

Original Research Article

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Diagnosis of Subclinical Endometritis in Murrah Buffaloes through Cytobrush Technique

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ABSTRACT

The objective of this study was to find out microbial cause of repeat breeding in ten repeat breeder Murrah buffaloes at Government Livestock Farm, Sector-II, Hisar. The cytological samples were obtained from these buffaloes' uterus using cytobrush technique and relating the findings to the presence of polymorphonuclear cells, identification of bacteria and antibiotic sensitivity pattern is reported. The subclinical endometritis was declared on the basis of presence of $\geq 10\%$ polymorphonuclear cells in endometrial smears. Besides *Bacillus* (5), mixed infection of *Staphylococci* + *E. coli* (3), *Bacillus* + *E. coli* (1), and *Proteus* + *Bacillus* (1) was also recorded. There was highest prevalence of *Bacillus* (46.66%) followed by *E. coli* (26.67%), *Staphylococci* (20.0%) and *Proteus* (6.66%). The bacterial isolates reported in the present study showed highest sensitivity (60%) towards Moxifloxacin and Enrofloxacin followed by Chloramphenicol and Gentamicin (33.3%) and least towards Ceftriaxone and Cefoperazone (13.3%). All the isolates showed resistance towards Penicillin-G, Ofloxacin, Amoxicillin, Oxytetracycline, Ampicillin, Neomycin, Amikacin, Cloxacillin and Cephalexin. The findings of the present study revealed that cytobrush technique could preferentially be used to detect subclinical endometritis in clinical practice.

Keywords

Buffalo, Cytobrush, Cytology, Endometritis, Murrah, Subclinical.

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Introduction

Uterine infections in dairy animals cause infertility in acute cases and sub-fertility in chronic cases. Consequently, uterine infection reduces conception rate, increase calving to conception interval and contribute for increased culling rate (Herath *et al.*, 2006; Sheldon *et al.*, 2009). During parturition, relaxation of the vulva and cervical dilation allow for the entry of bacteria into the uterus (Azawi, 2008) and hence, bacterial contamination of the uterus postpartum is

common. Among the various bacteria that cause uterine infection the most common bacteria include *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Prevotella* sp. (Griffin *et al.*, 1974; Sheldon *et al.*, 2002) and are acquired from the faeces and faecal contamination of the coat, bedding and environment. Repeat breeding is a major cause of infertility in the dairy animals. Among various aetiologies of repeat breeding,

subclinical endometritis is an important cause and is often undiagnosed. Diagnosis of subclinical endometritis as early as possible may reduce the cost of rearing the buffaloes at dairy farm or individual farmer. The various methods for diagnosing endometritis involve uterine biopsies, lavage and swabs are existing but these may cause irritation and distortion of cells (Singh *et al.*, 2016). An inconsistent success following conventional therapies is achieved due to lack of diagnostic standards (Kasimanickam *et al.*, 2005). Therefore, most of the recent studies have been focused on sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis at field level. A novel approach for uterine cytological examination by using cytobrush technique which is considered consistent and reliable method in dairy animals (Barlund *et al.*, 2008 and Honparkhe *et al.*, 2014). Under field conditions cervico-vaginal discharge hanging from vulvar lips is generally collected for assessment of uterine health which in majority of cases gives false reflection of uterine environment. Keeping this in view, the present study was designed to investigate the efficacy of cytobrush technique in diagnosing subclinical endometritis in repeat breeder Murrah buffaloes at an organized farm.

Materials and Methods

Ten apparently healthy, cyclic pleuriparous Murrah buffaloes which had been inseminated consecutively in more than three estrous cycles with semen of elite bulls and exhibited conception failure at Government livestock farm, Sector II, Hisar were selected for the study. The animals had also been administered GnRH agonists at the time of artificial insemination to ensure ovulation at proper time. The cervico-vaginal discharge of all the animals was apparently normal. All selected animals were examined per-rectally

and administered Cloprostenol 500 µg three days earlier to induce the estrus and it also allowed the passage of cytobrush assembly with ease. The cytobrush technique was applied in all the buffaloes for endometrial cytology for confirmation of subclinical endometritis.

The cytobrush assembly (especially fabricated for bovine contains intrauterine catheter and a stilette attached with cytobrush (Fig. 1) was introduced into vagina and then through the cervix to body of the uterus and then screwed gently in both directions. Immediately after removal from reproductive tract, the cytobrush was smeared on clean sterilized glass slides. The slide was fixed in methanol for one minute and stained with Field's stain with slight modification (3-4 dips in Soln. A and B). The slide was observed first under microscope under 100 X and then under oil immersion (1000 X magnification) by counting 100 cells to determine the percentage of polymorphonuclear cells. Each slide was read by two persons and the average of the readings was considered. A buffalo was considered to be suffering from subclinical endometritis, if the proportion of polymorphonuclear cells (PMNs) was greater than 10 per cent as suggested by Kasimanickam *et al.*, (2004). The cytobrush samples were immediately dipped into sterile tubes containing the brain heart infusion (BHI) broth and transported to the laboratory within 1 to 2 hours and kept in an incubator for 24 hours at 37 °C. The BHI broth was then checked for the turbidity and using a sterilized loop the broth culture was streaked onto BHI and selective media viz., 5% defibrinated sheep blood agar plate and MacConkey lactose agar plate and incubated aerobically at 37°C for 16-18 hours. The microbes were identified by colony morphology, Gram staining and primary biochemical tests. The standard disc diffusion method (Bauer *et al.*, 1966) was employed for carrying out

antimicrobial sensitivity test using 15 antimicrobials viz., ampicillin (25 mcg), ceftriaxone (10 mcg), amoxicillin (10 mcg), cephalexin (30 mcg), cloxacillin (30 mcg), chloramphenicol (25 mcg), neomycin (30 mcg), ofloxacin (5 mcg), enrofloxacin (10 mcg), cefoperazone (75 mcg), amikacin (10 mcg), penicillin G (10 units), gentamicin (30 mcg), moxifloxacin (5 mcg) and oxytetracycline (30 mcg) (Hi media, Mumbai). Zones of inhibition were measured and the results were interpreted as sensitive (S) and resistant (R) using the chart provided by manufacturer.

Results and Discussion

The examination of slides under microscope at 100 X and then under oil immersion (1000 X magnification) by counting 100 cells to determine the percentage of polymorphonuclear cells that revealed presence of $\geq 10\%$ polymorphonuclear cells (Table 1, Fig. 2a and 2b).

Kasimanickam *et al.*, (2004) found $>18\%$ neutrophils at 20-33 days postpartum or $>10\%$ neutrophils at 34-47 days postpartum in uterine samples as an indicative of subclinical endometritis whereas, Gilbert *et al.*, (2005) found 5% neutrophils at 40 to 60 days postpartum as an indicator of subclinical endometritis in cattle, while Barlund *et al.*, (2008) used a neutrophil threshold value of 8% at 28-41 days postpartum in cattle to declare endometritis. Various research studies have supported that endometrial cytology by cytobrush technique is most efficient and early diagnostic technique when used along with microbial assay for diagnosis of subclinical endometritis (Honparkhe *et al.*, 2014; Bajaj *et al.*, 2015). The subclinical endometritis was declared on the basis of clear mucus discharge and $\geq 10\%$

polymorphonuclear cells (PMN) whereas clinical endometritis diagnosed on the basis of mucopurulent discharge Kasimanickam *et al.*, (2004).

In the present study, a total of 15 organisms were isolated from ten samples. Besides *Bacillus* (5), mixed infection of *Staphylococci* + *E. coli* (3), *Bacillus* + *E. coli* (1), and *Proteus* + *Bacillus* (1) was also reported. There was highest prevalence of *Bacillus* (46.66%) followed by *E. coli* (26.67%), *Staphylococci* (20.0%) and *Proteus* (6.66%).

The bacterial isolates reported in the present study showed highest sensitivity (60%) towards Moxifloxacin and Enrofloxacin followed by Chloramphenicol and Gentamicin (33.3%) and least towards Ceftriaxone and Cefoperazone (13.3%). All the isolates showed resistance towards Penicillin-G, Ofloxacin, Amoxicillin, Oxytetracycline, Ampicillin, Neomycin, Amikacin, Cloxacillin and Cephalexin (Table 2).

Similar to our findings, Singh *et al.*, (2016) also reported highest prevalence of bacilli (47.94%) followed by *E. coli* (24.65%) and *Staphylococcus* (12.32%) in the subclinical endometritis in dairy cattle. While in another study, *E. coli* was the most prevalent pathogen followed by *Klebsiella*, *Proteus*, *Pseudomonas* and *Clostridium spp.* (Udhayavel *et al.*, 2013). Moges *et al.*, (2013) reported higher prevalence of *A. pyogenes* followed by *Streptococcus Sp.* and *E. coli*, *S. aureus*, *Klebsiella Spp.* and *C. fetus*. In a study conducted by Sahadev *et al.*, (2017), most common single isolate observed was *E. coli* (45.71%) in 16 out of 35 cows followed by *Staphylococcus* (42.86%), *Proteus spp.* and *Enterobacter sp.* (5.71%).

Fig.1 Cytobrush assembly and cytobrush



Fig. 2a and 2b Microscopic examination of Polymorphonuclear cells

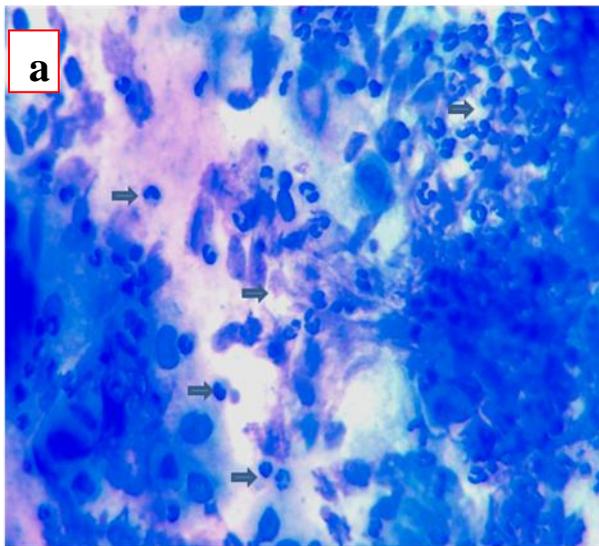


Fig. 2a: Polymorphonuclear cells (Arrows) in field's staining X100.

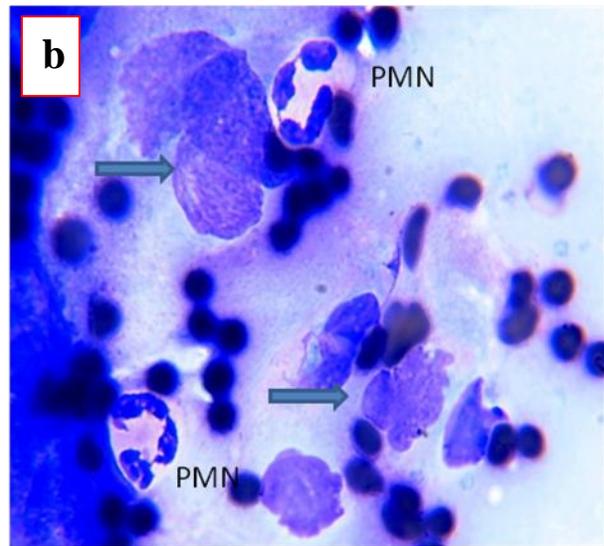


Fig. 2b: Polymorphonuclear cells and endometrial cells (Arrows) in field's staining X1000.

Table.1 Microscopic count of Polymorphonuclear cells (X1000)

Sr. No.	Buffalo No.	Polymorphonuclear cell count (%)
1	2667/355	12
2	2675CM/167	14
3	2672/339	12
4	2676/194	35
5	2674CM/212	10
6	2673CM/310	25
7	2671CM/113	20
8	2670CM/317	12
9	2669CM/072	15
10	2668CM/187	15

Table.2 Bacteriology of subclinical endometritis and *in vitro* antimicrobial sensitivity of the isolates in Murrah buffaloes

Bacteria	No. of isolates	No. of isolates sensitive to														
		P	Of	Amx	Ctr	O	Amp	N	Ak	Cox	Cpz	Mox	Ex	C	G	CN
Bacillus	7	0	0	0	1	0	0	0	0	0	1	6	6	5	5	0
<i>E.coli</i>	4	0	0	0	1	0	0	0	0	0	1	2	2	0	0	0
Staph.	3	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Proteus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	15	0	0	0	2	0	0	0	0	0	2	9	9	5	5	0
% sensitivity		0	0	0	13.3	0	0	0	0	0	13.3	60	60	33.3	33.3	0

P-Penicillin; Of-Ofloxacin; Amx-Amoxycillin; Ctr-Ceftriaxone; O-Oxytetracycline; Amp-Ampicillin; N-Neomycin; Ak-Amikacin; Cox-Cloxacin; Cpz-Cefoperazone; Mox-Moxifloxacin; Ex-Enrofloxacin; C-Chloramphenicol; G-Gentamicin, CN-Cephalexin

In an another detailed study, Gani *et al.*, (2008) taken 51 uterine biopsy samples collected from 14 normal fertile and 37 repeat breeding cows for bacteriological examinations to find out the prevalence of bacterial flora and their role in repeat breeding syndrome. They found among 37 that suffered from repeat breeding, 23 samples (62.2%) yielded 54 bacterial isolates; where *Staphylococcus* was predominant 14 (37.8%), followed by *Bacillus* 13 (35.1%), *E. coli* 11 (29.7%), *Pseudomonas* 7 (18.9%) while Gram negative minute rod shaped bacteria was 9 (24.3%).

Muneer *et al.*, (1991) and Bhat and Bhattacharyya (2012) reported oxytetracycline to be the best antibiotic for the treatment of metritis in cows. Findings of present study showed highest sensitivity towards Moxifloxacin and Enrofloxacin followed by Chloramphenicol and Gentamicin and least towards Ceftraixone and Cefoperazone against isolates of bacteria causing subclinical endometritis in Murrah buffaloes.

Indiscriminate use of antibiotics, irregular doses of antibiotics or under dosing of antibiotics may lead to development of resistance in microbes which creates major challenge for pharmaceuticals and veterinary practitioners. Therefore, antibiotic sensitivity

should be carried out to find the most effective drug.

The present study clearly indicated that there is high degree of correlation between polymorphonuclear cells and bacterial infection in the uterus. If PMN cell count is more than 10%, the chances of subclinical endometritis are highly evident. There was highest prevalence of *Bacillus* (46.66%) followed by *E. coli* (26.67%), *Staphylococci* (20.0%) and *Proteus* (6.66%).

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