Original Article

Epidemiology of Bovine Mastitis in Selected Major Milk Shade Districts of Arsi Zone, Ethiopia

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Abstract

The data for the study on the epidemiology of bovine mastitis were collected from November 2015 to February 2016 to investigate the prevalence of mastitis in administrative towns of three selected districts of Arsi zone, Oromia Regional State, namely Iteya, Sagure, and Asella, Ethiopia; and to analyze the association between the risk factors and mastitis occurrence. Sample cows were selected and examined for clinical mastitis and at the same time, milk samples were evaluated both clinically and using the California Mastitis Test (CMT). Some parameters of the cows which have biological relation with this disease together with other potential risk factors were collected along with primary data collection. The analysis of the primary data showed that overall mastitis prevalence in the locations covered by the study was 28.6% of which 83.3% was subclinical, while 16.7% were clinical mastitis cases. Analysis related to the investigation of the existence of an association between risk factors and mastitis revealed that there was a significant association between the occurrence of mastitis and two of the risk factors evaluated, breed and age of the cow. The other risk factors investigated (i.e. parity, location, and lactation stage had no significant association with mastitis occurrence). Since mastitis is a complex disease that results from the interaction of different factors, it may be difficult to limit it to only some given factors. Environment and management factors and their combination may also have an additional impact on the epidemiology of the disease. A prevalence with the indicated magnitude together with the added effect of a lower level of awareness of the society about milk-associated zoonoses implies that this disease causes significant public health problems. In addition, mastitis may cause silent economic loss through unnoticed production loss along with reduced milk yield from subclinical and clinical (e.g. blind teats) cases. Due to a lack of awareness about mastitis and/or negligence, farmers in all corners of the country are suffering from economic and public health problems imposed by mastitis. This lack of awareness about mastitis-associated problems and/or negligence could be a possible reason for the use of raw milk which is a common scenario particularly in pastoral areas and the selling of adulterated milk which is becoming increasingly practiced in Ethiopia which is also true in these specific localities.
Keywords: Bovine; Epidemiology; Mastitis; Prevalence; Risk factors

1. INTRODUCTION

1.1. Background

The dairy industry is facing a great set back due to the high prevalence and incidence of mastitis in dairy cows. In addition to causing huge economic losses, this disease also possesses the risk for the transmission of zoonotic diseases, such as tuberculosis, brucellosis, leptospirosis, and streptococcal sore throat. Mastitis is becoming a major health and production constraint in dairy farming. Dairy animals are seen as frequently affected by this disease (Rahmeto *et al*., 2016).

Up to now, several pieces of research have been conducted but the problem persists (Rahmeto *et al*., 2016). This may be because of an absence of intensive research that deeply investigates the epidemiology of the disease which can aid in planning to develop tools for effective prevention and/or control measures to be taken and overcome the demand-supply imbalance which becomes a reason for adulteration and deterioration of quality of milk.

1.2. Problem Statement

The frequent occurrence of cases of mastitis, reduction in milk yield that does not support the consumer's demand, milk quality problem, and fear of zoonoses (diseases shared by animals and people)

1.3. Objective

General Objective

- ❖ To investigate the prevalence of mastitis across the study sites and to identify if there is an association of the disease with risk factors.

Specific Objectives

- ❖ To screen cows with subclinical mastitis and identify cows with clinical mastitis among study animals
- ❖ To investigate the prevalence of mastitis across the study sites
- ❖ To assess the association of mastitis with some of the host and environmental factors

2. METHODOLOGY

2.1. Study Area and Study Population

This observational study on the epidemiology of bovine mastitis was conducted on selected three districts of the Arsi zone which has relatively larger dairy herds, and cows have a higher milk yield compared to other Ethiopian, and that is relatively located close to the school complex, the former School of Agriculture, Adama Science and Technology University. In each of the districts, the study was limited only to their administrative towns which are again potential dairy sites within the districts.

2.2. Selection and sampling of animals

The study was designed so that all lactating cows in each of the districts at the time of the survey were included in the sampling process. Besides, dry cows were selected to widen the scope for the investigation of subclinical mastitis. Based on these a total of 126 local and crossbreed cows were sampled. The following parameters were recorded for each animal at the time of sampling; age, breed, parity, stage of lactation, and milk yield.

2.3. Data collection method

Data about mastitis among the sampled dairy cows were gathered by collecting a reasonable amount of milk (2 ml from each quarter) poured on each of the four plates of the California Mastitis Test (CMT) paddle. The collected milk samples were clinically examined for the presence of abnormalities in milk, such as color change, change in odor, and presence of a clot. The milk was further screened by CMT procedure for subclinical mastitis by adding an equal amount of the CMT reagent, 4% NaOH into each of the four shallow cups in the CMT paddle. Clinical mastitis was established by clinical examination of the cow`s udder and teat for gross abnormalities like the asymmetry of an udder and blind teat, before milk sample examination. Examination of milk in case of clinical mastitis can be conducted by visualization of milk for the presence of abnormal flakes. Subclinical mastitis is inapparent during clinical examination of cow and is apparent only during milk analysis. A CMT reagent (4% NaOH in distilled water with an indicator, coloring dye of 1% bromothymol blue) which is equal in amount to the collected milk sample on each well of the paddle, was added to the milk. The paddle was tilted and swirled for a moment (15

seconds) and watched for the formation of a precipitate. Depending on the size of the precipitate, if any or absent, subclinical mastitis is roughly graded as 0, trace, +1, +2, and +3, where 0 and trace are regarded as negative for subclinical mastitis and the rest are considered as positive for the test, with the level of infection differs in the same order given. Cows having at least one quarter with a CMT score of at least 1+ were defined as positive (NMC, 1999; Quinn *et. al.*, 2004).

The test of subclinical mastitis by the California Mastitis Test (CMT) procedure is an indirect proof test. This test procedure induces the formation of clots in the milk sample after the reagent is added. The clot (precipitate) is formed as a result of the reaction between the added reagent and DNA of somatic cells that may present in milk if there is any inflammation of the mammary gland. An induced clot is formed in milk only when there are somatic cells (white blood cells), even if there are some which may complicate this reaction. These somatic cells are further present in milk only when there is inflammation of the udder which is due to either pathogenic or physical or chemical agents. That is, the presence of a clot in milk implies the presence of somatic cells, and these further imply the presence of an inflammation which in this case is called mastitis. So, based on the presence and level of the clot formed, the test result was interpreted as negative and positive with different levels of positivity which corresponds to the level of somatic cells in milk. (0 = 0-200,000 somatic cells per ml of milk, trace = 150,000-500,000, 1 = 400,000-1,500,000, 2 = 800,000-5,000,000, 3 = >5,000,000) (Miller and Kearns, 1967). The present study was hoping to search for pathogenic agents as the cause of mastitis which are the dominant cause or sometimes maybe the only cause of the disease.

2.4. Data Analysis

The data were recorded in the Microsoft Excel spreadsheet and coded before statistical analysis. Descriptive statistics and logistic regression analysis were computed. In the logistic regression analysis, the outcome variable was the presence of mastitis, while the predictors were the different host and environmental risk factors. The association between the dependent variable, cow mastitis status (0 = negative and 1 = positive), and categorical

independent variables (breed, age, parity, location, and lactation stage) was assessed using

logistic regression analyses. All the statistical analyses were performed using R-statistics,

declaring significant results at $P < 0.05$.

3. RESULTS AND DISCUSSION

A survey on the epidemiology of bovine mastitis to investigate the magnitude of mastitis among different risk factors and its association with the factors showed the under mentioned results. The study also showed that mastitis was a significant problem for dairy cows in the study area.

3.1. Prevalence of Mastitis

Prevalence of mastitis was computed at different levels such as overall prevalence for the total sample from the three study locations. The prevalence for each location, and for the tested risk factors, and also the prevalence of clinical and subclinical mastitis are described in Table 1. The overall prevalence of mastitis was calculated as the proportion of mastitis-positive cows (clinical and subclinical) against the total number of cows investigated. A cow was denoted as positive for mastitis if at least a single teat (quarter) with clinical mastitis or CMT positive result was detected.

Table 1: Prevalence of mastitis at a different level of risk factors

Risk Factor	Category	No of Cow Examined	No of Cow with Mastitis	Prevalence of mastitis	Subclinical	Clinical	P*
Location	Iteya	18	6	33.3	6	0	
	Sagure	21	6	28.6	4	2	
	Asella	87	24	27.6	20	4	
Breed	Local	22	2	9.1	2	0	
	Cross	104	34	32.7	28	6	
Lactation Stage	<3mth	21	12	57.1	6	6	
	[3,6) mth	9	0	0	0	0	
	[6,9) mth	33	6	18.2	6	0	
	[9,12) mth	18	9	50.0	9	0	
	>=12 mth	45	9	20.0	9	0	
Parity Number	1	36	9	25.0	7	2	
	2	32	8	25.0	8	0	
	3	33	11	33.3	9	2	
	>=4	25	8	32.0	6	2	
Age	[3,6) year	33	6	18.2	6	0	
	[6,9) year	72	21	29.2	21	0	
	>=9 year	21	9	42.9	3	6	
Prevalence (%)					23.8	4.8	28.6

P* = overall prevalence

3.2. Association of Mastitis with Risk Factors

In the logistic regression analyses conducted, statistical significance was set at $P < 0.05$. Several cow-level and environmental factors like the breed of the cow, age of the cow, parity number of the cow, location (study site), and lactation stage of the cow were considered in the logistic regression analysis for the presence of their association with mastitis. Among those factors, the breed was found to be significantly ($P < 0.05$) associated with the presence of mastitis while age had a significant statistical association with mastitis just at 0.05 indicating a slight association. On the other hand, parity, location, and lactation stage did not have a significant effect ($p > 0.05$) on the occurrence of mastitis.

Excluding the non-significant factors and rerunning the logistic regression only for the significant risk factors, breed and age, yield the following out put (Table 2).

Table 2: Multivariable Regression Analysis for the Potential Risk Factors of Mastitis

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-5.1638	1.6602	-3.110	0.00187	**
Age	0.6250	0.3196	1.956	0.05050	.
Breed	1.6117	0.7761	2.077	0.03785	*

```
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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Therefore, the regression equestion, that is the final regression model in this case become:

Ln(odd of mastitis) = -5.1638 + 1.6117*Breed + 0.6250*Age

But the Logistic regression analysis of the effect of breed alone on the occurrence of mastitis showed that mastitis occurrence was significantly influenced ($p < 0.05$) by the breed of the cow. The chance of occurrence of mastitis is significantly higher in crossbred cows than in local breed cows. Mastitis in crossbreed cows increases 4 times than in local breeds. The odds ratio for the breed is 4.857143, which is $\exp(1.5805) = 4.857384$(**Appendices**)
Ln(odd of mastitis) = -3.8830 + 1.5805*Breed

There is also a significant effect of the age of cows alone on the incidence of mastitis. Because the p-value for age is 0.05365 which is approximately equal to 0.05 level of significance.

Ln(odd of mastitis) = -2.1069 + 0.6081*Age

For every one-year increase in age of the cow, the odds of incidence of mastitis increases by 83.7 units. Because $\exp(0.6081)$ =1.836938 (**Appendices**). This implies that the age of the cow has a significant effect on the incidence of mastitis, which means that when the age of the cow increases by one year, the odds of incidence of mastitis increases by 84 units. That means there is a positive relationship between the age of cow and odds of incidence of mastitis (also seen from the logistic regression model, positive coefficient of age). The odds of getting mastitis increase by 84% as the age of cow increase by one unit (year).

There were different researches conducted so far in Ethiopia on mastitis. The research output of some of these researches was consistent with the present study while others were not. Beyene *et al*., 2017 have shown that at the analysis of chi-square and univariable logistic regression, four factors namely breed, lactation status, age and number of parity were statistically significant while at multivariable logistic regression breed, age and lactation status showed to be the main risk factors that contribute for occurrence of mastitis.

Rahmeto *et.al.,* 2016 have noted that in the multivariable logistic regression model, breed, parity, and stage of lactation have a significant effect on mastitis occurrence. The occurrence of bovine mastitis and lactation stage was significantly ($p<0.05$) associated. That is, higher infection in cows in early lactation stage followed by late and medium lactation stages. Mastitis prevalence was found to be higher in early lactation and lower in mid-lactation stages. The early lactation stage infection might be due to the carryover of infection from the dry period. Rahmeto *et al*., 2016 also showed that animals over 8 years old were more frequently affected with mastitis and those younger than 5 years were rarely affected. Mastitis is more common and has a serious impact on older lactating animals than in first lactation heifers Also the highest prevalence of mastitis was observed in animals with parity of more than 6, followed by 3-6 and 1-2 parity.

Mulugeta and Wassie, 2013 also found that all the potential risk factors considered for a univariate logistic regression, i.e. age, parity, breed, and lactation stage were found significant (P<0.05). However, age became insignificant when tested with multivariate logistic regression. The prevalence of mastitis varied significantly (p<0.05) among breeds. It was also appreciated that the lactation stage and parity significantly (p<0.05) influenced the occurrence of mastitis.

4. CONCLUSIONS AND RECOMMENDATIONS

The frequent occurrence of cases of mastitis, reduction in milk yield that does not support the consumer's demand, milk quality problem, and fear of zoonoses are some of the problems that call attention for the research. The source of mastitis infection may be regarded as contagious or environmental. Contagious pathogens spread during milking. Environmental agents are opportunistic invaders from the cow's environment.

Prevalence and distribution of mastitis might be due to differences in the breeds of cattle reared, age of the animal, parity, the geographical locations, and lactation stage. These variables were subjected to logistic regression analysis and accordingly, the logistic regression model revealed that the breed of the cow among the variables entered remained significant predictors of mastitis in the cows ($p < 0.05$). Age of the cow is slightly significant ($p = 0.05$). The remaining factors analyzed didn't show statistical significance at the 0.05 significance level.

Mastitis causes huge economic losses and also possesses the risk for the transmission of zoonotic agents. It is one of the diseases that cause silent public health and economic loss. Unless preventive and other control measures were in place it may continue to negatively influence human and other animals' health. The big problem in Ethiopia is the absence of clear and comprehensive and frequently updated epidemiological data on animal and human diseases that aid in designing an effective control measure. This is true for mastitis as well.

❖ There should be a comprehensive and frequently updated epidemiological data on mastitis in the study area for that matter in Ethiopia as a whole
❖ The epidemiological data obtained regularly should be properly analyzed and lead for action
❖ Evaluation and monitoring measures should always be in place for every control measure chosen and effected in every situation

❖ A method in which information-based amendments for the already effected control measure and initiation of new control measure based on need should be in place to control mastitis

❖ Awareness creation and frequent communication of information on mastitis among stakeholders should be implemented

5. Conflict of Interests

The author declares that there is no conflict of interest regarding the publication of this paper.

6. Acknowledgments

7. REFERENCES

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8. APPENDICES

1. Logistic regression analysis for the association between mastitis and breed of the cows and Odds ratio for breed

```
> fit<-glm(Infection~Breed,family=binomial(link="logit"),data=gesho)
> summary(fit)
```

Call:
glm(formula = Infection ~ Breed, family = binomial(link = "logit"),
    data = gesho)
Deviance Residuals:
    Min   1Q Median   3Q   Max
-0.8898 -0.8898 -0.8898   1.4953   2.1899

Coefficients:
          Estimate Std. Error z value Pr(>|z|)
(Intercept) -3.8830   1.4976 -2.593 0.00952 **
Breed   1.5805   0.7704   2.052 0.04021 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 150.76 on 125 degrees of freedom
Residual deviance: 144.86 on 124 degrees of freedom
AIC: 148.86
Number of Fisher Scoring iterations: 4

```
> exp(1.5805)
[1] 4.857384
```

2. Logistic regression analysis for the association between mastitis and age of the cows and Odds ratio for age

```
fit<-glm(Infection~Age,family=binomial(link="logit"),data=gesho)
summary(fit)
```

Call:
glm(formula = Infection ~ Age, family = binomial(link = "logit"),
    data = gesho)

Deviance Residuals:
    Min   1Q Median   3Q   Max
-1.0600 -0.8293 -0.7321   1.2996   1.8442

Coefficients:
          Estimate Std. Error z value Pr(>|z|)
(Intercept) -2.1069   0.6640 -3.173 0.00151 **
Age   0.6081   0.3151   1.930 0.05365 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 150.76 on 125 degrees of freedom
Residual deviance: 146.90 on 124 degrees of freedom
AIC: 150.9
Number of Fisher Scoring iterations: 4

> exp(0.6081)
[1] 1.836938

3. Logistic regression analysis for the association between mastitis and all the risk factors

> fit<-glm(Infection~Age+Breed+Lactation+Parity+Location,family=binomial(link="logit"),data=gesho)
> summary(fit)

Call:
glm(formula = Infection ~ Age + Breed + Lactation + Parity +
    Location, family = binomial(link = "logit"), data = gesho)

Deviance Residuals:
    Min   1Q   Median   3Q   Max
-1.3801 -0.8841 -0.6564   1.1605   2.1921

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -4.73753   1.88514 -2.513   0.0120 *
Age   0.62735   0.32359   1.939   0.0525 .
Breed   1.58463   0.78831   2.010   0.0444 *
Lactation   -0.05195   0.14166 -0.367   0.7138
Parity   0.09269   0.18808   0.493   0.6221
Location   -0.16765   0.28832 -0.581   0.5609

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 150.76 on 125 degrees of freedom
Residual deviance: 140.08 on 120 degrees of freedom
AIC: 152.08
Number of Fisher Scoring iterations: 4

4. Odds ratio for the risk factors for mastitis (STATA output)-for comparison with R

logistic infection age breed lactation location parity

Logistic regression   Number of obs   =   126
                                        LR chi2(5)   =   10.68
                                        Prob > chi2   =   0.0581
Log likelihood = -70.042061   Pseudo R2   =   0.0708

----
  infection | Odds Ratio Std. Err.   z   P>|z| [95% Conf. Interval]
------------+--
        age | 1.872646   .6059864   1.94 0.053   .9931339   3.531048
      breed | 4.877469   3.846169   2.01 0.044   1.039854   22.87793
  lactation | .9493721   .1344852   -0.37 0.714   .7192135   1.253185
   location | .8456531   .2438246   -0.58 0.561   .4805824   1.488047
     parity | 1.097124   .2063574   0.49 0.622   .7588462   1.586199
      _cons | .0087602   .0165182   -2.51 0.012   .0002175   .3528054