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Chapter

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

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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).

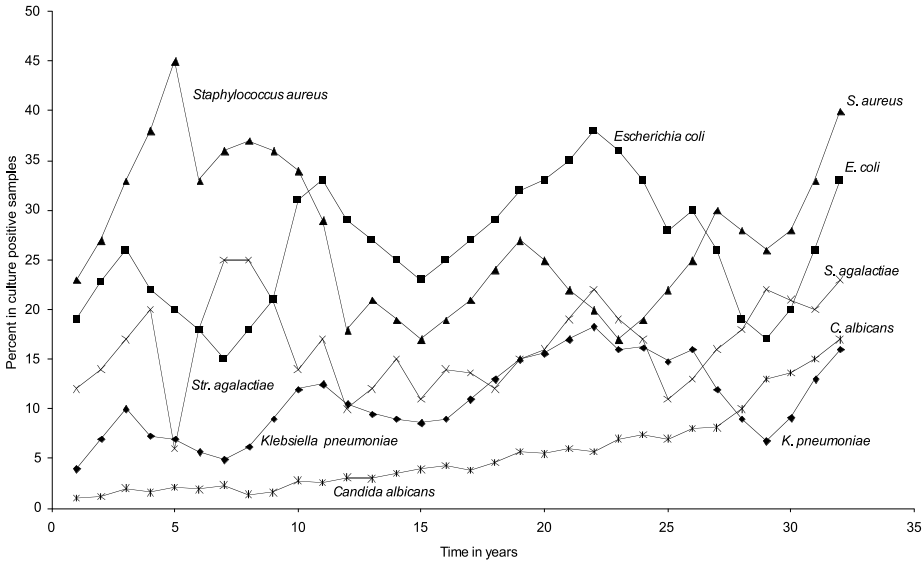
Eighteen per cent (354/1964) of the samples came from 186 cows in 31 smallholder dairy herds in the Ilala district, currently estimated to have 196 smallholder dairy herds with 615 dairy cows (MOAC, 2001); 236 (12%) of the 1964 quarter milk samples from the smallholder dairy herds were negative, while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

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