Mastitogenic bacteria isolated from dairy cows in Kenya and their antimicrobial sensitivity

Authors:
George K. Gitau[1]
Royford M. Bundi[1]
John Vanleeuwen[2]
Charles M. Mulei[1]

Affiliations:
[1]Department of Clinical Studies, University of Nairobi, Kenya

[2]Department of Health Management, University of Prince Edward Island, Canada

Correspondence to:
George Gitau

Email:
gkgitau@uonbi.ac.ke

Postal address:
PO Box 29053–00625, Nairobi, Kenya

Dates:
Received: 30 Oct. 2012
Accepted: 14 May 2013
Published: 26 Feb. 2014

How to cite this article:
Gitau, G.K., Bundi, R.M., Vanleeuwen, J. & Mulei, C.M., 2014, 'Mastitogenic bacteria isolated from dairy cows in Kenya and their antimicrobial sensitivity', *Journal of the South African Veterinary Association* 85(1), Art. #950, 8 pages. http://dx.doi.org/10.4102/jsava.v85i1.950

There is limited epidemiological knowledge on udder health in Kenyan dairy cattle that would aid in a pro-active approach towards mastitis prevention. The study objectives were: (1) to investigate the prevalence and distribution of clinical and subclinical mastitis in dairy cattle in Mukurwe-ini and Nakuru Districts, Kenya, and (2) to determine the antibacterial sensitivity of the organisms causing bovine mastitis in these districts. The study involved field-screening of milk samples from 241 dairy cows on 128 farms by use of the California Mastitis Test (CMT) and, if CMT-positive, followed by bacteriological culture of the major causative agents and their respective antibiotic sensitivity to eight commonly used antibiotics. All participating farms were visited twice during the study period. The results obtained during the first and second visits showed the prevalence of clinical mastitis to be very low: 0.9% and 0.5%, respectively; 56.0% and 65.0% of cows were CMT-positive on at least one quarter and 49.6% and 58.7% of cows were culture-positive, respectively. There was no significant difference in mastitis prevalence between Nakuru and Mukurwe-ini districts ($p > 0.10$). *Staphylococcus aureus* was isolated in 68.0% and 77.0% of samples during the first and second visits, respectively. Other frequently isolated agents included *Streptococcus agalactiae*, and other *Streptococcus* spp., *S. aureus* and *S. agalactiae* were most sensitive to gentamycin and norfloxacin, and least sensitive to cotrimazole and ampicillin. Knowing the prevalence of mastitogenic organisms and their antibiotic sensitivities could improve treatment efficacy and cow longevity.

Introduction

Background

With its frequent occurrence, mastitis is a very costly disease of the dairy industry, due to reduced milk production during the infection and often after infection, medications used and their associated withdrawal times, reduced fertility and premature culling (Erskine, Wagner & DeGraves 2003; Harmon 1994). Valuable components of the milk, such as lactose, fat and casein, are also decreased (Girma 2001; Shitandi & Kihumbu 2004).

Many infectious agents have been implicated as causes of mastitis in cattle (Owen *et al.* 1997; Radostits 2001), with over 135 different microorganisms having been isolated (Hawari & Fowzi 2008). The agents can be categorised into host-adapted pathogens, the most common organisms being *Streptococcus agalactiae* and *Staphylococcus aureus* (CSA 2004; Lim *et al.* 2007), and environmental pathogens, primarily coliforms and environmental Streptococci that are frequently found in the cow's environment (Quinn *et al.* 2002).

The frequencies and distributions of the various microbial causes of udder infections on any given farm will determine the severity of their costs to farmers (Gill *et al.* 1990). In Kenya, the most common organisms reported to cause udder infections are *Streptococcus*, *Staphylococcus*, *Escherichia coli*, *Trueperella* and *Pseudomonas* species (Gitau *et al.* 2011; Gitau *et al.* 2012). However, because these Kenyan results were from laboratory submissions, it is unclear how often these organisms are currently infecting cows epidemiologically. Knowledge of the most common organisms and their antibiotic sensitivity is also needed so that one can determine the potential antibiotic regimen to use even before the laboratory results on culture and sensitivity tests are available (Godden *et al.* 2007).

Mastitis is considered to be one of the major reasons for antibiotic use in dairy animals (Hogan & Smith 2003). Identification of the mastitis causing pathogens and their antibiotic sensitivity patterns is needed not only to treat and control mastitis effectively (Ruegg 2004; Siamak *et al.* 2001), but also to support public health concerns on judicious use of antibiotics in developed and developing countries (Dhakal *et al.* 2007). Costly treatment failure in mastitis is often due to indiscriminate use of antibiotics without testing *in vitro* sensitivity, and can result in development of resistance to antimicrobial drugs (Health Canada 2003; Silva *et al.* 2005).

The objectives of this study were to investigate the prevalence and distribution of mastitis in dairy cattle in Mukurwe-ini and Nakuru Districts, Kenya, and to determine the antibacterial sensitivity

of the organisms causing bovine mastitis in these districts to aid better decision-making in mastitis treatment and control efforts in Kenya and other similar dairy production areas. Knowledge of the prevalence of mastitogenic organisms and their antibiotic sensitivities could improve treatment efficacy and cow longevity and aid in a pro-active approach towards mastitis prevention in Kenya and possibly elsewhere in sub-Saharan Africa.

Materials and methods

Study area

The study was carried out in Mukurwe-ini District of Nyeri County and Nakuru District of Nakuru County between 21 June 2010 and 31 August 2010. Nyeri County is one of the five Counties of Central Province and forms part of Kenya's central highlands according to the constitution of Kenya (National Council for Law Reporting 2010). It covers an area of about 3300 km^2 and is situated between longitudes 36°E and 38ºE and between the equator and latitude 0º38′S. The main physical features of Nyeri County are Mt. Kenya (5199 m) to the east and the Aberdare Range (3999 m) to the west (Ministry of Planning and National Development 2010a). Dairy farming is an important enterprise in Nyeri County, with many farmers practising zero-grazing, where pastures are cut and carried to the cattle (Ministry of Livestock Development 2008a).

Nakuru County is one of the 14 counties of the Rift Valley Province. The county covers an area of about 7230 km^2 and is located between longitudes 35°28′E and 35°36′E and latitudes 0°12′S and 1°10′S (Ministry of Planning and National Development 2010b). Dairy farmers in the area practise both zero-grazing and semi-zero-grazing, where cattle are housed but allowed to graze at certain times (Ministry of Livestock Development 2008b).

Study design

Selection of study area, farms and animals

The study was carried out on 64 smallholder dairy farms using zero-grazing in Mukurwe-ini District and on an equal number of dairy farms using both zero-grazing and pasture-grazing in Nakuru District.

In Nakuru, simple random selection was employed at farm level using a sampling frame of the dairy farms provided by the District Livestock Production Officer. In Mukurwe-ini, a convenient sampling method was used for logistical reasons, as the research was conducted alongside another project (Dohoo *et al*. 2012, 2013). The other project included 32 farms with biogas digesters recently constructed on the farm by a non-governmental organisation and a reference group of 32 randomly selected farms without biogas digesters in the region, matched on age of the participant, family size, and number of cows. Due to the similarity of farming practices across farms in the district, the sample was considered fairly representative of the populations in the district. The study farms were visited twice during the study period to increase the sample size and determine the consistency of the results, as the researchers were on the farms for other reasons.

Within the herds, cows were eligible for the study if they were lactating. All cows were identified with an ear tag (cows without an ear tag were given one on the first visit). For farms with less than five lactating cows, all the cows were selected to participate. For the farms that had more than five lactating cows, the lactating cows were systematically randomly selected by examining alternate cows in the crush, starting with the first one. Fewer than 10 cows were examined on each farm. Of the 128 farms, 68 farms had only one lactating cow, whilst four farms had six or more cows. There were 33, 13, 10 and zero farms having two, three, four and five cows, respectively. There were 113 and 128 cows sampled in Mukurwe-ini and Nakuru Districts respectively.

Milk sample collection and handling

Milk samples from individual quarters of all selected milking cows on the participating farms were screened using the California Mastitis Test (CMT). The CMT results were interpreted subjectively as negative, trace, 1+, 2+, or 3+ based on the viscosity of the gel formed after mixing the reagent with milk, as described by Radostits (2001). Clinical mastitis was defined as milk that appeared abnormal with or without other local or systemic signs. Subclinical mastitis was defined as milk that appeared normal but had a CMT $\geq$ 1 (Radostits *et al*. 2000). The project graduate student (R.B.) was trained in the interpretation of the CMT results (based on Radostits 2001) and was responsible for CMT interpretation of all samples, eliminating inter-rater differences. Results for each quarter were recorded.

From quarters that were CMT-positive ($\geq$ 1 CMT), milk samples were collected for culture after routine teat cleaning and disinfection using 70% alcohol, as described by the National Mastitis Council (1999). If more than one quarter was CMT-positive in the same cow, a composite milk sample was taken from all CMT-positive quarters. The composite samples taken from different quarters were comprised of one milk stream from each quarter, which was considered to be nearly the same volume. The first stream of milk from each quarter was discarded prior to sampling for both CMT and cultures. The milk samples were refrigerated for a maximum of 96 h (four days) until they were transported in a cool box with ice packs to the Clinical Microbiology Laboratory, Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, for bacterial culture.

Bacterial identification and antibiotic sensitivity tests

At the laboratory, bacteriological cultures were performed on the milk samples according to the *Laboratory handbook on bovine mastitis* (National Mastitis Council 1999). A 10 µL aliquot of each milk sample was streaked onto the surface of 5% sheep blood and MacConkey agar plates. The plates were incubated at 37 °C for 18 h – 24 h. The laboratory plates were read for growth of micro-organisms. Those plates having no growth after 24 h were re-incubated further for

up to four days, after which, if there was still no growth, the conclusion of no growth was assigned. Where growth occurred, the cultures were first studied macroscopically for both abundance and colonial morphology. The cultures were thereafter stained and examined microscopically for Gram reaction, and biochemical tests were used to determine the genus and species of pathogens in the sample. The targeted organisms during culture were mainly *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *T. pyogenes*, and *Proteus* spp.

Samples were classified as having significant growth if the growth was considered of 'probable significance' or 'highly significant' based on National Mastitis Council Guidelines for significance (National Mastitis Council 1987). Samples that had two colony types were considered mixed growth, and samples with three or more colony types were considered contaminated.

All Gram-negative rods were divided into lactose and non-lactose fermenters, based on their growth characteristics on MacConkey agar; lactose fermenters had pink-red colonies, whilst non-lactose fermenters were colourless and/or translucent colonies. The lactose fermenters were subjected to the citrate fermentation test to determine whether they were *E. coli* or *Klebsiella*. Those that tested negative with the citrate test were classified as *E. coli,* and those that tested positive was classified as *Klebsiella.*

The small to medium-sized colonies that were haemolytic or non-haemolytic on 5% sheep blood agar, and yielding Gram-positive cocci, were subjected to catalase and coagulase tests. All colonies that tested negative for catalase were identified as Streptococci. Those that tested positive for catalase were further tested with rabbit plasma for coagulase activity, and those found to be positive (ability to produce coagulase enzyme that clots rabbit plasma) were confirmed to be *S. aureus.* Those that tested negative were confirmed to be coagulase-negative Staphylococci. The catalase-negative Streptococci were further tested with bacitracin and those testing negative were classified as *S. agalactiae.*

The bacterial isolates were tested for antibiotic sensitivity through a panel of antimicrobial drugs on the disks available at the laboratory, using a panel of 8 locally available chemotherapeutics: ampicillin, gentamycin, ceforclor, cotrimazole, kanamycin, tetracycline, norfloxacin and streptomycin. Commercially available drug-impregnated paper disks, either singly or combined, as in Multodisk® (Oxoid), Mastring-S® (Mast Laboratories) and Octodics® (HI Media), were applied onto the surface of 5% sheep blood agar and MacConkey agar that were inoculated uniformly with the pathogen and then incubated overnight at 37 °C. The effectiveness of a drug was determined by measuring the diameter of the zone of inhibition around the disc – the larger the diameter, the more effective the drug was considered to be. The following standard criteria were used to summarise the various sensitivity classes for each of the antibiotics used:

- A zone diameter of 0 mm – 8 mm scored 0 or 'R' for resistant.
- A zone diameter of 9 mm – 15 mm scored + or slightly sensitive.
- A zone diameter of 16 mm – 22 mm scored ++ or sensitive.
- A zone diameter of 23 mm and above scored +++ or very sensitive.

Data handling and storage

A Microsoft Excel 1997–2003 spreadsheet file was developed based on CMT and laboratory culture results. The Microsoft Excel spreadsheet file was exported to a statistical package (Genstat 2008) for all statistical analyses.

Data analysis

The results of the CMT were converted to a binary outcome where any animal testing CMT-negative (CMT of 0 or trace) in the field was considered as infection-negative, whilst those testing CMT-positive (CMT of 1, 2, or 3) were considered as infection-positive. For the purpose of comparison of the results, laboratory culture results were classified so that the samples that yielded organisms on culture were considered infection-positive, whilst the cows that were CMT-negative (as no sample was collected) and the milk samples that yielded no organism after laboratory culture were considered to be infection-negative. This was performed for the results of the first and second visits separately, and later for the results of the first and second visits combined.

Two-by-two tables of districts versus infection status were constructed using the CMT and laboratory culture results, as described by Dohoo, Martin and Stryhn (2009) for the calculation of the probability of infection (prevalence) in each district. A Chi-square was computed to establish whether there was an association between the district and infection status (Dohoo, Martin & Stryhn 2009). If there was a significant association, a relative risk was calculated (a strength of association) to compare the risk between the two districts (Dohoo *et al.* 2009).

Descriptive statistics were computed for the laboratory culture results to establish the frequencies and percentages of quarters and cows infected, based on the clinical signs (clinical mastitis), CMT score (subclinical mastitis), and organisms isolated (culture-based mastitis), for the first and second visits. Descriptive statistics were also computed for antibiotic sensitivity, based on the sensitivity of different organisms isolated, as well as the overall sensitivity for all organisms isolated. These statistics were performed for the two districts combined and later for each district separately.

Results

The estimated prevalence of clinical mastitis was very low at 0.9% (two of 234 cows) and 0.5% (one of 214 cows) for the first and second visits, respectively.

Table 1 shows the CMT results, by level of CMT score, during the first and second farm visits. Based on the CMT, the

overall quarter prevalence of subclinical mastitis was 30.5% and 34.3% during the first and second visits, respectively (scores 1, 2 and 3 combined). A CMT score of 1 was the most common score, making up two thirds of the CMT-positive quarters. The differences between visits were not statistically significant ($p > 0.05$).

Table 2 shows the distribution of cow infections, by number of CMT-positive quarters, during the first and second visits. Of the 130 and 139 cows with CMT-positive quarters, over one third were test-positive on only one quarter during the first and the second visits, respectively, whilst just over 20% tested positive on all 4 quarters during both the first and the second visits. Some of these cows with 4 positive quarters may not have had mastitis. Again, the differences between visits were not statistically significant ($p > 0.05$).

Table 3 shows the CMT results and the combined CMT and laboratory culture results of the first and second farm visits. The estimated prevalence of subclinical mastitis based on CMT results increased from first to second sampling in Nakuru District first from 52% to 69%, whilst this prevalence stayed the same in Mukurwe-ini District at 61%. Overall, there was no significant difference in subclinical mastitis prevalence between the two districts based on the CMT results of the first sampling ($\chi^2 = 2.56$, $p = 0.11$) or the second sampling ($\chi^2 = 1.30$, $p > 0.05$).

The estimated prevalence of subclinical mastitis based on culture results increased from first to second sampling in Nakuru District from 47% to 60%, whilst this prevalence only increased from 53% to 57% in Mukurwe-ini District. Overall, there was no significant difference between the two districts based on the culture results for the first sampling ($\chi^2 = 0.832$, $p = 0.505$) or the second sampling ($\chi^2 = 0.178$, $p > 0.050$).

Table 4 provides a summary of the cow-level culture results for the two combined districts during the first, second, and both visits combined. *S. aureus* was the most prevalent organism at 68.5% and 77.7% during the first and second visits, respectively, followed by *S. agalactiae* and other Streptococci. Of the CMT-positive samples collected from the first and second visits, 11.5% and 9.4%, respectively, did not yield any organism after laboratory bacterial culture.

Table 5 shows the cow-level sensitivity of the *S. aureus* isolates to antimicrobials during the first and second visits in Mukurwe-ini and Nakuru Districts combined. During the first visit, *S. aureus* was most sensitive to gentamycin at 88%, followed by norfloxacin at 83%. The highest resistance was seen for cotrimazole and ampicillin at 76% and 57%, respectively. During the second visit, the antimicrobial sensitivity patterns were similar to those from the first visit.

TABLE 1: Distribution of quarter California Mastitis Test scores for both the first and second farm visits from Mukurwe-ini and Nakuru Districts, Kenya, June 2010 to August 2010.

CMT score	First visit		Second visit	
	f	%	*f*	%
Score 0 or trace	639	68.9	553	64.8
Score 1	194	20.9	200	23.5
Score 2	79	8.5	84	9.9
Score 3	10	1.1	10	1.2
Non-functional quarters	6	0.6	5	0.6
Total quarters	**928**	**100**	**852**	**100**

f, Frequency; CMT, California Mastitis Test.

TABLE 2: Distribution of cow infections, by number of quarters infected, based on California Mastitis Test results for the first ($n = 130$) and second ($n = 139$) farm visits from Mukurwe-ini and Nakuru Districts, Kenya, June 2010 to August 2010.

Number of quarters	First visit		Second visit	
	CMT-positive	%	CMT-positive	%
1 quarter	46	35.4	52	37.4
2 quarters	37	28.5	43	30.9
3 quarters	19	14.6	15	10.8
All 4 quarters	28	21.5	29	20.9
Total number of cows	**130**	**100**	**139**	**100**

CMT, California Mastitis Test.

TABLE 3: Results of the California Mastitis Test and laboratory cultures at the cow-level of the first and second dairy farm visit in Mukurwe-ini and Nakuru Districts, Kenya, June 2010 to August 2010.

Test and/or laboratory culture	Nakuru		Mukurwe-ini		Total cows	
	n	%	*n*	%	*n*	%
First sampling						
CMT+	64	52	66	61	130	56
CMT−	60	48	42	39	102	44
CMT+ and Lab Culture+	58	47	57	53	115	50
CMT− or Lab Culture−	66	53	51	47	117	50
Total	**124**	**–**	**108**	**–**	**232**	**–**
Second sampling						
CMT+	79	69	60	61	139	65
CMT−	36	31	38	39	74	35
CMT+ and Lab Culture+	69	60	56	57	125	59
CMT− or Lab Culture−	46	40	42	43	88	41
Total	**115**	**–**	**98**	**–**	**213**	**–**

n, number of cows; CMT, California Mastitis Test.

TABLE 4: Laboratory culture results at the cow-level for the first and second dairy farm visits from Mukurwe-ini and Nakuru Districts, Kenya, June 2010 to August 2010.

Organism	First visit		Second visit		Combined visits	
	n	%	*n*	%	*n*	%
Staphylococcus aureus	89	68.5	107	77.0	196	72.9
Streptococcus agalactiae	10	7.7	4	2.9	14	5.2
Other Streptococci	6	4.6	7	5.0	13	4.8
Coagulase-negative Staphylococci	4	3.1	0	0.0	4	1.5
Corynebacterium bovis	3	2.3	3	2.0	6	2.2
No growth	15	5.0	14	2.0	29	10.8
Mixed growth (Staphylococci and Streptococci)	2	11.0	3	10.1	5	1.9
Klebsiella	1	1.5	1	2.2	2	0.7
Total organisms	**130**	**0.8**	**139**	**0.7**	**269**	**100**

n, number of cows.

Table 6 shows the cow-level sensitivity of the *S. agalactiae* isolates to antimicrobials during the first visit in Mukurwe-ini and Nakuru Districts combined. *Streptococcus agalactiae* was most sensitive to norfloxacin at 90%, followed by gentamycin at 70% and ampicillin at 50%. The highest resistance was seen for cotrimazole, kanamycin and ceforclor at 80%, 40% and 40%, respectively. During the second visit, the antibiotic sensitivity patterns were similar.

Discussion

This study has provided prevalence data for clinical and subclinical mastitis in dairy cattle in two districts in Kenya known for their dairy farming, based on field-screening with the CMT and bacteriological culture of CMT-positive milk samples. The sensitivity to antibiotics of the main microbes isolated was also determined.

The prevalence of clinical mastitis at the two points in time of sampling was quite low (< 1%). These findings were in agreement with Harmon (1994) and Gitau *et al*. (2003), who concluded that the subclinical form of bovine mastitis is 15–40 times more prevalent than the clinical form of bovine mastitis.

The frequency and distribution of subclinical mastitis amongst the sampled dairy farms indicated that it was very common and widespread, both at the quarter level (Table 1 and Table 2) and at the cow level (Table 3). The percentage of positive quarters (Table 1) was lower than the Egyptian findings of Attia, El-Rashidy and Metias (2003), who reported 75% and 30% of quarters with the CMT score of ++ and +++, respectively. This difference could be because of the subjective nature of the CMT interpretations by those carrying out the CMT test in the field or from real differences in the epidemiology of mastitis between the two studies.

The overall quarter prevalence of subclinical mastitis in this study was lower than the 37.0% reported by Nessru, Teshome and Getachew (1997) but higher than that reported by Biffa, Debela and Beyene (2005) and Haftu *et al.* (2012), who reported a prevalence of 17.9% and 28.2%, respectively. The cow-level prevalence of subclinical mastitis estimated in the present study was lower than that reported by Biru (1989) and Bishi (1998) in dairy cows on farms around Addis Ababa, Ethiopia, who reported a mastitis prevalence of 67.4% and 69.8%, respectively. On the other hand, our findings were higher than the reports of Nessru *et al*. (1997) and Haftu *et al.* (2012), who reported a prevalence of 33.0% and 34.0% in Ethiopia, respectively. The variability in the prevalence of bovine mastitis amongst the reports could be attributed to differences in the sampling schemes, management of the farms in the study areas, breeds considered, or the technical methods used by the investigators.

The percentage of cows positive on the laboratory bacterial cultures in this study (Table 3) was lower than the cow-level prevalence reported in Egypt by Morcos *et al*. (1991) at 66.8% and in Algeria by Ghazi and Niar (2006) at 81.4%. The non-significant differences in the prevalence of subclinical mastitis based on laboratory culture results between Mukurwe-ini and Nakuru Districts in Kenya may be explained by the similarity in the smallholder dairy management in these regions.

In the present study, the most common bacterium isolated (Table 4) was *S. aureus* (73.2%). This finding was higher than the 36.0% of isolates found by Haftu *et al.* (2012) in Ethiopia, but similar to studies by Omore *et al*. (1996) and Gitau *et al*. (2011) in Kenya. Shekimweri, Kurwijila and Mgongo (1998) and Gitau *et al*. (2003) in Tanzania and Zanzibar, respectively, reported higher prevalence of *S. aureus* mastitis in the smallholder dairy sector. This high prevalence may be partly attributed to the fact that *S. aureus* is one of the most prevalent bacteria of subclinical mastitis in dairy cows and in this investigation, it may have been spread by milkers' hands (no farmers used gloves) and wash cloths,

TABLE 5: Sensitivity results against antimicrobials for *Staphylococcus aureus* isolated from subclinical mastitis at the cow-level during the first (n = 89) and second (n = 108) farm visits in Mukurwe-ini and Nakuru Districts, Kenya, June 2010 to August 2010.

Outcome measure	Ampicillin		Gentamycin		Ceforclor		Cotrimoxazole		Kanamycin		Tetracycline		Norfloxacin		Streptomycin	
	f	%	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%
First visit																
Resistant	51	57	0	88	34	38	68	76	7	8	15	17	0	0	2	6
Slightly sensitive	36	40	11	12	40	45	19	21	68	76	61	69	15	17	82	92
Sensitive	2	2	78	0	15	17	2	2	14	16	13	15	74	83	5	2
Second visit																
Resistant	50	46	1	1	6	6	65	6	1	1	21	19	0	0	14	10
Slightly sensitive	15	14	11	10	94	87	29	0	91	84	82	76	7	6	83	77
Sensitive	43	40	96	89	8	7	14	27	16	15	5	5	101	94	11	13

f, Frequency.

TABLE 6: Sensitivity results against antimicrobials for *Streptococcus agalactiae* isolated from subclinical mastitis at the cow-level during the first farm visit (n = 10) from Mukurwe-ini and Nakuru Districts, Kenya, June 2010 to August 2010.

Outcome measure	Ampicillin		Gentamycin		Ceforclor		Cotrimoxazole		Kanamycin		Tetracycline		Norfloxacin		Streptomycin	
	f	%	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%
Resistant	3	30	0	70	4	30	8	80	4	40	2	20	0	90	2	20
Slightly sensitive	2	20	3	30	3	30	1	0	4	40	4	40	1	10	6	60
Sensitive	5	50	7	0	3	40	1	1	2	2	4	40	9	0	2	20

f, Frequency.

which are considered the main tools in the distribution of microorganisms from teat to teat and from cow to cow, in addition to lack of hygiene, as reported by El-Balkemy *et al*. (1997). Small herd sizes would limit the potential of exposure to *S. aureus* amongst cows on the same farm. However, most of the dairy farms were in close proximity to other dairy farms, especially in the Mukurwe-ini District, and neighbours frequently provide milking assistance to each other in these close-knit dairying communities, with limited attention to biosecurity, thereby increasing the number of other cows to which a cow is exposed. Poor treatment success and limited use of dry cow therapy would also contribute to chronic infections that would act as a reservoir (Radostits 2001). Minimal use of post-milking teat dip by the farmers (22%) was also likely contributing to the spread of this contagious pathogen in the present study.

The other moderately prevalent isolated organisms in the present study were *S. agalactiae*, coagulase negative *Staphylococcus* spp. and other *Streptococcus* spp. (Table 4). This agrees with the findings of Dego and Tareke (2003) and El-Attar, Salama and Abd El Samie (2002), who reported that Staphylococci and Streptococci caused up to 90% of bovine mastitis in Egypt and Ethiopia.

The pathogens isolated were generally more sensitive to gentamycin and norfloxacin, with high resistance to ampicillin and cotrimoxazole, but the sensitivity was moderate too low for the other antibacterial drugs under study (Table 5 and Table 6). This is in agreement with Kumar and Sharma (2002) and Haftu *et al.* (2012) in India and Ethiopia, respectively, as well as a study carried out by De Oliveira *et al*. (2000) that showed a high resistance to drugs routinely used for *S. aureus* mastitis therapy in 11 different western countries. The moderate to low sensitivity to some antimicrobials could be explained by the fact that there has been availability of tetracyclines and beta-lactams for many years in the areas under study, whilst gentamycin and norfloxacin were only introduced recently.

In the present study, 10.4% of the CMT-positive milk samples did not yield any organisms after laboratory bacterial culture (Table 4), in agreement with the findings reported by Sori, Zerihun and Abdicho (2005) of 9.8%. The reasons why the CMT-positive milk samples did not yield bacterial growth were probably failure to isolate organisms by the culture techniques employed (selective media for mycoplasma, *Haemophilus* spp. and fungi were not employed), as suggested by Ismail and Hatem (1998); short-lived recent infections that have been cleared by treatment or by the cow; infections that are characterised by intermittent shedding of bacteria, such as *S. agalactiae, S. aureus,* and *Mycoplasma;* and other reasons for having an elevated CMT, unrelated to mastitogenic microbes.

The study had some limitations as the CMT only provides an indication of increased somatic cell counts in milk, for which mastitis is the main cause. However, the concentration of white blood cells in colostrum is elevated, and the concentration of glandular cells in milk can be increased with low milk production volumes (due to a relatively constant rate of glandular cell shedding). Milk production levels are affected by many factors other than mastitis, including parity, days-in-milk, nutrition, stress or concomitant disease (Radostits 2001). All cows in the study were clinically healthy upon examination. Also, in our statistical analyses of risk factors associated with CMT status (unpublished data), breed, milk production volume, days-in-milk and parity were not significantly associated with CMT status. Furthermore, nearly 90% of CMT-positive cows had positive culture results. If the CMT scores were due to a systemic cause, the CMT scores on all quarters would likely be the same. There were only 10 cows that had CMT scores equal on all 4 quarters. Of these 10 cows, only two had no growth on the culture. Therefore, the authors are reasonably confident that the results provided by the CMT represented udder infections, although there is a possibility of a small number of false positives.

California Mastitis Test-negative cows were not cultured in this study owing to financial constraints. This may have contributed to the low prevalence of bacteriological cultures if some of the CMT-negative cows were false negatives. However, false negatives could have occurred only if the undetected bacterial infections produced a limited inflammatory response at the time of the sampling.

Whilst this epidemiological study provides useful information for dairy farmers and animal health personnel treating dairy cows for mastitis, the results should not be over-interpreted. The cows and farms were probably reasonably representative due to the large number of farms that were randomly selected (Nakuru District) or quasi-randomly selected (Mukurwe-ini District). However, the samples were taken during the cool dry season, during which environmental mastitis is less likely to occur, and therefore the results probably underestimate environmental mastitis prevalence.

The high prevalence of *S. aureus* infections found in this population of dairy cows indicated that additional preventive measures need to be taken to limit the spread of this host-adapted pathogen. It is recommended that cows with normal-looking milk that is rejected, based on an alcohol test, should be tested with a CMT, and if CMT-positive, should be cultured to determine the pathogen, if possible. Cows with known infections should be milked last when there is more than one lactating cow on a farm, and a teat with a known infection should be milked last, after the other teats are milked. Latex gloves should be worn when milking the cows, and post-milking teat dip should be routinely used if possible. Infected quarters with positive cultures for *S. aureus* should be treated as soon as possible, either with extended lactation therapy or dry cow therapy, using an antibiotic that is likely to be effective (Barkema, Schukken & Zadoks 2006).

Conclusion

From this epidemiological study of 241 cows on 128 Kenyan dairy farms, each visited twice between June 2010 and

August 2010, the cow-level prevalence of clinical mastitis was estimated to be below 1%, but the prevalence of subclinical mastitis was generally high for the smallholder farms in Mukurwe-ini and Nakuru Districts, with only small differences between districts.

From the total number of samples collected in the two districts during the first and the second visits combined, *S. aureus* was the most prevalent organism isolated, followed by *S. agalactiae* and other Streptococci, whilst 10% of samples had no growth. The isolated *S. aureus* and *S. agalactiae* were most sensitive to gentamycin and norfloxacin, and least sensitive to cotrimoxazole and ampicillin.

These findings provide information about dry season mastitis for improved treatment efficacy and pro-active approaches towards mastitis prevention in Kenya and possibly elsewhere in sub-Saharan Africa. Sampling in both the dry and rainy seasons would provide a more complete understanding of udder health throughout the year.

Acknowledgements

The study was sponsored by Farmers Helping Farmers, a Canadian non-governmental organisation, Veterinarians without Borders (*Vétérinaires sans Frontières*) Canada, XXIII World Veterinary Congress Foundation, the University of Prince Edward Island, and the University of Nairobi. Athumani Mwashando is hereby acknowledged for his contribution in the laboratory processing of the samples, and Laura Bourque and Vionna Kwan for their field technical support.

Competing interests

The authors declare that they have no financial personal relationship(s) that may have inappropriately influenced them in writing this article.

Author's contributions

J.V. (University of Prince Edward Island) was the project leader. G.K.G. (University of Nairobi) and R.M.B. (University of Nairobi) were in charge of local coordination and study activities whilst J.V. was not in Kenya, and of statistical analyses. C.M.M. (University of Nairobi) was in charge of bacteriological cultures and provided graduate student supervision.

References

Barkema, H.W., Schukken, Y.H. & Zadoks, R.N., 2006, 'The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis', *Journal of Dairy Science* 89, 1877–1895. http://dx.doi.org/10.3168/jds.S0022-0302(06)72256-1

Attia, E.R.H., El-Rashidy, A.A. & Metias, K.N., 2003, 'Comparative study between electrical conductivity, California mastitis test and somatic cell count for rapid diagnosis of subclinical mastitis in lactating cows', *Proceedings of the 7th Scientific Congress, Egyptian Society for Cattle Diseases*, 07–09 December 2003, Assiut, Egypt.

Biffa, D., Debela, E. & Beyene, F., 2005, 'Prevalence and risk factors of mastitis in lactating dairy cows in southern Ethiopia', *International Journal of Applied Research in Veterinary Medicine* 3, 189–198.

Biru, G., 1989, 'Major bacteria causing bovine mastitis and their sensitivity to common antibiotics', *Ethiopian Journal of Agricultural Science* 11, 4–6.

Bishi, A.S., 1998, 'Cross-sectional study and longitudinal prospective study of bovine clinical and subclinical mastitis in peri-urban and urban dairy production system in Addis Ababa Region, Ethiopia', MSc thesis, Addis Ababa University and Free University of Berlin, Germany.

CSA, 2004, *The 2001/02 Ethiopian Agricultural Sample Enumeration (EASE), Executive Summary*, Addis Ababa, Ethiopia, May 2004.

Dego, O.K. & Tareke, F., 2003, 'Bovine mastitis in selected areas of southern Ethiopia', *Tropical Animal Health and Production* 35, 197–205. http://dx.doi.org/10.1023/A:1023352811751 PMid:12797409

De Oliveira, A.P., Watts, J.L., Salmon, S.A. & Aarestrup, F.M., 2000, 'Antimicrobial sensitivity of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States', *Journal of Dairy Science* 83, 855–862. http://dx.doi.org/10.3168/jds.S0022-0302(00)74949-6

Dhakal, I.P., Dhakal, P., Koshihara, T. & Nagahat, H., 2007, 'Epidemiological and bacteriological survey of buffalo mastitis in Nepal', *Journal of Veterinary Medical Science* 69, 1241–1245. http://dx.doi.org/10.1292/jvms.69.1241, PMid:18176019

Dohoo, I.R., Martin, S.W. & Stryhn H., 2009, *Veterinary epidemiologic research*, 2nd edn., AVC Inc., Charlottetown.

Dohoo, C., Guernsey, J., Critchley, K. & Van Leeuwen, J.A., 2012, 'Pilot study on the impacts of biogas digesters on respiratory health in women on Kenyan smallholder dairy farms', *Journal of the Environment and Public Health* 636298, Epub 28 August 2012. http://dx.doi.org/10.1155/2012/636298

Dohoo, C., Van Leeuwen, J.A., Guernsey, J. & Critchley, K., 2013, 'Impact of biogas digesters on wood utilization and self-reported back pain on rural Kenyan smallholder dairy farms', *Global Public Health* 8, 221–235. http://dx.doi.org/10.1080/17441692.2012.758299, PMid:23305236

El-Attar, A.A., Salama, M.E. & Abd El-Samie, M.M., 2002, 'Incidence of mastitis in lactating cows and buffaloes kept under different management conditions in Ismailia province', *Veterinary Medical Journal* 2, 583–589.

El-Balkemy, A., Esmat, M., Afaf, M., Azza, N. & Farag, E., 1997, 'Evaluation of screening tests used for the detection of subclinical mastitis', *Proceedings of the 4th Scientific Congress, Egyptian Society for Cattle Diseases*, 07–09 December 1997, Assiut University, Egypt.

Erskine, R.J., Wagner, S. & DeGraves, F.J., 2003, 'Mastitis therapy and pharmacology', *Veterinary Clinics of North America: Food Animal Practice* 19, 109–138. http://dx.doi.org/10.1016/S0749-0720(02)00067-1

GenStat, 2008, Discovery Edition 3.0, VSNi International, Hemel Hempstead.

Ghazi, K. & Niar, A., 2006, 'Incidence of mastitis in various bovine breedings in Tiaret area (Algeria)', *Assiut Veterinary Medicine Journal* 52, 198–205.

Gill, R., Howard, W.H., Leslie, K.E. & Lissemore, K., 1990, 'Economics of mastitis control', *Journal of Dairy Science* 73, 3340–3348. http://dx.doi.org/10.3168/jds.S0022-0302(90)79029-7

Girma, T., 2001, 'Prevalence of mastitis at Alemaya University dairy farm', *Journal of the Ethiopian Veterinary Association* 5, 17–21.

Gitau, G.K. Bundi R.M., Vanleeuwen J. & Mulei C.M., 2012, 'Evaluation of Petrifilms™ as a diagnostic test to detect bovine mastitis organisms in Kenya', *Tropical Animal Health and Production* 45, 883–886. http://dx.doi.org/10.1007/s11250-012-0286-y, PMid:23108587, PMCid:3574565

Gitau, G.K., Wabacha, J.K., Mulei, C.M., Ndurumo, S. & Nduhiu, J.K., 2011, 'Isolation rates and antimicrobial sensitivity patterns of bovine mastitis pathogens in peri-urban area of Nairobi, Kabete, Kenya', *Ethiopian Veterinary Journal* 15, 1–13. http://dx.doi.org/10.4314/evj.v15i1.67680

Gitau, G.K., Waridi, M., Makame, H.A., Saleh, M.M., Muhamed, R.A., Mkola, A.P. *et al.,* 2003, 'Occurrence of high udder infection rates in dairy cows in Ungunja Island of Zanzibar, Tanzania', *Journal of Applied Research in Veterinary Medicine* 1, 73–76.

Godden, S., Lago, A., Bey, R., Leslie, K., Ruegg, P. & Dingwell, R., 2007, 'Use of on-farm culture systems in mastitis control programs', *Proceedings of the National Mastitis Council,* pp. 1–9, Visalia, CA, Inc., Madison. PMid:17715875

Haftu, R., Taddele, H., Gugsa, G. & Kalayou, S., 2012, 'Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia', *Tropical Animal Health and Production* 44, 1765–1771. http://dx.doi.org/10.1007/s11250-012-0135-z, PMid:22476790

Harmon, R.J., 1994, 'Physiology of mastitis and factors affecting somatic cell counts', *Journal of Dairy Science* 77, 2103–2112. http://dx.doi.org/10.3168/jds.S0022-0302(94)77153-8

Hawari, A.D. & Fowzi, A., 2008, 'Prevalence and distribution of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Jordan', *American Journal of Animal and Veterinary Science* 3, 36–39. http://dx.doi.org/10.3844/ajavsp.2008.36.39

Health Canada, 2003, 'Antimicrobial resistance. Keeping it in the box!', *Health Policy Research Bulletin* 6, 1–2.

Hogan, J. & Smith, K.L., 2003, 'Coliform mastitis', *Veterinary Research* 34, 507–519. http://dx.doi.org/10.1051/vetres:2003022, PMid:14556693

Ismail, T.M. & Hatem, M.E., 1998, 'Prevalence of subclinical mastitis in a dairy cattle herd in the eastern region of Saudi Arabia', *Proceedings of the 8th Scientific Conference, Faculty of Veterinary Medicine,* 15–17 November 1998, Assiut University, Egypt.

Kumar, R. & Sharma, A., 2002, 'Prevalence etiology and antibiogram of mastitis in cows and buffaloes in Hissar, Haryana', *Indian Journal of Animal Science* 72, 361–363.

Lim, G.H., Leslie, K.E., Kelton, D.F., Duffield, T.F., Timms, L.L. & Dingwell, R.T., 2007, 'Adherence and efficacy of an external sealant to prevent new intramammary infections in the dry period', *Journal of Dairy Science* 90, 1289–1300. http://dx.doi.org/10.3168/jds.S0022-0302(07)71617-X

Ministry of Livestock Development, 2008a, *Nyeri District Livestock Production Office, Annual Report 2008*, Republic of Kenya, Government Printers, Nairobi.

Ministry of Livestock Development, 2008b, *Nakuru District Livestock Production Office Annual Report 2008,* Republic of Kenya, Government Printers, Nairobi.

Ministry of Planning and National Development, 2010a, *Nyeri District Strategic Plan 2005–2010*, Republic of Kenya, Government Printers, Nairobi.

Ministry of Planning and National Development, 2010b, *Nakuru District Strategic Plan 2005–2010*, Republic of Kenya, Government Printers, Nairobi.

Morcos, M.B., Shoukry, S., Zaki, E.R., Reyad, E.M. & El-Sawah, H., 1991, 'Studies on subclinical mastitis in a commercial dairy herd in western Nubaria', *Journal of the Egyptian Veterinary Medicine Association* 51, 261–267.

National Council for Law Reporting, 2010, First schedule: Article 6 (1), *The Constitution of Kenya*, Republic of Kenya, Government Printers, Nairobi.

National Mastitis Council, 1987, *Laboratory and field handbook on bovine mastitis,* 1st edn., National Mastitis Council Inc., Arlington.

National Mastitis Council, 1999, *Laboratory and field handbook on bovine mastitis,* 2nd edn., National Mastitis Control Council, Madison.

Nessru, H., Teshome, Y. & Getachew, T., 1997, 'Prevalence of mastitis in cross-bred and zebu cattle', *Ethiopian Journal of Agriculture Science* 16, 53–60.

Omore, A., McDermott, J.J., Arimi, S., Kyule, M. & Ouma, D., 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. http://dx.doi.org/10.1016/S0167-5877(96)01054-9

Owen, W.E., Ray, C.H., Watts, J.L. & Yancey, R.J., 1997, 'Comparison of success and results of antibiotic therapy during lactation and results of antibiotic susceptibility tests for bovine mastitis', *Journal of Dairy Science* 80, 313–317. http://dx.doi.org/10.3168/jds.S0022-0302(97)75940-X

Quinn, P.J., Carter, M.E., Markey, B.K. & Carter, G.R., 2002, *Veterinary microbiology: microbial diseases, bacterial causes of bovine mastitis,* 8th edn., Mosby International, London.

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 edn., W.B. Saunders, London. PMCid:1476169

Radostits, O.M., 2001, *Herd health: food production medicine,* 3rd edn., W.B. Saunders, London.

Ruegg, P.L., 2004, 'Lactation therapy and milk quality', *Proceedings of the National Mastitis Control Council Annual Meeting,* Charlotte, NC, 2004, pp. 4–7, National Mastitis Council, Inc., Verona.

Shekimweri, M., Kurwijila, L. & Mgongo, F., 1998, 'Incidence, causative agents and strategy of control mastitis in smallholder dairy herds in Morogoro, Tanzania', *Journal of Agricultural Science* 1, 28–36.

Shitandi, A. & Kihumbu, G., 2004, 'Assessment of the California mastitis test usage in small holder dairy herds and risk of violative antimicrobial residues', *Journal of Veterinary Science* 5, 5–9. PMid:15028880

Siamak, P.Y., Sorum, Y., Larsen, H.J.S. & Gogstad, S., 2001, 'Rapid method for detection of gram-positive and negative bacteria in milk from cows with moderate or severe clinical mastitis', *Journal of Clinical Microbiology* 39, 3228–3233. http://dx.doi.org/10.1128/JCM.39.9.3228-3233.2001, PMCid:88323

Silva, B.O., Caraviello, D.Z., Rodrigues, A.C. & Ruegg, P.L., 2005, 'Evaluation of Petrifilm for the isolation of *Staphylococcus aureus* from milk samples', *Journal of Dairy Science* 88, 3000–3008. http://dx.doi.org/10.3168/jds.S0022-0302(05)72980-5

Sori, H., Zerihun, A., & Abdicho, S., 2005, 'Dairy cattle mastitis in and around Sebeta, Ethiopia', *International Journal of Applied Research in Veterinary Medicine* 3, 332–338.