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Prevalence and Determinants of Mastitis and Milk-borne Zoonoses in Smallholder Dairy Farming Sector in Kibaha and Morogoro Districts in Eastern Tanzania

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Summary

A study was carried out to establish the prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farms in Kibaha and Morogoro districts (Tanzania). A total of 57 herds comprising 114 milking cows in Kibaha and 48 herds consisting of 96 milking animals in Morogoro were included in the study. A questionnaire survey was used to assess the socio-economic determinants of mastitis, whereas California mastitis test (CMT) and microbiological assessment of milk was carried out to establish the status of mastitis and responsible aetiological agents. Seroconversion for brucellosis was evaluated using enzyme-linked immunosorbent assay (ELISA). Animals were also tuberculin-tested using a single comparative intradermal method and milk samples were cultured for isolation of Mycobacterium species. Based on CMT, the cow-based prevalence of subclinical mastitis was 82.4% in Kibaha and 62.4% in Morogoro. Of the 919-quarter milk samples cultured, 8.2% were positive for aerobic bacteria with predominant isolates being Staphylococcus epidermidis (2.8%), Staph. aureus (1.7%), Streptococcus agalactiae (1.2%) and Staph. intermedius (1.1%). There was a strong association between CMT positivity and bacteriological isolation [relative risk (RR) = 2.60; P = 0.02]. Fungal growth was observed in 21.8% (n = 881) of the samples and the isolates were yeast (19.2%), *Mucor* (2.5%) and Aspergillus (0.1%). Bucket feeding of calves was associated with increased risk of a quarter being CMT positive (RR = 1.24; P = 0.000), while residual calf suckling was associated with decreased risk of positivity (RR = 0.86; P = 0.015). Earth floor was associated with increased risk of CMT positivity at quarter level (RR = 1.13; P = 0.041) and Jersey breed was identified as a risk factor to mastitis. The prevalence of bovine tuberculosis was 0.4% (n=259) and 1.7% (n = 181) in Kibaha and Morogoro, respectively. Similarly, the prevalence of brucellosis was 1% (n = 208) in Kibaha and 1.9% (n = 104) in Morogoro. Findings from this study have demonstrated a high prevalence of subclinical mastitis and existence of health risks to milk consumers despite the low prevalence of tuberculosis and brucellosis in the study herds.

Introduction

The smallholder dairy industry in Tanzania that emerged in 1980s in an attempt to contribute to the milk supply after

parastatal farms failed to meet consumers' demands (Anon., 1997; Sumberg, 1997) contributes significantly to poverty alleviation and reduction of malnutrition. It provides a regular source of household income and food and self-employment particularly to women. In some parts of the country such as Iringa region, farmers consider the sector as their main source of income (Leslie et al., 1999). However, despite the important role of the industry, farmers in Tanzania have continued to experience sub-optimal performance of their animals. In the Southern Highlands of Tanzania, the average milk yield per cow was found to be 5.7 l/day (Karimuribo, 1999) and such low yield is attributed to poor management and diseases (Schepers and Dijkhuizen, 1991; Nangwala and Kurwijila, 1995; Kambarage et al., 1996). Mastitis is considered to be one of the most important diseases in the dairy industry. For instance, in the USA, it was observed that subclinical mastitis contributed to about 40% of the total milk losses (Janzen, 1970).

In Tanzania, mastitis that occurs in either clinical or subclinical forms is also a common problem in 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). The disease is also prevalent in large and medium scale dairy farms (Kinabo and Assey, 1983; Kambarage et al., 1996).

The high prevalence of udder infections poses great health risks if farmers do not observe the drug withdrawal periods. Milk consumers also are at great risk of contracting milkborne zoonotic infections. In Tanzania this may be facilitated by the lack of policies and strategies for the control of tuberculosis (Kazwala, 1996; Ngotonie, 1999) and brucellosis (Weinhaupl et al., 2000; Mtui-Malamsha, 2001). Consumer preference for raw milk may also predispose consumers to these diseases. Diseases such as tuberculosis and brucellosis and, the presence of atypical mycobacteria in milk may have great influence on morbidities and severity of infections such as HIV/AIDS syndrome, which is now a pandemic in some developing countries including Tanzania. Information on the prevalence of tuberculosis and brucellosis in smallholder dairy animals in Tanzania is scanty thus calling for studies to elucidate the zoonotic risks associated with milk consumption.

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In most studies carried out in Tanzania (Kinabo and Assey, 1983; Shekimweri, 1992; Kambarage et al., 1996; Karimuribo, 2002), investigations on the aetiological determinants of mastitis were centred on conventional bacteriological causes such as *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp., *Arcanobacter pyogenes*, etc. The information regarding the involvement of other micro-organisms such as anaerobic bacteria, fungi and *Mycoplasma* in udder infections in Tanzania is lacking. Therefore, the aim of the present study was to establish the aetiological agents of mastitis; the risk factors for the disease and the prevalence of tuberculosis and brucellosis in the small holder dairy sector in the periurban and urban areas of Kibaha and Morogoro districts in eastern Tanzania.

Materials and Methods

This study was conducted between April and November 2001 in Kibaha and Morogoro districts in the Coast and Morogoro regions, respectively. A total of 57 and 48 smallholder dairy farms comprising of 144 and 96 milking animals in Kibaha and Morogoro, respectively, were randomly selected for this study. The type of animals that were included in the study is shown in Table 1.

A questionnaire survey was carried out to assess the knowledge, attitudes and practices of farmers towards mastitis. Information on the age, breed and milk production of individual animals was also collected. During farm visits, observational assessments on milking hygiene practices, calfrearing systems, housing system and hygiene were carried out. Additional observational assessments were made on the type of house floor that was categorized as either concrete, earth or wooden and on the state of house floor, which was categorized as either muddy, dusty, dry or wet.

Animals were clinically examined for evidence of pain, swelling and changes in milk including presence of clots, change of colour and consistency as indicators of clinical mastitis. Evidence for subclinical mastitis for each quarter was checked at cow side using California mastitis test (CMT). Milk samples were collected aseptically for microbiological examination and were stored in cool boxes during shipment to the laboratory where they were cultured for isolation of anaerobic and aerobic bacteria using standard procedures (Carter et al., 1991). Bacterial isolates were identified by Gram-stain and biochemical tests using standard procedures (Carter et al., 1991; Roger and Edmondson, 2000). Biochemical identification of Staphylococcus isolates was carried out using rabbit plasma coagulase test and lactose, glucose, arginine, maltose, raffinose and mannitol fermentation tests (Carter et al., 1991). The Sabourad dextrose agar (SDA) was used for cultivation of fungi.

Table 1. Percentage distribution of cattle breeds kept by smallholder dairy farmers in Kibaha and Morogoro districts (Tanzania). Crosses were F1, F2 or F3

Breed	Kibaha % (<i>n</i> = 144)	Morogoro $\%$ $(n = 92)$		
Friesian crosses	51.4	47.8		
Ayrshire crosses	29.9	30.4		
Jersey crosses	2.0	3.3		
Boran	8.3	13.0		
Tanzania shorthorn Zebu	8.3	2.2		
Unclassified	0	3.3		

Isolation of mycobacteria from milk samples involved decontamination, neutralization, centrifugation and inoculation procedures according to Scottish Mycobacterium Reference Laboratory (SMRL) protocol. All procedures were carried out in a class one safety cabinet (Howorth Particulate, Bolton, UK). Milk samples were decontaminated using equal volume of 4% sodium hydroxide (PARK Scientific Ltd, Northampton, UK) then neutralized with 14% potassium dihydrogen ortho-phosphate (BDH Laboratories Supplies, UK) buffer containing 0.1% phenol red indicator (Fison Scientific Equipment, Loughborough, UK). After centrifugation at 3000 g for 20 min, the supernatant was decanted into a container with cresol soap, then sterilized at 121°C for 30 min. Sediments were inoculated onto Lowensten-Jensen medium with pyruvate and on Lowensten-Jensen medium with glycerol as described by Collins et al. (1985) and Groothuis and Yates (1991). Cultures were incubated at 37°C for 12 weeks and observation for growth was performed on days 3 and 7, and then on weekly basis. While all negative cultures were discarded after 12 weeks, positive cultures were subcultured onto both media for identification purposes. After 2 weeks, these subcultures were identified using Ziehl-Neelsen staining, oxygen preference test, Tween hydrolysis test, and pigmentation and temperature tests (Collins et al., 1985; Groothuis and Yates, 1991). The medium used for culture of *Mycoplasma* was based on a modified Hayflick medium (Friis, 1975) that has been adopted from the formula being used by Dr Niels Friis at the Danish Veterinary Laboratory (Copenhagen, Denmark). In addition, this medium has been used by other workers (Bitsch et al., 1976; Friis, 1975) and also referred to by Gourlay and Howard (1983). It has been recommended as a good medium for isolation of bovine mycoplasmas from various specimens including Mycoplasma bovis from mastitic milk, which was the major focus of this work. The isolation medium contained 3500 ml distilled water; 75 g PPLO broth (Difco, Detroit MI, USA); 180 ml yeast extract (Sigma, St Louis, MO, USA); 1.0 g ampicillin; 10 ml 0.6% w/v phenol red, and 15% horse and swine serum at pH 7.0. The medium was enriched with 0.5 mg/ml L-arginine; 0.5 mg/ml urea; 0.2 mg/ml MgSO₄ (Sigma) and 0.15 mg/ml D-cycloserine (Fluka Biochemika, Buchs, Switzerland). The solid medium was prepared using standard aseptic bacteriological procedures using purified agar (L28; Oxoid, Hampshire, UK). The cultural procedures were carried out as described by Kusiluka et al. (2000). As, there were no isolates of mycoplasmas, no further procedures for identification of isolates were carried out.

Susceptibility of all aerobic isolates to antimicrobial agents was carried out using amoxicilin, neomycin, oxytetracycline, penicillin G, chloramphenicol, streptomycin and cephalexin sensitivity discs (NCCLS, 1994). Briefly, the bacterial isolates were cultured on Mueller–Hinton agar and antimicrobial discs were placed onto the surface before incubation at 37°C for 24 h. The results were read after 24 h and the presence of an inhibition zone of specific diameter around the discs was considered for interpretation of resistance or susceptibility to a corresponding antibiotic. A bacterial isolate with an inhibition zone of equal or > 13 mm was considered susceptible and an isolate with inhibition zone of <13 mm was considered resistant.

Blood from all animals above 1 year of age were subjected to ELISA test for *Brucella* antibodies as described by Perrett et al. (2001). Animals were also tested for tuberculosis using

single comparative intradermal tuberculin test (SCITT) using avian- and bovine-purified protein derivatives (PPDs), kindly supplied by the Central Veterinary Laboratory (Addlestone, Surrey, UK). Each dose of 0.1 ml of both avian and bovine containing 2500 IU was administered intradermally in the cervical region at a distance of 12.5 cm apart. Prior to injection, the skin fold thickness at each site was measured using a pair of callipers (Barr-Knight Engineers, Glasgow, UK). After 72 h, the skin fold thickness at each site was measured again. Bovine positive reactors (bovine biased) and avian reactors (avian biased) were obtained using the following formulae: $(Bov_{72} - Bov_0) - (Av_{72} - Av_0)$ and $(Av_{72} - Av_0) -$ (Bov₇₂ – Bov₀), respectively (Shirima et al., 2003), where Bov₇₂ is skin fold thickness after 72 h of inoculation of bovine tuberculin, Bovo is skin fold thickness before inoculation of bovine tuberculin, Av₇₂ is skin fold thickness after 72 h of inoculation of avian tuberculin and Av₀ is skin fold thickness after 72 h of inoculation of Avian tuberculin. Whereas a skin fold thickness of <3 mm was regarded negative to tuberculin, a skin fold thickness of 3 mm was regarded as doubtful reaction and a skin fold thickness of 4 mm or greater as a positive reaction.

Data analysis

Collected data were entered in Epi Info databases (Coulombier et al., 2001) and the association between mastitis and risk factors was determined using 2X2 contingency table in Epi Info 6 Statcalc program (Coulombier et al., 2001). Strength of the association between a risk factor and mastitis was examined by relative risk (RR). Statistical difference between proportions was determined using Epi Info 6 Epitable program, with a critical probability of P=0.05. The Epitable program was also used to calculate 95% confidence interval (CI) of the proportions and exact binomial 95% CI values were used.

Definition of outcome variables

An animal was considered to be positive for clinical mastitis if it had observable changes in the milk such as presence of pus, clots, flakes or blood in milk or changes of udder including swelling or painful quarter or asymmetrical udder. Mastitis was defined at either cow or quarter level. A cow was considered to have subclinical mastitis if it was positive in at least one of her quarters scoring ≥1 + during CMT. A cow was also considered to be positive for subclinical mastitis if at least one of her quarter milk samples submitted for culture had bacterium or fungus isolated. Mastitis at the quarter level was defined by either a quarter CMT score of ≥1 + or isolation of mastitis bacterium or fungus from quarter milk sample submitted for culture.

Results

Milking practices

Different practices were applied before, during and after milking in the two districts as shown in Fig. 1. The proportion of farms in which farmers were washing hands with soap before milking was significantly higher (P < 0.001) in Kibaha than in Morogoro. The percentage of farmers in

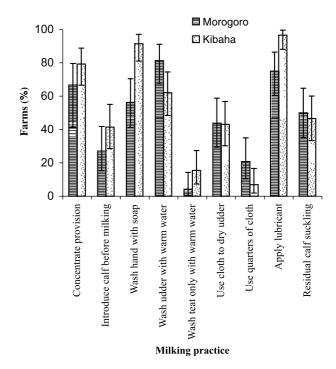


Fig. 1. Milking practices applied by farmers in Morogoro and Kibaha districts (Tanzania). Error bars represent 95% confidence intervals of the proportions.

Kibaha introducing calves to the dams before milking; washing the udder or teats; using cloth to dry the teats/ udder; applying and practising residual calf suckling were comparable (P > 0.05) with those in Morogoro. The rate of using single cloth for each quarter was significantly higher in Morogoro than in Kibaha area, whereas the proportion of farmers who reported to apply teat lubricant in Kibaha district was significantly higher (P = 0.001) than that of Morogoro district. Most farmers (52.8%) used milking salve and 34.9% used cooking oil.

Prevalence of mastitis

The percentage of farmers who reported to have seen clinical cases of mastitis in the last 2 years was 80.2% (95% CI = 71.3, 87.3) and there was no significant difference (P > 0.05) between Kibaha (84.5%) and Morogoro (75.0%). Of the 57 and 48 farms, 34.5 and 20.8%, respectively, had at least one case of clinical mastitis during the year 2000 in Kibaha and Morogoro districts. Farm based prevalence of clinical mastitis during the year 2001 (i.e. the period up to the first day of farm visit) was 22.4 and 25.0% in Kibaha and Morogoro, respectively. It was also apparent that a low proportion of farmers (15.1%, 95% CI = 8.9, 23.4%) in both areas were aware of subclinical mastitis.

The prevalence of subclinical mastitis at cow level based on CMT, aerobic culture and fungal culture in the two study sites (combined) were 74.5% (95% CI = 68.2, 80.2), 20.5% (95% CI = 15.6, 26.2) and 45.6% (95% CI = 39.1, 52.1), respectively. The proportion of cows in Kibaha that were CMT positive (82.4%) was significantly higher (P < 0.001) than that in Morogoro (62.4%). In contrast, the cow level prevalence based on isolation of aerobic bacteria was

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Table 2. Types of bacteria isolated from quarter milk samples from dairy cows in Kibaha and Morogoro districts (Tanzania)

Bacterial isolate	Kibaha % (n = 544)	Morogoro $\%$ $(n = 375)$	Overall $\frac{9}{0}$ ($n = 919$)
Streptococcus agalactiae	0.6	2.1	1.2
Arcanobacter pyogenes	0	0.5	0.2
Staphylococcus epidermidis	3.9	1.3	2.8
Staphylococcus hyicus	0.2	0	0.1
Staphylococcus intermedius	0	2.7	1.1
Staphylococcus saprophyticus	0	0.8	0.3
Staphylococcus aureus	1.3	2.4	1.7
Micrococcus spp.	0.9	0.3	0.7
*1	92.6	86.7	89.8

significantly higher (P = 0.039) in Morogoro (27.1%) than in Kibaha (16.1%). The prevalence of fungal infections in Kibaha (49.3%) and Morogoro (40.0%) districts were comparable (P > 0.05). The overall point prevalence of clinical mastitis on the first day of farm visit was 2.5%.

Of the 919-quarter milk samples cultured, 8.2% were positive for aerobic bacteria comprising *Staph. epidermidis* (2.8%), *Staph. aureus* (1.7%), *Strept. agalactiae* (1.2%), *Staph. intermedius* (1.1%), *Micrococcus* spp. (0.7%), *Staph. saprophyticus* (0.3%), *Arcan. pyogenes* (0.2%) and *Staph. hyicus* (0.1%) (Table 2). The majority of bacterial isolates showed susceptibility to amoxicilin, cephalexin and neomycin, and were susceptible to oxytetracycline, penicillin, chloramphenicol and streptomycin (Table 3).

Fourteen per cent of the milk samples (n=109) were positive for atypical mycobacteria and the isolates were M. gordonae (4%), M. smegmatis (2%), M. fortuitum (2.7%), M. phlei (2%), M. flavescens (2%) and M. avium intracellular (1%). All milk samples were negative to anaerobic bacteria and Mycoplasma species. The overall prevalence of fungal infection in the two districts was 21.0% (95% CI = 18.4, 23.8) and rate of fungal infections in the two study areas was comparable (P > 0.05) (Table 4). The isolates were yeast (18.5%), Mucor spp. (2.4%) and Aspergillus spp. (0.1%).

Prevalence of zoonoses

The prevalence of bovine tuberculosis based on tuberculin testing was 0.4% (n=259) and 1.7% (n=181) in Kibaha and Morogoro, respectively, while that of brucellosis was 1% (n=208) in Kibaha and 1.9% (n=104) in Morogoro.

Table 4. Fungal isolates from quarter milk samples from dairy cows in Kibaha and Morogoro districts (Tanzania)

Fungal isolate	Kibaha % (<i>n</i> = 542)	Morogoro $\%$ $(n = 373)$	Overall % (n = 915)	
Mucor spp. Aspergillus spp.	2.2 0.2	2.7 0.0	2.4 0.1	
Yeast	19.9	16.4	18.5	

Association between mastitis and risk factors

The risk of a quarter being CMT positive increased with bucket feeding (RR = 1.24; 95% CI = 1.10,1.39; P = 0.000) but was reduced by residual calf suckling (RR = 0.86; 96% CI = 0.75,0.97; P = 0.015). It was also observed that earth floor was associated with increased risk of subclinical mastitis at a quarter level (RR = 1.13; 95% CI = 1.01,1.27; P = 0.041) and that the Jersey breed was a risk factor to udder infections (RR = 1.39; 95% CI = 1.12,1.72; P = 0.030).

Discussion

This study has shown high prevalence of subclinical mastitis in smallholder dairy farms in Morogoro and Kibaha districts, and low prevalence of clinical mastitis when compared with previous studies in smallholder (Shekimweri, 1992; Omore et al., 1996; Karimuribo, 1999) and large-scale (Kinabo and Assey, 1983) dairy farms in Tanzania. The high prevalence of subclinical mastitis may partly be attributed to improper milking hygiene, poor house hygiene and lack of use of postmilking teat dipping. Contamination of milkers' hands and cloths, washing the whole udder and improper drying of the teats or udder have been reported to increase the incidence of Staphylococcus species which also appears to be common in areas where the treatment with penicillin has been practised intensively (Lafi and Hilat, 1998). In addition, the practice of using the cloth to dry the udder, if improperly used, has been observed to be a risk factor for mastitis (Blowey and Edmondson, 2000; Karimuribo, 2002). However, proper milking hygiene practices such as use of an individual towel for each cow is an important control measure for mastitis (Torgerson et al., 1992) as are pre-milking and post-milking teat disinfections (Neave et al., 1966). Galton et al. (1986) demonstrated that pre-milking dipping with iodophor, hypochlorite or dodecylbenzenesulphonic acid preparations decreased the total bacterial counts in milk particularly when

Table 3. Drug susceptibility profiles for bacteria isolated from quarter milk samples from dairy cows in Kibaha and Morogoro districts (Tanzania)

Bacterial isolates tested	Antimicrobials used for susceptibility testing in %						
	Amoxicilin	Neomycin	Oxytetracycline	Penicillin G	Chloramphenicol	Streptomycin	Cephalexin
Staphylococcus aureus	93.8	87.5	56.3	62.5	56.3	62.5	75
Staphylococcus intermedius	70	60	50	50	60	70	80
Streptococcus agalactiae	90.6	90.6	36.7	54.5	81.8	54.5	54.5
Staphylococcus epidermidis	96.2	100	50	84.6	69.2	69.2	80.8
Staphylococcus saprophyticus	100	100	33.3	33.3	100	66.7	66.7
Arcanobacter pyogenes	0	50	100	0	0	50	50
Micrococcus spp.	66.7	100	33.3	33.3	33.3	50	50
Staphylococcus hyicus	100	0	0	100	100	0	0

the teats were thoroughly wiped afterwards. The improper milking hygiene practised by farmers in this study may be due to poor or lack of knowledge about subclinical mastitis and its economic implications on milk production.

Staphylococcus species was the mainly mastitis pathogen isolated in the current study and this tallies with what has been reported in other studies (Mahlau and Hyera, 1984; Mbise et al., 1985; Miltenburg et al., 1996; Lafi and Hilat, 1998; Karimuribo, 2002). In this study the prevalence of *Staph*. epidermidis was high despite of the fact that its role as a putative udder pathogen is not well established. However, Radostits et al. (1994) reported that it can be an opportunistic agent in cases of severe devitalization of the udder tissue by other pathogens. Coliform species were not isolated in this study in contrast to other studies (Kambarage et al., 1996). It is possible that freezing of milk prior to culture may have reduced the isolation rate for E. coli as it also applies to Act. pyogenes (Schukken et al., 1989). Most of the isolates showed susceptibility to amoxicilin, cephalexin and neomycin but variable degrees of resistance to penicilin G, oxytetracycline, chloramphenicol and streptomycin, which are frequently used for treatment of mastitis and other diseases in the study areas.

Isolation of fungi in the 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; the latter being commonly practised by farmers in the study areas. The presence 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.

In this study, it was observed that residual calf suckling was a protective factor for CMT positivity and this is probably because of the fact that in addition to enhanced complete emptying of the udder during suckling, the calf saliva also contains a lysozyme that has an antibacterial activity (Karimuribo, 2002). The fact that earth floor is difficult to clean and favours survival and proliferation of micro-organisms on the udder and teats including their hair, may explain its role as a risk factor for mastitis observed in this study (Roger and Edmondson, 2000). However, in a recent study that was carried out to assess the effect of hair removal from the teats and the udder on mastitis and bacterial count in the milk revealed that such a practice had no influence on the infection rate and bacterial contamination of milk (Silk et al., 2003). However, it is worthy noting that their study was carried out in a country where house hygiene is good including use of concrete floor and where the milking practices are of good standards. Thus, in countries where animal house hygiene and milking practices are substandard, hair removal from the teats may have some influence on mastitis and bacterial count in milk. However, further studies are needed in order to verify this.

The prevalence of bovine tuberculosis and brucellosis in this study was low. Similar observations in Tanzania were made by Weinhaupl et al. (2000) in dairy animals in Dar es Salaam in which the prevalence of bovine tuberculosis in dairy animals was 0.9% and in pastoral animals in Lugoba was 0.6%. Shirima (1999) also observed a similar trend in smallholder dairy animals in other parts of the coastal areas of Tanzania. However, the low prevalence of brucellosis in smallholder dairy cattle may be due to confinement of animals and

low stocking rates that leads to reduced contamination of pastures.

This study has revealed subclinical mastitis as an unfamiliar problem amongst farmers in Morogoro and Kibaha districts. Because of its insidious nature, the subclinical mastitis might be among the causes of sub-optimal milk production that is evident in many smallholder farms. If milk production in the study area has to be improved, creation of awareness and control of mastitis through proper milking hygiene such as use of hot water to rinse the towels, individual towels for each cow and quarter, post-milking teat disinfection and proper treatment of mastitis cases is important. Creation of awareness about tuberculosis and brucellosis to farmers, milk vendors and milk consumers is also useful in order to reduce the health hazards associated with milk consumption, despite the low prevalence rates observed in this study.

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