

Study on Dystocia with History of Uterine Infections and Calving Hygiene along with Subclinical Endometritis in Dairy Cows in and Around Gondar, Northwest Ethiopia

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Abstract: The aim of this study was to assess dystocia with history of uterine infections and calving hygiene on the characteristics of the intrauterine environment in dairy cows by cytological tests were performed on intrauterine perfusion fluid along with subclinical endometritis conducted between January 2012 to September 2013. The percentage of polymorph nuclear cells (neutrophil) was calculated. It was found that increase in the number of neutrophils correlated with increase subclinical endometritis. During the study period 147 apparently healthy 3rd trimester pregnant cows were selected in dairy farms in and around Gondar, North Western Ethiopia. Questionnaire survey and regular follow up were conducted to determine subclinical endometritis in dairy cows. Abnormal parturition, uterine infections and calving hygiene were statistically significant ($P < 0.05$) for subclinical endometritis. In conclusion, subclinical endometritis in postpartum dairy cows resulting in substantial economic losses due to decreases in both milk production and fertility.

Key words: Calving Hygiene • Cow • Dystocia • Gondar • Subclinical endometritis • Uterine Infection

INTRODUCTION

Abnormal parturition technically called dystocia is the main cause for uterine infection. The prevalence of subclinical endometritis depends on the occurrence of early post-partum uterine diseases [1]. Difficult calving predisposes to subclinical endometritis for several reasons. Firstly, there is a higher than normal incidence of retained fetal membranes in animals that suffered dystocia. Secondly, there is often damage to maternal tissues causing devitalisation. The vulval seal may be damaged. Thirdly, the obstetrical interventions to correct the dystocia increase the load of pathogens within the uterus [2].

The two symptoms of dystocia are extended calving periods (over 8 hours) and evidence that the fetus is not oriented properly for a normal birth. If the cow has not delivered in the specified time or the calf is malpresented, veterinary assistance is often indicated. If there is reason to suspect a problem, the individual examining the cow should observe strict sanitation practices. These include tying up the tail, thoroughly cleaning the cow's vulva and

anal area and the examiner's hands and arms with clean warm water, soap and an antiseptic. A sterile plastic sleeve also should be worn to avoid contamination of the reproductive tract [3].

After parturition, bacteria from the animal's environment contaminate the uterine lumen of most cattle. Infection persists in the uterus of many animals for more than three weeks. These animals have lower conception rates, take longer to conceive and are more likely to be culled for infertility than unaffected animals. So, subclinical endometritis is an expensive condition for vets and farmers to manage [4].

Many management factors affect the incidence of subclinical endometritis. Season of the year may also affect the incidence; cows calving during the winter or spring are more prone to subclinical endometritis than those calving at other times [2].

A dirty unhygienic calving environment predisposes to the disease. This is probably the explanation for the effect of season of year. Since cows calving in the winter or indoors in the spring are likely to be in a more heavily contaminated environment [3].

A high prevalence of uterine disease such as subclinical endometritis impairs the reproductive performance of high yielding dairy cows due to persistent bacterial infection which leads to inflammation and damage to the endometrium thereby, prolonging uterine involution and impairing fertility [5,6]. Therefore, the present study was designed to assess the effect of dystocia, history of uterine infections and calving hygiene along with subclinical endometritis in dairy cows.

MATERIALS AND METHODS

Study Area: The study was conducted in urban and peri urban areas of Gondar town dairy farms which are located North West part of Ethiopia in Amhara regional state. Gondar town is found about 727 km from the capital city Addis Ababa. It is located 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 was 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.

Clinical Examination: In each cow a clinical examination of the reproductive tract was performed by vaginal examination and transrectal palpation of the uterus and the ovaries. Cows with vaginal discharge were diagnosed as affected by clinical endometritis and excluded from 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 endometritis by 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, 14]. 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 neutrophils 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 $\geq 3\%$ 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) was used. In this chi-square test, confidence interval and logistic regression were calculated. The Generalized Linear Model was utilized to analyze the effect of selected factors on the amount of neutrophils. Multiple logistic regression and Kaplan-Meier survival analysis were applied to analyze the relationship between the amount of neutrophils with parturition, history of uterine infections and calving hygiene. 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

Abnormal parturition, uterine infections and calving hygiene were statistically significant ($P < 0.05$) for subclinical endometritis. The last parturition of 36.47% ($n=31$) of the selected cows was normal and that of 85.48% ($n = 53$) was abnormal. The amount of neutrophils was higher in cows with abnormal parturition (Table 1). Abnormal parturition was associated with an increase in the amount of neutrophils in the uterus ($P = 0.001$).

Of the selected cows, 64.44% ($n = 29$) had a history of postpartum uterine infections and 37.25% ($n= 38$) had no previous uterine infections. Metritis, pyometra and endometritis categorized as uterine infections. The amount of neutrophils was higher in cows with a history of uterine infections than that of cows without previous uterine infections (Table 1).

Calving hygiene area 62.69 % ($n= 42$) had poor environment and the amount of neutrophils was higher as compared to good calving hygiene (Table 1).

DISCUSSION

In the present study subclinical endometritis was $>$ or equal to 3 which is in line with the previously documented in Ireland 4% [8]. Various percentages of PMNs in the endometrial cytology samples ($>8\%$, $>11\%$, $>14\%$, $>15\%$ and $>18\%$) were evaluated to determine the most appropriate threshold for the diagnoses of subclinical endometritis in dairy cows between 28 and 41 days postpartum. A threshold of $>15\%$ PMNs was found to be the most appropriate. In a related study Sheldon *et al.* [4] showed that a threshold of $>18\%$ was the lowest PMN percentage which was significantly associated with an elevation of three endometrial pro-inflammatory cytokines, IL-6, IL-8 and TNF- α in cows sampled between 28 and 41 days postpartum. These threshold PMN percentages are greater than what has been reported previously where the most appropriate threshold was $>8\%$ PMNs for defining subclinical endometritis-positive disease status in cows sampled between 28 and 41 Barlund *et al.* [8] and 25 Dourey *et al.* [9] days postpartum using 150 and 270 day pregnancy status as the outcome, respectively. Kasimanickam *et al.* [10] reported that a threshold of $>18\%$ PMNs was the most appropriate for cows examined

Table 1: Analysis of results of cows' abnormal parturition, history of uterine infections and calving hygiene examined 40-60 days postpartum for subclinical endometritis by uterine cytology.

Variables	No of examined	Positive for subclinical endometritis	Incidence%	Chi-square	p-value
parturition					
Normal	85	31	36.47	51.97	0.000
Abnormal	62	53	85.48		
History of uterine infections					
Present	45	29	64.44	14.48	0.01
Absent	102	38	37.25		
Calving hygiene					
Poor	79	42	62.69	12.34	0.03
Good	51	23	34.33		
V. Good	17	2	2.99		
Over all incidence	147	67	36.47		

between 20 and 33 DIM and that >10% should be used for cows examined between 34 and 47 DIM. <15%) did not benefit from intrauterine treatment. Other investigators have reported that cows which were diagnosed as endometritis-negative LeBlanc *et al.* [11] or subclinical endometritis-negative Kasimanickam *et al.* [12], Barlund *et al.* [13] showed that cows with cytological evidence of clinical endometritis (PMNs >8%) became pregnant 24 days later than non-diseased (PMNs<8%) cows, which was much higher than the findings in the current study and the 88-day difference reported by Gilbert *et al.* [14]. Cows that experienced dystocia had a longer interval to first service which is likely due to a delayed resumption of cyclicity post calving. Cows that experienced dystocia also required more inseminations and therefore also had longer calving intervals [8].

CONCLUSIONS

Subclinical endometritis has been implicated as the most common cause of failure of conception and impaired reproductive performance.

RECOMMENDATIONS

The owner needs to prepare the calving area which should be clean, dry, quiet and isolated to keep the prepartum cow close for the owner to notice and provide help if the cow shows signs of difficulty during the birth. In general the operation of a dairy farm for maximum profit includes good feeding, breeding, care and management, as well as, good record keeping and a dairy health program.

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REFERENCES

1. Sheldon, I.M., G.S. Lewis, S. Leblanc and R.O. Gilbert, 2006. Postpartum uterine disease in cattle. *Theriogenology* 65: 1516-1530.
2. Noakes, D.E., 2001. *Arthur's Veterinary Reproduction* 8th.ed. China. Saunders Elsever, pp: 402.
3. Noakes, D.E., T.J. Parkinson and G.C.W. England, 2009. *Arthur's Veterinary Reproduction and Obstetrics*. 8th.ed. China: Sanders, 384: 407-408.
4. Sheldon, J.M., M. Bushnell, J. Montgomery and A.N. Rycroft, 2004. Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. *Vet. Rec.*, 155: 383-387.
5. Gilbert, R.O., S.T. Shin, C.L. Guard and H.N. Erb, 2002. Incidence of endometritis and effects on reproductive performance of dairy cows. *J Dairy Sci.*, 85: 2223-2236.
6. Kasimanickam, R., T.F. Duffield, R.A. Foster, C.J. Gartley, K.E. Leslie, J.S. Walton and W.H. Johnson, 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*, 62: 9-23.
7. Central Statistical Authority (CSA), 2008. Report on livestock and livestock characteristics; Volume II, Agriculture Sample Survey 2008/09.
8. Barlund, C.S., T.D. Carruthers, C.L. Waldner, C.W. Palmer, 2008. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology* 69: 714-723.
9. Dourey, A., M.G. Colazo, P.P. Barajas and D.J. Ambrose, 2011. Relationships between endometrial cytology and interval to first ovulation and pregnancy in postpartum dairy cows in a single herd. *Res. Vet. SciIn. Press*.
10. Kasimanickam, R., T.F. Duffield, R.A. Foster, C.J. Gartley, K.E. Leslie, J.S. Walton and W.H. Johnson, 2005a. The effect of a single administration of cephalixin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. *Theriogenology*; 63: 818-830.
11. LeBlanc, S.J., T.F. Duffield, K.E. Leslie, K.G. Bateman, G.P. Keefe, J.S. Walton, W.H. Johnson, 2002b. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.*, 85: 2223-2236.
12. Kasimanickam, R., T.F. Duffield, R.A. Foster, C.J. Gartley, K.E. Leslie, J.S. Walton, W.H. Johnson, 2005a. The effect of a single administration of cephalixin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. *Theriogenology*, 63: 818-830.
13. Barlund, C.S., T.D. Carruthers, C.L. Waldner and C.W. Palmer, 2008. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology*, 69: 714-723.
14. Gilbert, R.O., Shin, T. Sang, C.L. Guard, E.N. Hollis and M. Frajblat, 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology*, 64: 1879-1888.