Incidence, Causative Agents and Strategy of Control of Mastitis Among Smallholder Dairy Herds in Morogoro, Tanzania

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Abstract

A study on the incidence, causative agents and strategy for control of mastitis was conducted on thirty urban and peri-urban small holder dairy farms in Morogoro. California Mastitis Test (CMT) was used in screening for mastitis. A total of 125 lactating cows were screened. The CMT positive quarter milk samples were taken for bacteriological examination and Microscopic Somatic Cell Count (MSCC). Incidence of clinical mastitis was 2.4% while that of subclinical mastitis was 60%. Infectious mastitis accounted for 63% of CMT positive cases. Major causative agents of infectious mastitis were ***Staphylococcus aureus*** *(17%) and* ***Streptococcus spp.*** *(14.5%). Non infectious mastitis accounted for 37% of all CMT positive udder quarters. A trial conducted on the different farm holdings and involving 40 cows in their last trimester was done to evaluate the use of dry cow therapy with or without accompanying subsequent use of pre- and post-milking udder sanitization with an iodophor disinfectant. Both Dry Cow Therapy (DCT) followed by routine udder hygiene (DCT-RH) treatment or full milking hygiene (DCT-FH) had significant effect (P=0.05) on reducing the infection rate which decreased gradually after calving. There was total elimination of* ***Staphylococcus spp.*** *whereas* ***Streptococcus spp.*** *were reduced by 67% within 8 weeks post calving. The effect of No Dry Cow Therapy followed by full milking hygiene alone (NDCT + FH) was not statistically significant but there was a 66% reduction in the proportion of infected quarters over a period of eight weeks. The study demonstrated that smallholder dairy farmers could achieve significant reduction of mastitis cases in their dairy herds through use of a combination of dry cow therapy and use of pre- and post-milking teat dip.*

Keywords: Mastitis, Incidence, Causative agents, Morogoro, Tanzania

Introduction

Worldwide, about 50% of the dairy cows in a farm have some form of mastitis (McDonald 1979). Experiences from large scale dairy farms show that mastitis is also common in Tanzania. Studies conducted in large scale dairy farms in Morogoro, Dar es Salaam, Arusha, Moshi,Mbeya and Iringa regions showed that the average annual incidence of clinical mastitis was between 2.2% and 2.8% while the incidence for subclinical mastitis was between 40% and 71.6% (Kinabo and Assey, 1983; Msanga *et al* 1989; Mahlau and Hyera. 1984). Furthermore, the commonest bacteria isolated were *Staphylococcus aureus* and *Streptococcus spp.* (Kinabo and Assey, 1983; Msanga *et al* 1989).

There is apparently no information on incidence rates of mastitis in the small holder dairy farms in Tanzania. Most of these farmers have little knowledge on dairy husbandry and thus the economic significance of mastitis on their dairy enterprises (Mchau, 1995). Hence, the extent of mastitis as a problem in small holder dairy farms in Tanzania, is not well known. This is ironical, bearing in mind that smallholder dairy farmers own about 60% of the es-

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timated 300,000 herd of dairy cattle in the country (Mtumwa and Mwasha, 1995). This study was therefore conducted in order to reveal the significance and establish the actual level of mastitis among smallholder dairy farms. Secondly, the study sought to evaluate the prophylactic effect of use of dry cow therapy alone or in combination with subsequent use of disinfectant udder wash and post milking teat dip on prevention of mastitis infection of dairy cows under smallholder farmer own management.

Materials and Methods

Animals and their management

Animals used in the study were lactating cows of the Friesian, Jersey and Ayrshire crossbred with indigenous zebu from 30 smallholder dairy farms. The animals were generally grazed with little supplementation with cut grass or concentrate. In addition, lactating cows were supplemented at the time of milking and they were all hand milked. While most of the farmers practised pre-milking teat sanitization with warm water only, none of the farmers practised either post-milking teat dipping in a sanitizing solution or dry cow therapy at drying off.

Study 1: Baseline screening for mastitis

In order to reveal the actual level and significance of the mastitis problem, all 30 smallholder dairy farms were visited and animals were screened for mastitis by using California Mastitis Test (CMT), (Schalm 1960). Milk samples were submitted to the Veterinary Microbiology laboratory for bacteriological examination and/or Microscopic Somatic Cell Counts (MSCC).

Sampling procedure

Milk samples were obtained at the morning milking between 06.00 and 07.30 a.m. Hands were washed with the Iosan$^{(R)}$ (CIBA-GEIGY Ltd. Co. Basle, Switzerland), an iodophor disinfectant solution, before handling the udder. The udders were then washed with 0.5% Iosan$^{(R)}$ disinfectant solution, and thoroughly dried with another clean piece of cloth. Teats were disinfected by 1% Iosan$^{(R)}$ solution. First milk was examined on a strip cup for visual evidence of clinical mastitis. Teat ends were cleaned thoroughly with cotton swabs soaked in 70% ethanol. A separate alcohol swab was used for each teat.

Approximately 10 ml of fore milk was collected from each quarter in a sterile universal sample bottle. California Mastitis Test (Schalm, 1960) was then performed on a portion of milk from each quarter. The rest of the milk samples were stored on ice and transported to the laboratory for additional tests of Microscopic Somatic Cell Count. Another portion was submitted for bacteriological culture within two to three hours post collection.

California Mastitis Test (CMT) Procedure

The California Mastitis test was carried out according to a procedure described by Marth (1978).

Bacteriological examination of milk samples

The CMT positive samples (+1 and above) were examined according to standard bacteriological procedures (IDF 1981). A sample (0.05 ml) was streaked onto one half of blood agar (BA) plate using a sterile wire loop of 6 mm. The blood agar was prepared according to manufacturers instructions (Oxoid Ltd. Basingstoke Hampshire, England). An additional sample (0.05 ml), was streaked on one half of MacConkey agar plate (Oxoid Ltd. Basingstock Hunts, England) to aid detection of coliform bacteria as recommended by Smith *et al.* (1982). Both plates were incubated at 37° C and growth recorded after 24 hrs and 48 hrs. Growth on primary culture media was identified tentatively by colony morphology and haemolytic characteristics. Additional routine biochemical tests were used to identify isolates according to IDF (1981).

Direct microscopic leucocyte count

Milk leucocyte counts were done on all CMT positive samples (+1 and above). The direct leucocyte count was carried out as de-

scribed by IDF (1979) with the exception that the stain used was a modified Newman Lampart Stain. This modified stain was prepared by mixing 54 ml of 95% ethanol and 40 ml of trichloroethane instead of tetrachloroethane in a 500 ml flask. The rest of the procedure was as described by IDF (1979).

Study 2: Dry cow therapy and full udder hygiene

Dry cow therapy (DCT) treatment

The study was conducted parallel to the baseline study 1. Forty cows in their last trimester were selected randomly from a pooled list of cows of different farm households that had been confirmed pregnant by rectal palpation. The animals were divided randomly as they calved into two groups namely, the control (n = 20) and the experimental, Dry Cow therapy group (n = 20). At the time of drying off, the experimental cows were given a long acting antibiotic infusion (cloxacillin benzathin (Phenix Pharmaceuticals-Netherlands), with one injector into each quarter once.

Milk samples were taken religiously from all the 40 cows at the time of drying off and every two weeks after parturition for a period of two months and tested for mastitis by the CMT. Occurrence of subclinical mastitis was recorded and classified according to CMT findings.

Full udder hygiene (FH) sub-treatment

This part of the trial was carried out parallel to the dry cow therapy study. The two groups from the dry cow therapy study were further sub-divided into full hygiene (i.e. pre-and post milking teat disinfection) and routine hygiene (pre-milking udder wash with warm water only). Iosan(R) was used as pre-milking udder wash and post milking teat dip at the concentration of 0.5% and 3%, respectively. CMT and bacteriological examination of milk samples from all 40 cows were performed every two weeks for two months.

Statistical analysis

The baseline data (study 1) was analysed using SPSS statistical package to obtain means and pertcentages.The data obtained and derived variables from Study 2 were subjected to analysis of variance (ANOVA) for a two factorial design experiment using methods described by Snedecor and Cochran (1980). The statistical model employed was:

$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$

Where:

Y_{ijk} = CMT recording taken for cow *k* under level *i* of dry cow therapy and level *j* of hygiene

μ = overall mean

A_i = the effect of level *i* of dry cow therapy

B_j = the effect of level *j* of hygiene

$(AB)_{ij}$ = the interaction of level *i* of dry cow therapy and level *j* of hygiene

e_{ijk} = the random error component specific for the cow *k* on dry cow therapy *i* and hygiene level *j*

Duncan's New Multiple Range Test (Snedecor and Cochran, 1980) was used to locate significant mean differences among treatment groups.

Results

Incidence of Mastitis

The results of mastitis screening are presented in Fig. 1. Out of 125 cows studied, three (2.4%) had clinical mastitis while 122 (97.6%) cows were apparently healthy. The apparently healthy cows had their quarter milk samples taken for California Mastitis Test (CMT) and results are presented in Table 1. Seventy five (60%) cows had positive CMT scores (i.e. +1 and above) reflecting high somatic cells in one or more quarters, an indication of presence of subclinical mastitis. On a quarter basis out of 485 quarters examined 159 (32.8%) had subclinical mastitis of which 100 (12.2%) were infectious. The rest (20.6%) which were CMT positive but yielded no bacteria isolates were classified as having non-specific mastitis .

The 159 CMT positive quarter samples were cultured on blood agar and bacteria were

isolated from one hundred (63%) samples (Table 1).

Common pathogens isolated were *Staphylococcus spp* (25.2%), *Streprococcus spp* (14.5%) and *Bacillus spp* (12.6%).

Effect of Dry Cow Therapy and Udder Hygiene

Results on udder infection rate of the experimental cows is shown in Table 2. There was a significant difference among treatments (P=0.05). At the 5% level, only Dry Cow Therapy treatment mean (highest) was significantly different from the control group mean (lowest, Table 2).

The effect of dry cow therapy on the CMT score was highly significant (P= 0.01, Table 2). There was a general improvement in the infection status of the udder as indicated by the change of CMT value from 4.2 to 4.5 (Table 2). However, a sudden increase of CMT value

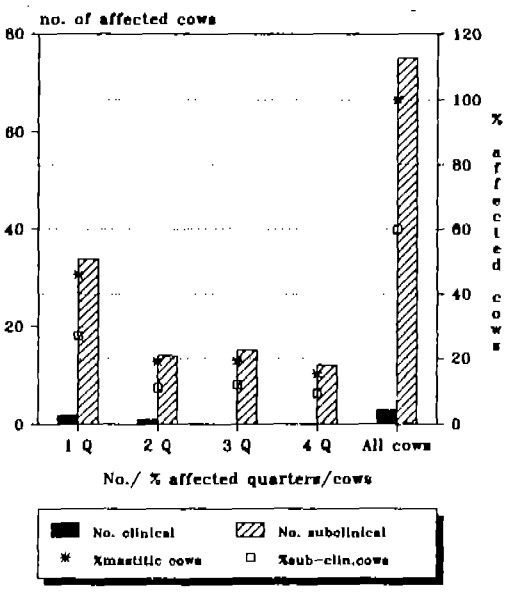

Figure 1: Frequency distribution of CMT +ve quarters

Table 1: Prevalence of bacteria isolates from CMT positive quarter milk samples of cows

Bacteria	No of samples	Proportion of bacteria +ve samples (%)	Proportion of total samples (%)
Staphylococcus spp (total)	40	40.0	25.2
Staphylococcus aureus	27	27.0	17.0
Other staphylococci - Coagulase -ve	13	13.0	8.2
Streptococcus (total)	23	23.0	14.5
- *alpha* - haemolytic	15	9.4	
- *betta* - haemolytic	2	1.3	
- non - haemolytic	6	3.8	
Escherichia spp	8	8.0	5.0
Bacillus spp	20	20.0	12.6
Others[2]	9	9.0	5.7
Total	100	100.0	63.0

Note:

[1]Total CMT positive samples submitted were 159

2Others include *Pseudomonas aeroghosa, Corynebacteria pyogenes and Proteus spp*

(4.2 to 4.83) was observed from day 0 (the beginning of experiment and drying off time) to 2 weeks (after calving) which decreased gradually to 4.5 at 8 weeks time.

Type of bacterial isolates are shown in Table 3. Dry Cow Therapy treatment eliminated Staphylococcal infections whereas Streptococcal infections were reduced by 67% (Table 3).

The level of *Staphylococcal* and *Streptococcal* infection at the beginning and end of the study was the same in the control group (Table 3). However, there were new infections and

some infections were eliminated during the study. The proportion of infected quarters decreased from 27.5% at day 0 to 7.5% at the end of the experiment in Dry Cow Therapy treatment but there was no change in the control group (Table 4).

Although there was an apparent improvement in the infection status as indicated by the increase of the CMT value from 4.1 to 4.2, the effect of "full hygiene" treatment was not significant (Table 5).

Udder infection status in the DCT-FH group improved gradually from 52.5% to 30% CMT positive cases at the end of the 8 week period. The proportion of bacteria infected quarters decreased from 30% at day 0 to 10% during the same period.

Results of SCC taken to collate the results of CMT value are presented in Table 4 together with the prevalence of bacteria isolates. There was a general decrease in MSCC in all groups. The change in somatic cell counts were 28.5, 32.0, 18.6 and 5.8 % respectively for DCT-RH, NDCT-FH, DCT-FH and the control (NDCT-RH) groups . The corresponding improvement in CMT score was respectively, 6.7, 3.7, 15.9 and 10.8%.

Discussion

Incidence of mastitis among smallholder dairy herds

Whereas the incidence of clinical mastitis are discernible by the average farmer, most cases of subclinical mastitis go unnoticed, until they become clinical. In order to institute effective mastitis control measures and strategies at the farm level, the causes of this insiduous disease has to be elucidated for each particular environment.

The incidence of both clinical and sub-clinical mastits in the smallholder dairy herds was similar to the levels which have been reported for large scale dairy farms by other workers (Kinabo and Assey, 1983; Msanga *et al* 1989; Mahlau and Hyera. 1984) who reported incidences of 2.2 - 2.8 and 40% to 71.4 % for clinical and subclinical mastitis, respectively. Hamir *et al* (1978) reported similar incidences of clinical mastitis (2.3 - 3.0 %) in Kenya but the incidence of sub-clinical mastitis was slightly lower (48%). Results in this study have shown that about 21% of all udders tested had infectious mastitis. These results are higher than what has been reported in other countries such as Norway, 6.5%, (Bakken, 1981) and Britain, 9.6%, (Wilson and Richards (1980). This is not surprising given the fact that none of the farmers surveyed were practising recommended hand milking hygiene, especially the use of udder disinfectants.

Causative agents

Out of the 159 udder quarters diagnosed to have had mastitis, 37.1 % were apparently not due to bacterial infections. These could have been due to trauma caused by poor milking practices. Bacteria could be isolated from 63% of the CMT positive quarters (Table 1). The main bacterial agents were *Staphyloccocus* (25.2%) and *streptococci* (14.5%) and *Bacillus spp* (12.6%).

A high frequency of *Staphylococci* with *Staphylococcus aureus* constituting 17.0% and *Streptococcus spp* (14.5%) from quarter milk samples has also been observed by various workers (Hamir *et al* 1978; Mahlau and Hyera. 1984). Kinabo and Assey (1983) reported a frequency of 21% for *Staphylococcus aureus* while Mbise *et al* (1983) reported a frequency of 36.5% and 18.7% for *Staphylococcus aureus* and *Streptococcus spp* respectively. Similarly Msanga *et al* 1989 isolated *Staphylococcus aureus* from 20% of the milk samples collected from the Lake Zone at the Veterinary Investigation Centre (VIC) Mwanza. *Staphylococcus spp* was isolated from most of the clinical and subclinical cases of mastitis probably because it is commonly found on the skin and cuts, tick bites and the warm water used to wash udders. Similar to *Staphylococcus*, *Streptococci* reside in the cow's environment and cause mastitis where the hygiene is poor.

Effect of dry cow therapy and udder disinfection

To reduce the economic losses incurred by most farmers due to mastitis, subclinical cases have to be tackled on a routine basis through prophylactic measures. The effect of introduc-

Table 2: Mean udder infection status[1] of 40 experimental and control cows on the basis of CMT[2] score at the begining and during 8 weeks of the treatment

Treatment (n)		Sampling period (in weeks)					Treatment mean
		0[3]	2	4	6	8	
DCT-FH	Mean	3.8	4.3	4.5	4.4	4.5	4.29^{*ab}
(n=10)	± S.D.	(± 1.3)	(± 1.1)	(± 0.9)	(± 0.09)	(± 0.8)	
DCT-RH	Mean	4.2	4.8	4.7	4.4	4.5	4.53^{**b}
(n=10)	± S.D.	(± 0.1)	(± 0.5)	(± 0.6)	(± 0.9)	(± 0.8)	
NDCT-FH	Mean	4.1	4.1	4.2	4.2	4.2	$4.17NS^{ab}$
(n=10)	± S.D.	(± 1.2)	(± 1.2)	(± 1.1)	(± 1.1)	(± 1.1)	
NDCT-RH	Mean	3.7	3.8	4.2	4.1	4.2	3.99^{a}
(n=10)	± S.D.	(± 1.3)	(± 1.4)	(± 1.2)	(± 1.1)	(± 1.1)	

Note:

[1]Infection status depends on the CMT scores given Quality values for calculation purposes as follows:

CMT scores	Numerical Quality value
Negative	5
Trace	4
1	3
2	2
3	1

[2]CMT - California Mastitis Test

[3]Day 0 - The start of the experiment

** Highly significant at P = 0.05

NS Not significant at P= 0.05

Treatment means with common superscripts in the same column are not significantly different

A = DCT-FT (dry cow therapy and full hygiene)

B = NDCT-FH (No dry cow therapy and full hygiene)

C = DCT-RH (dry cow therapy and routine hygiene)

D = NDCT-RH (No dry cow therapy and routine hygiene) - Control

ing dry cow therapy and pre- and post-milking udder disinfection under the farmers' own management demonstrated that significant reductions in overall incidence of mastitis and the infectious types of mastitis is achievable under smallholder farmer conditions. The effect of dry cow therapy alone (DCT-RH) although significant, declined steadily from two weeks post calving to the end of the experimental period. This is probably due to the effect of dry cow therapy on the infection rate, which decreases after calving in the absence of measures to prevent new infections, during lactation. It has been reported by; MacMillan *et al.* (1983) that dry cow Therapy eliminates many established infections and prevents most new infections in the dry period, consequently reducing the incidence of new infections in the subsequent lactation. In the absence of hygienic measures which are important in reducing the rate of new infection during lactation, the effect of Dry Cow Therapy decreases with time, and the infection rate picks up. Results in Table 4, show that the effects of Dry Cow Therapy decreased at an increasing rate. The change of CMT value from 4.4 to 4.5 (Table 4) could be attributed to spontaneous recovery. Griffin *et al.* (1983) have reported a spontaneous recovery in 20% of the infected cows.

Results from "full hygiene" (NDCT-FH) without dry cow therapy treatment in this study are somehow difficult to interpret. The reduction of infection and proportion of infected quarters observed in the study is higher compared to what has been reported in literature (Kingwill *et al* 1970; Seymaour, 1989) if the study period (8 weeks) is taken in consideration. This is probably because there has never been any hygiene measures practised in the dairy farms under study such that the introduc-

Table 3: Prevalence of bacteria isolates from CMT[a] positive quarter samples of 40 cows in four treatment groups during 8 weeks of udder hygiene treatment

Bacteria	Sampling Period (in weeks)																				% Reduction (1 - 8 weeks)			
	0[b]				2				4				6				8							
Treatment	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
CMT positive	21	18	16	23	14	16	5	19	10	14	8	15	12	16	15	19	12	16	12	18	43	11	25	22
Infectious	12	9	11	5	6	7	0	3	4	6	1	6	4	2	1	4	4	3	3	5	67	67	25	0
Staphylococcus spp.	7	1	4	1	4	-	2	-	4	1	2	-	4	-	1	-	4	-	1	1	67	100	75	0
Streptococcus spp.	4	6	4	2	1	4	1	-	-	-	2	1	-	1	-	1	-	2	-	2	100	67	100	0
Escherichia spp.	1	3	-	-	1	-	1	1	-	-	1	4	-	-	-	2	-	1	-	1	100	67	100	100
Others[c]	-	1	1	2	-	-	1	1	-	-	-	1	-	-	-	1	-	-	-	1	100	100	100	50

Note:

A = DCT + FH (Dry cow therapy and full hygiene)

B = NDCT + FH (No dry cow therapy and full hygiene)

C = DCT + RH (Dry cow therapy and routine hygiene)

D = Control (NDCT + RH = No dry cow therapy plus routine hygiene)

a = California Mastitis Test

b = start of the experiment

c = included Bacillus spp, Proteus spp and Actinomyces

Table 4: Proportion of infected and non-infected CMT positive quarters during weeks of prophylactic udder disinfection treatment of dry cow therapy treated and untreated cows

Treatment	Bacteria infested CMT positive quarters at week (%)						
	CMT + ve quarters at start (%)	0	2	4	6	8	CMT positive quarters at week 8 (%)
DCT-FH (n=40)	52.5	30.0	15.0	10.0	10.0	10.0	30.0
DCT-RH (n=40)	40.0	27.5	0	2.5	2.5	7.5	30.0
NDCT-FT (n=40)	45.0	22.5	17.5	15.0	5.0	7.5	40.0
NDCT-RH (n=40)	57.5	12.5	7.5	15.0	10.0	12.5	45.0

Note:

DCT-FH (Dry cow therapy and full hygiene)

NDCT-FH (No dry cow therapy and full hygiene)

DCT-RH (Dry cow therapy and routine hygiene)

Control (NDCT-RH = No dry cow therapy plus routine hygiene)

tion of pre- and post-milking teat disinfection practice produced a tremendous change within such a short period. Neave *et al.* (1969) in England studied both full and partial hygiene for 12 months and reported a 50% reduction in the rate of infection, but there was no appreciable decrease in the percentage of infected quarters for both hygiene practices.

The gradual improvement in the infection status throughout the study is probably due to the complimentary effects of Dry Cow Therapy and hygiene in eliminating the existing infections and prevention of new infection in the dry period and prevention of new lactation infections respectively. There was a complete elimination of Streptococcal infections whereas Staphylococcal infection was reduced by 43%

Table 5: Relationship between Mean CMT score, mean somatic cell counts and prevalence of bacteria isolated in udder quarters of cows on different udder hygiene treatment with or without dry cow therapy

Treatment	Mean CMT numerical value	Mean SCC/ml x 10^4	Number of bacteria isolated for Staphylococcus spp	Streptococcus spp.	Escherichia spp.	Others
DCT-RH						
Week 1	4.2	144	1	5	3	1
Week 8	4.5	103	0	2	1	0
% Change	+6.7	-28.5				
NDCT-FH						
Week 1	4.08	125	4	4	0	1
Week 8	4.23	85	1	0	0	0
% Change	+3.7	-32.0				
DCT-FH						
Week 1	3.76	188	7	4	1	0
Week 8	4.47	153	4	0	0	0
% Change	+15.9	-18.6				
NDCT-RH (Control)						
Week 1	3.73	208	1	2	0	2
Week 8	4.18	196	1	2	1	1
% Change	+10.8	-5.8				

Note:

A = DCT + FH (Dry cow therapy and full hygiene)

B = NDCT + FH (No dry cow therapy and full hygiene)

C = DCT + RH (Dry cow therapy and routine hygiene)

D = Control (NDCT + RH = No dry cow therapy plus routine hygiene)

Others include Bacillus spp.; Proteus spp. and Actinomyces

CMT scores	CMT numerical value
Negative	5
Trace	4
1	3
2	2
3	1

(Table 3). Persistence of Staphylococcal infection may be related to variations in the pathology of the infections which in turn, can be a function of genetics, either bovine or bacteria. Staphylococci have the capacity to penetrate tissue producing deep seated foci (Jain 1979). Persistence of Staphylococcal infections have been reported by Dodd and Griffin (1975). Whatever the reasons for persistence are, the probability of an individual infection being eliminated by therapy is related to the severity of the infection and its duration at the time the therapy is given.

The somatic cell counts were generally high in all treatment groups in relation to the CMT value. For example in Dry Cow Therapy treatment at the beginning of the study CMT value was 4.2 which was equivalent to trace in CMT scores, (Table 4) but the mean somatic cell counts was 1,443,753 cells/ml. This is higher compared to Schalm's (1960) CMT grading system which indicate a range of 150,000 to 550,000 cells/ml for trace class. This is probably because at the beginning of the study, somatic cell count was high (Cullen 1968; Schultz 1977). At the end of the study (8 weeks post partum) somatic cell counts had decreased but still they were high in relation to the CMT value. It is difficult to explain these results but early lactation high somatic cell count have been reported (Natzke *et al.* 1975). However,

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milk somatic cell count is affected by many factors.

Conclusions

This study has shown that the magnitude of both clinical and subclinical mastitis in small scale farms as well as the role of *Staphylococcus aureus* and *Streptococcus spp.* as causative agents was similar to the situation existing on most large scale dairy farms in Tanzania. Both udder disinfection at milking and dry cow therapy at drying off could be undertaken by smallholder dairy farmers with positive results on the reduction of mastitis infection rate. These practices therefore should be more actively promoted among smallholder farmers in Tanzania.

Acknowledgement

The authors wish to acknowledge the financial support by the Norwegian Agency for Development Co-operation (NORAD) for this study.

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