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Occurrence of Subclinical Endometritis due to Bacterial Infection and Bacterial Isolation in Repeat Breeder Buffaloes of Jabalpur

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ABSTRACT

The present study was designed to evaluate the occurrence of subclinical endometritis due to bacterial infection in repeat breeder buffaloes. One hundred sixty three repeat breeder buffaloes were screened in various organised farm in and around Jabalpur (M.P). All the selected repeat breeder buffaloes were subjected for screening by physical examination, per rectal examination, cervico-vaginal mucus examination, Whiteside test and endometrial cytology. All the animals found positive for subclinical endometritis were subjected to bacterial isolation. On the basis of endometrial cytology by cytobrush technique 19.63 per cent repeat breeder buffaloes were diagnosed to be suffering from subclinical endometritis. Endometrial cytology by cytobrush technique revealed polymorphonuclear cell percentage in repeat breeder buffaloes found positive and negative for subclinical endometritis to be 9.70±0.80 and 2.19±0.09 per cent, respectively. The difference between the repeat breeder buffaloes found positive and negative for subclinical endometritis for PMN percentage was significant ($p<0.05$). Total 17.17 per cent repeat breeder buffalo were positive for subclinical endometritis due to bacterial infection. Among these 28 bacterial isolates 12 (42.85%) samples yielded single while 16 (57.15%) samples yielded mixed isolates. Among the 28 bacterial isolates 20 (45.45%) *Staphylococcus* species was most prevalent followed by 12 (27.27%) of *Streptococcus* species, 8 (18.18%) of *Bacillus* species and 6 (13.64%) of *E. coli*. It was concluded that occurrence of subclinical endometritis due to bacterial infection was 17.17 per cent and *Staphylococcus* species (45.45%) was most prevalent among the bacterial isolates obtained.

HIGHLIGHTS

- Occurrence of subclinical endometritis due to bacterial infection was 17.17 per cent.
- *Staphylococcus* species (45.45%) was most prevalent among the bacterial isolates obtained.

Keywords: Subclinical endometritis, endometrial cytology, bacterial isolates, Occurrence

Buffalo population globally estimated is around 207 million (FAO, 2020) out of which 109.85 million are being reared in India while 10.30 million in Madhya Pradesh. The total contribution of buffalo in Madhya Pradesh is 7.92 million tonnes out of total 17.11 million tonnes of total milk production. Buffalo when compared with cattle have significantly longer productive life, still the productivity of buffaloes remains low mainly because of poor management of health, nutrition and breeding (Warriach *et al*., 2015). Anoestrus and repeat breeding are the two major causes of infertility in buffaloes (Singh *et al*., 2008).

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A repeat breeder affect the production of farm severely as it may be associated with subclinical endometritis, delayed ovulation and corpus luteum deficiency, which cause failure of fertilization or embryonic mortality (Parkinson, 2009). Subclinical endometritis is an inflammatory process of the endometrium characterised by a high proportion of polymorphonuclear cells (PMNs) in the uterus without any clinical signs of endometritis but reduced reproductive performance of the affected cows. Subclinical endometritis leads to increase in inter-oestrus period, repeat breeding and also reduction in conception as well as pregnancy rates. Since last decade's variety of diagnostic methods are developed for diagnosis of endometrial infections with diagnostic accuracy like ultrasonography and endometrial cytology (Wagener *et al.,* 2017).

Prevalence of subclinical endometritis in postpartum buffaloes (35 days postpartum) by endometrial cytology has been reported to be 33.70 per cent (Nehru *et al*., 2018). Singh *et al*. (2018) in their study on subclinical endometritis in estrual buffaloes reported an incidence of 26.10 per cent for subclinical endometritis due to bacterial infection.

Quantitative analysis of uterine infection is of utmost importance, as it signifies the extent and level of bacterial contamination also the speculations of quantitative differences in the bacterial count of genital or the reproductive tract of repeat breeder and normal animals have been reported by several workers. Uterine culture is the most important tool to determine the causative agent of uterine infection. *E. coli*, *Bacillus* species, *Streptococcus* species, *Staphylococcus* species, *Pseudomonas* species and *Micrococcus* species like bacteria species have been isolated from cervical mucus of cows suffering from subclinical endometritis (Kumari, 2017).

Keeping above facts in the mind, the present study was designed with the specific objectives to study the occurrence of subclinical endometritis due to bacterial infection in repeat breeder buffaloes in and around Jabalpur.

MATERIALS AND METHODS

A survey was carried out in 163 repeat breeder buffaloes reared at organized dairy farms in Jabalpur during the period of six months (July-December 2022). Apparently healthy repeat breeder buffaloes having body condition score (BCS) between 3-5 in 5-point scale from 3rd to 4th parity were selected for the study. The buffaloes were stall fed and kept in cemented shed with brick floor under intensive housing system supplemented with mineral mixture. Seasonally available green fodder with wheat straw and concentrate mixture were fed to all animals. During morning and evening, clean drinking water was provided ad lib. The feeding system of private farms in Jabalpur area was almost similar. The calving and breeding history of animals were recorded from the animal owners in the prescribed proforma. All the animals after obtaining history were subjected to physical and gyneco-clinical examination (per rectal examination). Per rectal examination for location of uterus, asymmetry of uterine horns, palpable ovarian structures, cervix, cervical Os condition, any other apparent anatomical abnormality, presence or absence of discharge its nature if discharge present. Cervico-vaginal mucus (CVM) *i.e*., clear, cloudy, thick and turbid was collected aseptically by syringe and pipette method and was kept in sterile test tubes and Whiteside test was performed. All the Animals found positive for Whiteside test were subjected to endometrial cytology by cytobrush technique. Endometrial cytology was carried out according to Senosy *et al.* (2012). A total of 300 cells were counted under the binocular microscope at X400 and X1000 (oil immersion) and per cent PMN cells were calculated. The threshold cut off value for PMN percentage for diagnosis of subclinical endometritis by cytobrush technique was >5% PMNs (Gilbert *et al.,* 2005).

The endometrial samples of animals found positive for subclinical endometritis by endometrial cytology were used for culture isolation of bacteria culture as per standard procedures.

The data ware analysed statistically by analysis of variance (ANOVA). The means were compared using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Repeat breeding leads to decrease in milk production and reduces the number of calves born per female leading to economic losses to farmer (Mohyuddin *et al*., 2019). Among several aetiological agents, subclinical endometritis is one of the major cause of repeat breeding and is often remains undiagnosed (Dutt *et al*., 2017).

Based on cervicovaginal mucus (CVM) characteristics, rectal examination, Whiteside test and endometrial cytology, 19.63 per cent (32/163) repeat breeder buffalo were positive for subclinical endometritis. When these 32 buffaloes were subjected for bacterial isolation, 28 (87.50%) out of 32 repeat breeder buffaloes were positive for bacterial isolates. Hence total 17.17 per cent (28/163) repeat breeder buffalo were positive for subclinical endometritis due to bacterial infection while 04 (12.50%) were negative for bacterial isolates.

The findings of the present study are slightly higher than the findings of Choudhary (2022) who reported incidence of subclinical endometritis in repeat breeder buffaloes to be 15.30 per cent (28/183). Pothmann *et al.* (2015) reported prevalence of subclinical endometritis in repeat breeder cows as 12.70 per cent. Higher prevalence of subclinical endometritis was reported by Gahlot *et al*. (2017) 23.08 per cent in postpartum buffaloes. Nehru *et al*. (2018) also reported higher prevalence of subclinical endometritis to be 33.70 per cent in postpartum buffaloes on the basis of PMN count. Similarly, Manjhi *et al.* (2018) reported higher prevalence of subclinical endometritis in cows *i.e*. 28.00 per cent. However, Janowski *et al.* (2013) and Behera *et al*. (2015) reported the high prevalence of repeat breeding due to subclinical endometritis in cows to be 40.20 and 38.29 per cent, respectively. Bajaj *et al.* (2018) reported the incidence of subclinical endometritis as 26.00 per cent in postpartum buffaloes. The prevalence of this condition depends upon the occurrence of early postpartum uterine disease, the time of examination and diagnostic technique. However, newer techniques like ultrasonography and endometrial cytology are playing important role for early diagnosis of sub-clinical endometritis.

Endometrial cytology using cytobrush technique

PMNs are the predominant inflammatory cell type found in the endometrial fluid pool and determining the relative proportion of PMNs has been observed to be a predictor of fertility. Polymorphonucler cell percentage of >5 per cent in endometrial cytology sample by cytobrush technique is as an indicator of subclinical endometritis in cattle (Gilbert *et al*., 2005) which was used as the threshold parameter in the present study. One hundred sixty three repeat breeder buffaloes were subjected to endometrial cytology by cytobrush technique and results were recorded in the form of per cent polymorphonuclear cell. The detailed results of endometrial cytology by cytobrush technique are depicted in the table 1.

Endometrial cytology by cytobrush technique in repeat breeder buffaloes revealed polymorphonuclear cell (PMN) percentage to be 9.70±0.80 and 2.19±0.09 per cent, respectively. The difference between the repeat breeder buffaloes found positive and negative for subclinical endometritis for PMN percentage was significant ($p<0.05$). Fibroblasts count was recorded as 3.88±0.36 and 0.13±0.03 per cent in repeat breeder buffaloes found positive and negative for subclinical endometritis. The difference between the repeat breeder buffaloes found positive and negative for subclinical endometritis for fibroblasts was significant ($p<0.05$).

The PMN percentage findings in samples found positive for subclinical endometritis by cytobrush technique are higher than the cut-off values of Gilbert *et al*. (2005) ≥5%. The PMN percentage more than cut off values (>5%) in endometrial cytology samples is indicative of inflammatory process. According to Vieira Neto *et al*. (2014) high uterine PMN population or counts are well known to be associated with poor reproductive outcome. Presence of RBCs in endometrial cytology samples in not of that much concern as according to Cocchia *et al*. (2012) the rigid brush of cytobrush might be responsible for high amount of RBC contamination in the endometrial cytology samples and this blood contamination must be

Table 1: Endometrial cytology findings in repeat breeder buffaloes for diagnosis of subclinical endometritis

Repeat breeder Buffaloes (n=163)	PMN (n)	Endometrial cell (n)	Erythrocyte (n)	Fibroblasts (n)	PMN %
Repeat breeder buffaloes found positive for Subclinical endometritis (n=32)	29.09^{a}±2.39	263.84^{b}±2.43	3.19±0.43	3.88^{a}±0.36	9.70^{a}±0.80
Repeat breeder buffaloes found negative for subclinical endometritis (n=131)	6.57^{b}±0.27	289.62^{a}±0.34	3.68±0.18	0.13^{b}±0.03	2.19^{b}±0.09

Mean values bearing different superscripts (a, b) in a column differ significantly ($p<0.05$); *Average of mean number per 300 cells counted.

taken as a negligible observation and in this study also their difference is non-significant. According to Bajaj *et al.* (2018) high involvement of fibroblast is indicative of severe degree of infection while the marginal count post treatment is related to healing.

Bacterial culture and isolation

Endometrial cytology sample of all the 32 repeat breeder buffaloes found positive for subclinical endometritis were subjected to bacterial isolation. Different types of bacterial isolates were obtained from uterine (endometrium) samples of subclinical endometritic repeat breeder buffaloes are depicted in table 2.

A wide variety of bacteria, both Gram positive and Gram negative aerobes and anaerobes were obtained from bovine uterus. Out of 32 uterine samples obtained from repeat breeder found positive for subclinical endometritis, 28 (87.50%) samples were found positive for bacterial isolates. Among the 28 bacterial isolates 20 (45.45%) *Staphylococcus* species was most prevalent followed by 12 (27.27%) of *Streptococcus* species, 8 (18.18%) of *Bacillus* species and 6 (13.64%) of *E. coli*.

Findings of present study are similar to the findings of Gani *et al*. (2008) who reported *Staphylococcus* species 37.80 per cent as highly prevalent bacteria followed by *Bacillus* species 35.10 per cent, *E. coli* 29.70 per cent, *Pseudomonas* species 18.90 per cent while Gram negative minute rod shaped bacteria was 24.30 per cent. However, Udhayavel *et al*. (2013) reported that *E. coli* and *Klebisella spp*. are more commonly isolated in endometritis in cows. Sahadev *et al*. (2017), found that the most common single bacterial isolate in 16 out of 35 cows was *E. coli* (45.71%) followed by *Staphylococcus* (42.86%), *Proteus* species and *Enterobacter* species (5.71%).

However, Dutt *et al*. (2017) in Murrah buffaloes reported maximum prevalence of *Bacillus* (46.66%) followed by *E. coli* (26.67 %), *Staphylococci* (20.0%) and *Proteus* (6.66%). Singh *et al*. (2016) also reported similar pattern of bacterial isolates in which prevalence of *Bacillus* (47.94%) was highest followed by *E. coli* (24.65%) and *Staphylococcus* (12.32%) in subclinical endometritic cows. The higher prevalence of *A. pyogenes* followed by *Streptococcus* species and *E. coli*, *S. aureus*, *Klebsiella* species and *C. fetus* were reported by the Moges *et al.* (2013).

Although 04 endometrial cytology samples were found negative for presence of bacterial isolates, under such circumstances this condition is related be non-infectious subclinical endometritis or cytological endometritis. As per Barlund *et al*. (2008) two populations of postpartum animals exist which apparently appear normal. One with impaired uterine clearance (good volume of fluid in lumen) and other with increased inflammatory response (lower or no fluid volume in lumen). Animal with increased inflammatory response may be without infection or infection might be spontaneously cleared off by their natural defence mechanism on time and hence can be graded as animals with "cytologic endometritis", while animals with impaired uterine clearance may harbour bacterial load and infection (infective endometritis). Again, the degree of infection was depending on type of non-specific bacteria that are colonizing and its load. Therefore, if endometrial cytology with higher PMN per cent cut off threshold with respect to day postpartum is coupled with microbial assay it were definitely aid in detecting infective versus cytologic endometritis more accurately. The availability of any bacteria within the uterus is affected by several factors like the host immunity; failure of uterine defence mechanism, animal's environment, retained placenta and imbalance in host uterine microbes etc.

Table 2: Bacterial isolates obtained from endometrial cytology sample of repeat breeder buffaloes suffering from subclinical endometritis

Total samples analysed	Bacterial isolates		Positive Bacterial Isolates			Types of bacterial isolates			
	Positive	Negative	Single	Mixed	Total	*Streptococcus* sp.	*Staphylococcus* sp.	*E. coli*	*Bacillus* sp.
n=32	28 (87.50)	4 (12.50)	12 (42.85)	16 (57.15)	44	12 (27.27)	20 (45.45)	6 (13.64)	8 (18.18)

Figures in parenthesis indicate percentage.

CONCLUSION

Based on the above study, it can be concluded that occurrence of subclinical endometritis due to bacterial infection was 17.17 per cent. Endometrial cytology by cytobrush technique in repeat breeder buffaloes revealed polymorphonuclear cell (PMN) percentage to be 9.70±0.80 and 2.19±0.09 per cent, respectively. *Staphylococcus* species (45.45%) was most prevalent among the bacterial isolates obtained.

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