detected. However, detection of mammary quarters with subclinical mastitis is more difficult because signs are not readily apparent. Consequently, subclinical mastitis, which is the most prevalent form of the disease, often goes undetected. Many subclinical IMI tend to persist, often resulting in elevated milk somatic cell counts (SCC) and decreased milk production, which may lead to development of clinical mastitis and the opportunity for certain mastitis pathogens to spread from infected mammary quarters to uninfected mammary quarters. Mastitis occurring in clinical and subclinical forms is an important animal health constraint that affects both the amount and quality of milk produced by the smallholder dairy herds (Kapaga *et al*., 1995; Karimuribo *et al*., 2003; Mdegela *et al.*, 2004; Kivaria *et al*., 2004). Compared with other diseases, mastitis is ranked low in priorities by the national veterinary authority and consequently has received little attention in Tanzania. Extension efforts have therefore been focused on the treatment of clinical cases rather than tackling the disease from the control point of view.

Aetiology

Mastitis pathogens are categorized as either contagious or environmental. Contagious pathogens live and multiply on and in the cow's mammary gland and are spread from cow to cow primarily during milking. Contagious pathogens include: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* species, and *Corynebacterium bovis* (Radostits *et al*., 2000, Quinn *et al*., 2000). Environmental pathogens reside in the environment where cows live. The most frequently isolated environmental pathogens are streptococci other than *Streptococcus agalactiae*, commonly referred to as environmental streptococci, and gram-negative bacteria (Hogan *et al*., 1999). Environmental streptococcus species involved in bovine mastitis include *Streptoccus uberis*, *Streptococcus dysgalactiae* ssp. *dysgalactiae*, *Streptococcus equinus*, *Streptococcus equi*, and the enterococcus species (Radostits *et al*., 2000). Among the environmental streptococci, *Streptococcus uberis* appears to be the most prevalent (Jayarao *et al*., 1999). Gram-negative bacteria involved in bovine mastitis include *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, enterobacter spp. and citrobacter spp. Among the gram-negative mastitis pathogens, *Escherichia coli* and *Klebsiella* spp. appear to be the most prevalent

(Hogan *et al*., 1999). In addition to these udder pathogens, our results (Chapters 2 and 3) indicate that fungal and mycotic infections to be an important cause of mastitis in the smallholder dairy herds. Contagious pathogens are capable of establishing sub-clinical infections, which are manifested as an elevation in the somatic cell count (SCC) of milk from the affected quarter (Blowey and Edmondson, 2000; Radostits *et al*., 2000), and therefore a positive (≥ +1) CMT score. In contrast, the course of environmental mastitis is more often acute compared to contagious mastitis and therefore the signs of environmental mastitis are more frequently clinical (Dopfer *et al*., 1999; Radostits, 2001; Bradley, 2002). However, 'contagious-environmental' classification may not be as clear-cut as previously thought. Indeed, the epidemiology of udder pathogens is better represented by a sliding scale where the balance of contagious and environmental transmission shifts gradually, than by a species based dichotomy (Zadoks, 2003).

Sources

The primary sources are firstly animal carriage and subclinical IMI. The main significant Staphylococci reservoirs are subclinical and chronic IMI, and teat and udder lesions (Radostits *et al*., 2000). Coagulase negative staphylococci (CNS) and *Staphylococcus aureus* can also be cultured from healthy teat skin (Burriel, 1997; Scott and Murphy, 1997). Other bacteria also have animal primary sources: *Streptococcus agalactiae* are carried in the adults' and suckling young's mouth, nasopharynx and tonsils (Scott and Jones, 1998). Other primary sources are environmental: Enterobacteria and Enterococci are found particularly in the litter and *Pseudomonas* spp. especially in water or a humid environment. *Aspergillus fumigatus* and other fungi are isolated from mouldy forage, wet bedding, litter, and air (Perez *et al*., 1998; Hogan *et al*., 1999; Quinn *et al*., 2000). *Streptococcus uberis* is known to have mixed reservoirs: infected animals, litter, and the environment. The accessory sources are, for Staphylococci, housing, bedding, feedstuffs, air, insects, udder cloths, humans (hands), other animals, etc. (Albenzio *et al*., 2003; Burriel, 1998; Sevi *et al*., 2001). *Streptococcus agalactiae* can be found on the teat skin of cows soon after lambing (Radostits *et al*., 2000) and in the environment of diseased animals: grass, water, straw bedding.

Prevalence and Persistence

Clinical mastitis

Surveys of the prevalence of mastitis in most countries, irrespective of the cause, show a comparable figure of 50% among dairy cows and a quarter infection rate of 25% (Radostits *et al*., 2000). On the other hand, in a Sudan study, Abdelrahim *et al*., (1989) reported 70.3% and 44.1% infection rates in cows and quarters, respectively. Kapaga *et al*., (1995), in a study on bovine mastitis in smallholder herds in the Dar es Salaam region, found cow infection rates of 12%. In their study of mastitis in smallholder dairy herds in the Iringa region of Tanzania, Karimuribo *et al* (2003) reported a prevalence of 19%. Our prevalence figures for clinical mastitis in the smallholder dairy herds were ≤ 5% (Chapters 3 and 4).

Subclinical mastitis

Subclinical mastitis is believed to be more prevalent than clinical mastitis in most countries (Schukken *et al*., 1995). The prevalence of subclinical mastitis on farms could range from 19 to 78% (Tuteja *et al*., 1993). Of great economic significance is the fact that subclinical mastitis may cause between 15 and 45% reduction in milk production in affected lactating cows (Dohoo and Meek, 1982). Our study has shown that subclinical mastitis is not known to the smallholder producers (Chapter 4), which implies that the disease is not detected and no attempts are made to eliminate it during lactation. California Mastitis Test (CMT) based studies conducted in smallholder dairy herds in Tanzania (Kapaga *et al*., 1995; Karimuribo *et al*., 2003; Mdegela *et al*., 2004; Kivaria *et al*., 2004) have indicated the prevalence of subclinical mastitis to be ≥ 80% at both cow and quarter levels, respectively.

Transmission

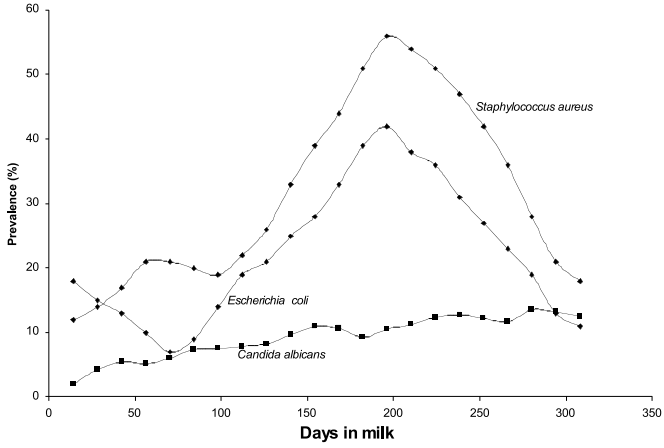
Spreading mainly occurs during udder massage, udder washing and milking. Udder pathogens are also transported passively by milkers' hands and udder towels. Transmission is also possible by "milk-robber" calves (buccal carriage) and may be important for staphylococci and streptococcus mastitis. Penetration of the udder by organisms is performed via the teat duct. In cases of systemic infections, haematogenous colonization is frequent (mycoplasmosis and brucellosis).

Persistence of intramammary infections

The persistence of individual, clinical IMI during lactation depends on the milking time hygiene level and the general husbandry practices. In the smallholder herds mastitic animals are not often treated correctly, and acute cases may become chronic for several months or more (1.5 to more than 30%). Fibrotic mammary glands are frequently (≥ 70%) observed, particularly in animals of ≥ 2 parity, and culling of animals with chronic mastitis is rarely (≤ 3%) done. The persistence of subclinical IMI during lactation is variable according to the causative pathogen but is generally high, since *Staphylococci aureus* represent the more frequent one (Chapter 3).

The incidence of clinical IMI varies with the lactation stage. A high incidence at drying-off (mycotic infections) or at parturition (coliform mastitis) is frequently observed in the majority of the animals, in relation with environmental contamination and/or poor hygiene practices (Chapters 4-6). On the contrary, the higher rates of staphylococcus mastitis are observed at the middle of the lactation. In our study, higher rates have been observed towards the end of the lactation, but prevalence seems to increase throughout the lactation cycle (Figure 1). Such observations must however, be cautiously interpreted; the incidence is difficult to differentiate from prevalence (chronicity or relapse), and older animals may be the more resistant ones.

Figure 1. Persistence of the commonly isolated udder pathogens from quarter-milk samples of the smallholder dairy cows with subclinical mastitis in the Dar es Salaam, region.



Risk factors associated with IMI persistence

Udder infection persistence is due to the lack of precocious IMI detection and systematic application of control programmes: teat antiseptics, antibiotherapy or culling. In smallholder dairy herds, udder palpation and California Mastitis Test (CMT), post-milking teat antiseptics are not practiced at all; while only 4% of the farmers practice drying-off (Chapters 4-6). Extra-mammary bacterial persistence is firstly due to hygienic conditions. Secondly, high stocking density, particularly in zero grazed herds may result in large air concentrations of total microorganisms, mesophilic or coliform bacteria and staphylococci. These effects are probably associated with incorrect ventilation and high temperatures and relative humidity. The multiplication of various bacteria on the skin (and in the litter) can be subsequently enhanced (Albenzio *et al*., 2002; Sevi *et al*., 1999; Sevi *et al*., 2001).

Risk factors such as management practices (poor teat and udder hygiene, poor environmental hygiene, sanitation, large herd size, use of hand washcloth) and diet (selenium and vitamin E deficiency) amongst others have been reported to be important in the prevalence and epidemiology of subclinical mastitis (Bartlett *et al*., 1992; Chassagne *et al*., 1998). Teat dipping and dry cow therapy have also been found to play key roles in preventing subclinical and clinical mastitis (Hogan *et al*., 1994; Lam *et al*., 1995). A striking feature of the smallholder dairy cows is the high prevalence of teat and udder lesions (Chapters 4-6). Teat lesions, regardless of cause, are frequently colonized by staphylococci and streptococci species (Mulei, 1999). Consequently, high new infection rates and increased numbers of mastitis cases are common squeals in herds where teat lesions are prevalent.

Overall, our results indicate (Chapters 4-6) that the risk factors for mastitis in cows that have been determined in other countries (Radostits *et al*., 2000) also apply in the smallholder dairy settings. However, the most notable factors were the general lack of knowledge on dairy cattle husbandry by the smallholder producers, and the paucity of livestock extension services. The absence in Tanzania, of udder health programmes consisting of regular visits by the veterinarian may, therefore, be a risk factor for mastitis which may be associated with a relative lack of awareness by the dairyperson of the importance of the principles of mastitis control. Lack of knowledge and awareness are undoubtedly the most important environmental risk factors contributing to IMI, but difficulty to quantify. Knowledge and awareness of mastitis influence farmers' perceptions and decisions which in turn will affect preventive

and treatment regimes, such as post-milking teat disinfection, dry cow therapy, hygiene, ventilation, feeding, milking, housing, bedding. 96% of farmers do not use dry cow therapy because they believe that if they use it, the cow will produce less in the subsequent lactation. Similarly many farmers do not cull cows with chronic mastitis, because they expect a female calf; farmers do not observe the full course of antibiotic treatment and they tend to change therapy if the clinical case do not improve fast enough, but they do not do that at all in the same stage of clinical mastitis or in an adequate manner.

Diagnosis

There are three levels where the detection of mastitis and assessment of milk quality is required: individual cow level in the herd, a more large scale testing for the bulk milk, and finally, testing of milk supplies in the dairy plant. Clinical mastitis is easy to detect but also cows with subclinical mastitis should be identified. Bacteriological sampling is not feasible as a routine test to detect mastitis. Tests for indicators of inflammation are therefore necessary as screening tests to identify quarters with IMI and to select cows for subsequent bacteriological sampling (Fthenakis, 1996: Radostits, 2001). The golden standard to measure inflammation is the cytological investigation; milk somatic cell count (SCC).

Milk SCC has been used extensively as an indicator of IMI since the nineteen-sixties and CMT scores are directly related to average somatic cell counts. The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the SCC and microbiological status of the quarter (Berriatua *et al*., 2001; Burriel, 1998). The final definition of mastitis with agreed thresholds is still under debate; suggestions for definitions can be seen in Table 1. The original limit for SCC of a healthy quarter is 500,000 cells mL^{-1} (Burriel, 1998). The threshold levels for SCC were based on a population mean plus two times standard deviation for one measurement of the foremilk from an individual quarter. The definition was a guide for diagnosis, even though 50% of truly infected quarters could at any time have a cell count less than the cut-off point of 500,000 cells mL^{-1} (Burriel, 1998). The threshold of 500,000 cells mL^{-1} for a quarter has not been relevant for a long time (Omore *et al*., 1996).

Table 1. Criteria for diagnosis of mastitis, based on the results from the quarter milk samples

Results from the milk sample		
Microbial growth	Inflammation (e.g. >500,000 SCC mL^{-1})	Diagnosis
Yes	No	Latent infection
No	Yes	Non specific
Yes	Yes	Mastitis

At present, the only indirect mastitis tests which can be used (in the context of the smallholder dairy producers) as the "cow side" test is the California Mastitis Test (CMT), the misclassification of this test may range between 25-50% (Fthenakis, 1996). CMT has an advantage of being very inexpensive and the only cow-side test with real-time results for selection of the quarters for subsequent bacteriological examination. However CMT is not a test for large-scale monitoring purposes unless automatised. The ability of CMT to identify quarters with IMI has been evaluated extensively, with variable results (Esnal *et al*., 1994; Haenlein, 2002).

Factors which will affect CMT scores of a cow include the number of infected quarters and the kind of infection (*Streptococcus agalactiae* is a more potent stimulator of SCC than *Staphylococcus aureus*). Lactation stage affects the SCC so that immediately after parturition SCC is high, but decreases fast to the normal level within 4-5 days after calving (Albenzio *et al*., 2002). Towards the end of the lactation period, SCC increases slightly (Rodostits *et al*., 2000). According to more recent studies, the physiological effects affect very little the SCC of truly healthy cows (Charffeddine *et al*., 1997). It was shown by Albenzio *et al*., (2002) that SCC decreases to a low level within 3 days in healthy quarters but remains high in the infected ones; thus quarter SCC can be used early post partum to detect new IMIs. Similarly older cows have higher SCC than young ≤ 4 years. Milking frequency also affects milk SCC. A shift from a two times a day to three times a day milking was clearly shown to decrease bulk milk SCC and the proportion of high SCC cows (Boscos *et al*., 1996); on the contrary very short milking intervals (4hrs and less) were found to increase SCC (Bergonier *et al*., 1996). SCC declines as milk yield increases (Emanuelson *et al*., 1991). These observations, coupled with the very high background level

of somatic cells in low yielding dairy cows, lead us to question whether a standard CMT score of ≥ +1 for positivity is an appropriate cut-off for smallholder dairy cows in Tanzania (Chapter 7).

Future research prospects

Although there is currently sufficient, published information with regard to the epidemiology of bovine mastitis available to start a prevention and control programme, there is an important lack of information with regard to epidemiology of bovine mastitis in smallholder dairy herds. Therefore, design of appropriate udder health programmes should be accompanied by research efforts to further fine-tune the available information. Future research on bovine mastitis in Tanzania should concern with the following areas.

Farmers' awareness of the mastitis problem

Although it is difficult to estimate the effect of dairy extension services, any improvement in udder health is unlikely to be attained without an intensive dairy extension services. The main focus of such services should be to increase the farmers' awareness of the problem of mastitis, and to make them conscious of possible risk for producing milk of insufficient quality. A major concern is the level of knowledge of farmers, herd attendants and their livestock field officers. For the livestock field officers it is difficult to stay up-to-date when science is advancing. Farmers and herd attendants need to improve their level of knowledge, attitude and motivation towards udder health. The best way to update their knowledge and to motivate them will be education in groups. In addition, herd health programmes should be incorporated in our university and agricultural colleges' curricula. Such programmes should focus more on preventive than treatment skills as is the case now. Post-academic courses in udder health focusing on the design of a herd-level and herd specific approach, should be developed and offered to the farmers, attendants and livestock field officers (course design will depend on the target group).

Alternative to antibiotic treatment

The current practices of non-observance of drug withdrawal periods and incomplete dosing by the smallholder farmers necessitate for a search of an alternative mastitis therapy, which would leave no

undesirable residues in milk. Studies should also focus on: a) the options of reducing the use of antibiotics; b) the role of micro-organisms other than bacteria, in the epidemiology of bovine mastitis; c) resistance patterns of udder pathogens towards the commonly used antimicrobials.

The improvement of diagnosis,

The inflammatory and immune processes of the low yielding cows are not completely understood. This point must be underlined since SCC are the only large scale available screening tests to be used for control programmes, and since maximum levels are held as standards by the dairy industry or health officials. Progress in this field could be achieved by characterizing bacteriologically negative quarters with high and low SCC, at different lactation stages, from cytological, biochemical and histopathological points of view.

Risk factors and economic studies

Risk factor studies are needed to evaluate different strategies for bovine mastitis control. Important tools for risk factor studies of bovine mastitis are knowledge about persistence of the udder pathogens, the characteristics of diagnostic tests involved, and the (within and between herds) transmission characteristics of the udder pathogens. On the basis of risk factor studies, a national-wide prevention and control programme can be developed and evaluated. No studies are available to estimate the total costs of mastitis (prevention, subclinical and clinical) in smallholder dairy herds. The economic relationship between cost of prevention measures and revenues of udder health programmes should also be studied. It is acknowledged that the five-point mastitis control plan may increase the incidence of environmental udder pathogens. To make further developments in mastitis control possible, a continuous and critical analysis of the results of udder health programmes is essential.

References

Abdelrahim, A. I., Shommein, A. I., Suliman, H. B. and Shaddad, S. A. I., 1989. Prevalence of mastitis in imported Friesian cows in Sudan. Review of Production and Veterinary Medicine for Tropical Countries. **42**: 512-514

Albenzio M., Taibi L., Caroprese M., De Rosa G., Muscio A., Sevi A., 2003. Immune response, udder health and productive traits of machine milked and suckling ewes, Small Ruminant Research. **48**: 189-200.

Albenzio M., Taibi L., Muscio A., Sevi A., 2002. Prevalence and etiology of subclinical mastitis in intensively managed flocks and related changes in the yield and quality of ewe milk, Small Ruminant Research. **43**: 219-226.

Ameh J.A., Tari I.S., 1999. Observations on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri, Small Ruminant Researc. **35**: 1-5.

Bartlett, P. C., G. Y. Miller, S. E. Lance, D. D. Hancock, and L. E. Heider, 1992: Managerial risk factors of intramammary infection with *Streptococcus agalactiae* in dairy herds in Ohio. American Journal of Veterinary Research. **53**: 1715-1721.

Bergonier D., Lagriffoul G., Berthelot X., Barillet F., 1996. Facteurs de variation non infectieux des comptages de cellules somatiques chez les ovins et les caprins laitiers, in: Rubino R. (Ed.), Proceedings of Somatic cells and milk of Small Ruminants, International Symposium, Bella, Italy, Wageningen Pers, Netherlands, pp. 113–135.

Berriatua E., Ziluaga I., Miguel Virto C., Uribarren P., Juste R., Laevens S., Vandamme P., Govan J.R.W., 2001. Outbreak of subclinical mastitis in a flock of dairy sheep associated with *Burkholderia cepacia* complex infection, Journal of Clinical Microbiology. **39**: 990-994.

Blowey, R. and P. Edmondson. 2000. Mastitis control in dairy herds, Farming press books, Ipswich, UK.

Boscos C., Stefanakis A., Alexopoulos C., Samartzi F., 1996. Prevalence of subclinical mastitis and influence of breed, parity, stage of lactation and mammary bacteriological status on Coulter Counter Counts and California Mastitis Test in the milk of Saanen and autochthonous Greek goats, Small Ruminant Research. **21**: 139-147.

Bradley, A.J. 2002. Bovine mastitis: An evolving disease. The Veterinary

Journal. **164:** 116-128

Burriel A.R., 1997. Dynamics of intramammary infection in the sheep caused by coagulasenegative staphylococci and its influence on udder tissue and milk composition, Veterinary Record. **140**: 419-423.

Burriel A.R., 1997. Dynamics of intramammary infection in the sheep caused by coagulasenegative staphylococci and its influence on udder tissue and milk composition, Veterinary Record **140**: 419-423.

Burriel A.R., 1998. Isolation of coagulase-negative staphylococci from the milk and environment of sheep, Journal of Dairy Research. **65**: 139-142.

Charffeddine N., Alenda R., Carabano M.J., 1997. Genetic parameters for somatic cell score within first lactation, and across lactations in Spannish Holstein-Frisian cattle, p32 in: Proc. 48th Ann. Meet. Eur. Association of Animal Production, Vienna, Austria.

Chassagne, M., J. Barnouin, and J. P. Chacornac, 1998: Biological predictors for early clinical mastitis occurrence in Holstein cows under field conditions in France. Preventive Veterinary Medicine. **35**: 29-38.

density on ewes' milk yield, udder health and microenvironment, Journal of Dairy Research **66**: 489-499.

Dohoo, I. R., and A. H. Meek, 1982: Somatic cell counts in bovine milk. Canadian Veterinary Journal **23**: 119-125.

Dopfer, D., Barkema, H. W., Lam, T. J. G. M., Schukken, Y. H., Gaastra, W. 1999. Recurrent clinical mastitis caused by Escherichia coli in dairy cows**.** Journal of Dairy Science **82:** 80-85

Emanuelson, U and Fuke, H., 1991. Effect of milk yield on relationship between bulk milk somatic cell count and prevalence of mastitis. Journal of Dairy Science. **74**(**8**):2479-83.

Esnal A., Romeo M., Extramiana B., Gonzalez L., Marco J.C., 1994. Mamitis en la oveja Latxa: eficacia del tratamiento y dinámica de infección durante el período seco, in: XIX Jornadas Científicas Sociedad Española Ovinotechnica Caprinotechnica, Burgos, España,

Fthenakis G.C., 1996. Use of somatic cell counts or of indirect tests in milk for the diagnosis of subclinical mastitis in ewes, in: Rubino R. (Ed.), Proceedings of Somatic cells and milk of Small Ruminants, International Symposium, Bella, Italy, Wageningen Pers, The Netherlands, pp. 27–29.

Haenlein G.F.W., 2002. Relationship of somatic cell counts in goat milk

to mastitis and productivity, Small Ruminant Research. **45**: 163-178.

Hogan, J. S., K. L. Smith, D. A. Todhunter, P. S. Shoenberger, R. D. Disnmore, M. R. Cantell, and C. S. Gabel, 1994: Efficacy of dry cow therapy and a *Propionibacterium acnes* products in herds with low somatic cell count. Journal of Dairy Research. **77**: 3331-3337.

Hogan. S. J., Gonzalez R. N., Harmon, J. R., Nickerson, S.C., Oliver, S. P., Pankey,.J. W. and Smith, L. K. 1999. Laboratory Handbook on Bovine Mastitis. Published by National Mastitis Council, Inc., W D Hoard, Fort Atkinson, USA.

Jayarao B. M., Gillespie B. E., Lewis M. J., Dolen H. H., Oliver S. P., 1999. Epidemiology of *Streptococcus uberis* intramammary infections in a dairy herd. Zentralbl Veterinarmed B. **46**(**7**):433-42.

Kapaga.A.M., Weinhaupl.I. and Baumann, M.P.O. 1995. Risk Factors and Mastitis Prevalence in Dairy Cattle in The Region of Dar Er Salaam- Tanzania. Livestock Production & Diseases. Proceedings of The 8th Conference, Institute of Tropical Veterinary Medicine. Berlin.Germany.

Karimuribo, E., Fitzpatrick, J.L.; Bell, C.E.; Swai, E.S.; Kambarage, D.M.; Ogden, N.H. & French, N.P. 2003. Mastitis in smallholder dairy farms in Tanzania: From risk to intervention and knowledge transfer. In: Reid, S.W.J. & Menzies, F.D. (Editors). Proceedings of Society for Veterinary Epidemiology and Preventive Medicine held at Warwick, UK, 31st March-2nd, April, 2003: pp 83-94.

Kivaria, F. M., Noordhuizen, J. P. T. M., Kapaga, A. M., 2004. Risk Indicators Associated with Subclinical Mastitis in Smallholder Dairy Cows in Tanzania. Tropical Animal Health and Production, **36**:581- 592

Lam, T. J., J. H. Van Vliet, and Y. H. Schukken, 1995. Udder disinfection and mastitis in cattle: a literature review. Tijdschrift v. Diergen. **120**: 392–399.

Mdegela, R. H., L. J. M. Kusiluka., A. M. Kapaga., E. D. Karimuribo., F. M. Turuka., A. Bundala., F. Kivaria., B. Kabula., A. Manjurano., T. Loken., D. M. Kambarage., 2004. Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in eastern Tanzania. Journal of Veterinary Medicine B. **51**: 123–128.

Ministry of Agriculture and Food security (MOAF). 2006. National sample census of agriculture 2002/2003; Smallholder agriculture. Volume III: Livestock sector-national report. Central Printing

Works (Ltd), Dar es Salaam.

Mulei C.M. 1999. Teat lesions and their relationship to intramammary infections on small-scale dairy farms in Kiambu district in Kenya. Journal of the south African Veterinary Association. **70** (**4**): 156-157

Omore. A.O., J.J. McDermott., S.M. Arimi., M.N. Kyule., and D. Ouma., 1996. A longitudinal study of milk somatic cell counts and bacterial culture from cows on smallholder dairy farms in Kiambu district, Kenya. Preventive Veterinary Medicine. **29**: 77-89

Perez V., Corpa J.M., Garcia Marin J.F., Aduriz J.J., Jensen H.E., 1998. Mammary and systemic aspergillosis in dairy sheep, Veterinary pathology **35**: 235–240.

Quinn, P.J., Carter, M. E., Markey, B. K. and Carter, G.R. 2000. Clinical veterinary microbiology. London, Mosby-year book Europe limited, pp. 120-121

Radostits, O. M., 2001. Herd Health: Food Animal Production Medicine, 3rd edition. Philadelphia, W. B. Saunders.

Radostits. O.M., Gay. C. C., Blood, D.C., Hinchcliff. K. W. 2000. Veterinary Medicine; A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edition. pp. 603-660. W. B. Saunders. London.

Schukken. Y.H., T. J. Lam., M. Nielen., H. Hogeveen., H., W. Barkema., and F. J. Grommers. 1995. Subclinical mastitis on dairy farms in the Netherlands: epidemiological developments. Tijdschrift van Diergeneeskunde (in Dutch). **120:** 208-213

Scott M.J., Jones J.E., 1998. The carriage of *Pasteurella haemolytica* in sheep and its transfer between ewes and in relation to mastitis, Journal of Comparative Pathology **118**: 359–363.

Scott P.R., Murphy S., 1997. Outbreak of staphylococcal dermatitis in housed lactating Suffolk ewes, Veterinary Record. **140**: 631–632.

Sevi A., Massa S., Annicchiarico G., Dell'Aquila S., Muscio A., 1999. Effect of stocking

Sevi A., Taibi L., Albenzio M., Annicchiarico G., Muscio A., 2001. Airspace effects on the yield and quality of ewe milk, Journal of Dairy Research. **84**: 2632–2640.

Tuteja, F. F., M. P. Kapur, A. Sharma, and A. K. Vinajaka, 1993: Studies on bovine subclinical mastitis: Prevalence and microflora. Indian Veterinary Journal **70**: 787–791.

Walshe, M.J., Grindle, J., Nell, A. and Bachmann, M., 1991. Dairy development in sub sahara Africa: A study of issues and options. World bank Tech. Pap. No 135, pp 55. World Bank, Washington.

Winrock International., 1992. Assessment of Animal agriculture in sub-sahara Africa. Winrock International, Morrilton, AR.

Zadoks, R. N. 2003. Contagious and Environmental pathogens: from dichotomy to sliding scale. International Dairy Federation IDF, Mastitis newsletter N^{O} 25, Brussels, Belgium. pp 16-17.

Summary

Kivaria F. M.

Population Studies, Animal Diseases Research Institute
P. O. Box, 9254, Dar es Salaam, Tanzania
fredkiv@yahoo.com

Bovine mastitis is the most common and costly production disease affecting dairy cows. Literally mastitis is inflammation of mammary gland tissue. Mastitis is a major concern to dairy producers and the food industry because it affects milk quantity and quality, cow health, food safety and public health. Mastitis may be caused by biological, chemical and physical agents. This dissertation deals with mastitis caused by biological agents (bacteria, fungus and mycotic agents). The choice of control measures is often based on a classification of the udder pathogens as environmental or contagious. Environmental pathogens are present in the environment of the dairy cow, and the cow may be exposed to such pathogens at any time. Infected udders form the main reservoir of contagious pathogens, and exposure of cows to contagious pathogens is largely limited to the milking process. The aim of the research described in this thesis was to contribute to knowledge necessary to design herd-specific udder health control programmes for the smallholder producers in the Dar es Salaam region of Tanzania. To achieve this, a combination of field and laboratory methods (**Chapter 1**) to mastitis epidemiology is used.

In **Chapter 2**, the aetiology and temporal distribution of bovine clinical mastitis among the smallholder dairy cows are determined through a retrospective study, for the period November 1971-December 2002. Environmental udder pathogens were the predominant isolates, followed by the contagious and the miscellaneous (Candida species) udder pathogens. Contrarily to a steady increase of clinical *Candida albicans* mastitis, the prevalence of both contagious and environmental udder pathogens remained over the years above 10%. It was concluded that udder health in smallholder dairy herds is of sub-optimal standard.

In **Chapter 3**, a prevalence study involving 69 smallholder dairy herds and 230 dairy cows in the Dar es Salaam region was implemented to elucidate the prevalence and cause of subclinical intramammary infections (IMI), and the sensitivity of the isolated udder pathogens to the commonly used antimicrobials. The observed prevalence of clinical mastitis was 4.5 cases per 100 cows, whereas the prevalence of sub-clinical mastitis, defined by positive (≥ +1) CMT score or bacteriologically positive culture was 90.3% and 90.7% respectively. The most important micro-organisms detected from quarter samples were (in descending order of abundance) *Staphylococcus aureus*, *Streptococcus agalactiae*, *Candida albicans*, *Streptococcus pyogenes*, *Escherichia coli*,

Arcanobacterium pyogenes and *Pseudomonas aeruginosa*. A higher prevalence of antibiotic resistance was observed for the commonly used antimicrobials than for the newly introduced antimicrobials. It was concluded that udder health is poor and that intensive farmer education programmes are required to improve udder health in the smallholder dairy sector in the study area.

Information on the risk factors of bovine mastitis is of great importance as this is required for the design and implementation of appropriate prevention and control strategies. Chapters 4-to-6 deal with risk factors for subclinical (Chapter 4) and clinical (Chapters 5 and 6) mastitis. In **Chapter 4**, a questionnaire and CMT (California mastitis test) based cross-sectional study involving 182 lactating cows from 62 smallholder herds, was conducted between June and September 2002. The study objectives were to establish the prevalence of subclinical mastitis and the associated risk factors. Three field procedures (clinical, farm and farm-records inspection) based on the principles of herd health management were followed. Clinical inspection of the udders indicated that 3.8% and 90.3% of the cows had clinical and subclinical mastitis, respectively. The inspection of farm-records revealed a recorded annual prevalence of 62.4 cases of clinical mastitis per 100 lactating cows, during the previous 12 months. Subclinical mastitis was never detected, hence was never recorded before by either the farmer or the visiting livestock extension officer. Only 5% of dairymen were aware of the presence of subclinical mastitis. Farm inspection indicated that water scarcity, barn size, residual calf suckling, the use of single udder-towel and dairy-labourers to be the most significant risk indicators for subclinical mastitis.

In **Chapter 5**, a questionnaire based 18-months longitudinal study involving 317 cows on 87 smallholder dairy herds was conducted between July 2003-and- March 2005 with farm visits at an interval of 14 days. The objective was to elucidate the risk factors associated with the incidence rate of clinical mastitis among the smallholder dairy cows in the Dar es Salaam region. Over the study period, 937 new clinical mastitis episodes were recorded at the quarter level. This figure represented an incidence rate of 38.4 per 100 quarter-years at risk. The total number of clinical mastitis episodes at the cow level was 1472, representing an incidence rate of 43.3 per 100 cow-years at risk over the study period. The incidence rate of clinical mastitis was significantly associated with cow factors (body

condition score, parity, stage of lactation, and udder consistency), housing (floor type) conditions and milking (cow and udder preparation) practices.

Using the data set described in chapter 5, management related risk factors for the pathogen specific incidence rate of clinical *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*), *Candida albicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) mastitis were longitudinally studied in **Chapter 6**. The final multivariate Poisson regression models indicated that the incidence rate for clinical *S. aureus* and *S. agalactiae* mastitis was significantly related to residual calf suckling, lack of dry cow therapy, and hired herd labourers, respectively. Whereas, the incidence rate of clinical *C. albicans* and *E. coli* mastitis was significantly associated with gradual drying off, and dirty barn floors, respectively. Failure to use soap for hand preparation, leaving some teats for calf to suckle, use of udder towels, and water shortage were significantly associated with clinical mastitis caused by the four study pathogens. In view of the current results (Chapters 4-to-6) we can conclude that udder hygiene among the smallholder dairy herds is generally poor.

In addition to clinical mastitis, subclinical mastitis should be efficiently detected. Bacteriological sampling is not feasible as a routine test to identify subclinical mastitis, and indirect tests of mastitis are more suitable for selecting cows with intramammary infections for subsequent bacteriological sampling. Indicators of inflammation in the milk which can be determined using rapid, reliable and easy routine techniques can be used for the early detection of mastitis. The measuring of the somatic cell count (SCC) in milk is the standard method. At present, the only indirect mastitis test which can be used as the "cow side" test (by the smallholder producers) is the California Mastitis Test (CMT). And the limit for SCC of a healthy quarter is 500,000 cells mL^{-1} corresponding to a CMT score of ≥ +1. However, given the very high background level of somatic cells in low-yielding dairy cows, this threshold may not apply to low-yielding dairy cows. **Chapter 7** was undertaken to investigate the clinical utility of CMT for screening of *Staphylococcus aureus* subclinical mastitis in low-yielding smallholder dairy cows in Tanzania. Based on our results and practical considerations, it was concluded that CMT score of ≥ +2 is the best cut-off to reliably identify *Staphylococcus aureus* intramammary infections in low-yielding dairy cows in Tanzania.

Mastitis affects the quality of milk and can be a potential health risk to the consumers, particularly in the smallholder settings where informal milk markets and consumption of raw milk are common practices. In **Chapter 8**, the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region was evaluated in a cross-sectional study conducted between August-and-October 2003. A total of 128 milk samples and corresponding water samples were collected from randomly selected milk selling points in the study area. The mean TBC was 8.2×10^6 cfu $mL^{-1} \pm 1.9 \times 10^6$ cfu mL^{-1}, and major bacterial isolates from the milk samples were (in the descending order) *Escherichia coli, Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterobacter aerogenes* and *Enterococcus faecalis*. In most cases, the organisms identified in milk corresponded to those isolated from the corresponding water samples. 79% of milk samples were positive to the CMT and 7% were positive for antimicrobial residues. In a random effect model, water microbial quality, frequency of cleaning the milk containers, frequency of milk supply, milk storage time and the type of containers, and mixing of fresh and previous milk were significantly associated with the mean LogTBC. Whereas, in the fixed effect model, in addition to these indicators, water shortage, water source and the refrigerator condition were significantly associated with LogTBC. It was concluded that the milk sold in the Dar es Salaam region is of poor quality and is of public health hazard significance.

The Tanzania urban and peri-urban dairying originated in the mid 80s as a source of extra income for the monthly salaried civil servants. However, over the years the sub-sector has proved to be useful to the town dwellers. Some case studies have shown that urban dairying contributes as much as 60% to the total household income. The dairy sub-sector also offers employment for the household and hired labour, food security and spreading of risks. Thus the 1997 national agriculture policy support smallholder dairying as a key development pathway for creating employment, catalysing agricultural development and reducing the growing deficits in domestic demand for milk partially influenced by urbanisation. Despite its significance, the urban and peri-urban smallholder dairy sector is faced with important technical and socio-economic constraints. **Chapter 9** was conducted to explore the prospects and constraints of the smallholder dairy sector, and to suggest possible solutions for the identified constraints. The study revealed that the sector has opened up a number of supporting businesses, creating primary

(family members and hired labourers) and secondary (veterinary-inputs and animal feed stockists) employment for the people engaged in it. But, the sector's potentials are limited by a number of technical and socio-economic constraints. Which among many others, include the general lack of awareness of mastitis. For instance, only 5% of the producers were aware of subclinical mastitis. It is indicated that the system would have a future if these technical, and socio-economic constraints are removed through research and technology transfer with appropriate political support.

In **Chapter 10** the results from the preceding chapters are discussed in relation to cow factors and management. Finally some recommendations for future research are given.

Samenvatting

F. M. Kivaria

Population Studies, Animal Diseases Research Institute
P. O. Box, 9254, Dar es Salaam, Tanzania
fredkiv@yahoo.com

Mastitis is de meest voorkomende en kostbare productie-aandoening van melkvee. Letterlijk betekent mastitis ontsteking van het uierweefsel. Mastitis is een grote zorg voor melkveehouders en de zuivelindustrie, omdat het de melkhoeveelheid en melkkwaliteit aantast, de diergezondheid en het welzijn, voedselveiligheid en volksgezondheid. Mastitis kan worden veroorzaakt door biologische, chemische en fysische agentia. Dit proefschrift handelt over mastitis veroorzaakt door biologische agentia (bacterieen, fungi en mycotische agentia). De keuze van beheersmaatregelen is vaak gebaseerd op een classificatie van de uierpathogenen als omgevingsgebonden of besmettelijk.

Omgevingsgebonden pathogenen bevinden zich in de omgeving van de koe, en de koe kan te allen tijde worden blootgesteld aan zulke kiemen. Geinfecteerde uiers vormen het belangrijkste reservoir voor besmettelijke pathogenen, en blootstelling van koeien aan deze kiemen is met name beperkt tot het melkwinningsproces. Doelstelling van het onderhavige onderzoek is bij te dragen aan de kennis die nodig is om een bedrijfsspecifiek uiergezondheidsprogramma voor kleinschalige veeteelt in het Dar es Salaam gebied te kunnen ontwerpen. Om dit te bereiken is een combinatie van veldwerk en laboratoriummethodieken binnen de epidemiologie van mastitis gevolgd (**Hoofdstuk 1**).

In **Hoofdstuk 2** werden de etiologie en verdeling in de tijd van klinische rundermastitiden op kleinschalige melkveebedrijven bepaald middels een retro-spectieve studie van november 1971 tot december 2002. Omgevingspathogenen waren de meest voorkomende uierpathogenen, ge

volgd door de besmettelijke pathogenen, en tenslotte de menginfecties (Candida species). In tegenstelling tot een gestage toename van klinische *Candida albicans* mastitiden, bleef de prevalentie van zowel besmettelijke als omgevingspathogenen over de jaren heen boven de 10%. De conclusie was dat uiergezondheid op kleinschalige melkveebedrijven op een suboptimaal niveau verkeert.

Een prevalentiestudie met 69 kleinschalige melkveebedrijven en 230 koeien in de Dar es Salaam regio werd uitgevoerd om de prevalentie en oorzaak van subklinische uierinfecties (IMI) vast te stellen, alsmede de gevoeligheid van de geisoleerde pathogenen voor de meest voorkomende antimicrobiele middelen (**Hoofdstuk 3**). De waargenomen prevalentie van klinische mastitis was 4.5 gevallen per 100 koeien, terwijl die voor subklinische mastitis, zoals vastgesteld middels een CMT score > +1 of

een bacteriologische positieve uitslag, 90.3% en 90.7% was. De meest belangrijke micro-organismen uit kwartiermelkmonsters waren (in afnemende volgorde) *Staphylococcus aureus*, *Streptococcus agalactiae, Candida albicans, Streptococcus pyogenes, Eschericia coli, Arcanabacterium pyogenes* en *Pseudomonas aeruginosa*. Een hogere prevalentie van antibiotica-resistentie werd gevonden voor de meest voorkomende antimicrobiele middelen ten opzichte van de recentelijk geintroduceerde middelen. De conclusie was dat de uiergezondheid matig is en dat intensieve trainings- en coachingsprogramma's voor kleinschalige boeren nodig zijn om de uiergezondheid te verbeteren in het onderzoeksgebied.

Informatie over de risicofactoren van mastitis is van groot belang, daar dit noodzakelijk is voor het juiste ontwerp en de juiste uitvoering van preventieve en op beheersing gerichte uiergezondheidsprogramma's. De Hoofdstukken 4 tot 6 handelen over de risicofactoren voor subklinische (**Hoofdstuk 4**) en klinische mastitiden (**Hoofdstukken 5 en 6**). In Hoofdstuk 4 wordt een op een enquete en CMT (California Mastitis Test) gebaseerd dwarsdoorsnede-onderzoek gepresenteerd, waarin 182 lacterende koeien van 62 kleinschalige bedrijven deelnamen tussen juni en september 2002. De onderzoeksdoelen waren de prevalentie van subklinische mastitis vast te stellen, alsmede de daaraan geassocieerde risicofactoren. Drie veldprocedures (klinische inspectie; bedrijfsinspectie en inspectie van administratie) zoals uitgevoerd in het kader van veterinaire bedrijfsadvisering werden hiertoe gehanteerd. De klinische inspectie van de uiers wees erop dat 3.3% en 90.3% van de koeien een klinische respectievelijk subklinische mastitis hadden. De inspectie van de bedrijfsadministratie duidde op een prevalentie van 62.4 gevallen van klinische mastitis per 100 lacterende koeien gedurende de voorafgaande 12 maanden. Subklinische mastitis was tevoren nooit waargenomen, dus ook niet genoteerd door noch de boer noch de veterinair technicus. Slechts 5% van de boeren gaf aan dat ze wisten van het bestaan van subklinische mastitis. Bedrijfsinspectie duidde op gebrek aan water, stalafmetingen, nazuigen van uier door kalveren, gebruik van een enkele uierdoek en bedrijfsarbeiders als meest belangrijke risicofactoren voor subklinische mastitis.

In **Hoofdstuk 5** is een op een enquete gebaseerde, 18 maanden durende longitudinale studie met 317 koeien van 87 kleinschalige bedrijven beschreven die liep van juli 2003 tot maart 2005, met bedrijfsbezoeken elke 14 dagen. Het onderzoeksdoel was de risicofactoren voor de

incidentie van klinische mastitis op kleinschalige melkvee-bedrijven in de Dar es Salaam omgeving vast te stellen. Tijdens de onderzoeksperiode zijn 937 nieuwe klinische mastitiden op kwartierniveau vastgesteld. Dit cijfer stelt een *incidence rate* voor van 38.4 per 100 kwartier-jaren at risk. Het totaal aantal klinische mastitis episoden op koeniveau in de onderzoeksperiode was 1472, oftewel een *incidence rate* van 43.3 per 100 koe-jaren at risk. Deze *incidence rate* was significant geassocieerd met koefactoren (lichaamsconditie; pariteit; lactatiestadium; uierconsistentie), huisvestingsfactoren (vloertype) en melkwinning (koe en uiervoorbereiding).

Met gebruikmaking van de dataset uit hoofdstuk 5 werden aan management gerelateerde risicofactoren voor de pathogeen-specifieke incidentie van klinische mastitis t.g.v. *S. aureus, Str. Agalactiae, Candida albicans*, en *E.coli* longitudinal onderzocht (**Hoofdstuk 6**). De uiteindelijke multivariate Poisson regressie modellen lieten zien dat de incidenties van klinische mastitis t.g.v. *S.aureus* en *Str. agalactiae* significant waren geassocieerd met nazuigen van de uier door kalveren, ontbreken van droogzettherapie, ingehuurde arbeiders respectievelijk, terwijl de incidenties van klinische *C.albicans* en *E.coli* mastitis significant waren geassocieerd met gradueel droogzetten en vuile stalvloeren respectievelijk. Het niet gebruiken van zeep om handen te reinigen, het aan kalveren overlaten van spenen om na te zuigen, het gebruik van uierdoeken, en gebrek aan water bleken significante factoren te zijn geassocieerd met klinische mastitis t.g.v. de vier in onderzoek betrokken pathogenen. In het licht van de verkregen resultaten (Hoofdstukken 4 tot 6) werd geconcludeerd dat de uierhygiene rondom het melken op kleinschalige melkveebedrijven in het algemeen matig is.

Behalve aan de detectie van klinische mastitis dient nadrukkelijk aandacht te worden geschonken aan de efficiente detectie van subklinische mastitis. Bemonsteren voor bacteriologisch onderzoek is als routine om subklinische mastitis vast te stellen niet uitvoerbaar, en indirecte testen op mastitis zijn meer geschikt om koeien met een IMI te selecteren voor nader bacteriologisch onderzoek. Indicatoren van ontsteking in de melk die kan worden vastgesteld middels snelle, betrouwbare en gemakkelijke routinetesten, kunnen worden benut voor de vroegdetectie van mastitis. Het bepalen van het somatische celgetal (SCC) in de melk is een standaardmethode. Vandaag de dag is de enige indirecte mastitistest die kan worden gebruikt als *cow-side test* door kleinschalige melkveehouders de CMT. De grens die is gesteld voor het

onderscheid tussen gezonde en zieke kwartieren bedraagt 500.000 cellen/ml, overeenkomend met een CMT uitslag van >+1. Echter, gegeven het hoge achtergrondniveau van somatische cellen in laagproductieve koeien, is deze grenswaarde mogelijk niet van toepassing op laagproductieve koeien. **Hoofdstuk 7** beschrijft het onderzoek naar het klinische nut en bruikbaarheid van de CMT als screenmethodiek voor *S.aureus* subklinische mastitiden in laagproductieve koeien van kleinschalige bedrijven in Tanzania. Gebaseerd op de onderzoeksresultaten en praktische overwegingen is de conclusie dat een CMT score van >+2 in de gegeven omstandigheden de beste grenswaarde is om betrouwbaar S.aureus IMI in laagproductieve koeien vast te stellen.

Mastitis tast de kwaliteit van de melk aan en kan een mogelijk gezondheidsrisico vormen voor de consument, in het bijzonder in het kader van de kleinschalige bedrijven waarbij informele circuits en consumptie van rauwe melk gangbaar zijn. In **Hoofdstuk 8** werd de hygienische kwaliteit en het daaraan verbonden potentiele volksgezondheidsgevaar van rauwe melk, verkocht door kleinschalige boeren in de Dar es Salaam regio, geevalueerd middels een dwarsdoorsende-onderzoek van augustus tot oktober 2003. In totaal werden 128 melkmonsters en bijbehorende watermonsters verzameld bij at random geselecteerde verkooppunten in het onderzoeksgebied. Het gemiddelde TBC (*totale bacterie count*) bedroeg 8.2×10^6 cfu/ml +-1.9×10^6 cfu/ml; belangrijkste bacteriologische isolaten waren (in afnemende volgorde van belangrijkheid): *E.coli, B. cereus, St aureus, Str agalactiae, Enterobacter aerogenes* en *Enterococcus faecalis*. In de meeste gevallencorrespondeerden de micro-organismen gevonden in de melkmonsters met die in de bijbehorende watermonsters. Van de melkmonsters was 79% CMT-positief en 7% was positief op antibioticum-residuen. In een random-effect model waren microbiele kwaliteit van het water, frequentie van het schoonspoelen van de melkcontainers, de frequentie van melkaanvoer, melkopslag-tijd, en type melkcontainer significant geassocieerd met het gemiddelde LogTBC. Bovendien bleken in een fixed-effect model ook nog water tekort, waterbron en de koelkast omstandigheden significant van invloed op de LogTBC te zijn. De conclusie was dat de melk die verkocht wordt in de Dar es Salaam regio van matige kwaliteit is en een volksgezondheidsgevaar in zich draagt.

De melkveehouderij rondom stedelijke gebieden in Tanzania dateert van midden tachtiger jaren, als een bron van aanvullende inkomsten voor de ambtenaren. Echter, in de loop van de tijd bewees deze subsector ook van nut te zijn voor andere mensen. Sommige case-studies hebben aangetoond dat melkveehouderij in en rondom stedelijke gebieden zoveel als 60% bijdraagt aan het totale huishoud-inkomen. Deze activiteit biedt ook werk aan leden van de huishouding en aan ingehuurd personeel. De nationale landbouwpolitiek ondersteunt sinds 1997 de kleinschalige veehouderij als een cruciale factor op het ontwikkelingspad middels het verschaffen van werk, het stimuleren van de landbouwontwikkeling, en het terugdringen van en beantwoorden aan de door verstedelijking toenemende vraag naar melk. Ondanks deze belangrijke rol wordt de kleinschalige veehouderij met essentiele technische en sociaal-economische beperkingen geconfronteerd. **Hoofdstuk 9** beschrijft het onderzoek naar de beperkingen en mogelijkheden van de kleinschalige veehouderij-sector; mogelijke oplossingsrichtingen worden aangegeven. Het onderzoek liet zien dat de sector een aantal ondersteunende ondernemingen heeft voortgebracht, die primair (familieleden en ingehuurd personeel) en secundair (inputs van veterinairen en nutritionisten) werk hebben gecreeerd. Maar de mogelijkheden voor de sector worden beperkt door een aantal technische en sociaal-economische beperkingen. Een daarvan betreft het algemene gebrek aan kennis en bewustzijn omtrent mastitis; zo was slechts 5% van de boeren zich ervan bewust dat subklinische mastitis ook zou kunnen voorkomen. Er zijn indicaties dat de kleinschalige veehouderij welzeker toekomst heeft indien deze technische en sociaal-economische beperkingen door middel van onderzoek en kennisoverdracht, met de noodzakelijke overheidssteun kunnen worden weggenomen.

In **Hoofdstuk 10** worden tenslotte de resultaten van de voorgaande hoofdstukken in relatie met koe-factoren en management nog eens belicht vanuit een totaaloverzicht. Dit hoofdstuk sluit af met een aantal suggesties voor toekomstig gewenst onderzoek.

Acknowledgements

Although I personally bear full responsibility for all the information in this thesis because they all have come from me and through me, a work of this magnitude with the responsibility to be so accurate could only have been done with a team of supportive co-workers who committed themselves to assist me by arduous labour with loving devotion and commitment to excellence. Many friends have participated in the team–all of whom deserve to be commanded and thanked. Painfully I confront the fact that I cannot acknowledge all individually by names. Necessarily, however, I have to be transparent about key facilitation without which this thesis would not have been a product for the public.

My highest gratitude belongs to my mentor and a friend, Professor Dr. Jos P. T. M. Noordhuizen. He gave me the opportunity and the support to pursue my scientific ideas and follies, wherever they took me. Jos had worked next to me through the whole project, labouring beyond anyone while serving as supervisor and editor. His exceptional gift for leadership, along with his vast knowledge of Veterinary Epidemiology and experience, coupled with our one-mindedness academically, plus his writing skills, have made for a most effective partnership.

Gratitude in abundance must be given to Professor Dr. Hans Heesterbeek for all the discussions, constructive criticism and help during the project. Hans you immensely shaped this thesis through thorough conceptualisation of scientific content and critique, I am a proud product of your refereeing.

Mrs Dr. Mirjam Nielen, I want to thank you for the long-term support and inspiration, for looking at things from the different angle. I immensely benefited from your vast experience in statistics, which ensured that the models were appropriately applied. I counted on your skills to ensure adherence to the technical details that go with serious thesis .

Thank you to Dr Ingrid Leemans, IFS-scientific programme coordinator. Ingrid you immensely shaped my first and second IFS applications for research funds through critical evaluation of scientific content and they both went through. And it is not all: through your many advises, I am an admirable disciple of that art of getting more from less.

Many thanks to my friend Jean De Gooijer of the "Bureau Internationale Contacten" (BIC) for overseeing the administrative,

financial affairs and my socio-welfare while I am in Utrecht. Jean you proved to be a friend.

The ADRI staff, painfully I am unable to mention you individually by names: Collectively and individually, you frequently gave me a push over many hurdles I had to go through to this end. Dr. Paul Mkonyi, you deserve special mention for your "Continous yelling", which were humorous with a therapy towards emptying my "filled recycle bin".

The Kinenekejo family, I counted on you most dearly. My family and friends are dearly thanked for their patience, support and advice during all these years. My brothers Frank Timothy Kinenekejo and Prof. Jumanne Abdallah Maghembe, and my sister Mary Kinenekejo I am fully aware that reaching this end would have been more difficulty for me without the timely support and encouragement you offered.

My mother's advice has always been very dear to me and will be remembered as a great gift. What my mother has done for me is unique and full of love, trust and confidence in life. I am very thankful for that gift as well and will try to pass it on.

A special word of thanks is certainly in order for my beloved friend and wife, Asinath, who assisted me with her prayers and encouragement, and endured my times of isolation. Asinath, if there is one person full of kindness, intelligence and warmth, who invests herself during work and off-work hours in order to get serious work combined with a personal life that is you. Thank you for your support and for being such an example of a wonderful wife.

Most especially, my thanks to my son Joshua and my daughter Margaret, you always had only new ways of supporting and encouraging me on, even during some difficulty moments we had to go through in our family so far. To you, I dedicate this thesis.

All the above mentioned individuals made this effort a joyous marathon for me to run. I pray God's blessing on them all in response to their devotion to this work, and HE THAT GLORIETH, LET HIM GLORY IN THE LORD.

Fredrick Mathias Kivaria

Curriculum vitae

Biography

Fredrick M. Kivaria was born on March 16, 1968 in Mwanga district of Kilimanjaro region, Tanzania. He attended primary and secondary schools in Dar es Salaam region, between 1974 and 1987. He studied for Diploma in Animal Production between 1988 and 1990 at Uyole Agriculture College in Mbeya region. Between June 1990 and July 1991, he worked with the Ministry of Agriculture at Uyole Agriculture Centre as a tutorial assistant; teaching sheep and goat husbandry to students and farmers. In September 1991 he joined the Faculty of Veterinary Medicine of the Sokoine University of Agriculture for a BVSc, which he completed in November 1996. After his BVSc, he rejoined the Ministry of Agriculture as a Veterinary Research Officer at the Animal Diseases Research Institute. He was awarded in 1998 a fellowship by The Netherlands University Fellowship Programme to study Veterinary Epidemiology and Herd Health Economics between September 1998-April 2000 at Utrecht University. His MSc thesis was entitled "Endemic stability for *Theileria parva* infections in Ankole calves of the Ankole ranching scheme, Uganda". From 2002 to 2006, he was awarded a research grant by the International Foundation for Science (IFS), the Dutch Academy for Science (KNAW) and the Tanzanian Government to undertake studies on bovine herd health, epidemiology, and udder health in urban and peri-urban based smallholder dairy herds. The research described here is part of these studies, and it was undertaken in collaboration with the Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands. He will continue with his epidemiological studies at the Animal Diseases Research Institute, but focusing more on livestock and rural development as his contribution in fighting rural poverty.

List of related publications

Publications

F. M. Kivaria and A. M. Kapaga (2002). Review of current problems and shortcomings in the Tanzanian animal health information system with suggestions on improvement. Onderstepoort Journal of Veterinary Research, 69: 305-314

F. M. Kivaria (2003). Foot and mouth disease in Tanzania: An overview of its national status. Veterinary Quarterly 25 (2): 72-78

R. H. Mdegela, L. J. M. Kusiluka, A. M. Kapaga, E. D. Karimuribo, F. M. Turuka, A. Bundala, **F. M. Kivaria**, B. Kabula, A. Manjurano, T. Loken, D. M. Kambarage (2004). Prevalence and Determinants of Mastitis and Milk-borne Zoonoses in Smallholder Dairy Farming Sector in Kibaha and Morogoro Districts in Eastern Tanzania. Journal of Veterinary Medicine Series B 51, 123-128

F.M. Kivaria, C. Heuer, F. Jongejan, J. Okello-onen, T. Rutagwenda, F. Unger and W. Boehle (2004). Endemic stability for Theileria parva infections in Ankole calves of the Ankole ranching scheme, Uganda. Onderstepoort Journal of Veterinary Research, 71: 189 – 195

F. M. Kivaria., J. P. T. M. Noordhuizen., A. M. Kapaga (2005). Risk indicators associated with Staphylococcus aureus subclinical mastitis in smallholder dairy cows in Tanzania. In. H. Hogeveen (editor) Mastitis in Dairy production; current knowledge and future solutions. Wageningen Academic Publishers, Wageningen, The Netherlands.

F. M. Kivaria., M. R. Ruheta., P. A. Mkonyi., P. C. Malamsha. (2005). Epidemiological aspects and economic impact of bovine theileriosis and its control: a preliminary
assessment with special reference to Kibaha district, Tanzania. The Veterinary Journal. doi: 10.1016/j.tvjl.2005.08.013

F. M. Kivaria (2006). Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Tropical Animal Health and Production. 38: 291-299

Articles in Newsletters

E. D. Karimuribo, A. M. Kapaga., L. J. M. Kusiluka., R. H. Mdegela., F. M. Turuka., A. Bundala., **F. M. Kivaria** and D. M. Kambarage. (2003). Quality of milk and hygiene of containers on smallholder dairy farms in Kibaha and Morogoro districts, Scientific papers

presented for the workshop on collaborative research for food security. Research Abstracts, No 2; TARP II SUA Project

F. M. Kivaria, (2004) An investigation on cattle morbidity and mortality attributed to theileriosis in Lugoba ward of Bagamoyo district, Tanzania, Animal Health News Letter, A quarterly Newsletter of the Ministry of Water and Livestock development, Tanzania. No 9, October-December 2004

F. M. Kivaria, (2005) Veterinary epidemiology, animal health and international trade: challenges and opportunities, Animal Health News Letter, A quarterly Newsletter of the Ministry of Water and Livestock development, Tanzania. No 10, January-March 2005

F. M. Kivaria, (2005) The traditional Maasai animal identification practices and their role in 'traceability', Animal Health News Letter, A quarterly Newsletter of the Ministry of Water and Livestock development, Tanzania. No11, April-June 2005

F. M. Kivaria, (2005) The role of traceability of livestock and livestock products in public health and global trade, Animal Health News Letter, A quarterly Newsletter of the Ministry of Water and Livestock development, Tanzania. No 11, April-June 2005

F. M. Kivaria, (2005) Commercialisation and delivery of animal health services in Tanzania: New options for old problems. Animal Health News Letter, A quarterly Newsletter of the Ministry of Water and Livestock development, Tanzania. No12, September-December 2005

F. M. Kivaria, (2006) Pastoral coping mechanisms to drought and floods. Animal Health News Letter, A quarterly Newsletter of the Ministry of Water and Livestock development, Tanzania. No13, January-March 2006.