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Original Article

Prevalence of Mastitis, Risk Factors and Its Causative Agents Western Hararghe Oromia, Ethiopia

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ABSTRACT

A cross sectional investigation was conducted from May 2018 to June 2019 to determine the prevalence of bovine mastitis, potential associated risk factors for the incidence of mastitis in lactating cows and to isolate the major bovine mastitis causative agents in West Harrerghe, Zone Oromia Ethiopia. A total of 151 lactating dairy cows were examined clinically to quantify the incidence of clinical mastitis. The prevalence of subclinical mastitis was detected by using California Mastitis Test (CMT). The overall prevalence of mastitis at cow and quarter level was 36.5% and 23.4%, respectively. In the study the incidence rate sub clinical mastitis was higher (36.5%) while clinical mastitis accounts only 9.3% of the overall prevalence. Out of 604 examined quarters, 3.9%of them were clinically affected, 2.2% quarters were blind and 19.5% were positive to CMT test. The prevalence of mastitis was statistically significant (P<0.05) with associated risk factors, previous mastitis history, Teat lesion stage of lactation and parity. The result shows that Staphylococcus species, Streptococcus species and Echerichia coli were the major bacteria genera causes of bovine mastitis in the study area, with percentages of 51.7, 20.9 and 20.3, respectively. The present study showed that mastitis is a major problem of dairy animals in the study area and it hinders the development of national dairy sector. Contagious bacteria were the dominant cause for the disease called mastitis. Therefore strict implementation of hygienic production, practices, cow therapy and awareness

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creation among dairy cow owners about the disease and its treatment could undertook to reduce mastitis incidence and its risk on dairy sector.

Keywords: bacteria clinical mastitis, clinical mastitis, prevalence, isolates, risk factor

INTRODUCTION

Ethiopia holds large potential for dairy development mainly due to its large livestock population and relatively favorable climate for improved high-yielding animal breeds (Abebe *et al.,* 2014). The livestock population census showed that Ethiopia has about 56.71 million cattle population. From the total population 98.66% are local breeds and the remaining are hybrid and exotic breed (CSA, 2015). Agricultural sector in Ethiopia engaging 80% of the population, contributes52% of the gross domestic product (GDP) and 90% of the foreign exchange (Aynalem *et al.,* 2011). The livestock sub-sector alone contributes 12% of the total and over 45% of the agricultural GDP, and over 85% and 90% of the farmer and pastoralist incomes, respectively, are generated from livestock (MOA, 2010).

From livestock sub sector, dairy sector is a development tool because it widens and sustains pathways out of poverty through securing assets of the poor, improving smallholder productivity and increasing market participation by the poor (ILRI, 2007). Hence, development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation, improved nutrition and household income (Mohamed *et al.,* 2004). However the dairy sector has not been fully exploited and promoted in Ethiopia, due to lack of modern animal husbandry and management, limited skilled manpower in dairy technology and marketing, inadequate distribution systems and limited health program of dairy cow affect the development of the sector and milk production (CSA, 2007).

Lack of efficient husbandry and management system causes for a number of dairy cow diseases, such as bovine tuberculosis, brucellosis and mastitis, from those diseases which potentially infect and affect the wellbeing of dairy cattle population is mastitis, which is the common and costly disease causing loss in milk yield, quality and treatment cost for dairy farmers (Nibret *et al.,* 2011).It is one of the serious diseases of dairy cows that generally involve interplay between management practices and infectious agents; having different causes, degrees of intensity and variations in duration and residual effects. Moreover, mastitis is economically important and expensive disease of dairy production. It can be caused by bacteria, viruses, yeast or fungi (Lidet *et al*., 2013). It is the major concern of the dairy industry worldwide for number of reasons, such as effects on milk composition, yield, and quality of dairy products; it is also considered as welfare concern due to the pain cows' experience, especially during an episode of acute, severe mastitis (Leslie and Petersson-Wolfe, 2012).

In Ethiopia, the available information indicates that bovine mastitis is one of the most frequently encountered diseases of dairy cows (Hagos, 2015). According to Yien (2014) different studies conducted in different parts of Ethiopia showed variable prevalence of mastitis depending on the type of farm and managements systems. Prevalence of clinical and sub clinical mastitis were recorded in different parts of Ethiopia ranges from 1.2 to 25.1% and 19 to 56% (Mungube, 2001;Workineh *et al.,* 2002; Lemma *et al*., 2001; Nibret *et al.,* 2011) respectively. Moreover it affects milk

yield as well as quality by damaging the epithelial cell that is responsible for the synthesis of milk components and by increasing the microbial load of the milk. It changes the enzymatic action of somatic cells or microorganisms in the infected mammary gland (FAO, 2014).

Mastitis prevalence had been reported by several authors in different parts of Ethiopia (Lakew *et al.,* 2009; Gebreyohannes *et al.,* 2010; Yien, 2014; Zenebe *et al.,* 2014; Rahmeto*et al*., 2016). However, in some parts of Ethiopia and particularly in the study area, the disease is insufficiently investigated and information relating to its distribution and risk factors is limited and also continuous consumer demands for milk and milk products, periodical assessment is required to offer safe and good quality milk for consumption to reduce public health hazards due to poor quality milk and milk products. In other word, there is limited study undertaken so far on prevalence of mastitis, risk factors and its effect on milk quality in the selected District of West Harerghe Zone, which requires an intervention in order to create awareness about the disease, route of transmission and use of conventional drugs, to reduce the prevalence of mastitis and improve milk quality. Therefore this study was designed to assess prevalence of mastitis and risk factors and its causative agents west harerghe zone with the following objectives:-

- To assess the prevalence of clinical and subclinical mastitis
- Identifying mastitis associated risk factors in the study area and,
- To determine mastitis causative agents.

MATERIALS AND METHODS

Description of Study Area

The study was conducted in two districts of West Hararghe Zone of Oromiya National Regional State, Ethiopia. West Hararge is one of the Zones in the Ethiopian Region of Oromiya. West Hararghe takes its name from the former province of Hararghe. West Harerge is bordered on the south by the Shebelle River which separates it from Bale, on the southwest by Arsi, on the northwest by the Afar Region, on the north by the Somali Region and on the east by East Hararghe. Chiro is the town of West Hararghe Zone and 325 km far from the capital city of Ethiopia, Addis Abeba.

Study Procedure

The study was carried out in two parts survey and laboratory analysis. The survey part was conducted on assessing the risk factor of mastitis and screening mastitis using CMT reagent while in laboratory part, microbial analysis conducted to determine mastitis causative agents by collecting CMT positive milk samples.

Sample Size and Sampling Method

Purposive sampling procedure were applied to select 2 districts (Meiso and Tullo) based on their milk production potential from West Harerghe Zone and also 3kebeles were selected purposively from each district. A total of 120 households (60 from each district and 20 from each kebeles) that own at least one lactating cows were selected randomly to carry out the survey and to perform screening of mastitis case with CMT. Besides, 30 CMT positive milk samples were collected from previously surveyed districts (15 from each district) for microbial analysis (bacteriological identification).

Data Collection

A semi structured questionnaire were used to gather required information about the prevalence of mastitis and its risk factors, the assessment were focused on production system practiced in the area, livestock holding preference, herd structure, milking practice, animal related risk factors (breed, parity and calving date), house and hygienic practices (housing and cleaning of barn and frequency of cleaning, washing of udder and milking equipment, source of water and milking procedures) in the study districts.

Following to this physical observation of udder and cows were examined visually and then by palpation to detect fibrosis inflammatory swellings, visible injury, tick infestation, atrophy of the tissue and swelling of supra mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as swelling, firmness, and blindness with the help of veterinarian and animal health technicians. Moreover information relating to the previous health history of the mammary quarters, and other data related with risk factors (host and environmental risk factors) were collected from the owners of the cows by using semi structured questionnaire for and filed observation of the researchers.

California mastitis test (CMT)

The California Mastitis Test was carried out for each quarter to diagnose the presence of subclinical mastitis based on the method described by Quinn *et al.,* (2004). Proper milking procedures were applied then after the stripping milk was discarded and a few streams of (fore) milk from each quarter were milked into four plastic dishes set on a paddle. Mix the milk in the cup of the paddle with an equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane and a positive gelling reaction occurs in a few second with positive samples. The test result were interpreted based on the thickness of gel formed by CMT reagent and milk mixture and scored as 0(negative), T (trace), 1(weak positive), 2(distinct positive) and 3(strong positive) (ICAR, 2011).

Bacteriological Isolation and Characterization

Bacteriological examination of the milk were carried out for CMT positive results following standard procedures set by Hogan *et al*. (1999) Quinn et al. (2002) and Quinn *et al*. (2004) was followed. The milk samples collected from 30 mastitis positive cows were labeled and transported to Oda Bultum University Microbiology Laboratory in an icebox and the milk sample were kept at room temperature (25°C) for 15 min and vortex before plating on the standard bacteriological media (on MacConkey agar and blood agar). Plates were incubated aerobically at 37°C for up to 72 h and checked for any bacterial growth. Suspected colonies were identified morphologically, microscopically and biochemically according to Quinn et al. (2004) and NMC (2004).

Data Analysis

Data were coded, cleaned and entered into Microsoft Excel computer software. Statistical analysis was carried out by using SPSS version 20. Both the questionnaire and CMT data will be analyzed on the given statistical package software. Descriptive

statistics presented in frequency and percentage; odd ratios were used to determine the association between different risk factors like host (parity and stage of lactation) and environment with mastitis prevalence. For all odd ratio test confidence level was at 95% and statistical analysis is consider significant at $P<0.05$.

RESULTS AND DISCUSSION

Prevalence of Mastitis

The prevalence of mastitis detected in this study was presented in table 1. A total 151 cows were diagnosed with either clinical or subclinical mastitis based on the medical examination and California mastitis test (CMT) correspondingly. The incidence of clinical mastitis were 9.3%, from the total clinical positive cows the higher proportion of prevalence were found in tullu district which counts about 9 (5.9%) and the remaining prevalence rate 5(3.4%) were acquired from meiso district. This difference might be due to the temperature variation which is a detrimental factor for the growth of bacteria that cause mastitis.

Out of the total examined lactating animals 41(27.2%) had subclinical mastitis; the prevalence of sub clinical mastitis were nearly double in tullu district 26(17.3%) to meiso district prevalence level 15(9.9%). The overall prevalence recorded in the current study was 36.5%. This is in line with Jirata and Indalem Telila (2016), Tesfanesh *et al.* (2018) and Umer *et al.* (2015), who reported that the overall prevalence of mastitis 34.3% %, 36.1%, and 38% respectively. However, the findings of the current study is much lower than the findings of Biniam *et al.* (2018), Mekibib *et al.* (2010) Zeryehun *et al.* (2013), who reported prevalence rate between 58.2% 74.7% from different part of the country. On contrary the result of the current study was higher than Bitew *et al.* (2010) and Endale et al. (2016) who reports a prevalence rate of 28% and 32.92 % in and around Bahir Dar and wolaita Sodo town respectively. The higher prevalence in the present study might be due to poor hygienic and husbandry practices, lack of awareness about the disease and its treatment.

The prevalence of clinical mastitis in this study is in agreement with the reports of Umer *et al.* (2015), and Ararsa *et al.* (2013) who reported a prevalence level of 7.3% and 7.8% from West Arsi Zone and Holleta. Besides the current result was lower than the prevalence recorded by Zeryehun *et al.* (2013) and Demelash *et al.* (2005), who reported prevalence rate of 19.6% and 16.11% from central and south Ethiopia respectively. However, it was higher than that reported by Mekonnen *et al.* (2012), Bekele *et al.* (2012), Rediet *et al.* (2013), Bitew *et al.* (2010) and Biniam *et al.* (2018) who reported a prevalence of 3.9%, 3.3%, 5.9% , 3% and 6.77% respectively, from different part of the country.

The prevalence of sub clinical mastitis in the present study agrees with the findings of Islam *et al*. (2011), Mulugeta Yohannis and Wassie Molla (2013) and Berhe *et al.* (2019) 29%, 26.9% and 27.89% narrated from bangladish, Wolaita Sodo and Western Tigray in that order.

On the contrary the result of this study was lower than the results reported by Zeryehun *et al.* (2013), Mekonnen *et al.* (2012) and Nibret *et al.* (2011), who reported the prevalence of 55.1, 54.4 and 56%, respectively from different part of Ethiopia, The lower prevalence rate of sub clinical mastitis (SCM) observed in this study might be

due to the discrepancy of vulnerability difference within animal breed , management practice like, sanitation and housing conditions, milking procedures (suckling, lack of milking order and dung smear can create an opportunity for contagious type of mastitis) and environmental conditions may perhaps liable for this deviation.

The result of the study shows that the prevalence of sub clinical mastitis were about three times higher than the rate of clinical case, and this finding is in line with several previous studies from different part of Ethiopia (Rahmeto *et al.,* 2016; Umer *et al.,* 2015; Berhe et al, 2019). While the farmers have no awareness about clinical mastitis case; due to evident in the teat they treat through washing with warm water or by providing antibiotic. In contrarily sub clinical mastitis had no visible symptom and difficult to identify by farmers and it is critical issue for dairy producers. In addition in line with the report of Bayush and Abera (2018) high prevalence of sub-clinical mastitis may be due to improper milking hygiene, poor house hygiene, lack of post milking teat dipping and practicing of milk by contact labors use of lubricant, absence of order in milking cows of different ages.

Table 1. Prevalence of clinical and sub clinical mastitis at cow

Forms of mastitis	NAE	AAT (n=67)	AAM (n=84)	Over all	
				NAA	(%)
Clinical	151	9(5.9%)	5(3.4%)	14	9.3
Sub clinical	151	26(17.3%)	15(9.9%)	41	27.2
Total	151	35(23.2%)	20(13.3%)	55	36.5

NAA= Number of animals affected, NAE = number of animal examined, N = number of animal, AAT = animals affected in tullu, AAM = animals affected in meiso**.**

Prevalence of clinical and subclinical mastitis at quarter level

A total of 604 quarters, from 151 lactating cows were inspected. Among these 591 (97.8%) quarters were active and subjected for clinical and sub clinical investigation and the remaining 13(2.2%) of them were blind. The result is in line with the findings of Girma *et al.* (2012) who reported 2.2% of quarter was blind; on the other hand the result of this study was higher than the findings of Bekele *et al.* (2012) and Muhammed et al. (2017) reported from Hawassa Town and Borena district. Blindness of the teat could be due either to lack of awareness to detect the disease in early stage and deficiency of treatment or due to external like tick and torn, which is in agreement with the report of Tesfaye (2016) in and around Debrezeit, Ethiopia.

The quarter level prevalence of clinical mastitis (3.9%) of the current study was in agreement with the finding of Girma *et al.* (2012) in Doba district, Alemu *et al*. (2013) in Goder, who reported 3.90% and 2.8 % of prevalence rate respectively. On contrary this finding was higher prevalence rate than the outcome of Bitew et al. (2010) who reported a null prevalence rate. This might be due to the variation in environ, the husbandry and management activities in these area.

The quarter level prevalence of sub clinical mastitis (19.5%) recorded in the current study was higher than finding of Girma *et al*. (2012), Yibrah andTsega (2016), Biniam *et al*. (2018) who reported quarter prevalence rate of 4.13%, 18.23% and 12.3% from Doba district, Sidamo zone and bahir dar, but lower than the report made by Kifle and Tolossa (2008) in Selale, Ethiopia, Zeryehun *et al*. (2013) in and around Addis Ababa,

Ethiopia, who reported 63.1 and 62.3%, respectively. This might be due to variation in milking and hygienic practice, animal breed and environment.

Table 2: Quarter level prevalence of clinical and sub clinical mastitis

Quarter	Quarters examined	SCM Affected quarters	CM affected quarters	Blind Quarter
LF	148	17(11.5%)	4(2.7%)	3(2.0%)
LR	147	30(20.4%)	5(3.4%)	4(2.7%)
RF	151	28(18.5%)	3(2.0%)	0
RR	145	40(27.6%)	11(7.6%)	6(3.8%)
Over all	591	115 (19.5%)	23(3.9%)	13(2.2%)

LF = left front, LR = left rear, RF = right front, RR =right rear, SCM = subclinical mastitis, CM = clinical mastitis

Risk factors affecting the prevalence mastitis

Previous history for mastitis prevalence

The association between previous mastitis history, teat lesion and gross milk quality of the study is presented in table3. According to the result recorded in the current study, the prevalence of subclinical mastitis was significantly higher in cows which had previous mastitis history (56.25%). This is similar with the report of Hagos (2015), Yein (2014) and Muhammed (2017). Almost half of the cows (47.4%) with teat lesion had mastitis positive, this is finding was lower than the finding reported by Belayneh *et al*. (2013).

Gross milk quality were used as an indicator for the presence of mastitis in this study and all cows which had a blood tinged and clot milk were mastitis positive. Therefore higher prevalence was observed in animals which had teat lesion and poor gross milk quality. This could be due to lack of awareness, medical treatment and poor hygienic and sanitary practice performed in the study area.

Table3: Previous history for mastitis prevalence

Variables	NAE	Over all		OR(p-value)
		NAA	(%)	
Previous mastitis history				
Yes	16	9	56.25	
No	135	37	27.4	1.2(0.793)
Teat lesion				
Present	19	10	52.6	
Absent	132	36	27.3	0.64(0.507)
Gross milk quality				
Watery	23	6	26.2	
Blood tinged	9	9	100	3.1(0.4)
Clot	3	3	100	0.7(.000)
Normal	116	28	24.1	0.58(.000)

NAE = number of animal examined, NAA= Number of animals affected, N = number of household, OR= Odd ratio.

Animal related risk factor

Parity and stage of lactation were considered as fundamental host related risk factors that influenced prevalence of mastitis (table4). The output of multinomial logistic

regression shows that the prevalence of mastitis in the study area was significantly affected by stage of lactation and parity ($p<0.05$).

In the current study higher cow level mastitis prevalence were observed in late lactation (34.6%) followed by late lactation (28.5%). Despite result of the current study was contrary with the findings of Dinao *et al*. (2016); Zenebe *et al*.(2013); Shiferaw and Telila (2016) who reported higher prevalence of mastitis were observed in early stage of lactation from different parts of Ethiopia. The result was similar with findings reported by Belayneh *et al*. (2013); Adane *et al*. (2012); Sisay *et al.* (2012), Zenebe *et al.* (2013) and Tuke M, *et al*. (2017) reported form different parts of Ethiopia. The highest prevalence of observed in late lactation in this study might be due to long exposure time of milking, presence of milk residue or absence of complete milking because of suckling only before milking practice in this stage of lactation and dung smear of the teat and udder.

Parity was one of the intrinsic risk factor that affects mastitis prevalence. According to the result of the study the incidence of mastitis was significantly higher in cow's parity number greater than four (56.3%) (OR- 2.845). The incidence of mastitis prevalence increased with parity observed in this study was similar with the findings Tuke M, et al. (2017); Nibret *et al.* (2017) Tesfanesh (2018); Amare, *et al.* (2018) and Berhe *et al.* (2019) who reported from deferent part of Ethiopia. This might be due to the increased opportunity of infection with time, the extended period of infection and parity was strongly related and the decrement of immunity in old animals; this conditions increase could the prevalence rate.

Table4. Host (cow) related risk factor

Variables	Category levels	NAE	Over all		OR(P- value)
			NAA	Percentage	
Calving date					
	1-60 days	42	12	28.5	
	61-120 days	32	6	18.6	0.948 (0.907)
	>120 days	77	28	36.4	2.732 (0.157)
Parity					
	1calves	28	3	10.8	
	2 calves	53	9	17	1.409(0.604)
	3 calves	38	16	42.1	1.586(0.563)
	≥4calves	32	18	56.3	2.845(0.118)

CD = Calving date, CMP = Clinical mastitis positive, SCMP= Subclinical mastitis positive, NAE = number of animals examined**,** AEPU = Animals examine in peri urban production system, AEM = Animals examine in mixed farming, OR= Odd ratio.

Microbiological Culture (Bacterial Isolation)

According to the result all samples were positive for different bacterial species in microbiological culture. Staphylococcus species was primary bacterial species isolates in this study that accounts 46.6% followed by E.coli with 30% incidence. Out of the examined samples, 14 samples were positive for Staphylococcus species. This indicates that Staphylococcus species were the significant mastitis causative agents.

The bacteria isolated in the present study (Staphylococcus, Streptococcus, Escherichia and Other gram positive *cocci* and rods) agree with the findings of Zenebe *et al.* (2013); Zenebe N. *et al*. (2014); Umer *et al.,* 2015; Birhanu *et al*. (2017); Amare, *et al.* (2018)and Tesfanesh (2018) who noted Staphylococcus, Streptococcus, and Escherichia as major mastitis causative agents. Radostits *et al*. (2000) stated that Satphylococcus *aureus* is well adapted to persist in the udder and usually establishes a mild subclinical infection of long duration, from which milking utensils and the persons were facilitate transmission to healthy animals, mainly during milking procedures. All the experimental samples were positive for microbial growth of different bacteria species. This shows that households have a shared problem in hygienic and management practices they could not minimize the mastitis causing micro-organisms.

Staphylococcus species were the leading pathogen that accounts 46.6% from all bacterial isolates in the current study. The prevalence Staphylococcus species in the present study is agreed with the finding of Umer *et al., (*2015). Besides the current result is higher than the findings of Adane *et al*. (2012); Abunna *et al*. (2013); Tesfaheywet *et al.* (2013) and Amare, et al. (2018) who reported 29.2% 21.13%, 28.8%, 34% bacterial isolates respectively form deferent part of the country. The relative high prevalence of Staphylococcus species in the current study show the absence of dry cow therapy and treatment of infected animals in the study area. The incidence of *Streptococcus* species were 30% of the total in the study area. The result was comparable with the findings reported by other authors Hawari and Al-dabbas (2008) and Atyabi *et al*. (2006) reported relative prevalence of 26.2% and 33.54% *Streptococcus* species in Jordan and at farms around Tehran.

E.coli comes about with the prevalence rate of 16.7% of the isolated bacteria genera. The finding is in line with that noted by Amare, *et al*. (2018) at in and around Gondar town, Ethiopia. However the current result was lower than the finding of Zenebe *et al*. (2014) in Adigrat. On the other hand lower results were presented by Hagos *et al.* (2015) at Dire Dawa, Birhanu *et al.* (2017) in Bishoftu Town. *E. coli* is originated from wet environment or fecal contamination and its prevalence in the present report could be related to hygienic status practiced at the study area.

Generally prevalence of mastitis recorded in the study was relatively higher in Tullu district than Meiso district; this might be due to the temperature difference of the area. The farmers should improve the hygienic and management practices performed though out milk production line and seek veterinary advice and treatment for their cows to reduce mastitis prevalence rate.

Table 5: Bacteria isolated from mastitis affected quarters

Bacterial isolate	Frequency	Percentage
Staphylococcus species	14	46.6
Streptococcus species	9	30
Escherichia coli	5	16.7
Other gram positive cocci and rods	2	6.7
Total sample	30	100

CONCLUSION AND RECOMMENDATION

Milk production plays an invaluable role to build a sustainable economy for developing countries like Ethiopia. However there are a number of factors that hinder dairy industry and mastitis is one of the most important economical diseases of dairy animals and have been a cause for great loss of productivity through poor milk quality, reduced milk yield, and due to culling of cows. The overall prevalence of mastitis at cow and quarter level noted in the current study was 36.5% and respectively. Fifty five (36.5) of cows were mastitis positive however only 14(9.3) of them were clinically affected. Besides, out of 604 examined quarters 2.2% were blind and 5.4% of quarters were clinically positive. The result of the current study illustrates that; risk factors like mastitis record, teat lesion, stage of lactation and parity had significant effect on the incidence of mastitis. The present study shows high prevalence of bovine mastitis with Staphylococcus and Streptococcus species to be the main bacterial isolates in the study area. Based on the results of this study, we recommend implementation of rigorous mastitis control program, improved milking hygiene, prevention of skin lesions, culling of chronic mastitis carrier animals, and treating of clinically infected cows in the study area.

Based on the above conclusion, the following points are forwarded as recommendations

- Establishing strong and committed extension service to create public awareness about the disease,
- Implementation of better management and hygiene practice through introduction of hard and fast animal treatment
- Implementation of proper milking procedure like hand and udder washing, disinfecting as well as milking infecting animal last and culling of chronically infected animal to reduce direct transmission of mastitis.

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