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Cytological Study of Subclinical Endometritisin Respect of Age, Parity, Farm Scale and body Condition Score in Dairy Cows in and Around Gondar, Northwest Ethiopia

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Abstract: The objective of this study was to investigate the incidence of subclinical endometritisinrespect ofage, parity, farm scale and body condition scorein dairy cows. A total of 147apparently healthy 3rd trimester pregnant cows were selected with no signs of clinical endometritis were examinedfrom January 2012 to September 2013. Questionnaire survey and regular follow up were conducted to determine subclinical endometritis in dairy cows. Age and parity were statistically significant ($P<0.05$) for subclinical endometritis. Older cows greater than 6 years were more affected sub clinical endometritis 38 (71.70%) than younger cows 13 (29.55%), the difference was statistically significant. The incidence of sub clinical endometritis in cows primiparous was 21 (40.38%) and multiparous was 46 (48.42%), the difference is statistically significant. However,body condition score and farm scale showed no significant variation with regard to subclinical endometritis.

Key words: Age · Body Condition Score · Cytology · Gondar · Parity · Subclinical Endometritis

INTRODUCTION

A high level of reproductive efficiency requires each cow to be bred successfully and calve with a calving interval that maximizes the output of milk within the herd. Under normal circumstances, however, microbial contamination of the uterus is a frequent finding in postpartum dairy cows [1].

Interestingly, multiparous cows have increased bacterial contamination ~50 days after calving compared to primiparous cows [2]. Milk production has a detrimental effect on leukocyte function; therefore, leukocytes from multiparous cows are expected to be more severely affected because of greater milk yields. In fact, phagocytic activity of neutrophils in older cows is more markedly reduced after calving compared to younger cows. Therefore, increased levels of pro-inflammatory cytokine production in the uterine endometrium might help to prevent subclinical endometritis; however, because multiparous cows have greater demands for milk yield, they might be less able to clear an infection completely and, therefore, might be more likely to have subclinical endometritis. Another important factor that might be involved in the susceptibility to subclinical endometritis is the circulating levels of immunoglobulins. Immunoglobulins work as opsonins, which greatly enhance phagocytic capacity. Primiparous cows have lower immunoglobulin content in colostrums, which indicates lower circulating immunoglobulin levels; therefore, phagocytosis might not be optimal in early lactation in primiparous cows [3].

New techniques have been described for the diagnosis of subclinical endometritis. The inflammation of the endometrium is characterized by the proportion of polymorphonuclear (PMN) cells in a cytological sample taken from clinically healthy cows. Cytological samples obtained by flushing the uterine lumen [4-6].

Cows with a higher condition score at calving were less prone to subclinical endometritis and conceived more successfully to first service.Cows calving in a higher body condition score produced more milk, fat and protein in the first 90 days of lactation. Body condition score represents a subjective assessment of the tissue reserves of dry and lactating cows (Edmonson and others 1989).Therefore, the objective of the study was to identify the effect of age, parity, farm scale and body condition score in dairy cows in and around Gondar.

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MATERIALS AND METHODS

Study Area: The study was conducted in urban and peri urban areas of Gondar towndairy farms which are located North West part of Ethiopia in Amhara regional state. Gondar town is found about 727 km from the capital cityAddis Ababa.It islocated at latitude, longitude, altitude of 12.3-13.8°N, 35.3-35.7°E and 2200m.s.l, respectively. The annual mean minimum and maximum temperature of the area vary between 12-17°C and 22-30°C, respectively. The area is located under woynadega, agro-climatic zone and receives a bimodal rainfall the average annual precipitation rate being 1000 mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April and May while the long ones extend from June through September [7].

Sample Size: A sampling frame i.e. the list of the dairy farms was acquired from the urban agricultural development office at the beginning of the study. Dairy farms / cows were selected from this list using a stratified sampling procedure to ensure the selection of proportional and representative sampling of dairy farms and cows.

Questionnaire Survey: A systematic question was designed and instituted to obtain relevant and reliable information about their animals.The questionnaire were checked for clarity of the questions prior the interview, respondents were briefed to the objective of the study.Following that, the actual questionnaires were presented.

Regular Follow Up: About 147 pregnant cows were randomly selected in and around Gondar that were expected to give birth within the study period. These cows were subjected to different clinical and gynecological examinations including rectal palpation and findings were recorded once a week.

Body Condition Scoring: The Body Condition Scoring (BCS) was determined according to Richard [9]. For all cows under the study their body condition were grouped from 0-5.Body condition score 0 stands for cows with the poorest body condition while score 5 for cows with the best condition.

Clinical Examination: In each cow a clinical examination ofthe reproductive tract was performed by vaginal examination and transrectal palpation of the uterusand the ovaries. Cows with vaginal discharge were diagnosedas affected by clinical endometritis and excludedfrom the study. In addition, cows which had received systemic or intrauterine antibiotic therapy within 6 days prior to enrollment were not selected for the study. Pregnancy diagnosis was performed by transrectal palpation of the uterus and its contents post insemination.

Cytological Samples: The cows were examined between 40 and 60 days after calving for the presence of subclinical endometritisby using the lavage technique. Collected samples were centrifuged and a drop of sediment was streaked onto a clean microscopic slide and stained with Giemsa.

Subclinical endometritis was determined using endometrial cytology [12]. To minimize contamination of the sample, the vulva and perineum were cleaned with water and soap properly. The uterus was lavaged by infusing 50 ml of 0.9% sterile sodium chloride solution with 50 ml syringe attached to a 52 cm sterile plastic infusion rod. The uterus was then manipulated and massaged through rectum for about 10 seconds and some of the infused fluid was aspirated into the syringe via the same sterile plastic infusion rod by negative pressure aspiration and retracted to recover the fluid. No special effort was made to retrieve the fluid if it did not flow freely.

As much fluid as possible was recovered by negative pressure aspiration into the syringe and transferred to the 10 ml sterile test tube without any preservative. The uterine samples were put into the icebox and brought to the Faculty of Veterinary Medicine, Microbiology laboratory within 2 hours of collection and centrifuged at 800 rpm for 5 min. A drop of sediment was streaked on to a clean microscope slide and air-dried. Then the slide was fixed with methanol and stained with Geimsa for 45 min and examined under a microscope at 400× magnification. Initially the whole slide was assessed and a representative area was selected to determine the PMN % among all other cells was estimated. The percentage of neutrphils PMN % was determined by counting 80-100 cells on a representative field of vision. The threshold value for the proportion of PMN indicated samples with = 3% neutrophils were categorized as subclinical endometritis and cows were characterized as suffering from subclinical endometritis. The counted cells contained epithelial cells, neutrophils, large mononuclear cells (Presumed to be macrophages) and small mononuclear cells (Presumed to be lymphocytes). The samples that did not contain epithelial cells were considered not taken from uterus and rejected for the study.

Data Management and Statistical Methods: Data collected from the longitudinal follow up study were entered in Microsoft excel. For analysis of the data statistical package for social science (SPSS)(version 18) wasused.In this chi- square test, confidence interval and logistic regression were calculated.The Generalized Linear Model was utilized to analyse the effect of selected factors on the amount of neutrophils. Multiple logistic regression and Kaplan-Meier survival analysis were applied to analyse the relationship between the amount of neutrophils with age, parity and body condition score. A probability of $P < 0.05$ was set as the significance level. The Confidence Interval (CI) was set at 95%. The Receiver Operating Characteristic (ROC) analysis was applied to determine the most appropriate cutoff point for percentage of neutrophils in samples.

RESULTS

In the present study the samples which ranged from 3% to 15% of PMN cells were correlated with subclinical cases of endometritis. Hence, the endometrial samples which contain PMN cells of 3% and above were considered as positive for subclinical endometritis based on endometrial cytology when using a threshold of 3% and above.

Age and parity were statistically significant ($P<0.05$) for subclinical endometritis. Older cows greater than 6 years were more affected by sub clinical endometritis 38 (71.70%) than younger cows 13 (29.55%) (x^2=51.97; $P<0.05$), the difference was statistically significant (Table 1). The incidence of sub clinical endometritis in cows primiparous was 21 (40.38%) and multiparous was 46 (48.42%) (x^2=14.48; $P<0.05$), the difference is statistically significant. Body condition score was also statistically significant (Table 1). However, farm scale showed no significant variation with regard to subclinical endometritis (Table 1).

DISCUSSION

In the present study, multiparous cows 48.42% emaciated cows 40.40% and cows above 6 years of age 71.70% were the most affected by subclinical endometritis which is higher than the previous finding by Takele*et al.* [10] that were recorded 19.23% in and around Nazareth town. This could be due to the repeated exposure of the genital tract of multiparous cows to environmental pathogenic microorganisms then causing gradual decrease in the efficiency of immune mechanism due to ageing.

The current study revealed an overall incidence of 46% (67/147) of subclinical endometritis infection in dairy cows with a Polymorph nuclear cells(PMN) level of = 3 neutrophiles in the uterine sample set as a threshold value indicative forsubclinical endometritis(SCE). This incidence of SCE was similar with Belachew and Fekadu [11] in Debrezeit which was 47.5% and 30.5% respectively. However, in the cited study samples were taken 4 and 8 weeks postpartum period separately whereas samples in this study were taken 4 up to 8weeks postpartum cows together. An explanation for the higher prevalence of SCE in this study could be the difference in the time arrangement. Gilbert [4] in USA reported a prevalence of subclinical endometritis of 53% at 40 to 60 days postpartum and Dubuc*et al.* [12] in Québec which was the

Table 1: Analysis of results of cow's age, parity, farm scale,body condition score and pregnancy status examined 40-60 days postpartum for subclinical endometritis by uterine cytology.

Variables	No of examined	Positive for subclinical endometritis	Incidence%	Chi-square	p-value
Age					
2-3	44	13	29.55	51.97	0.000
4-6	50	16	32.00		
>6	53	38	71.70		
Parity					
primiparous	52	21	40.38	14.48	0.01
multiparous	95	46	48.42		
Farm scale					
small	54	25	46.30	0.30	0.85
medium	66	29	43.94		
large	27	13	48.15		
Body condition score					
1 and 2	99	40	40.40	10.24	0.03
3 and 4	48	27	56.25		
Pregnancy status					
pregnant	72	7	9.72	70.63	0.000
non pregnant	75	60	80.00		

prevalence of subclinical endometritis of 56%. In other studies, subclinical endometritis has been reported as 43% for cows between 20 and 33 days in milk (DIM) 45% for cows between 34 and 47 DIM [13]. The variation of SCE in the current study compared to the above mentioned cited studies could be due to the difference in the management system of dairy cows.

The incidence of SCE in this study was 46% also higher than the incidence of 13.4% SCE reported by Singh [14] in Germany. However, samples in this study were taken 4 up to 8 weeks postpartum period, whereas samples in the cited study were taken four hours after breeding. The higher incidence of SCE in this study compared to the above cited study could be the difference in the time of sampling.

The incidence of subclinical endometritis in cows having body condition score 1 and 2 was 40.40% which is lower than cows having body condition score 3 and 4 56.25% and body condition score was significant (P = 0.03). The prevalence of subclinical endometritis in cows having body condition score (BCS)-2 was 73% which was not statistically significant (p = 0.554) was higher than cows having BCS-3 of 62.6% which is agree to Belachew and Fekadu [11] DebreZeit which was the prevalence of 64.5% in BCS>2.5 and 28.6% of in BCS>2.5 which was significant (P = 0.035).

CONCLUSIONS

A herd health program is critical in maintaining uterine health and identifying potential problem areas. Routine postpartum examinations will help to identify problems early so that effective therapy can be administered in problem situations.

RECOMMENDATIONS

The study showed that direct association of subclinical endometritis with age and parity. Therefore, proper herd health management and proper feeding is very important and also detailed studies should be conducted to identify their etiology, distribution and prevalence.

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