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Bovine Mastitis Prevalence, Aetiology, Therapeutics and Control in Tatton Agriculture Park, Egerton University

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A B S T R A C T

The study was accomplished to evaluate bovine mastitis therapeutics and control in Tatton Agriculture Park (TAP), Egerton University. Causative agents were investigated through culture and identification then subjected to commonly used antimicrobials to determine their sensitivity hence identify drug of choice. A total of 41 dairy cows or 164 quarters were sampled from TAP. Of all the samples, 34.1% were California Mastitis Test (CMT) positive for sub-clinical mastitis while 82.9% yielded bacterial growths. When cultured 21.4% of the CMT positive were without growths (false positive) while 85.2% of those that tested CMT negative yielded bacterial growths (false negative). The most prevalent bacterial species were *Staphylococcus aureus* (58. 8%). The CMT was not a fully reliable test for detection of sub-clinical mastitis, therefore, should be accompanied by a bacteriological test for accurate diagnosis. The study showed that the causative organisms were most sensitive to Tetracycline, Gentamycin, Enrofloxacin, Sulfamethoxazole, Ceftifour and Streptomycin, with the least effective drugs being Ampicillin, Neomycin and Cloxacillin. Most drugs used on the farm were Terrexine, Intramammary tubes (multiject), Adamycin, Penstrep, and Neomycin. The incidence rate of mastitis in TAP was at 82.9%. The specific causative agents were *Staphylococcus aureus*, *Streptococcus agalacteae*, and *Escherichia coli*. *Staphylococcus ssp* accounted for 58.8% of the mastitis cases while *Streptococcus ssp* accounted for 11.8%, and *Escherichia coli* accounted for 8.9%. Mixed infections of *Staphylococcus ssp*, *Streptococcus ssp* and *Escherichia coli* accounted for 20.5% of the cases of mastitis infection. It is concluded that the most effective method for the prevention of mastitis is through the establishment of good husbandry practices, sanitation, sound milking procedures, including post-milking, teat dipping and treatment during the non-lactating period and culling of chronically infected cows.

Introduction

The dairy industry in Kenya contributes 80% of the milk, and is dominated by small holder farmers (Odero-Waitituh, 2017) who are mostly women. This small-scale dairy sector contributes considerably to reduction of poverty and malnutrition, and provides a steady income for the households as well as women empowerment (Karimuribo *et al.*, 2006). Mastitis is a multi-etiological and difficult disease characterized by swelling of mammary glands (FAO, 2014). It remains to be the main problem in dairy production.

Different causal organisms including bacteria, mycoplasma, fungi and yeasts are the major pathogens and these are usually distributed in the environment. Some of the particular organisms are Streptococci (*Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis);* Staphylococci (*Staphylococcus aureus);* Coliforms (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa);* Mycoplasmas, Leptospiras, Macrococci, Corynebacterium (*Corynebacterium pyogenes*), Mycobacterium and Yeast and fungi. Most mastitis cases are due to defective management -poor milking procedures, overstocking, chilling of the udder, poor use of milking machine or the change from hand to machine milking (Current concepts of Bovine mastitis, 1996).

Mastitis presents in two forms depending on presence or absence of clinical signs, as clinical mastitis or sub-clinical mastitis. Commonly used methods of assessing impacts of mastitis in Kenya are observational structures and clinical trials. The latter are based on random allocation of animals or farms provide a better level of process in testing alternative control methods than do observational method when groups are self-selected by a farmer or investigator and that's more susceptible to confounding effects (Madison, 1999).

Milk production in Kenya in the showing a wide range of 1300 Kgs to 4000 Kgs per cow per year has been reported (Peeler and Omore 1997; Omore *et al.,* 1999), but this changes with the level of intensification and agro-ecological areas (Muia *et al.,* 2011), with the highest being 4575kg/cow/year in high potential areas (Mugambi *et al.,* 2015).

The yield per animal is low as equated to worlds best of 9000 litres per year (Techno serve, 2008) but it can be enhanced with noble management (USAID/GoK 2009).

Mastitis is a worldwide problem affecting animal health, quality of milk (Ondiek *et al.,* 2018) and economics of milk production, including huge financial losses (Sharma *et al.,* 2012). Probable losses from decreased milk yield alone amount to well over $1 billion yearly, and when all other expenses are computed, it transforms to approximately $185 per cow (Philpot and Nickerson, 2000).

Bovine mastitis leads to varying degrees of economic losses (Van Soest *et al.,* 2016; Hogeveen and Van Der Voort, 2017; Aghamohammadi *et al.,* 2018). The normal case of clinical mastitis results in an entire economic monetary value of $444, with $128 in direct costs and $316 in indirect costs. Direct costs comprised diagnostics ($10), therapeutics ($36), non-saleable milk ($25), veterinary service ($4), labor ($21), and death loss ($32). Indirect costs included succeeding milk production loss ($125), premature culling and replacement cost ($182), and future reproductive cost ($9) (Rollin *et al.,* 2015).

The loss is in addition to developing of resistance to antibiotics (Saini *et al.,* 2012, Oliver and Murinda, 2012) particularly penicillin, pirlimycin and florfenicol. ampicillin, cefoxitin, chloramphenicol, trimethoprim-sulfamethoxazole combination, sulfisoxazole, streptomycin and kanamycin tetracycline and sulfisoxazole; cloxacillin, penicillin-novobiocin combination, and cephapirin used for dry cow therapy, and ceftiofur used in lactating cow therapy applied as systemically or intramammary infusion (Saini *et al.,* 2013). Some strains of microorganisms may displayresistance to several drugs, even multidrug resistance (Yoshimura *et al.,* 2002; Nunes *et al.,* 2007; Oliviera *et al.,* 2012; Fernandes *et al.,* 2016).

High mastitis prevalence 34-82% reported by Ondiek *et al.,* (2013) is due to the absence of surveillance on the control program, poor

hygiene during milking and infrequent preventive measures. Since mastitis prevalence is normally high during the long rainy season and in dry cows, there is a need for control and prevention of mastitis at this particular time. This study was set up to find the best management practices for control of mastitis in TAP, and investigate the incidence rate, the specific causative agents and effective drugs for mastitis treatment.

Materials and Methods

Milk sample collection

Sterile 100ml Boujou bottles were labeled prior to sampling. Hands were washed with soap under running tap water, and teats were washed in sanitizing solution, dried with towels, and the first two squirts of milk discarded from each teat before sampling.

The sterile Boujou bottle was opened under the lease and held at an angle to prevent any foreign material from falling into the openings. Two squirts of milk were collected from each quarter beginning from the closest quarters and working towards the ones furthest away. The Boujou bottle was then closed before removing it from beneath the teats. Samples were refrigerated until the time of analysis in the laboratory.

Culturing of samples

Bunsen burner was used to sterilize the air around the operating area and to sterilize the inoculation wire loops. The inoculation loop was cooled using prepared solidified agar and the bottle containing the sample was then gently swirled to mix the milk. The Boujou bottle was opened and a loopful of milk taken from each sample bottle and streaking done for dilution. For each sample, inoculation was done on both Nutrient and MacConkey agar, labeled and incubated at 37^{o}C for 24 hours to allow the growth of bacterial colonies present in the milk samples.

Identification of isolates

Bacterial colonies were identified according to colony characteristics and cultural characteristics which were reported as heavy pure growth, heavy mixed growth, scanty growth, moderate mixed or no growth.

Gram staining was carried out as follows: Bacterial smear was made on a clean glass slide. It was then air dried and fixed by passing it over a flame 3 times, stained with crystal violet for 30 seconds, and washed with plenty of distilled water. Lugol's iodine was poured over the slide for 30 seconds (a mordant), then the slide was washed with acetone to decolorize it, counterstained with carbolfuchsin stain for 30 seconds, then washed and dried. It was examined using x100 oil immersion objective lens where, gram-positive organism appeared blue or purple and gram-negative was pink or red (Black and Black, 1999).

Sensitivity test

To evaluate the antimicrobial resistance a wire loop was heated, cooled and a pure colony picked and streaked in a nutrient agar plate while a mixed culture was also picked and platted on a different plate.

These plates were incubated at 37^{o}C for 24 hours after which they were observed for growths and inhibition zones. To determine the most effective drugs to clear all cases of mastitis infections caused by microbes the following antimicrobial agents were used; Tetracycline, Gentamycin, Enrofloxacin, Sulfamethoxazole, Ceftifour, Streptomycin, Ampicillin, Neomycin and Cloxacillin. The percentage of sensitivity to pathogens was also computed and presented in a table 4.

Results and Discussion

The CMT test revealed a positive prevalence of 14 cows or 34.1% cases while 27 cows or 65.9% of the 41 cows were negative. Of the 27 CMT negative cases, 23 or 85.2% had growths and only 11.1% did not result to growths. However, of the CMT positive cases, 21.4% were false positive and had no growth whereas 78.6% were true positive. A total of 64.4% of the samples showed false results of either False ‾ or False + (Table 1 and 2).

The bacterial pathogens isolates associated with mastitis cases in TAP include: *Staphylococcus ssp, Streptococcus ssp,* mixed infections (*Staphylococcus ssp, Streptococcus ssp* and *Escherichia coli)* and *Escherichia coli* (Table 3).

Therapeutic management of the milking cows is done by administration of anti-mastitis drugs either by injection or intramammary infusion. The drugs of choice include Tetracycline, Gentamycin, Enrofloxacin, Sulfamethoxazole, Ceftifour and Streptomycin (Table 4).

CMT is an indirect cow-side chemical test for estimating somatic cell numbers. The chemical reagents rapture the cell thereby releasing DNA responsible for the gel formation and viscosity (Schalm *et al.,* 1971). The ability of the test to predict the presence of bacteria depends on the level of the somatic cell in the quarter. According to the results stipulated in the table 2 it shows that, out of 41 samples, 14 were CMT positive of which 21.4% were a false positive. Of the 27 CMT negative samples, 85.2% were a false negative. They may have resulted from an experimental error and the unreliability of CMT reagent as an indicator of the presence of bacteria. Out of the 41 samples, 34 samples showed growth which represents 82.9% of the cases analyzed. Therefore, CMT cases should be accompanied with bacterial isolation for effective decisions on control and treatment of sub-clinical mastitis. The bacterial isolates, in this case, are important pathogens associated with mastitis in dairy production (Wilson *et al.,* 1995). *Staphylococcus ssp* account for 58.8% of the mastitis cases in Tatton Agriculture Park (TAP) while *Streptococcus ssp* accounts for 11.8% and mixed infections of *Staphylococcus ssp, Streptococcus ssp* and E. coli account for 20.5%. E. coli accounts for 8.9% of the cases of infections.

There is a need to determine efficient tool to use specific antibiotic which can efficiently stop and control bovine mastitis in dairy animals (Hossain *et al.,* 2017). Bovine mastitis is caused by numerous forms of gram positive and negative microbes and consist of infectious pathogens, environmental pathogens, minor and uncommon pathogens which can be treated by applying specific and selective antibiotics (Radostits *et al.,* 2000). The sensitivity in table 4 shows that the most effective drugs to clear all cases of mastitis infections are; Tetracycline, Gentamycin, Enrofloxacin, Sulfamethoxazole, Ceftifour and Streptomycin, the least effective drugs being Ampicillin, Neomycin and Cloxacillin. Gram negative pathogens were reported to be more sensitive to enrofloxacin and gentamicin and to a lesser extent sensitive to ampicillin and penicillin (Karthikeyan, 2003) which backed up the present outcomes in both the antibiotic sensitivity tests. Gram positive bacteria were more sensitive to Enrofloxacin, Sulfamethoxazole, Ceftifour, Cloxacillin. Penicillin, amoxicilin and Streptomycin, and less sensitiveto Ampicillin and Neomycin. The various drug resistance between the cultures involved in mastitis has also been shown by other researchers (Yoshimura *et al.,* 2002; Nunes *et al.,* 2007; Saini *et al.,* 2012; Oliver and Murinda, 2012; Oliviera *et al.,* 2012; Saini *et al.,* 2013; El-Hamid *et al.,* 2016; Gomes *et al.,* 2016).

Table.1 Cows, CMT reaction, cultures and microorganism identification in TAP

No.	Cow	CMT	Growth/No growth	Result Interpretation	Specific Microorganisms
1	Patricia 13	-	Growth	*False* _	*Staphylococcus*
2	Terry	-	Growth	*False* _	*Staphylococcus, E. coli*
3	Maiden 57	-	Growth	*False* _	*Staphylococcus*
4	Maiden 59	-	Growth	*False* _	*Staphylococcus*
5	Taveta 5	-	Growth	*False* _	*Streptococcus, Staphylococcus*
6	Buttercup 15	-	Growth	*False* _	*Staphylococcus*
7	Ngong 22	-	Growth	*False* _	*E. coli*
8	Lucy 19	-	Growth	*False* _	*Streptococcus, Staphylococcus*
9	Susan12	-	Growth	*False* _	*Staphylococcus*
10	Susan 11	-	Growth	*False* _	*Streptococcus, Staphylococcus*
11	Buttercup 17	-	Growth	*False* _	*Staphylococcus*
12	Maiden 67	-	Growth	*False* _	*Streptococcus*
13	Bushbaby	-	Growth	*False* _	*Staphylococcus*
14	Buttercup 16	-	Growth	*False* _	*Staphylococcus*
15	Maiden 59	-	Growth	*False* _	*Staphylococcus*
16	Maiden 60	-	Growth	*False* _	*Staphylococcus*
17	Kilo 18	-	Growth	*False* _	*Streptococcus*
18	Panzi 20	-	Growth	*False* _	*Staphylococcus*
19	Patricia 17	-	Growth	*False* _	*Staphylococcus*
20	Janet 26	-	Growth	*False* _	*Streptococcus*
21	Ngong 24	-	Growth	*False* _	*Staphylococcus*
22	Waterbuck	-	Growth	*False* _	*Streptococcus, Staphylococcus*
23	Maiden 64	-	Growth	*False* _	*Staphylococcus*
24	Betty	+	No growth	*False* +	-
25	Tembo 3	+	No growth	*False* +	-
26	Maiden 65	+	No growth	*False* +	-
27	Maiden 58	-	No growth	*True* _	-
28	Janet	-	No growth	*True* _	-
29	Maiden 70	-	No growth	*True* _	-
30	Julia 6	-	No growth	*True* _	-
31	Tembo 5	+	Growth	*True* +	*Streptococcus Staphylococcus*
32	Maiden 66	+	Growth	*True* +	*Staphylococcus*
33	Ngong 17	+	Growth	*True* +	*E. coli*
34	Tembo 6	+	Growth	*True* +	*Staphylococcus*
35	Kilo 20	+	Growth	*True* +	*Staphylococcus*
36	Ruth	+	Growth	*True* +	*Streptococcus, E. coli*
37	Patricia 14	+	Growth	*True* +	*Streptococcus*
38	Patricia 16	+	Growth	*True* +	*Staphylococcus*
39	Maiden 69	+	Growth	*True* +	*Staphylococcus*
40	Lucy 19	+	Growth	*True* +	*E. coli*
41	Kilo 20	+	Growth	*True* +	*Staphylococcus*

Key: CMT positive (+) gel formation; CMT negative (-) no visible change

Table.2 Results of bacterial growth and % distribution

Test used	No. of samples	% of growth	% of no growth	% of total samples
CMT positive	14	11 (78.6)	3 (21.4)	34.1
CMT negative	27	23 (85.2)	4 (14.8)	65.9
Total	41	34 (82.9)	7 (17.1)	100.0

Table.3 Mastitis microorganism prevalence (%) in TAP

Microorganisms	Number (n)	% prevalence
Staphylococcus spp	20	58.8
Mixed (*Staphylococcus spp,E. coli & Streptococcus spp*)	7	20.5
Streptococcus spp	4	11.8
Escherichia coli	3	8.90
Total	34	100

Table.4 Antibiotic sensitivity (%) of different microbial pathogens to different groups of antibiotics

Antibiotics	Percentage of sensitivity to pathogens		
	S. aureus	*S. agalactiae*	*E. coli*
Penicillin	86.8	100	4
Ampicillin	8.33	20	-
Amoxicillin	78.9	100	18
Tetracycline	70.4	60	40
Gentamicin	97.8	40	94.4
Neomycin	28.0	0	41
Cloxacillin	92.1	100	2
Streptomycin	71.1	66.67	84
Enrofloxacin	97.4	100	100
Erythromycin	51.8	80	20
Ceftifour	94.7	100	100
Sulfamethoxazole	99.3	98.6	88.9

According to TAP records, mastitis remains a bother to the farm, since it has raised the cost of production through treatment and discarding of milk. It is associated more with the environment because of the way milking and milking procedures are conducted. Most cases are reported during the drying off period, heavy rains, during shortages of waters for cleaning parlor and equipment, incomplete milking due to lack of experience by workers and the unhygienic situation in the farm. Once there is a positive case in the farm, they do diagnostic tests i.e. use strip cup and CMT kit, and then take a sample for culture and sensitivity in the laboratory.

After milking the workers forget to put the teats into teat dip thus encouraging

multiplication of bacteria hence relapse of mastitis especially at late lactation.

Some of the most commonly affected animals were Ngong 22, Maiden 65, Lucy 19, Tembo 3, Betty and Ngong 17 among others. The problem may persist due to the delayed treatment. Also the rotational employment of casual workers as animal health technicians, lead unclear and improper treatment records and hence problematic detection of mastitis. Lack of supply of detergents and use of few towels for all milking animals is the cause of the spread of infection among animals.

The routine usage of the strip cup to check for clinical mastitis should be augmented by CMT checks for all milking cows. Continuous testing of cultures for antibiotic susceptibility outline will be beneficial for selection of an appropriate antibiotic and as well identify the changing drifts of antibiotics resistance for developing antibiotic use approach (Hossain *et al.,* 2017).Most drugs used on the farm are Terrexine, Intramammary tubes (multi-ject) Adamycin, Penstrep, and Neomycin. The farm does not practice dry cow therapy and this may exacerbate the10% positive incidences of mastitis observed.

Recommendation and Control

The most effective method for the prevention of mastitis is through the establishment of good husbandry practices, sanitation, sound milking procedures, including post-milking, teat dipping and treatment during the non-lactating period and culling of chronically infected cows. Rotational use of the most effective drugs to treat mastitis infections, such as; Tetracycline, Gentamycin, Enrofloxacin, Sulfamethoxazole, Ceftifour and Streptomycin is advised.

The recommended control measures include: Maintaining clean and dry cow environment as much as possible; observing high standards of hygienic milking procedures, hand and machine – proper vacuum levels i.e. 40-50kPa and pulsations 56-60 P/minute; as well as having routine dry cow therapy/ post-milking intramammary infusion with effective germicidal agents.

The specific causative agents were; *Staphylococcus aureus*, *Streptococcus agalacteae*, and *E. coli*. The drug of choice for the above causative agents as per the sensitivity results were; Tetracycline, Gentamycin, Enrofloxacin, Sulfamethoxazole, Ceftifour and Streptomycin.

The incidence rate of mastitis in Tatton Agriculture Park stands at 82.9%. The CMT reagent is not a fully reliable test for detection of sub-clinical mastitis cases, therefore, should be accompanied by a bacteriological test for accurate diagnosis. The most efficient way for the prevention of mastitis is through the formation of noble husbandry practices, hygiene, comprehensive milking procedures, intervention during throughout the non-lactating period and removing of persistently septic cows in the herd.

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