



Effect of Bismuth Subnitrate Teat Canal Sealant with Ampicillin-Cloxacillin Combination in Control of Bovine Mastitis in Selected Farms in Kenya

Nashon Wavumbah Wanjala^{1*}, George K Gitau², Gerald M Muchemi¹ and Dennis N Makau³

¹Department of Public Health, Pharmacology and Toxicology University of Nairobi- Kenya

²Department of Clinical Studies, University of Nairobi-Kenya

³Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, USA

*Corresponding author: nashonwavumbah@gmail.com

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ABSTRACT

This study presents the results of effect of a combination of bismuth subnitrate teat canal sealant (Boviseal® -Bimeda® Animal Health, Ireland) and the antibiotic Ampicillin & Cloxacillin (Bovaclox® DC-Norbrook Laboratories Ltd-UK) when used during the dry period on occurrence of mastitis 100 days post-calving. The objectives of this study were: to determine the effect of Boviseal® teat canal sealant in combination with Bovaclox® DC in control of dairy cow mastitis 100 days after calving; to determine bacterial pathogens causing mastitis in the selected farms and to determine risk factors for occurrence of dairy mastitis in the selected farms. This controlled field trial was carried out in two Kenyan dairy farms: Chemusian Farm in Nakuru County and Gicheha Farm in Kiambu County. 156 dairy cows were used in the study. Healthy cows with no history of mastitis in their current lactation were recruited. They were randomly placed into either of the two study groups: the control and the test group. The Control group received Bovaclox® DC while the Test group received the Bovaclox® DC followed by Boviseal®. The cows were followed during the entire dry period and 100 days post-calving monitoring for mastitis occurrence. Cows in the control group were more susceptible to mastitis 100 days post-calving compared to cows in the treatment group ($P < 0.001$, $RR = 4.4$, $OR = 17.7$). Of the bacterial pathogens, coagulase negative Staphylococci (CNS) were the most common pathogens isolated from mastitic milk at 34.6 % followed by *Micrococcus* spp. (9.0%). Other bacteria isolated were *Streptococcus agalactiae* (3.8%), *Staphylococcus aureus* (1.9%); *Escherichia coli* (0.6%) and various bacterial mixtures. Results of logistic regressions at $P \leq 0.05$ showed that farm, position of the quarter, type of barn floor and type of treatment were significantly associated with occurrence of mastitis. Cows in Gicheha farm whose barn floor was earthen, those cows in the control group and hindquarters were risk factors for mastitis ($RR = 1.5$, 4.4 and 1.18 respectively). The results of this study showed that Bovaclox® DC + Boviseal® teat canal sealant combination applied during the dry period is more effective in controlling bovine mastitis 100 days post-calving compared to the use of Bovaclox® DC alone. The study thus recommends the use of Bovaclox® DC + Boviseal® dry cow combination for control of bovine mastitis.

Key words: Dry cow mastitis; Teat sealant-antibiotic; Kenya

INTRODUCTION

The livestock industry contributes to the growth of the economy of not only Kenya, but the globally (Muthami, 2011; Mihret *et al.*, 2017). Kenya has approximately 17.5 million cattle, with approximately 3.5 million *Bos taurus* cows and 14 million *Bos indicus* cows (KNBS, 2010). Growth of the dairy sector is limited by various factors including; diseases, poor access to the market, inadequate veterinary and livestock extension service providers and poor cattle nutrition among others (Munyori and Karanja, 2014). One of the major

production diseases affecting the dairy cattle is mastitis (Barlow, 2011, Gitau *et al.*, 2014; Gomes and Henriques, 2016).

Mastitis is defined as persistent inflammation of the udder tissues due to trauma or infection by microorganisms. In the dairy industry, mastitis is the most costly production disease (Gomes and Henriques, 2016; Viguer *et al.*, 2009; Youssif *et al.*, 2020). Microorganism- caused-mastitis can be attributed to various pathogens, ranging from bacterial, fungal to viral organisms. Of major importance are the bacteria, both gram positive (such as *Staphylococcus* and *Streptococcus*

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species) and gram negative including the coliforms such as *Escherichia coli* (Belayneh *et al.*, 2013; Blowey and Edmondson, 2010; Girma *et al.*, 2012). Viral infections such as foot and mouth disease and bovine herpes directly cause mastitis or erode the skin of the udder and predispose it to secondary bacterial infections resulting in mastitis (Wellenberg *et al.*, 2002). Based on clinical features, mastitis can be classified as either clinical or subclinical (Fox, 2009; Mdegela *et al.*, 2009). Mastitis can also be classified either as environmental or contagious mastitis (Fox, 2009; National Mastitis Council, 2015). Environmental mastitis is caused by pathogens commonly isolated from the environment of the cow, which includes milking machine, barn floor, soil, walkways, pasture and any surface with which the cow may be in contact with. Organisms that cause environmental mastitis include *Staphylococcus* species (excluding *Staphylococcus aureus*), *Streptococcus* species (excluding *Streptococcus agalactiae*), coliforms such as *Escherichia coli* and *Enterobacter* species, *Pseudomonas*, *Proteus*, Yeast and *Prototheca* among others. Contagious mastitis is caused by pathogens that spread from cow to cow. These pathogens primarily inhabit the udder and teat of cows. The major pathogens responsible for this type of mastitis are *Streptococcus agalactiae*, *Corynebacterium bovis*, *Staphylococcus aureus* and *Mycoplasma* (Gonzalez and Wilson, 2003; Breen *et al.*, 2009; Erskine, 2016; Sonmez and Erbas, 2017). Common predisposing factors for bovine mastitis are breed, high milk production, parity of the cow, poor hygienic status of the cow environment among others (Breen *et al.*, 2009; Ramírez *et al.*, 2014).

Mastitis not only reduces milk quality and quantity during lactation, but may occasionally result in fatalities of the affected animal (Gomes and Henriques, 2016a). Moreover, some mastitis causing microorganisms such as *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Listeria monocytogenes* are zoonotic (Mwinyelle and Alhassan, 2014; Vishnupriya *et al.*, 2014; Sharma *et al.*, 2017).

Commonly employed mechanisms for management of mastitis include the use of antimicrobials such as tetracyclines, sulphonamides and lincosamides among others (Oliver and Murinda, 2012). Prolonged use and misuse of these agents has contributed to antimicrobial resistance (AMR) in both livestock and humans (Oliver and Murinda, 2012; WHO, 2014). To mitigate the AMR challenge, there has been a shift to the use of more environmentally friendly interventions such as vaccines, internal teat sealants, recombinant mucolytic proteins e.g. lysostaphin and nanoparticles (Sankar, 2016).

Usually the teat canal remains patent during the early dry period, regardless of antibiotic use during this period. Provided the canal is open, pathogens easily enter the udder usually resulting into an infection. The use of internal teat sealant containing bismuth subnitrate in controlling bovine mastitis has been practiced in various places worldwide. Several studies have shown its efficacy in reducing prevalence of mastitis in dairy cows. The product is efficacious against clinical and subclinical mastitis as well as reducing the level of milk somatic cell counts (Cook *et al.*, 2005; Compton *et al.*, 2014). Rabiee and Lean (2013) demonstrated that use of bismuth

subnitrate alone or in combination with antibiotic dry cow therapy pre-calving reduces incidence of clinical mastitis post-calving by 29% and 48% respectively.

To the authors' knowledge, there are no documented studies in the efficacy of using bismuth subnitrate or its combination with an antibiotic containing dry cow intramammary in prevention and control of mastitis in Kenya. The objective of this study was to determine the effect of bismuth subnitrate teat canal sealant in combination with Ampicillin & Cloxacillin dry cow therapy in controlling bovine mastitis in selected farms in Kenya. More specifically this study aimed to determine the effect of bismuth subnitrate teat canal sealant in combination with Ampicillin & Cloxacillin dry cow therapy on control of dairy cow mastitis 100 days after calving, determine bacterial pathogens causing mastitis in the selected farms and identify risk factors associated with mastitis in dairy cows in the selected farms.

MATERIALS AND METHODS

Study area

The study was carried out in two dairy farms in Kenya; namely Chemusian and Gicheha. These farms were conveniently selected because they were willing to participate in the study, had a structured record keeping system and routinely had dairy cows for drying, which were target animals for this study. In both farms, records on individual animals including age, parity, barn floor type and disease management were computer-stored thus easily retrievable. Both farms had a resident veterinarian and animal health assistants who helped in monitoring of animal health during the entire study period.

Chemusian farm, located in Rongai Constituency, Nakuru County is approximately 19.7 km West of Nakuru Town and 200 kms west of the capital city, Nairobi. The farm has about 1000 dairy cows mainly of two breeds, Friesian and Ayrshire. Gicheha farm, which is in Kiambu County, is approximately 25kms north of the capital city of Nairobi. The farm has about 500 dairy cows predominantly Friesians and a few Ayrshire and Guernsey breeds.

Study design

The study was a randomized controlled field trial.

Sample size determination and allocation into study groups

Sample size was calculated as follows, using the formula by Naing *et al.* (2006):

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where

n= sample size

Z= Z statistic for a level of confidence (which is 1.96 at 95% CL)

P= expected prevalence or proportion (which is estimated at 0.5 since no study has been conducted on prevalence of mastitis in both farms).

d= precision (= 0.05).

Using this formula, the sample size for each farm was 384 animals. However, since the farms had a finite population of lactating cows for dry off at 200 for

Chemusian and 50 for Gicheha, the sample size was adjusted using the formula by Naing *et al.*, (2006):

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1) + Z^2P(1-P)}$$

Where

n' = sample size with finite population correction

N = Population size (200 for Chemusian and 50 for Gicheha)

Z = Z statistic for a level of confidence (1.96 at 95% CL),

P = Expected proportion (0.5)

d = Precision (0.05).

The calculated values were 133 and 44 cows for Chemusian and Gicheha farms respectively. In total 177 cows were included in the study. In each farm, the cows were randomly allocated to different treatment groups.

Criteria for cow selection into the study

All the cows in the study were healthy on physical and historical assessment. The animals were in their first or subsequent lactation with no case of mastitis in the current lactation. The California Mastitis Test (CMT) was used to check the health status of the udder and only the animals with a score of 0, indicating absence of mastitis were included in the study. The cows were in their dry period (60 days to calving) as indicated in the farm records.

Udder and hind leg hygiene scoring

Before administration of the study products, the cleanliness of individual cow's hind leg and udder were scored using the method proposed by Schreiner and Ruegg (2010). In this study, scores 1 and 2 were merged to indicate a clean score while 3 and 4 were merged to indicate a dirty udder and hind leg (Fig. 1).

Administration of Reference and Test products

A simple random approach was used for allocation of cows into either group. For each farm, the cows were allocated into either of the two study groups: Test group or Control group. The test group received bismuth subnitrate (Boviseal® - Bimeda® Animal Health, Ireland) and antibiotic Ampicillin & Cloxacillin (Bovaclox® DC-Norbrook Laboratories Ltd-UK) while the control group received the antibiotic Ampicillin & Cloxacillin alone. These products were infused aseptically.

Animal follow-up

Animals in the study were monitored for development of mastitis from the day of dry off to 100 days post-calving. Each quarter was examined for any clinical abnormalities including signs of inflammation of teat canal / teat cistern/udder cistern on the following occasions: prior to the administration of Test and Reference Products; study Day 7, 14, 30 (post administration of the Test and Reference Product); immediately prior to calving and on each day post calving until day 100. Any abnormal clinical observation including signs of inflammation was recorded in the data capture form. In the event that a case of clinical mastitis was suspected, such that clots or abnormalities are found in the foremilk, 8 squirts were stripped to empty the teat cistern. At this stage milk would be coming from the udder tissue. If at this stage the abnormalities / clots had

disappeared, then this was not clinical mastitis. If the abnormalities persisted, the cow was deemed to have clinical mastitis and treated by the farm veterinarian using recommended farm specific protocols.

Milk sample collection and transportation

Milk samples were collected for bacteriological examination if quarter or udder was clinically diagnosed as having mastitis through CMT and visual examination of the milk. Milk was also collected from each cow in the study at any day after calving (within 100 days post-calving) to determine the prevalence of subclinical mastitis for those animals which had not developed clinical mastitis. The milk sample was collected aseptically as per the National Mastitis Council (2015). Five milliliters composite milk was stripped into a properly labeled sterile test tube for each cow. The samples were transported in a cool box with ice packs to the University of Nairobi, Department of Public Health, Pharmacology and Toxicology laboratory for immediate bacteriological culture or stored at 4°C for culture within 48 hours.

Culture and identification of bacteria

The milk samples were inoculated on blood agar and bacteria identified morphologically and biochemically by coagulase and catalase production, indole, methyl red, Voges-Proskauer, citrate and Christie-Atkins-Munch-Petersen tests (Phillips, 2007).

Data management and analysis

All data collected were entered, cleaned and stored in MS Excel 2010 (Microsoft, Sacramento, California, USA). The data were analysed using Stata13.1 software (StataCorp LLC, College station, Texas, USA). The outcome of the study was presence/absence of bovine mastitis while the explanatory variables included type of treatment during dry period, animal age, barn floor type, breed, farm, and lactation number, daily milk production, milking frequency, management system, hind leg and udder hygiene score. Descriptive data analysis was performed, and summary statistics were presented in form of proportions of various variables such as microorganisms causing mastitis, breed, milking frequency, management system and mastitis cases. Chi square tests were used to evaluate level of association for each independent variable and the outcome using statistical frequency tables. The factors influencing occurrence of mastitis under the study were subjected to a univariate logistic regression at $P \leq 0.2$ to accommodate as many relevant factors in the multivariable logistic model. Those variables with significant association with mastitis at $P \leq 0.2$ were subjected to multivariate logistic regression at $P \leq 0.05$ in order to get a parsimonious model. Odds ratios and relative risks were calculated from the frequency tables in order to find out if a variable was a risk factor for mastitis at P value ≤ 0.05 .

RESULTS

Incidence of mastitis

Table 1 summarises the incidence of various categories of mastitis in Chemusian and Gicheha farms. In

Chemusian farm, the incidence of clinical and subclinical mastitis was 10.48% and 36.29% respectively. In Gicheha farm, the incidence of clinical and subclinical mastitis was 25% and 71.88% respectively. As shown in Table 2, there was significant difference in the incidence of mastitis between cows in the treatment and control groups in both farms at $P \leq 0.05$. Cows in the control group were 4 and 10 times more likely to develop mastitis than those in the test group in Chemusian and Gicheha farms respectively.

Clinical mastitis and Subclinical mastitis

The incidence of clinical mastitis was 10.48% and 25% in Chemusian and Gicheha respectively (Table 2). There was a significant difference in the incidence of clinical mastitis between the test and control group in Chemusian farm ($P=0.001$). Cows in the control group were 1.2 times more likely to develop clinical mastitis compared to those in the test group. In Gicheha farm, there was no significant difference in the incidence of clinical mastitis between the test and control group ($P=0.072$).

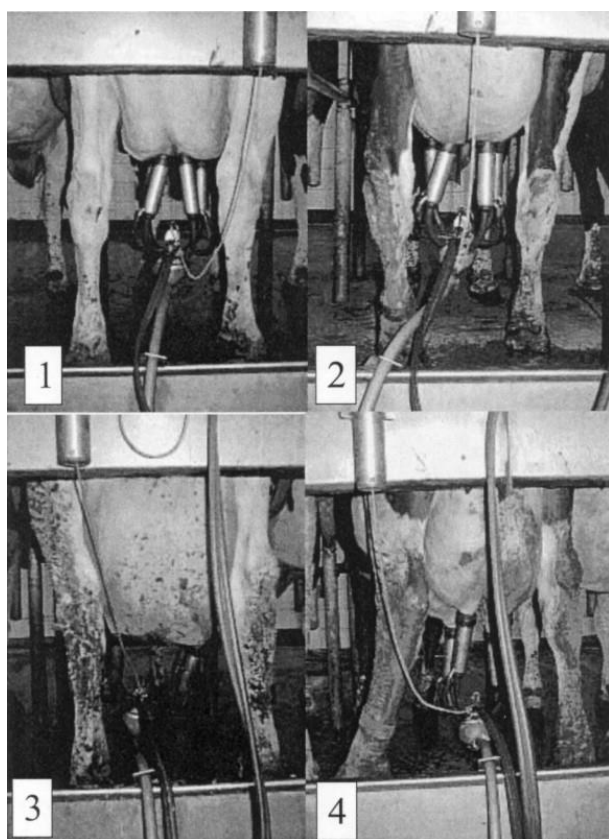


Fig 1: Udder cleanliness score. Picture number corresponds to the score for udder and leg by Schreiner and Ruegg, 2010.

Table 1: Number of cases and percentage of various categories of bovine mastitis in Chemusian and Gicheha Farms in 2017/2018.

Farm	Mastitis category	Number of cases (percentage)
Chemusian	Overall mastitis	59 (47.58)
	Clinical	13 (10.48)
	Subclinical	45 (36.29)
Gicheha	Overall mastitis	21 (65.63)
	Clinical	8 (25)
	Subclinical	23 (71.88)

The results of subclinical mastitis indicated significant difference in the incidence between test and control groups at $P \leq 0.05$ (Table 2). Animals in the control group were 2 and 2.2 times more likely to develop subclinical mastitis in Chemusian and Gicheha farms respectively compared to those in the test group. Hindquarters were 1.1 to 1.5 times more likely to develop mastitis compared to forequarters in Chemusian and Gicheha farms respectively.

Factors influencing the occurrence of mastitis in the selected farm

From univariable analysis, factors significantly associated with mastitis were farm, breed, barn floor and quarter position. These variables were fitted in the multivariable model from which barn floor and group were the two variables that significantly explained the difference in the incidence of mastitis. The variable farm and barn floor were collinear, since cows in Chemusian farm slept on a concrete floor while those in Gicheha farm slept on an earthen floor (Table 4)

Bacterial pathogens causing mastitis

The most common bacterial pathogens isolated from mastitic milk were coagulase negative Staphylococci (CNS) (34.6 %) and *Micrococcus* spp. (9.0%). Other bacteria isolated were *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli* and various bacterial mixtures as shown in Table 5. Pathogen distribution differed between the test and control groups. More bacteria pathogens were isolated from the control group than the test group ($P=0.001$).

DISCUSSION

From this study, a combination of Ampicillin & Cloxacillin (Bovaclox® DC) and bismuth subnitrate was more effective in controlling bovine mastitis 100 days post-calving compared to Bovaclox® DC alone. These findings were in agreement with studies done by Newton *et al.*, (2008), Runciman *et al.*, (2010), Berry and Hillerton, 2010, Golder *et al.*, (2016) and Bates *et al.*, (2016) who used the sealant with other antibiotics. In this study, animals in the control group were 4.4 times more likely to develop mastitis within 100 days post-calving compared to 1.9 times obtained from a study by Golder *et al.*, (2016). Bismuth subnitrate closes the teat canal during the dry period thus limiting entry and colonisation of mastitis causing pathogens in the udder. This greatly reduces incidence of both dry cow and post-calving mastitis (Woolford *et al.*, 1998). The teat sealant complemented the antibiotic function of Bovaclox® DC. It seals the teat canal thus limiting the number of bacteria and other mastitis causing pathogens entering the udder tissue. This explains why the test group animals (received both bismuth subnitrate and Bovaclox® DC) had a lower prevalence of mastitis compared to the control group.

The incidence of mastitis (clinical and subclinical) from this study was 51.8% (47.58% in Chemusian and 65.63% in Gicheha), slightly lower than that documented by Mekibib *et al.*, (2010) of 71.0% in Holeta town of Ethiopia and 74.7% reported by Abebe *et al.*, (2016). The difference in prevalence could be attributed to differences

Table 2: Comparison of mastitis in the Test and Control groups in Chemusian and Gicheha farms in 2017/2018

Farm	Category of mastitis	Group	Positive	Negative	Total	Pearson χ^2	P-value	Relative risk (odds ratio)
Chemusian	Overall	Control	49	13	62	49.18	<0.001	4(19.6)
		Test	10	52	62			
		Total	59	65	124			
	Clinical	Control	12	50	62	10.40	0.001	1.2(14.6)
		Test	1	61	62			
		Total	13	111	124			
	Subclinical	Control	36	26	62	25.43	0.001	2(8.2)
		Test	9	53	62			
		Total	45	79	124			
Gicheha	Overall	Control	15	1	16	11.22	0.001	10(25)
		Test	6	10	16			
		Total	21	11	32			
	Clinical	Control	5	11	16	0.685	0.414	
		Test	3	13	16			
		Total	8	24	32			
	Subclinical	Control	10	6	16	6.35	0.012	2.2(7.2)
		Test	13	3	16			
		Total	23	9	32			

Table 3: Comparison of occurrence of mastitis between hind and forequarters for cows in Chemusian and Gicheha farms in 2017/2018

Farm	Quarter	Mastitis			Pearson χ^2	P value	Relative risk (odds ratio)
Chemusian		Positive	Negative	Total	5.78	0.016	1.1(1.6)
	Hind	81	167	248			
	Fore	57	191	248			
Gicheha	Hind	38	26	64	4.5	0.034	1.5(2.1)
	Fore	26	38	64			

Table 4: Multivariate analysis of various factors influencing occurrence of mastitis in Chemusian and Gicheha Farms in 2017 and 2018

Variable	Estimate	95% confidence interval		P value
Breed				
Friesian	reference			
Others	0.348	-1.042	1.737	0.624
Barn floor				
Earthen	reference			
Concrete	-1.221	-2.252	-0.190	0.020
Group				
Test	reference			
Control	3.057	2.189	3.926	0.001
Constant	-1.744	-3.468	-0.020	0.047

Table 5: Table showing proportion of various bacteria isolated from mastitic milk from Chemusian and Gicheha Farms in 2017/2018.

Organism	Number	Percentage
CNS	54	34.6
<i>Micrococcus</i> spp.	14	9.0
<i>Streptococcus agalactiae</i>	6	3.8
<i>S.aureus</i>	3	1.9
CNS+ <i>Strep. Agalactiae</i>	3	1.9
<i>E.coli</i>	1	0.6
<i>Micrococcus+E.coli</i>	1	0.6
<i>Micro+Strep. Agalactiae</i>	1	0.6
No growth	73	46.8
Total	156	100

in production systems among other management factors. The prevalence of subclinical and clinical mastitis was 37.18% (36.29% in Chemusian and 40.63% in Gicheha) and 14.74% (10.48% in Chemusian and 25% in Gicheha) respectively. This observation concurred with several studies previously done in that showed that prevalence of subclinical mastitis is usually higher compared to clinical mastitis in a ratio of even up to 1:40 (Shaheen *et al.*, 2016). Mureithi and Njuguna (2016) had reported a

prevalence of 64% for subclinical mastitis in herds within Thika sub county of Kenya. In a study by Gitau *et al.*, (2014) in Mukurweini and Nakuru Districts of Kenya, the prevalence of clinical and subclinical mastitis was 0.7% and 32.4% respectively. Ndirangu *et al.* (2017) reported the prevalence of clinical and subclinical mastitis in Sahiwal cows of Kenya Agricultural and Livestock Research Organization (KALRO)-Naivasha as 6% and 54% respectively. Prevalence of subclinical mastitis is higher than that of clinical mastitis because subclinical mastitis is not easily detected at farm level by both farmers and animal health care providers. Therefore, most farms do not pay attention to subclinical mastitis because there are no obvious financial costs attributed to it thus limited control measures are implemented to curb it.

From this study, the most prevalent bacteria isolated from mastitic milk were the Coagulase-negative staphylococci. The high prevalence of CNS was also observed in studies by Pitkälä *et al.* (2004), Pyörälä and Taponen, (2009) and Vakkamäki *et al.* (2017) in Finland and Mpatwenumugabo *et al.*, (2017) in Ethiopia showing that the group is an emerging cause of subclinical mastitis. According to (del Pilar *et al.*, 2018) coagulase negative staphylococci are the most prevalent mastitis causing pathogens in Anaime Canyon, a dairy region in Colombia. This disagreed with a study done by Gitau *et al.* (2014) whose findings showed that *Staphylococcus aureus* is the commonest bacteria causing bovine mastitis in Mukurweini and Nakuru Districts of Kenya. Generally organisms in the staphylococcal group are the main pathogens causing mastitis in dairy cows as also documented by Ndirangu *et al.* (2017) in a study carried out at KALRO in Naivasha, Kenya. Coagulase negative staphylococci are emerging mastitis causing pathogens that are becoming the most prevalent pathogens isolated in mastitic milk in many countries (Taponen and Pyörälä, 2009).

From this study, the hind quarters were more likely to develop both clinical and subclinical mastitis compared to forequarters. This predisposition of the hind quarter was also observed by Vulić, (2000) and Khan and Muhammad (2005) in Faisalabad in Pakistan, Joshi and Gokhale, 2006, Tripathi *et al.* (2018) in India on cross breed cows, Hussain *et al.* (2018) in a study on dairy buffaloes in Pakistan. The increased risk of hind quarters may be partly because hind quarters are more frequently dirtied from dung and the floor. Furthermore, hind quarters are more vulnerable to direct trauma due to their closeness to the floor compared to forequarters.

This study showed that cows sleeping on concrete floor are less susceptible to mastitis compared to those sleeping on earthen floors. This is in agreement with a study by Hardenberg (2016) in Bihar, India. Kayesh *et al.*, (2014) reported 36.69% and 23.7% prevalence in subclinical mastitis for cows sleeping on earthen and concrete floors respectively in Bangladesh. This difference could be because concrete floors are easier to clean, thus environmental pathogens are washed off more easily than earthen floors.

Conclusions

Use of bismuth subnitrate teat canal sealant and Ampicillin & Cloxacillin combination during the dry period significantly reduces occurrence of mastitis compared to use of Ampicillin & Cloxacillin dry cow therapy alone. Subclinical mastitis is more common than clinical mastitis in both Chemusian Gicheha Farms. In these farms, earthen floor predisposes cows to mastitis more than concrete floors. Coagulase negative staphylococci pathogens are the most common mastitis causing pathogens in both Gicheha and Chemusian farm.

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