

## RESEARCH ARTICLE

# Bacterial Isolates from the Genital Aspirates of Cyclic, Acyclic, Endometritic and Pregnant Buffaloes

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### ABSTRACT

The study was carried out on 50 vaginal secretions/aspirates/discharge samples collected aseptically using syringe and pipette method from infertile (anestrus; endometritic, n = 6 each) buffaloes of villages nearby Anand and healthy cyclic (n = 5; proestus, estrus, metestrus, diestrus) as well as 3, 6 and 9 month pregnant (n = 6 each) buffaloes of University farm to identify the vaginal microorganisms based on routine cultural examination. In all 117 bacterial isolates were recovered from all 50 vaginal samples (100 %) of 35 buffaloes during different physio-pathological status. The bacteria isolated from vaginal mucus/aspirates of buffaloes during the follicular phase comprised *Corynebacterium* spp. as the most predominant isolate (28.57%) followed by *E. coli*, *Bacillus* Spp., *Staphylococcus* spp., *Streptococcus* spp., *Salmonella* spp., *Proteus* spp., and vaginal yeast, whereas during the luteal phase, the most predominant bacteria were *E. coli* (23.33%) followed by *Corynebacterium* spp., *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Klebsiella* spp. In acyclic buffaloes, the most predominant bacteria isolated were *Corynebacterium* spp. (21.43%) *Bacillus* spp., *Micrococcus* spp., *Pseudomonas*, *Staphylococcus* spp., *Streptococcus* spp., *E. coli* and *Salmonella* spp., whereas the endometritic buffaloes evinced the most predominant bacterial isolates as *Corynebacterium* spp. and *E. coli* (20.00% each) followed by *Bacillus* spp., *Salmonella*, *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Klebsiella* spp. The major bacteria isolated during the entire period of pregnancy were *E. coli*, *Micrococcus*, *Corynebacterium* Spp., *Bacillus* spp., *Staphylococcus* spp. and *Proteus*. This study concludes rich bacterial diversity in the vagina of buffaloes during different physio-pathological status.

**Keywords:** Acyclic, Bacterial isolates, Buffalo, Cyclic, Endometritis, Genital aspirates, Pregnant.

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### INTRODUCTION

Buffalo is the principal dairy animal in the developing countries of Asia and the mainstay of the Indian dairy industry. As on today, the buffalo population in India is 108.7 million, and its milk production is 66 million tonnes per year (Livestock Census, 2017). Fertility is one of the key determinants of the cow's performance warranting one calf every year for optimum economic performance. Infertility is one of the major problems which incur losses for the dairy industry (Sheldon *et al.*, 2009). The prevalence rate of uterine infection in buffalo is much higher than in cows (Moghaddam and Mamoei, 2004), and it is one of the most important reproductive disorders in buffalo (Melenzed *et al.*, 2004).

The normal microbial flora of the genital tract is composed of bacteria of the genera *Staphylococcus*, *Streptococcus*, and the coliform group (Hafez, 1993). According to previous reports (Otero *et al.*, 2000; Rocha *et al.*, 2004; Fernandez *et al.*, 2006), the normal vaginal microflora in bovines mostly comprise aerobic (*Staphylococcus*, *Streptococcus*, *Coli* forms) and anaerobic bacteria (*Lactobacillus*, *Fusobacterium*, *Peptostreptococcus*), and proportionately less fungi (*Aspergillus*, *Penicillium*). There are scattered reports of the routine cultural isolation of bacteria from the genital discharges of infertile bovine females. Still, not much is known about normal vaginal microflora of cyclic, acyclic, pregnant, and endometritic cattle or buffalo (Patel *et al.*, 2019). Hence,

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this study was aimed to assess the relative bacterial isolates from buffaloes under such physio-pathological conditions.

### MATERIALS AND METHODS

The study was carried out on vaginal secretions/aspirates/discharge from infertile (anestrus; endometritic) buffaloes of villages nearby Anand and healthy cyclic as well as pregnant

buffaloes of University farm, AAU, Anand from August 2018 to June 2019. The work was carried out on total 35 buffaloes covering five regular cyclic (proestrus, estrus, metestrus, diestrus) and six each acyclic, endometritic and 3, 6 and 9 months pregnant buffaloes. The samples of cervicovaginal mucus/discharge during estrus/endometritis, and vaginal washings/aspirates during other phases of the estrous cycle, *i.e.*, proestrus, metestrus, diestrus, and anestrus as well as pregnancy were collected aseptically using syringe and pipette method. For the collection of vaginal washing, the pointed end of glass pipette was connected to a 60 mL syringe with rubber junction and 30 mL sterile normal saline was first infused in the vaginal fornix, massaged per rectally for few minutes and again aspirated with the same pipette aseptically (Patel *et al.*, 2019). Isolation of bacteria was carried out as per Cruickshank (1965) by inoculation of samples on blood agar and MacConkey agar plates. Thereafter, the cultures obtained were subjected to detailed identification of organisms using Gram's staining and biochemical tests such as oxidase, KOH, and catalase tests.

## RESULTS AND DISCUSSION

Bacteria were recovered from all 50 vaginal samples (100%) of 35 buffaloes with different physio-pathological status. In all, 117 bacterial isolates were obtained from 50 samples. The details of bacterial isolates obtained on cultural examinations of vaginal discharges/aspirates collected from buffaloes of different reproductive status are furnished in Table 1.

### Bacterial Isolates from Vaginal Mucus/Aspirates of Cyclic Buffaloes

The bacteria isolated from vaginal mucus/aspirates of buffaloes during the follicular phase of estrous cycle comprised the most predominant bacterial isolate to be *Corynebacterium* spp. (28.57%) followed by *E. coli* (23.80%), *Bacillus* spp. (19.04%), *Staphylococcus* spp. (9.52%), *Streptococcus* spp., *Salmonella* spp., *Proteus* spp., and vaginal yeast (4.76% each), with an overall of 17.94% prevalence among the total 117 isolates, whereas the luteal phase of cyclic buffaloes, contributed 25.64% isolates. Among them, the most predominant bacteria were *E. coli* (23.33%), followed by *Corynebacterium* spp. (20.00%), *Bacillus* spp. (16.66%), *Staphylococcus* spp. (13.33%), and *Streptococcus* spp., and *Klebsiella* spp. (6.66% each). Thus, based on the total bacterial isolates obtained during the entire estrous cycle, the bacterial isolates for follicular and luteal phases constituted 42.00 and 58.00%, respectively.

The findings of the present study showed that the rate of bacterial isolation was higher in luteal phase than the follicular phase of estrous cycle. Low incidence of bacteria isolated during the follicular phase can be attributed to the bacteriostatic effect of estrogens, while during luteal phase, bacteria flourished in the vagina due to the luxuriant medium

provided by progesterone. Progesterone is known for its immunosuppressive effects during the luteal phase of the estrous cycle in female animals. The dominance of estrogen during the follicular phase of the estrous cycle increases the rate of migration of leucocyte into the uterine and vaginal lumen, and thus increases the bactericidal activity. The results of the present study corroborated with the findings of El-Jakee *et al.* (2008), Kavyashree (2013) and Patel *et al.* (2019), who also reported that the bacteria isolated during luteal phase were found to be higher than those found in follicular phase of estrous cycle. However, Vlcek and Svobodova (1985) reported contrary findings of 40.90% samples with bacterial isolates during the follicular phase and 35.36% during luteal phase of the normal estrous cycle in cows.

### Bacterial Isolates from Vagina of Acyclic and Endometritic Buffaloes

The bacterial isolates from the vaginal aspirates of acyclic buffaloes constituted 11.11% of the total isolates obtained in the study. The major isolates were *Corynebacterium* spp. (21.43%), *Bacillus* spp., *Micrococcus* spp., *Pseudomonas* (14.28% each), and *Staphylococcus* spp., *Streptococcus* spp., *E. coli* and *Salmonella* spp. (7.14% each), whereas the vaginal discharges of endometritic buffaloes evinced the most predominant bacterial isolates as *Corynebacterium* spp. and *E. coli* (20.00% each), followed by *Bacillus* spp., *Salmonella* and *Proteus* spp. (13.33% each), *Staphylococcus* spp., *Streptococcus* spp., and *Klebsiella* spp. (6.66% each), constituting 12.82% of the total bacterial isolates.

The bacterial isolates obtained from the vaginal aspirates of acyclic buffaloes in the present study corroborated with the report of El-Jakee *et al.* (2008), who isolated *E. coli*, *Klebsiella* spp., *Streptococcus* spp. and *Staphylococcus* spp. from the vaginal swabs of the anestrus buffalo-cows, and almost similar were the findings of Patel *et al.* (2019) in crossbred cows. Clinically acyclic buffaloes have the state of ovarian inactivity with an absence of circulatory estrogen. *E. coli*, *Corynebacterium*, *Pseudomonas*, *Bacillus* and *Proteus* spp. were the common isolates from the endometritic buffaloes in the present study. These findings are in consonance with the observations reported earlier by Azawi *et al.* (2008), Atchawaran *et al.* (2013), Barman *et al.* (2013) and Patel *et al.* (2019). However, in contrary to the present findings, Patel *et al.* (2009) reported only 55.55% samples showing bacterial growth in postpartum endometritic buffaloes. The uterine bacterial infection suppresses pituitary LH secretion and disturbs ovarian follicle growth and functions, which disturb ovulation in animals (El-Jakee *et al.*, 2008).

### Bacterial Isolates from Vagina of Pregnant Buffaloes

The bacterial isolates were obtained from the vaginal aspirates of buffaloes at 3, 6, and 9 months of gestation. The bacterial isolates at 3 months of pregnancy comprised *Bacillus* spp. and *Corynebacterium* spp. (21.42% each), *E. coli*

**Table 1:** Cultural isolates from vaginal discharges/ aspirates of buffaloes during different reproductive physio-pathological statuses

Stages of Sampling (n = Animals)	Distribution of Types of Organisms Isolated										Total Isolates		
	Bacillus Spp.	Corynebacterium	Staphylococcus	Streptococcus	Micrococcus	Pseudomonas	E. coli	Salmonella	Klebsiella	Proteus		Vaginal Yeast	
Proestrus (5)	No.	2	2	1	1	0	0	3	1	0	0	0	10
	%	20.00	20.00	10.00	10.00	0.00	0.00	30.00	10.00	0.00	0.00	0.00	8.54
Estrus (5)	No.	2	4	1	0	0	0	2	0	0	1	1	11
	%	18.18	36.36	9.09	0.00	0.00	0.00	18.18	0.00	0.00	9.09	9.09	9.40
Pooled	No.	4	6	2	1	0	0	5	1	0	1	1	21
	%	19.04	28.57	9.52	4.76	0.00	0.00	23.80	4.76	0.00	4.76	4.76	17.94
Metestrus (5)	No.	3	3	2	1	1	0	4	1	1	0	0	16
	%	18.75	18.75	12.5	6.25	6.25	0.00	25.00	6.25	6.25	0.00	0.00	13.67
Diestrus (5)	No.	2	3	1	1	0	1	3	0	1	1	0	14
	%	14.28	21.42	7.14	7.14	0.00	7.14	21.43	0.00	7.14	7.14	0.00	11.11
Pooled	No.	5	6	3	2	1	1	7	1	2	1	0	30
	%	16.66	20.00	10.00	6.66	3.33	3.33	23.33	3.33	6.66	3.33	0.00	25.64
Acyclic (6)	No.	2	3	1	1	2	2	1	1	0	0	0	13
	%	14.28	21.43	7.14	7.14	14.28	14.28	7.14	7.14	0.00	0.00	0.00	11.11
Endometritis (6)	No.	2	3	1	1	0	0	3	2	1	2	0	15
	%	13.33	20.00	6.66	6.66	0.00	0.00	20.00	13.33	6.66	13.33	0.00	12.82
3 Months (6)	No.	3	3	1	1	1	1	2	1	1	0	0	14
	%	21.42	21.42	7.14	7.14	7.14	7.14	14.28	7.14	7.14	0.00	0.00	11.96
6 Months (6)	No.	3	2	1	1	2	1	0	2	1	0	0	13
	%	23.07	15.38	7.69	7.69	15.38	7.69	0.00	15.38	7.69	0.00	0.00	11.11
9 Months (6)	No.	1	2	0	1	2	2	0	2	1	0	0	11
	%	9.09	18.18	0.00	9.09	18.18	18.18	0.00	18.18	9.09	0.00	0.00	9.40
Pooled	No.	7	7	2	3	5	4	2	5	3	0	0	38
	%	18.42	18.42	5.26	7.89	13.15	10.52	5.26	13.15	7.89	0.00	0.00	32.47
Total (50)	No.	20	25	10	8	8	7	18	10	6	4	1	117
	%	17.09	21.36	8.54	6.83	6.83	5.98	15.38	8.54	5.12	3.41	0.85	100



(14.28%), *Pseudomonas*, *Streptococcus* spp., *Staphylococcus* spp., *Micrococcus*, *Salmonella* and *Klebsiella* spp. (7.14% each), whereas the buffaloes having six months pregnancy had *Bacillus* spp. (23.07%), *Corynebacterium* spp., *Micrococcus* spp. and *Salmonella* spp. (15.38% each), *Staphylococcus* spp., *Streptococcus* spp. and *Pseudomonas* and *Klebsiella* spp. (7.69% each). Moreover, the bacteria isolated from the vagina of nine months pregnant buffaloes were *Corynebacterium* spp., *Micrococcus*, *Pseudomonas*, and *Salmonella* (18.18% each), *Bacillus* spp., *Streptococcus* spp., and *Klebsiella* (9.09% each).

Major bacteria isolated during the entire period of pregnancy were *E. coli*, *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp., *Staphylococcus* spp. and *Proteus* spp. Similar results were also reported by El-Zakee *et al.* (2008), Kavyashree (2013), and Patel *et al.* (2019). The rates of bacterial isolates found at three (11.96 %), six (11.11 %), and nine (9.40 %) months of pregnancy in buffaloes are suggestive of more or less constant or less fluctuating type of vaginal bacterial flora, with a trend of marginal decline.

From the study, it was concluded that the vaginal cavity of healthy cyclic, acyclic, endometritic and pregnant buffaloes shows the rich diversity of bacterial isolates according to ovarian/ endocrine status, signifying its role in physiopathology of reproduction in buffaloes.

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