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Bacterial Pathogens and Udder Infection Dynamics During the Early Lactation Period in Primiparous Cows in Ambo Town, Central Ethiopia

[1]Siraj Arga, [1]Getachew Tadesse, [2]Tesfaye Sisay Tessema and [3]Endrias Zewdu

[1]Department of Biomedical Sciences, School of Veterinary Medicine,
College of Health Sciences, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia
[2]Department of Microbiology, Epidemiology and Public Health, School of Veterinary Medicine,
College of Health Sciences, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia
[3]Department of Veterinary Laboratory Technology,
Faculty of Agriculture and Veterinary Science, Ambo University, P.O. Box 19, Ambo, Ethiopia

Abstract: Mastitis is a major disease in dairy cattle of Ethiopia. The objectives of this study were to identify the bacterial pathogens and estimate the incidence of mastitis and the spontaneous cure rate of infected glands in primiparous cows during the early lactation period. The study was conducted for four months and a total of 328 quarters were examined to detect clinical mastitis and subclinical mastitis by udder physical examinations and the California mastitis Test (CMT) respectively. California mastitis test positive milk samples were used to isolate and identify bacteria. The study revealed that the changes in glands infection status were significant ($p < 0.05$). The incidence rate of mastitis per gland month at risk was 26.06% and the spontaneous cure rate of infected quarters was 41.18%. The incidence of mastitis was higher in cows kept under an intensive management system than the semi intensive system ($p < 0.05$). The percentage of CMT positive glands that remained positive after a month and the percentage of CMT negative glands that remained negative after a month were 58.82% and 73.94% respectively. CMT negative quarters acquired new infections after a mean of ±SD 1.25±0.5 months. *Staphylococcus aureus* (27.55%), Coagulase negative staphylococci (CNS) [21.43%] and *Streptococcus agalactiae* (12.24%) were the predominant bacteria. We concluded that the prevalence of *Staphylococcus aureus* warrants serious attention and the application of antibacterial agents earlier before calving may help to reduce the incidence of mastitis during the early lactation period.

Key words: Bacterial Pathogens · Ethiopia · Incidence · Mastitis · Primiparae

INTRODUCTION

Mastitis affects the production performance of first-calving heifers and it is of a serious concern to dairy producers. Heifer mastitis causes economic losses due to a decrease in milk production, treatment costs and culling costs [1]. Nielsen *et a*l. [2] estimated a milk loss of 155 Kgs on 305 days lactation and Huijps *et al*. [1] estimated the cost of mastitis at £55/heifer present on a farm. Intramammary infections (IMI) before calving and incidences of mastitis immediately following calving have been reported [3].

The prevalence, incidence and types of pathogens vary among herds [4, 5]. The incidence of clinical mastitis in the peripartum period is relatively higher in heifers than in older animals [6] and there is a positive association between prepartum and postpartum infections [7].

The pathogens that cause mastitis in multiparae are also causes of both pre and postpartum intramammary infections in heifers [8, 9] and are classified as major and minor pathogens [6]. The major pathogens often cause clinical mastitis and the minor pathogens are most often associated with subclinical mastitis. Mammary gland pathogens can also be classified as contagious and environmental [10].

There are several works on the prevalence and risk factors associated with bovine mastitis in Ethiopia [11-13]. However, information on the incidence of mastitis is scarce. The objectives of this study were to identify the bacterial pathogens causing mastitis and estimate

Corresponding Author: GetachewTadesse, School of Veterinary Medicine, Addis Ababa University
P.O. Box 34, Debrezeit, Ethiopia.

the incidence and spontaneous cure rate in primiparous cows during the early lactation period in small holder farms at Ambo town, Central Ethiopia.

MATERIALS AND METHODS

Study Design: The study was carried out from November, 2010 to April, 2011. A total of 38 animals belonging to Ambo University and small holder dairy farms at Ambo town were used. The animals were predominantly crosses of the Zebu and Holstein-Friesian cattle (N = 34), aged above 18 months at first parturition and managed under an intensive or semi intensive management systems. Clinical examinations were done and milk samples were collected 2 weeks after parturition and monthly for four lactation months.

Physical Examination of the Udder: Mammary glands were examined by inspection and palpation. Swelling and pain reaction up on palpation, changes in the consistency of the udder, changes in the colour of milk and the presence of flakes in the milk were considered as indicators of clinical mastitis.

California Mastitis Test (CMT): The California mastitis test (CMT) was used to detect subclinical mastitis. The procedure and the interpretations were according to Quinn *et al*. [15]. The results were read as negative (0), trace (T), weak positive (+), distinct positive (++) and strong positive (+++). Reactions that included weak positive and above were considered as indicators of sub clinical mastitis.

Milk Sample Collection: Milk samples were collected as described by Quinn *et al*. [15]. Ten ml of milk was collected in sterile tubes. Samples were held in an ice box and transported to the Microbiology Laboratory of Ambo University. Samples that were not immediately processed were refrigerated at +4°C for 24-72 hours. Samples to be processed after 3 days were kept in a refrigerator at or below -20°C.

Isolation and Identification of Bacteria: Milk samples were inoculated on 5% sheep blood agar and MacConkey agar plates as described by Smith *et al*. [16] and Bradely and Green [17]. The plates were aerobically incubated at 37°C and examined for bacterial growth, morphology and haemolytic characteristics of colonies after 24-48 hours. Typical colonies were sub-cultured on blood agar and MacConkey agar and were incubated aerobically at 37°C for 24-48 hours. Colonies from pure cultures were Gram stained. Coliforms and Gram negative bacteria were identified by colony morphology and IMVIC (indole, methyl red, Voges-Proskaur and citrate) test. Differential tests were conducted according to Quinn *et al*. [15].

Statistical Analysis: The incidence rates were estimated at gland level and expressed in terms of gland month at risk according to Thrusfield [18]. The significance of changes in the infection status of quarters was assessed by the McNemer test. The Chi-squared test and the relative risk (RR) were used to evaluate risk factors. Alpha (á) was set at 0.05. The data were analysed by using Epi Info™ (version 3.5.1., Centre for Disease Control, USA).

RESULTS

Gland Infection Dynamics: The changes in quarters infection status were significant ($\chi^2 = 56.45$; df = 2; $p < 0.05$). The percentage of CMT positive glands that remained positive after a month and the percentage of CMT negative glands that remained negative after a month were 58.82% and 73.94% respectively (Table 1). The incidence rate of subclinical mastitis per gland month at risk was 26.06% (Table 2). The occurrence of new infections in cross bred cows was not significantly affected by blood level, quarter position and lactation month ($P > 0.05$) but by the management system ($P < 0.01$) (Table 3). The incidence rate was about four times higher in animals kept under an intensive management system than animals under the semi- intensive management (RR = 3.94; 95% CI = 1.29-11.97). The spontaneous cure rate of infected quarters was 41.18% and not significantly affected by blood level (50% vs. 75%; $P > 0.05$), lactation months ($P > 0.05$), management (intensive vs. semi intensive, $P > 0.05$) and quarter position (front vs. rear quarters; $P > 0.05$). The mean ± SD time that CMT negative quarters acquired new infections was 1.25±0.5 months.

Prevalence of Bacterial Pathogens: A total of 98 bacterial isolates were identified from milk samples. *Staphylococci* (48.98%) and *Streptococci* (21.42%) were the predominant genera (Table 4). The major bacterial species identified were *Staphylococcus aureus 27* (27.55%), Coagulase negative staphylococci (CNS) 21 (21.43%) and *Streptococcus agalactiae* 12 (12.24%).

Table 1: Changes in gland infection status between CMT positive and negative quarters

Preceding CMT results	Number of quarters	Gland status after a month: Number Positive (%)	Gland status after a month: Number negative (%)
Positive	34	20(58.82)	14(41.18)
Negative	142	37(26.06)	105(73.94)

Table 2: Incidence of subclinical mastitis per gland month at risk by lactation months

Lactation month	Gland months	Number of new infections	Incidence Rate (%)
Second	37	8	21.62
Third	40	9	22.5
Fourth	65	20	30.78
Total	142	37	26.06

Table 3: Potential risk factors associated with the incidence of subclinical mastitis

Risk factors		Gland Months	Incidence rate	χ^2	P-value
Blood level	75%	80	22	0.21	0.650
	50%	48	15		
Gland position	Front	66	20	0.13	0.719
	Rear	62	17		
Management	Intensive	95	34	7.25	0.007
	Semi intensive	33	3		
Lactation Month	Second	33	8	1.33	0.513
	Third	36	9		
	Fourth	59	20		

Table 4: Prevalence of bacterial pathogens during the early lactation period

Bacterial isolates	Number	%
Staphylococcus aureus	27	27.55
Cogulase negative satphylococci	21	21.43
Streptococcus agalactiae	12	12.24
Streptococcus dysagalactiae	6	6.12
Streptococcus uberis	3	3.06
Escherichia coli	6	6.12
Pseudomonas aeroginosa	6	6.12
Actinomyces pyogenes	4	4.08
Corynebacterium bovis	3	3.06
Enterococcus fecalis	3	3.06
Klebsiella pneumoniae	3	3.06
Bacillus spp.	2	2.04
Micrococcus spp	2	2.04
Total	98	100

DISCUSSION

The incidence rate of mastitis was higher in cows kept under the intensive management system compared to cows under the semi-intensive management system. This could be due to the restricted exercise of animals and the transmission of pathogens from infected cows to primiparae as animals were housed together. Higher risks of mastitis in cows with restricted exercises [19, 20] and in a combined housing management of heifers with multiparous cows [21] were reported.

The spontaneous cure rate was higher in animals kept under the intensive management system than the semi-intensive management system. The difference might be due to the relatively better feeding management practiced in the intensive system where animals are stall-fed with grass hay and supplemented with milling byproducts. The relationships between proper nutrition and resistance to infection during the dry and early postpartum periods were reported [22]. Some nutrients including Selenium, Vitamin E, Copper, Zinc and Vitamin A were associated with a decreased risk of mastitis in cattle [23, 24]. Piepers *et al.* [25] reported that lack of mineral/vitamin supplementation prior to calving were risk factors for intramammary infection caused by environmental major pathogens.

The imbalance between the incidence rate per quarter month at risk (26.06%) and the spontaneous cure rate (41.18%) could result in an increase in the prevalence rate during the early lactation period. With an incidence rate of 26.06% and a spontaneous cure rate of 41.18%, 59% of the new infections were likely to be transferred to the subsequent months resulting in pathogen buildup as lactation progressed.

Negative quarters could remain negative for a relatively longer period compared to positive quarters. The likelihood that a quarter not infected in the preceding month could remain negative in the subsequent month was about twice higher than the likelihood that an already infected gland could be free of infection (Table 1). The results are suggestive evidences that the application of antibacterial agents before the expected date of calving could help to reduce the incidence of mastitis during the early lactation period. The application of antimicrobials approximately two months prepartum was reported to have reduced the incidence of intra mammary infections by 59% and treatment in the third trimester generally reduced the occurrence of new prepartum intramammary infections and persisted up to calving with cure rates ranging from 80 to 100% for *Staphylococci* and *Streptococci* [26].

Blood level had no effect on the incidence rates of mastitis in cross bred cows. Cross bred cattle were more susceptible to mastitis than the local Zebu [12, 27, 28] and high yielding cows were reported to be more susceptible to mastitis relative to low yielding cows [29]. However, the similarities in the incidence rates between the cross bred animals in this study (50% vs. 75%) could be due to their similarities in their genetic make-ups and their responses to infections. Although high milk production was reported to be associated with the occurrence of mastitis [29], its effect in 50% and 75% cross animals appears negligible as both produced nearly similar amounts of milk, averaging 7 and 9 liters of milk per day respectively.

Staphylococci, Streptococci and coliforms were the major causes of subclinical mastitis in primiparous cows and the results were in accord with other studies on multiparous cows in Ethiopia [12, 13, 27, 28, 31-33]. In a study on the types of pathogens in primiparous and older cows, Waller *et al*. [34] reported a similar distribution of udder pathogens. Most mastitis pathogens associated with subclinical mastitis in lactating cows are the coagulase-negative and positive *Staphylococci*, the environmental *Streptococci* and coliforms [35].

Staphylococcus aureus was the predominant pathogen isolated from primiparous cows in the early lactation months. The results are in agreement with other reports in Ethiopia [12, 13, 28, 31-33, 36]. The absence of hygienic hand milking practices and keeping cows with chronic infections might be the factors that contributed most to the higher prevalence of the pathogens in Ethiopia. The higher prevalence of infections with *Staphylococcus aureus* poses a threat to public health and to production as the bacteria is contagious that results in infections ranging from subclinical to fatal mastitis.

Streptococci were the second most prevalent genera. *Streptococcus agalactiae* rank third on the list of pathogens identified. Similar results were reported from other studies in Ethiopia [12, 13, 28, 31, 32]. The streptococcal species cause problems during the postparturient period in heifers [37].

The coagulase negative *Staphylococci* (CNS) were the second most prevalent pathogens in primiparous cows. The results are comparable with the report of Mekonnen and Tesfaye [38] but lower than the reports of Almaw *et al*. [27], Argaw and Tolossa [39], Mekbib *et al*. [13] and Bitew *et al*. [28] in Ethiopia. The CNS has a higher prevalence in primiparous cows than in older cows, are of lesser pathogenicity than other principal pathogens and with infections that mostly remain subclinical and persistent [40].

The contagious pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*) have a prominent position as a cause of mastitis in primiparous cows in Ethiopia. In contrast, the use of therapeutic and prophylactic antibiotics treatments was reported to have decreased the prevalence of contagious pathogens [41]. The other bacterial species were of lesser frequency and similar distribution patterns reported in Ethiopia [12, 13, 28]. The variations in the preponderance of the bacterial species among the different studies could be attributed to differences at the animal level, the management practices and the agro-climatic heterogeneity of the country.

The incidence rates estimated in this study suggest that subclinical mastitis during the early lactation is a considerable problem in primiparous cows in Ethiopia. Infected quarters may produce up to 5% less milk for every additional 100 000 cells per ml in the milk [42] adding up to a cost two to three times that of the clinical disease [41]. We concluded that the high prevalence of *Staphylococcus aureus* warrants serious attention and research should focus on describing the prevalence, incidence, risk factors and economics of mastitis in primiparae in different agro-ecological setups in Ethiopia. The application of antibacterials before parturition could reduce the occurrence of mastitis during the postpartum period.

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