

Epidemiology and financial impact of bovine mastitis in an animal production and research center and small holder dairy farms in horoguduru wollega zone, western Ethiopia

Abstract

Mastitis is inflammation of parenchyma of the mammary gland regardless of the causative agent. The objective of this study was to determine the prevalence, risk factors and financial losses caused by mastitis in six small holder dairy farms and the Animal Production and Research Center of the Wollega University. Data was collected through a questionnaire on extrinsic and intrinsic risk factors and financial loss for one year production cycle. Sub-clinical mastitis prevalence was diagnosed using the California Mastitis test (CMT). Chi square, univariable and multivariable logistic regression methods were used to determine the risk factors of the disease. CMT's were done on 154 lactating cows. The result of the study showed a prevalence rate of 46.75%, with 36.36% subclinical and 10.39% clinical mastitis cases. The analysis of extrinsic risk factors showed the management system is poor for most farms. Multivariable logistic regression showed breed, age and lactation status are the main risk factors that contribute for occurrence of the disease. When all farms were put together, the disease caused total loss of 2,949.8USD (59,719.08ETB), with an average annual milk loss of 22.3% in cross breed and 2.24% in local zebu cattle. Results of this study showed a high mastitis prevalence rate resulting in significant milk production and economic losses. Factors identified which may have played a role include dairy cow management, breed, age and lactation status. Recommendations to address these problems should be made awareness creation for the dairy cow owners on housing and management of dairy cow; awareness creation for the dairy cow owners on sources of mastitis pathogen and transmission methods of mastitis causing agent; design and implementation of control methods and further investigation of the disease by researcher were recommended.

Keywords: cross breeds, financial impact, horoguduru animal production and research center, mastitis, risk factor, prevalence, shambu, california mastitis test

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Introduction

To increase milk production cross breeding of indigenous zebu with exotic breeds like Jersey with Zebu and Jersey with Zebu are done in many African countries. This results in a larger portion of the mixed breed cattle being used for milk production in urban areas. However, dairy production is subjected to diseases of intensification like mastitis and reproductive disorders.¹ Mastitis is inflammation of the parenchyma of the mammary gland regardless of the causative agent.²⁻⁴ It is characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue.^{3,5} The most important changes are discoloration of milk, the emergence of clots in milk; swelling, heat, pain and edema in the mammary gland of clinical cases.³ Isolation from the natural disease or experimental investigation showed that several species of bacteria, fungi, mycoplasmas and algae have been considered as causes of bovine mastitis though bacteria are the major ones.⁵

Mastitis also results in economic losses to the farmer through decreased milk production during remainder of lactation due to udder damage in case of subclinical infection. In addition to this it increases the culling and replacement rates leading to loss of genetic potential and deaths of diseased animals.⁴ The same authors stated that mastitis

causes 30% reduction in productivity per affected quarter and a 15% reduction in productive cow/lactation, which make the disease one of the most serious problems affecting the dairy industry worldwide.

In Ethiopia, previously many studies of bovine mastitis were concentrated in around large towns and in central Ethiopia.¹ There was not any study conducted on bovine mastitis in the present study area. The aims of the animal production and research center of Wollega University are to produce Horo breed crossed with Jersey as well as Horo crossed with Holstein Friesian and then distribute F1 crosses of these mixed breeds to the community. However, Data of veterinary clinic from those districts and Question from the society indicated Mastitis is the main problem for dairy cow.

However, the case report in the veterinary clinic and community problem appraisal indicates the presence of the disease and economic effect of mastitis in the area. Therefore, the objectives of the study were:

- i. To determine the prevalence of subclinical and clinical mastitis,
- ii. Investigate management and animal level risk factors that favor occurrences of mastitis and
- iii. To estimate financial losses incurred to the farm due to mastitis.

Material and methods

Study area

HoroGuduru Wollega Zone is located in the western part of Oromia regional state of Ethiopia. The livelihood of farmers in the area is depending on livestock rearing and crop production. In this study, two towns, namely; *Shambu* in Horo district and *Finch of Abayichoman* and *Horo guduru Animal Production and Research Center* in Guduru district were selected. Selection was based on the types of farming (Extensive, semi intensive and intensive grazing system; Housing system cows were housed on either earth floors without shade, a concrete floor with shade or a stone floor with shade) Milking system all animals were milked manually in those districts and the existences of smallholder dairy production and bovine mastitis was not studied before.

Study group

Animals in smallholder dairy farms of Shambu, Fincha, dairy farm of Wollega University Shambu Campus and Horoguduru Research Center of Wollega University were considered as target population and the lactating cows in those dairy farms were the study population. Milk samples were collected from 154 total lactating cows of different lactation status from the study population (Table 1).

Methods of data and sample collection

Questionnaire: A well designed questionnaire⁶ was developed and administered to the dairy owners or managers by the researcher in person. The managers or heads of the house hold were the active respondents for the data requested. Animal data were collected from records and through interviewing responsible personnel. To estimate financial losses due to mastitis, retrospective data over a period of 1 year on mastitis cases? Prevalence?, (one case?) treatment cost/animal breed? And milk discarded during the treatment were collected.

Collection and examination of milk sample: Milk samples were collected from total number of lactating cows in Shambu (n=39), Fincha (n=13), Wollega University Shambu campus (n=6) and HoroGuduru animal production and research center of Wollega University (n=96). Cows were sampled once only. Strict aseptic procedures like washing of teats with soap and water, drying with towel and application of 70% ethyl alcohol were used while collecting milk samples in order to prevent contamination with microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment.³ The California Mastitis Test (CMT) was carried out on every milk sample to determine the prevalence of mastitis at cow and quarter level.³ About 2ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15sec. The reaction was interpreted according to Quinn et al.,³ Pandey & Voskuil.⁷ The average duration of lactation of 240days for local zebu cows (with a range of 210-270days) and of 305days for crossbreds (with a range of 305-408days) were used as the average lactation lengths.⁸ The average milk off-take per lactation is estimated to be 323liters (L) (range of 276-376L) for local zebu cattle and 3694L (range of 3473-3915L) for crossbred cattle.⁸

Data analysis

All collected data were entered into Microsoft excel spreadsheet. Data was cleaned and descriptive statistics were performed on Microsoft excel spread sheet. The prevalence of clinical and subclinical

mastitis were the dependent variables while the herd level, cow level and quarter level variable were considered as independent variables. The independent variables at herd level included barn floor status (dust made, stone made and concrete made), milking hygiene (washing hands before and after milking (yes or no)) and grazing system (Extensive, semi intensive and intensive). While the independent variable at cow-level included the breeds (local and cross breed), age (2,5-6year, 6-9,5year and >9.5year), parity status (1-3 calf, 3-6 calf and >6calf lactation status (≤ 3 month, 3-6month and >6month)) and average milk yield. The total Prevalence was calculated as the sum of clinical mastitis and CMT positive sub clinical mastitis divided into the total lactating cows. Sub clinical mastitis is the inflammation of mammary gland that is clinically not apparent and infection is detected by milk diagnostic tests.⁵ Prevalence of Sub clinical mastitis was calculated by CMT positive but clinically non observable mastitis divide by total examined lactating cows. Clinical mastitis is characterized by swelling, heat, pain and edema in the mammary gland, discoloration of milk and the presence of clots in milk.³ Prevalence of Clinical mastitis was calculated by clinically observable mastitis divide by total examined lactating cow. The associations between dependent and independent variables were tested by chi-square test, Univariable logistic regression and multivariable logistic regression models. Odd ratio and confidence intervals were used to measure level of association.

Quarter was defined as CMT positive if it had a CMT score of 1+ or above. A lactating cow was defined mastitis positive if it had at least one quarter with a CMT score of +, ++, +++ or clinically observable signs like pus releasing, blood mixed milk or blinded teat.³ In this study nine factors (Washing of hand before milking start, washing of hand between milking of cows, use of towel, grazing type, washing hand between milking cows, breed, lactation status, age and number of parity) were considered as risk factors for occurrence of mastitis in the study area (Table 1) (Table 2). From those factors, four were statistically significant at analysis of chi-square and univariable logistic regression (breed, lactation status, age and number of parity). Based Grimes & Schulz⁹ after analysis of univariable logistic regression all factors that are considered biologically as predictor variables and have $P < 0.15$ were subjected to multivariable logistic regression analysis to construct the likely model ($P < 0.05$) by forward selection method. The overall fitness of the model was assessed using maximum likelihood (probability) estimation.⁶ From the four factors entered into multivariable, three factors (breed, lactation status and age) were statistically significant ($p < 0.05$) (Table 3). Those factors significant in univariable logistic regression ($P < 0.05$) and insignificant in multivariable logistic regression were considered as confounding. Whereas, those factors significant at all level of analysis (ch-square, univariable logistic regression and multivariable logistic regression were considered as risk factors for the occurrence of Mastitis in the study area).

In financial losses estimation, the total loss was calculated as

$$C = Md + Ct$$

Where, C: Total Financial Losses

Md: Milk discarded due to mastitis

Ct: Treatment cost.^{10,11}

Percentage of annual Production losses was calculated by dividing total annual production losses due to mastitis to total annual production without mastitis.

$$\text{Percentage of annual losses} = \left(\frac{\text{total annual production losses due to mastitis}}{\text{total annual production with out mastitis}} \right) \times 100$$

SPSS version 20 and SAS 9.2 software were used to analysis the data. In all cases the 95% confidence interval and 5% level of precision were applied.

Table 1 Descriptive statistics of cow level risk factors and prevalence of mastitis in smallholder dairy cows in HoroGuduruWollega zone

Variable	Tested cow	Test positive(%)	Prevalence	CI95%	x ²	P-value
Breed						
Local	89	34(38.20)		32.17-44.23	6.193	0.01
Cross	65	38(58.46)		49.23-67.69		
Lactation						
<3month	38	13(34.21)		28.81-39.61	9.764	0.044
3-6Month	34	13	38.24	32.19-44.27		
>6Month	82	46	56.1	47.24-64.95		
Age(in year)						
2.5-6	41	14	34.15	28.75-39.54	10.003	
6-9.5	46	17	36.96	31.12-42.79		0.007
>9.5	67	41	61.19	51.53-70.86		
Number of Parity						
3-Jan	77	28	36.36	30.6-42.1	9.26	0.01
6-Mar	61	32	52.46	44.2-60.7		
>6	16	12	75	63.2-86.7		

Table 2 Management system of smallholder dairy cows in HoroGuduruWollega Zone

Management system	Number of dairy farms	Number of tested cows (%)	Test positive (%)	x ²	P-value 95%
Washing hand between milking					
Yes	4	133 (86.36)	62 (46.62)	0.93	0.56
No	5	21 (13.64)	10 (47.62)		
Use of towel in cleaning teat after washing					
Common Towel		131 (85.06)	60 (45.8)	0.46	0.788
Separate Towel		18 (11.69)	9 (50)		
No use of Towel		5 (3.25)	3 (60)		
Washing hand before milking start in farm					
Yes	6	140 (90.91)	64 (45.71)	0.41	0.29
No	3	14 (9.09)	8 (57.14)		
Grazing type					
Extensive	2	99 (64.29)	42 (42.42)	2.93	0.23
Semi Intensive	6	54 (35.06)	29 (53.7)		
Intensive	1	1 (0.65)	1 (100)		
Floor type					
Concrete	4	49 (31.82)	24 (48.98)	2.02	0.36
Dust	1	96 (62.34)	41 (42.71)		

Table 3 Multivariable logistic regression of management and cow risk of smallholder dairy farm in HoroGuduru Wollega Zone

Parameter	B	S.E.	Walda	Exp(B)	95% C.I. for EXP(B)	P-Value -95%
Grazing system						
Extensive	Reference					
Semi intensive	2.941	2.419	1.478	18.93	0.165-226	0.224
Intensive	20.886	40192.97	0	0.001	0.001-0.01	1
Types of floor						
Concrete	Reference					
Stone made	0.147	1.655	0.008	1.158	0.045-29.6	0.929
Dust made	-2.063	1.631	1.601	0.127	0.005-3.1	0.206
Washing hand before milking						
Yes						
No	0.865	1.44	0.36	2.374	0.141-39.9	0.548
Breed						
Local breeds	Reference					
Cross breeds	3.283	1.003	10.711	26.647	3.73-45	0.001
Age (in year)						
2.5-6	Reference					
6-9.5	-2.699	0.806	11.229	0.067	0.014-0.326	0.001
>9.5	-2.214	0.652	11.521	0.109	0.030-0.392	0.001
Number of calving						
	Reference		2.23			0.328
>3-6	-0.967	0.776	1.554	0.38	0.083-1.74	0.213
>6	-1.14	0.767	2.21	0.32	0.071-1.437	0.137
Lactation						
<3month	Reference		6.856			0.032
3-6Month	-1.161	0.486	5.709	0.313	0.120-0.812	0.017
>6Month	-0.884	0.503	3.095	1.12	1.05-1.34	0.012

Result

The production system of dairy cows in the study area includes intensive, semi-intensive and extensive types. Our result revealed that 64.29%, 35.06% and 0.65% of the dairy cows were kept under extensive, semi intensive and intensive grazing system consecutively. The current study reported that 5.84%, 31.82% and 62.34% of the dairy cows are kept in housing in which the flooring consisted of stone earth with no roofing or a concrete floor with metal sheet roof (Table 2).

Prevalence

The current study revealed 72/154 (46.75%; CI95%, 39.37-54.13) cows had at least one quarter positive to mastitis. From the total of 616 quarters examined 141 (22.89%; 95%CI, 21.08-24.7) were positive to mastitis. From the total of 141 infected quarters, 94 quarters (15.26%; 95%CI, 14.05%-16.46%) were sub clinically positive to CMT and 47 quarters (7.63%; CI95%, 7.03%-8.23%) were clinically infected. The finding showed of the 37 clinically infected cows, 27 have single infected quarter and 10 cows have two clinically infected quarters. In this study there is no cow with more than two quarter infected with

mastitis. The study also found that of the total of clinically infected quarters 13(2.1%; 95%CI, 1.94%-2.28%) teats were blind. The Quarter level prevalence showed, that 5 cows with clinical mastitis had total blind teats (3.25%; 2.73-3.77 95%CI), 4 of these cows have single blind teats (2.61%; 95%CI, 2.19%-3.01%). However, there is no cow with more than two blind teats (Table 4).

Risk factors

Chi-square was used to analysis the presence of association between potential risk factors and occurrence of mastitis. The result showed that difference in breed, stage of lactation, age of animal and parity has a significant effect on the occurrence of mastitis in dairy cows (Table 1). The univariable logistic regression analysis showed that cross breed dairy cows were more than 2.3times more at risk of developing mastitis than local breed cows. The result also showed that an increase in age also significantly increase in prevalence of mastitis. Similarly, as the number of parities increased the risk of mastitis also increased (Table 5). Multivariable logistic regression showed that breed of animals, age and lactation status are the main risk factors and have cause effect relationship for prevalence of mastitis. However, the effect of number of parity was considered as bias.

Financial losses

Based on average duration of lactation of 240days for local zebu cows and 305days for crossbreds and average milk production per lactation is estimated to be 323liters (L) for local zebu cattle and 3694L for crossbred cattle.⁸ Using these reported values as standards then this study showed average annual milk losses of cross breed dairy cow to be 22.3% and 2.24% in local zebu dairy cows. The study also showed that milk discarded due to mastitis treatments was due to drug

withdrawal waiting periods. In this study the overall cost was highest due to the intra-mammary infusions which included 4 formulations. The costs of the drugs were 248.49USD (5,032ETB) for overall intra-mammary treatments, of which pen step represented 53.02USD (1,073.7ETB), 10% Oxytetracycline 3.58USD (72.54ETB) and 20% Oxytetracycline 0.78USD (15.84ETB). The total drug cost incurred to the dairy cow owners in this study was 305.88 USD (Table 6) (Table 7).

Table 4 Cow and teat level prevalence of sub clinical and clinical mastitis in HoroGuduruWollega Zone

Factor	CMT	Prevalence(%)	95%CI
Cow level			
Sub Clinical	56	36.36	30.62-42.11
Clinical	16	10.39	8.74-12.03
Total	72	46.75	39.37-54.14
Teat			
Subclinical	94	15.26	14.05-16.46
Clinical	47	7.63	7.03-8.23
Blind teat	13	2.1	1.94-2.28
Total	141	22.89	21.08-24.7
Clinically infected quarter			
	Number cow	Prevalence (%)	95%CI
One quarter	27	17.53	14.76-17.53
Two quarter	10	6.49	5.47-7.52
Three quarter	0	0	0
Four quarter	0	0	0
Blind teat			
One quarter	5	3.25	2.73-3.25
Two quarter	4	2.6	2.19-3.01
Three quarter	0	0	0
Four Quarter	0	0	0

Table 5 Univariable logistic regression analysis of cow level risk factors of mastitis in smallholder dairy cows in HoroGuduruWollega Zone

Variable	B	S.E.	Wald	P-value	Exp(B)	95% C.I. EXP(B)
Breed				0.014	2.28	1.19-4.37
Local	Ref					
Cross	0.823	0.333	6.1			
Age (in year)				0.008	1.6	1.35-1.85
2.5-6	Ref					
>6-9.5	1.112	0.414	7.22			
>9.5	0.99	0.395	6.27			
No of parity						
3-Jan	1.66	0.624	8.6	0.014	3	2.52-3.47
>3-6	1	0.632	7.06	0.008	0.19	
>6		0.577	2.51	0.113	0.368	
Lactation status						
<3month	Ref				1.28	1.08-1.48
3-6Month	0.899	0.408	4.86	0.028		
>6Month	0.725	0.417	3.02	0.082	0.407	

Table 6 Milk discarded during treatment and its losses in smallholder dairy cows in Horoguduru Wollega Zone

Breed	Number of animal treated	Total day of milk discarded	Amount of milk discarded (in liter)	Average losses due to milk discarded in ethiopia	Average losses due to milk discarded in USD
Cross Breeds	61	416	5038.37	52,903	2,612.50
Local Breeds	44	44	59.22	621.81	30.7
Total	105	460	5097.59	53,525	2,643.20

Table 7 Average cost incurred to the owner due to treating of clinical mastitis in Horoguduru Wollega zone

Name of drugs	unit	Total amount of drugs used for treatment	Cost in ethiopian birr	Cost in (USD)
Penstrip	ml	1481	1073.7	53.02
Oxytetracycline 10%	ml	186	72.54	3.58
Oxytetracycline 20%	ml	36	15.84	0.78
Intramammary infusion	Ampoule	272	5032	248.49
Total			6,194.08	305.88

Discussion

Prevalence

The total prevalence 72/154(46.75%, 95%CI, 39.37-54.14) is comparable with 52.78% overall prevalence reported by Sori et al.¹² from Sebeta Town of Ethiopia and 50% from most countries of the world.³ The result was lower than the 71% reported by Mekibab et al.¹³ from Holeta town, central Ethiopia. However; it was higher than the 23.18% report of Girma et al.⁹ from Hararge of eastern Ethiopia. That study was done on lactating Zebu cows. The clinical mastitis prevalence of 16/154 (10.39%, 95%CI, 8.74-12.03) is in agreement with the 11.9% reported by Biffa et al.¹⁴ In this study clinical mastitis is higher than 6.6% reported by Almaw et al.¹ and the 4.9% report by Moges et al.¹⁵ Our results showing 36.36% cow level subclinical mastitis is in agreement with 36.67% of Sebeta Sori et al.¹² 31.0% from Hawassa of the southern region¹⁶ and 40.6% of Batu and its surrounding area.¹⁷ A previous report of 48.6%¹³ on the prevalence of subclinical mastitis was higher than the shown in this study. The 141/616(22.89%, CI95% 21.08-24.7) prevalence of quarter level mastitis in our study is in agreement with 20.04% from Hawassa of southern Ethiopia¹⁶ and 10-25% from different parts of the world.³ The variation in prevalence of mastitis in the present study and different parts of the country was influenced by both intrinsic factors of dairy cow and environmental risk factors of mastitis.

Risk factors

Management Risk factor: In the analysis of management factors 62.34%, 31.82% and 5.84% of lactating cow were respectively housed on either earth floors without shade, a concrete floor with shade or a stone floor with shade. Muddy walking surface is one of the predisposing causes for mastitis in the dairy cow.^{3,4} In the current study there is no statistically significant difference in the different housing system (P>0.05). The prevalence of mastitis is 100%(1/1), 53%(29/54) and 42.42%(42/99) under Intensive, semi intensive and extensive management system. Even though higher prevalence were reported in this study it was not statistically significant different. The result is contras-

ting to the report of Sori et al.¹² who reported statistically significant difference between different grazing systems. A high prevalence of mastitis under intensive and semi intensive management was previously reported from Southern Ethiopia.¹⁴ The absence of significant difference might be due to small number of animals in intensive management system. The current study also reported a higher prevalence of mastitis 57.14% on farms that did not wash their hand before milking than those that did wash their hands before milking 45.71%.

Cow level risk factors

Lactation status: The prevalence of mastitis based on the stage of lactation was 34.21%, 38.24% and 56.1% in the 1st, 2nd and 3rd trimester of lactation. Statistically significant difference in prevalence of the disease in the 3rd trimester compared to the 1st and 2nd trimester (P<0.05) was similar to other findings in the country.^{15,17} The high prevalence of mastitis in older dairy cattle compared to young and adult cows is associated with an increased period of exposure of the udder during previous lactations.⁴

Breed: Prevalence of mastitis was 38.2%(32.17-44.23) in local breeds and 58.46%(CI95% 49.23-67.69) in cross breeds. There was a statistically significant difference in the prevalence of mastitis between breeds ($\chi^2=6.193$, P<0.01). In Ethiopia many studies showed statistically significant difference in mastitis between local and cross breeds.^{12,14,15,17} Cows with high milk yield is also more susceptible to mastitis where as low-yielding cows tend to be more resistant. Genetic improvement for milk yield is accompanied by gradual decline in genetic resistance to mastitis.³

Age: In the current study the prevalence of mastitis was 34.15% (CI95%, 28.75-39.54), 36.96% (CI95%, 31.12-42.79) and 61.16%(CI95%, 51.53-70.86) for young (2.5-6), adult (6-9.5) and >9.5(older) cows respectively. The statistical analysis showed that there is a significant difference between older and younger aged cows (P<0.01). In Ethiopia high prevalence of mastitis in old animals were reported by Biffa et al.¹⁴ Mekibib et al.¹³ Bedacha & Menghistu.¹⁷ The high prevalence of the disease in older animals related to the physiology of threat

canal which is more dilated and remains partially open due to years of repeated milking. This facilitates the entrance of environmental and skin-associated microorganisms leading clinical or sub clinical mastitis.^{3,18} Blowey & Edmondson⁴ also reported the high occurrence of mastitis in older aged cows compared to young and adult cattle. This could be due to damage of teat canals in old animals facilitates access of bacteria into the mammary gland.

Financial losses: In the current study mastitis causes both direct and indirect economic losses. Direct economic losses are associated with discarded milk during treatment and drug withdrawal, and cost of drugs of treatment. The indirect cost is related to decreased milk production because of udder damage. In Ethiopia many studies conducted were concentrated on determination of prevalence, risk factors and pathogen identification^{12–17} but did not report on its financial burden. This study shows that the disease caused a total loss of 2,949.08USD in a total of 89 local and 65 cross breed dairy cows.^{19–21} The disease caused annual production losses of 22.3% in cross breed cows and 2.24% in local breed lactating cows in the production cycle in one year. Other studies reported the economic effect of both direct and indirect effect of mastitis in different parts of the world.^{3,4}

Conclusion and recommendation

The study showed existence of subclinical and clinical mastitis in the area. Breed, age and lactation status were statistically significant risk factors for the occurrence of mastitis. The study also revealed that most lactating cows are kept in poor husbandry management. In this finding, 2,949.08USD losses were incurred due to discarding of 5,097.59Lt milk that resulted in average market price of 2,643.20USD and 305.88 USD due to expense to drugs. The understanding of farmers on cause and transmission of Mastitis is poor. As well, the farmers requires awareness on housing system, disease cause and transmission. Since there is no national or farm level controls program for the disease the following recommendations have been suggested. Awareness creation for the dairy cow owners on housing and management of dairy cattle, sources of mastitis pathogen and transmission methods of mastitis causing agent to reduce disease prevalence and prevent occurrence of disease. The policy makers should design control methods through vaccination for highly pathogenic agent and develop guideline for dairy production management in the country. Since this is the first report from the study area further investigation is recommended.

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Ethical issue

The aim of the study was to determine prevalence risk factor and economic importance of mastitis. It was designed and conducted through Questioner and detection of milk sample. Therefore, there is no any experiment done on animals under laboratory or field study. The response of the owners was based on their knowledge about the disease, personal willingness and critical reason to justify the clinical characteristics of the disease. The confidential issue of individuals were kept in secrete as to the standards.

Conflict of interest

Author declares that there is no conflict of interest.

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