

Journal brief

EPIDEMIOLOGICAL INCIDENCES OF CONTAGIOUS BOVINE MASTITIS ON SMALL HOLDER DAIRY FARMS IN UASIN-GISHU COUNTY, KENYA

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ABSTRACT

Milk and its products are key components of human food chain and in Uasin-Gishu it's the main source of livelihood. However, widespread and prevalent mastitis infection of dairy cows poses a threat to this source of livelihood. Bovine mastitis is a worldwide infection characterized by an inflammation of the mammary glands and swelling of udder tissues caused by bacteria, fungi and mycoplasma. Bovine mastitis is a public health burden that has immensely contributed to widespread antimicrobial resistance in cows and man, compromised milk quality and quantity thus rendering milk and its products unfit for human consumption. This study aimed at determining incidence of bovine mastitis on small holder farms in Moiben and Kapseret sub-counties of Uasin-Gishu County. This prospective cohort study involved 216 cows recruited on 81 small-holder farms. The cows were pre-screened using microbiological culture method and those found free of mastitis infection were recruited. The cows were then monitored for development of mastitis between January and October 2021. Sampling was done every 21 days and on any other day the farmer reported the cow to be sick. Bacterial growth occurred after culturing milk samples and the specific bacterial pathogens isolated and identified. Of the total 216 cows, 104(48.2%) developed mastitis, with the epidemiological distribution of mastitis in the two sub-counties reported as 67(31.0%) in Moiben and 37(17.2%) in Kapseret. *Staphylococcal*-mastitis was the highest 66(30.6%), followed by *Coli*-mastitis 11(5.1%), *Citrobacter*-mastitis 6(2.8%), *Micrococcal* and *Streptococcal*-mastitis 5(2.3%) each, while *Pneumococcal*-mastitis and *Pseudomonal*-mastitis was

lowest at 2(0.9%) each. These findings suggested a high incidence of contagious-bovine-mastitis, attributable to *Staphylococcus epidermidis* and *Staphylococcus aureus* as the main causative agents. Mitigation of contagious-bovine-mastitis unlike environmental mastitis is reliable to reduce incidence of the disease.

Keywords: Contagious bovine mastitis, small holder dairy farm, productivity

INTRODUCTION

Mastitis is distributed worldwide and transmitted mainly through contact with the milking machines, contaminated hands and materials. The disease has been reported in dairy farms in Europe with significant prevalence in countries like Britain, Denmark, Finland, Norway, New-Zeland, Australia, France, Poland and Sweden (Valde *et al.*, 2004; Uhlemann *et al.*, 2014). The disease has also been reported in the American continent (United States of America, Brazil, Mexico, Canada and Chile) where the dairy sub-sector struggles with massive drawback from the high prevalence and incidences of mastitis (Halasa *et al.*, 2007; Swinkels *et al.*, 2005). In 2015, for instance Mexico reported an estimated annual loss per cow due to decreased milk production of approximately \$140 to \$300 dollars (Ma.Fabiola *et al.*, 2015)

The Asian continent also experienced high incidences of Mastitis. Countries like China, India, Bangladesh, and Iran were among the Asian nations that were worst hit by bovine mastitis, with the dairy sub-sector witnessing reduced milk production (Varshney and Naresh, 2004). In both USA and India for example, the annual losses in the dairy sub-sector due to mastitis

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were approximately ~2 billion dollars and ~526 million dollars respectively (Varshney and Naresh, 2004).

African continent and a majority of the developing world also bore the brunt of mastitis. The disease was rampant in West Africa and the horn of Africa. The Food and Agriculture Organization of the United Nations (FAO, 2010) reported that small holder farmer herds (SFH) were the most affected. In these herds animal healthcare was haphazard; farmers neither practiced rational preventive medicine nor handled milk hygienically when milking (Jiménez-Jiménez *et al.*, 2011). Over time, Djibouti, Ethiopia and Eritrea; countries located in the Horn of Africa reported increased incidences of bovine mastitis. In Ethiopia, for instance, mastitis prevalence using California Mastitis Test (CMT) was 75.7% at herd level and 62.6% at cow level (Rahmeto *et al.*, 2016). Further, increasing prevalence of the disease in different Districts of Ethiopia (41.7%, 44.1%, 52.3%, 52.6%, 58%) was reported by (Edilu and Getachew, 2017)

The East and Central African region experienced many cases of contagious bovine mastitis. For instance, in Zimbabwe mastitis prevalence was 21.1% with *Staphylococcal*-mastitis at 43.9%, *Coli*-mastitis at 21.2%, *Streptococcal*-mastitis at 1.6% and *Pneumococcal*-mastitis at 15.5% (Simbarashe *et al.*, 2013). In Uganda the prevalence rate of mastitis was 61.3% (Byarugaba *et al.*, 2008) while in Rwanda it was 50.4% with *Staphylococcal*-mastitis at 51.5%, the infections were broadly reported on intensive farming systems (Mpatswenumugabo *et al.*, 2017).

In Kenya, bovine mastitis was rampant in many regions where dairy farming was practiced intensively, particularly in the former white highlands. For example, studies have documented predominant mastitis in the Mount Kenya region, Nairobi County, Kiambu County, North and South Rift regions - where prolonged and haphazard use of antibiotics have been a major handicap (Odongo *et al.*, 2012; Gitau *et al.*, 2011). In Kiambu and its environs the prevalence rate of mastitis was 93% of which *Staphylococcal*-mastitis was highest at 31.7% (Odongo *et al.*, 2012), the increased prevalence was due to low udder and teat hygiene. In Thika sub-County mastitis prevalence was 64.0% (Mureithi and Njuguna, 2016) while in Nakuru County and Mukurwe-ini in the central region mastitis prevalence was 58.7% with *Staphylococcal*-mastitis highest at 77% (Gitau *et al.*, 2014). This was also replicated in Embu and Kajiado

counties where mastitis prevalence was 74.4% with *Staphylococcus aureus* causing 15.7% and *Pseudomonas aeruginosa* causing 5.1% (Mbindyo *et al.*, 2020).

Normally an infection of mastitis higher than 40.0% is considered significantly high and appropriate preventive and control measures should be instituted (Levesque *et al.*, 2004). This study therefore aimed at determining incidence of bovine mastitis on small holder farms in Moiben and Kapseret sub-counties of Uasin-Gishu County.

MATERIALS AND METHODS

Study area

The study was conducted in Moiben and Kapseret sub-counties of Uasin-Gishu that lies in the Western lower region of North Rift and on the eastern side of the Lake Victoria basin. It has conducive environment and climate for Livestock farming and breeding. Over 90% of the dairy cows kept here are exotic breeds and cross breed comprising of pedigree high yielding dairy cows. Rainfall is bimodal ranging from 500 to 1500 mm with average temperature of 18.5 °C. The two study sub-counties are peri-urban and are densely populated, majority of farmers living close to Eldoret town practice intensive farming system while majority of farmers living far from the town practice semi-intensive and extensive farming system because of ownership of large tracts of land.

Study design

This study adopted prospective cohort study design. The study cows were recruited at farm level to determine the baseline results. Once all the cows tested negative for mastitis using microbiological culture method, they were recruited into the study and followed up between Jan. – Oct. 2021 to determine the bovine mastitis endpoints. The follow-up was done every 21 days or earlier should the farmer report a sick cow.

Study population and sample size determination

The study population of dairy cows in Moiben sub-County was 113,008 while in Kapseret sub-County was 98,012. Out of these; 147,714 (70%) were pedigree exotic breeds, 42,204 (20%) crossbreeds and 21,102 (10%) indigenous breeds as per County Directorate of Livestock production and County Integrated Development Plan (CDLP unpublished data, 2018; CIDP, 2013). The

overall sample size was 216 lactating dairy cows recruited on 81 farms with small holder herds of between 1-10 cows. The sample size was determined using Kasiulevicius formula, on determination of sample size in epidemiological studies (Kasiulevicius *et al.*, 2006).

Sampling design

The study employed multi-stage sampling design. This type of sampling comprised stratified, cluster and simple random sampling methods. Out of five sub-counties in Uasin-Gishu County two sub-counties were randomly selected – Moiben and Kapsaret. Then all administrative wards and locations in each sub-County were purposively selected. Farms with small holder herds (1-10 cows) were identified in these locations with the help of local Animal Health Officers. They were recruited and pooled milk samples aseptically taken into sterile vials for microbiological culture (Bourabah *et al.*, 2013). The farmer milked the first few drops of milk out then milked the mid-stream milk into sterile sampling vials. Milk samples that were not processed immediately within eight hours were moved to the laboratory and stored at (4-8) °C for one week or frozen at -20 °C until they were ready for culture.

Microbiological culture

Microbiological culture method was adopted from a study by Mahlangu *et al.*, 2018 and Mureithi Njuguna, 2016. Sheep Blood Agar (5%), MacConkey Agar and various biochemical testing media were prepared in accordance with the manufacturer's instructions (Himedia, India). The process of microbiological culture was carried out in a level II biosafety cabinet. Fresh milk was cultured onto two Blood agar plates and one MacConkey agar plate alongside standard American Type culture Collection (ATCC) control micro-organisms by streaking a loop-full of milk. One blood agar plate was incubated anaerobically at 37 °C for 18-24/72 hours while the second blood agar and MacConkey agar were incubated aerobically at 37 °C for 18-24/48 hours. Further incubation for up to 72 hours for anaerobic and 48 hours for aerobic incubation was allowed to rule out negative growth. Colony characteristics were scored; morphology, shape, colour, texture, odour and size. Gram stain was performed to distinguish between the colony shape of gram-negative and gram-positive micro-organisms. Gram-positive cocci organisms were subjected to catalase test and coagulase test to distinguish

between *Staphylococcus* species (catalase positive) and *Streptococcus* species (catalase negative). To identify different *Staphylococcus* species colonies were subjected to coagulase and mannitol salt agar testing. Gram negative rods were identified using biochemical tests and media. Indole and oxidase tests were used to identify *Escherichia coli* and *Pseudomonas aeruginosa*. Other gram-negative micro-organisms were inoculated on Triple Sugar Iron agar, Urea agar, citrate Simon's agar, EMB agar, Methyl red broth, Voges Proskauer agar and identified accordingly (Mahlangu *et al.*, 2018; Cheesbrough, 2006). The bacterial pathogens were then characterized based on the different types of mastitis they caused.

Statistical analysis

Microbiological culture data on microbial pathogens was obtained from the laboratory results, coded and entered on to MS excel (Microsoft, USA) spreadsheet. The data was then exported into SPSS version 20 (Microsoft, USA) software and processed. Pearson Chi-square (χ^2) was computed to establish statistical associations between different study variables. All the tests were two-tailed at P-value less than 0.05 and 95% CI for statistical significance. The data was then summarized in tables.

RESULTS AND DISCUSSION

The study showed overall epidemiological incidence of bovine mastitis in the study area as 104 (48.2%) (Table I). These results were in agreement with those documented in similar studies carried out in Rwanda and Ethiopia by (Mpatswenumugabo *et al.*, 2017; Edilu and Getachew, 2017). They also found a mastitis prevalence of 50.4% and 52.3% respectively. These results however, differed with the findings of (Rahmeto *et al.*, 2016; Mureithi and Njuguna, 2016; Byarugaba *et al.*, 2008) in the Southern Harhage district of Ethiopia, western Uganda and Thika Kenya; the trio in separate studies reported high prevalence rate of 62.6%, 61.3% and 64.0% at cow-level respectively. The high prevalence in the three East African countries could be attributable to poor animal husbandry practices and the CMT screening method they used which is lower in specificity and sensitivity compared to microbiological culture method that was used in current study and is superior (Rahmeto *et al.*, 2016). Low mastitis prevalence was reported in Zimbabwe at 21.1% (Simbarashe *et al.*, 2013). In Zimbabwe, teat

therapy was practiced regularly, frequent milking (thrice per day), practiced good udder hygiene and good cow housing/crush cleanliness. These management practices contributed significantly to low prevalence of mastitis.

The epidemiological distribution of mastitis in the two sub-counties was 67 (31.0%) in Moiben sub-County and 37(17.2%) in Kapseret sub-County. This was attributable to the fact that a majority of the farmers in Moiben practiced intensive farming unlike majority of farmers in Kapseret who embraced Semi-intensive farming system. Indeed, previous studies reported higher prevalence of mastitis among cows on intensive farming systems and spread faster - due to poor cattle housing, haphazard milking practices and low udder hygiene (Mpatswenumugabo *et al.*, 2017). The difference in distribution of mastitis in Moiben and Kapseret sub-counties showed no statistical significance, but the difference in disease distribution among wards in Moiben sub-County was statistically significant (P-value 0.005) relative to the distribution in wards in Kapseret sub-County (Table I). In Moiben sub-County prevalence was high in Kimumu ward 5(71.4%) and Moiben ward 23(69.7%) but low in Karuna ward 17(30.9%), while in Kapseret sub-County the frequency of distribution was almost constant (Table I).

Out of overall mastitis prevalence 104(48.2%) in both Moiben and Kapseret study area, *Staphylococcal*-mastitis attributable to *Staphylococcus* species was predominant 66 (30.6%), this was followed by *Coli*-mastitis at 11(5.1%), *Citrobacter*-mastitis at 6(2.8%), *Streptococcal*-mastitis and *Micrococcal*-mastitis at 5(2.3%) each. Other different types of bacterial mastitis reported prevalence rate of less than (2.0%) (Table II). These results were comparable to similar studies in the region for instance in Kiambu County the prevalence rate of *Staphylococcal*-mastitis was 31.7% (Odongo *et al.*, 2012); which also used microbiological culture method as the current study. However, higher prevalence rates were reported in Zimbabwe where *Staphylococcal*-mastitis was 43.9%, *Coli*-mastitis 21.2% and *Pneumococcal*-mastitis 15.5%) (Simbarashe *et al.*, 2013) and Rwanda *Staphylococcal* -Mastitis was 51.5% (Mpatswenumugabo *et al.*, 2017). The high prevalence was probably as a result of sampling during long rainy season, low udder hygiene and lack of teat therapy. In contrast, (Simbarashe *et al.*, 2013) recorded a low prevalence rate of environmental *Streptococcal*-mastitis (1.6%) as compared to ours (2.3%), the difference in prevalence may be due to the variance in animal husbandry practices on the two farms. There were observed low environment sanitation (49.4%) on our farms.

TABLE I- EPIDEMIOLOGICAL INCIDENCE OF BOVINE MASTITIS ON SMALL HOLDER FARMS IN MOIBEN AND KAPSERET SUB-COUNTIES, UASIN-GISHU COUNTY

Sub-County	Ward	Number Examined n=216	Mastitis culture results		Chi-square (χ^2)	P-Value
			Positive (%)	Negative (%)		
Moiben	Karuna	55	30.9	69.1	15.07	0.005
	Moiben	33	69.7	30.3		
	Tembelio	23	56.5	43.5		
	Sergoit	16	56.3	43.8		
	Kimumu	7	71.4	28.6		
Total		134	31.0	31.0		
Kapseret	Kapseret	27	48.1	51.9	7.11	0.130
	Ngeria	24	50.0	12(50.0)		
	Kipkenyo	12	66.7	4(33.3)		
	Langas	11	18.2	9(81.8)		
	Megun	8	25.0	6(75.9)		
Total		82	17.2	45(20.8)		
Grand Total		216	48.2	112(51.8)		

TABLE II- INCIDENCE OF BOVINE MASTITIS ATTRIBUTABLE TO SPECIFIC BACTERIAL PATHOGENS ON SMALL HOLDER FARMS (N=216) IN MOIBEN AND KAPSERET SUB-COUNTIES, UASIN-GISHU

Bacterial pathogen isolated	Type of mastitis	Sub-County		Number of total isolates	Incidence rate (%)
		Moiben isolates	Kapseret isolates		
<i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>	<i>Staphylococcal</i>	38	28	66	30.6
<i>Escherichia coli</i>	Coli	9	2	11	5.1
<i>Citrobacter freundii</i>	<i>Citrobacter</i>	6	0	6	2.8
<i>Streptococcus</i> species	<i>Streptococcal</i>	4	1	5	2.3
<i>Micrococcus</i> species	<i>Micrococcal</i>	3	2	5	2.3
<i>Serratia marcescens</i>	<i>Serratial</i>	4	0	4	1.9
<i>Proteus</i> species	<i>Proteus</i>	1	2	3	1.4
<i>Pseudomonas aeruginosa</i>	<i>Pseudomonal</i>	1	1	2	0.9
<i>Klebsiella pneumoniae</i>	<i>Pneumococcal</i>	2	0	2	0.9
Total		68	36	104	48.2

CONCLUSION AND RECOMMENDATIONS

The study findings indicate a high incidence of bovine mastitis (48.2%), of which contagious-bovine-mastitis attributable to combined *Staphylococcus epidermidis* and *Staphylococcus aureus* was highest (30.6%). Prevention and control of contagious-bovine-mastitis by way of maintaining good udder and teat hygiene and rational administration of sensitive antibiotic therapy is reliable to reduce disease incidence on small holder dairy farms in Moiben and Kapseret sub-counties, Uasin-Gishu County in Kenya and is recommended.

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REFERENCES

Bourabah, A., Ayad, A., Boukraa, L., Hammoudi, S.M. and Benbarek H. (2013). Prevalence and etiology of subclinical mastitis in goats of the Tiaret Region, Algeria. *Global Vet. vol. 11, no. 5, pp. 604–608, 2013.*

Byarugaba, D.K., Nakavuma, J.L., Vaarst, M. and Laker, C. (2008). Mastitis occurrence and constraints to mastitis control in smallholder dairy farming systems in Uganda; *Liv Res. for Rural Devpt 20 (1) 2008.*

Cheesbrough, M. (2006). District Laboratory practice in Tropical countries, second Edition Part 2; www.cambridge.org/9780521676311; ISBN-13 978-0-521-67631-1; Cambridge University press, Cambridge, UK.

Edilu, J.S. and Getachew, K.T. (2017). Cross-sectional study on bovine mastitis and its associated risk factors in Ambo district of West Shewa zone, Oromia, Ethiopia; *Vet World. 2017 Apr; 10(4): 398–402.; doi: 10.14202/vetworld.2017.398-402; PMID: PMC5422243.*

Food Agriculture Organization (FAO) (2010). Status of and prospects for smallholder milk production—a global perspective. In: Hemme T., Otte J., editors. *Pro-Poor Live-stock Policy Initiative.* Rome, Italy: Food Agriculture Organization; 2010.

Gitau, G.K., Royford, M., Bundi, J., Vanleeuwen, C. and Mulei, M. (2014). Mastitogenic bacteria isolated from dairy cows in Kenya and their antimicrobial sensitivity. *J. S. Afr. Vet. Assoc. vol.85 n.1 Pretoria Jan. 2014.*

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

Halasa, T., Huijps, K., Østerås, O. and Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: a review. *Vet. Quarterly. 2007;29(1):18–31.* doi: 10.1080/01652176.2007.9695224.

- Jiménez-Jiménez, R., Espinosa-Ortiz, V. and Alonso-Pesado, F. (2011). Globalization effects in family farms: a case of Mexican dairy production. In: Pachura P., editor. *The Eco. Geography of Globalization*. Rijeka, Croatia: In Tech; 2011.
- Kasiulevičius, V., Šapoka, V. and Filipavičiūtė, R. (2006). Sample size calculation in epidemiological studies. *Theory and practice*, Gerontologija 2006; 7(4): 225–231.
- Levesque, P., Mammite, M. and Lait, M., I (2004). Institut de Technologie Agroalimentaire, Campus de la Pocatiere. Quebec, Canada, 2004.
- Ma.Fabiola, L.G., José, E.B., Arianna, L.A., Mauricio, V.P., Aguayo, D.D., Carlos, C.P., Erika, A.M. and Abner, J.G. (2015). Molecular Detection and Sensitivity to Antibiotics and Bacteriocins of Pathogens Isolated from Bovine Mastitis in Family Dairy Herds of Central Mexico. *Biomed Res Int*. 2015; 2015 Mar 1. doi: 10.1155/2015/615153, PMID: PMC4359873.
- Mahlangu, P., Maina, N. and Kagira, J. (2018). Prevalence, Risk Factors, and Antibigram of Bacteria Isolated from Milk of Goats with Subclinical Mastitis in Thika East Subcounty, Kenya. *J. Vet. Med. Vol. 2018, Article ID 3801479, 8 pages* <https://doi.org/10.1155/2018/3801479>
- Mbindyo, C.M., George, C.G. and Mulei, C.M. (2020). Prevalence, Etiology, and Risk Factors of Mastitis in Dairy Cattle in Embu and Kajiado Counties, Kenya. *J. Vet. Med. Internal. Vol. 2020, Article ID 8831172, 12 pages* <https://doi.org/10.1155/2020/8831172>.
- Mpatwenumugabo, J.P., Bebor, L.C., Gitao, G.C., Mobegi, V.A., Iraguha, B., Kamana, O. and Shumbusho, B. (2017). Prevalence of Subclinical Mastitis and Distribution of Pathogens in Dairy Farms of Rubavu and Nyabihu Districts, Rwanda. *J. Vet. Med. Vol. 2017, Article ID 8456713.* <https://doi.org/10.1155/2017/8456713>.
- Mureithi, D.K. and Njuguna, M.N. (2016). Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya. *Liv. Res. for Rural Dev.* 28 (2).
- Odongo, M.O., Ndungu, T.N., Mulei, C.M., Macharia, M. and Nduhiu, J. (2012). Prevalence of Microbial causes of bovine Mastitis in the Kabete area of Kiambu County and its environs (2001-2010). *The Kenya Veterinarian 2012; Vol. 36 No. 1 December 2012; ISBN: 0256-5161*.
- Rahmeto, A., Hagere, H., Mesele, A., Bekele, M. and Kassahun, A. (2016). Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Vet Res.* 2016; 12: 270; 2016 Dec 3. doi: 10.1186/s12917-016-0905-3; PMID: PMC5135792.
- Simbarashe, K., Matope, G., Ndengu, M. and Pfukenyi, D.M. (2013). Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *J. Vet. Res. Vol. 80, No. 1*, <https://hdl.handle.net/10520/EJC134538>.
- Swinkels, J.M., Hogeveen, H. and Zadoks, R.N. (2005). A partial budget model to estimate economic benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis. *J. Dairy Sci.* 2005; 88(12):4273–4287. doi: 10.3168/jds.S0022-0302(05)73113-1.
- Uhlemann, A.C., Otto, M., Lowy, F.D. and DeLeo, F.R. (2014). Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*. *Infect. Genet. vol. 21, 563–574.* 10.1016/j.meegid.2013.04.030.
- Valde, J.P., Lawson, L.G., Lindberg, A., Agger, J.F., Saloniemi, H. and Østerås, O. (2004). Cumulative Risk of Bovine Mastitis Treatments in Denmark, Finland, Norway and Sweden. *Acta Vet Scand.* 2004; 45(4): 201–210; 2004 Dec 31. doi: 10.1186/1751-0147-45-201; PMID: PMC1820994.
- Varshney, J.P. and Naresh, R. (2004). Evaluation of a homeopathic complex in the clinical management of udder diseases of riverine buffaloes. *Homeopathy.* 2004; 93(1):17–20. doi: 10.1016/j.homp.2003.11.007.