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

Binal R Patel¹ *, MT Panchal², AJ Dhami³, RA Mathakiya⁴, BB Bhanderi⁵

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, Corynbacterium 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.

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, 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...
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Bacterial Isolates from Vaginal Mucus/Aspirates of Cyclic Buffaloes

The bacteria isolated from vaginal mucus/aspirates of buffaloes during the follicular phase comprised the most predominant bacteria to be Corynebacterium spp. (28.57%) followed by 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.

Bacterial Isolates from Vagina of Acyclic and Endometritic Buffaloes

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

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 leukocyte into the uterine and vaginal lumen, and thus increases the bactericidal activity. The results of the present study corroborated with the findings of Patel 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.

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.

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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.

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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

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

<table>
<thead>
<tr>
<th>Stages of Sampling (n = Animals)</th>
<th>Distribution of Types of Organisms Isolated</th>
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<tbody>
<tr>
<td></td>
<td>Bacillus Sp.</td>
</tr>
<tr>
<td>Proestrus (5)</td>
<td>No. 2</td>
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<tr>
<td>Estrus (5)</td>
<td>No. 2</td>
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<tr>
<td>Pooled</td>
<td>No. 4</td>
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<tr>
<td>Metestrus (5)</td>
<td>No. 3</td>
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<tr>
<td>Diestrus (5)</td>
<td>No. 2</td>
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<tr>
<td>Pooled</td>
<td>No. 5</td>
</tr>
<tr>
<td>Acyclic (6)</td>
<td>No. 2</td>
</tr>
<tr>
<td>Endometritis (6)</td>
<td>No. 2</td>
</tr>
<tr>
<td>3 Months (6)</td>
<td>No. 3</td>
</tr>
<tr>
<td>6 Months (6)</td>
<td>No. 3</td>
</tr>
<tr>
<td>9 Months (6)</td>
<td>No. 1</td>
</tr>
<tr>
<td>Pregnant</td>
<td>No. 7</td>
</tr>
<tr>
<td>Pooled</td>
<td>No. 7</td>
</tr>
<tr>
<td>Total (50)</td>
<td>No. 20</td>
</tr>
<tr>
<td></td>
<td>% 17.09</td>
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Vaginal Microbial Flora of Normal, Repeat
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of isolated bacteria from clinical endometritis uterine and
antimicrobial susceptibility in postpartum dairy cows. Chiang
Antibiogram of bacteria isolated from bovine endometritis. Vet.

(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., Corynbacterium
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 physio-
pathology of reproduction in buffaloes.

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cooperation while samples collected from their animals.

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