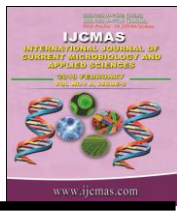
Int.J.Curr.Microbiol.App.Sci (2019) *8*(3): 536-542

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 03 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.803.065

Isolation of Bacteria from the Vaginal Aspirates of Cyclic, Acyclic, Endometritic and Pregnant Crossbred Cows

C.I. Patel[1], M.T. Panchal[1], A.J. Dhami[1]*, B.B. Bhanderi[2] and R.A. Mathakiya[2]

[1]Department of Animal Reproduction, Gynaecvology & Obstetrics, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat - 388 001, India
[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat - 388 001, India

**Corresponding author*

Keywords

Bacterial isolates, Cattle vagina, Estrus cycle, Acyclic, Endometritis, Pregnancy

Article Info

Accepted:
07 February 2019
Available Online:
10 March 2019

A B S T R A C T

A study was carried out on vaginal secretions/aspirates from infertile (acyclic and endometritic) crossbred cows from field and healthy cyclic as well as pregnant crossbred cows of University farm to identify the vaginal microorganisms based on routine cultural examination. The work was carried out on total 36 crossbred cows covering six each regular cyclic (proestrus, estrus, metestrus, diestrus), acyclic, endometritic and 3, 6 and 9 months pregnant animals. The samples of cervico-vaginal mucus/discharge during estrus/ endometritis, and vaginal washings during other phases of estrous cycle as well as pregnancy were collected aseptically using syringe and pipette method. The samples obtained were soon processed for cultural isolation on Blood agar and MacConkey agar, and identified using Gram's staining and biochemical tests. Bacteria were recovered from all 54 vaginal samples (100%) of cows with different physio-pathological status. During the follicular phase of estrous cycle, the most predominant bacteria isolated were *Bacillus* Spp., followed by *Corynebacterium* Spp., *Staphylococcus* Spp. and *Streptococcus* Spp., whereas during luteal phase the most predominant bacteria were *Staphylococcus* Spp. followed by *Corynebacterium* Spp., *Bacillus* Spp., *E. coli* and *Streptococcus* Spp. The most predominant vaginal bacterial isolates during pregnancy in descending order were *Staphylococcus* Spp., *Bacillus* Spp., *Streptococcus* Spp., *Klebsiella*, *E. coli* and *Pseudomonas*. The vaginal aspirates of acyclic cows contained *Streptococcus* Spp., *Bacillus* Spp. and *Staphylococcus* Spp., *Corynebacterium* Spp., *E. coli*, and *Micrococcus*, whereas in endometritis the major isolates were *Staphylococcus* Spp., *Bacillus* Spp., *Streptococcus* Spp., *E. coli*, *Salmonella* and *Corynebacterium*. The findings reflected rich bacterial diversity in the vagina of crossbred cattle with varied physio-pathological conditions.

Introduction

Optimum reproductive performance is essential for well managed and profitable dairy farms (Nebel and Jobst, 1998). Fertility of cow is affected by many specific and nonspecific pathogens of the genital tract. Cervical mucus discharge (CMD) is a

mechanical barrier against pathogens of the uterus. CMD of cows and heifers with abnormal appearance in estrous cycle is one of the factors that farmers or inseminators consider as a suppressor of reproductive performance (Mahmoudzadeh *et al.,* 2001). Endometritis in cows, characterized clinically by the presence of pus in the vagina (Sheldon *et al.,* 2002) is associated with lower first service conception rate, increased open days, and more culls for failure to conceive (LeBlanc *et al.,* 2002). According to various researchers (Fernandez *et al.,* 2006), the normal vaginal microflora mostly comprises aerobic bacteria (*Staphylococcus*, *Streptococcus* and coliforms), anaerobic bacteria (*Lactobacillus*, *Fusobacterium*, and *Pepto-streptococcus*) and proportionately less fungi (*Aspergillus* and *Penicillium*). *Enterobacteriaceae*, especially *E. coli* have been isolated from the urogenital tract of cattle in low numbers (Otero *et al.,* 2000). Nevertheless, *E. coli* is well known to cause endometritis and infertility in cattle (Sheldon *et al.,* 2002). The present work was aimed to know the normal bacterial flora from the genital discharges/aspirates of normal cyclic and pregnant cows, as well as those suffering from anestrus and endometritis to establish their relation with the status of reproduction.

Materials and Methods

The investigation was carried out on the vaginal secretions/aspirates of infertile crossbred cows under field from village Chikhodra of Anand taluka and healthy cyclic as well as pregnant crossbred cows of Livestock Research Station, AAU, Anand from August 2017 to June 2018. The work was carried out on total 36 crossbred cows covering six each regular cyclic (proestrus, estrus, metestrus, diestrus), acyclic, endometritic and 3, 6 and 9 months pregnant animals. The samples of cervico-vaginal mucus during estrus, and vaginal washings during other phases of estrous cycle, *i.e.*, proestus, metestrus, diestrus, and anestrus as well as pregnancy were collected aseptically, using sterilized 10 ml glass pipette and 60 ml syringe, employing recto-vaginal technique of Panangala *et al.,* (1978). Similarly the vaginal discharge samples from endometritic cows were also obtained aseptically. For obtaining vaginal mucus / discharge / washing, the pointed end of glass pipette was connected to a 60 ml syringe with rubber junction. For animals other than in estrus or endometritic, 30 ml sterile normal saline solution was first infused in the vaginal fornix, massaged per rectally for few minutes and was again aspirated with the same pipette aseptically.

The vaginal samples (n=54) obtained were processed for cultural isolation within an hour of collection. The aim of study was to identify the vaginal microorganism during different physio-pathological conditions of cattle based on routine culture of vaginal samples on blood agar and MacConkey agar plates (Cruickshank, 1965). The isolates obtained were subjected to detailed identification using Gram's staining and biochemical tests such as oxidase, KOH and catalase tests. The results of DNA extracted from all the vaginal samples and its metagenomics up to phyla, genera and species obtained using NGS and MG RAST library have been reported separately (Patel, 2018). The per cent frequency of various isolates obtained from different samples was worked out and is reported in this paper.

Results and Discussion

The details of vaginal bacterial flora of crossbred cows obtained on cultural examination during different phases of estrous cycle, anestrum, endometritis and 3, 6 and 9 months pregnancy are furnished in Table 1. Bacteria were recovered from all 54 vaginal samples (100%) of cows with different

physio-pathological status. In all, 170 bacterial isolates were obtained from 54 samples.

Bacterial isolates from vagina of cyclic cows

During the follicular phase (proestrus and estrus) of estrous cycle, the most predominant bacteria isolated were *Bacillus* Spp. (22.58%) followed by *Corynebacterium* Spp., *Staphylococcus* Spp. and *Streptococcus* Spp. (19.35% each), *E. coli* and *Salmonella* (6.45% each), and *Micrococcus* and vaginal yeast (3.23% each), making 18.24 per cent of the total 170 bacterial isolates. During luteal phase (metestrus and diestrus) of estrous cycle, the most predominant bacteria were *Staphylococcus* Spp. (19.05%) followed by *Corynebacterium* Spp. (14.29%), *Bacillus* Spp., *E. coli* and *Streptococcus* Spp. (11.90% each), *Micrococcus* (9.52%), *Salmonella* (7.14%), and *Klebsiella*, *Proteus* and vaginal yeast (4.76% each), making 24.71 per cent of the total 170 bacterial isolates. Thus, based on the total bacterial isolates of estrous cycle, follicular and luteal phase constituted 42.47 and 57.53 per cent, respectively. These findings supported the common consensus that the estrogenic phase of cycle inhibits vaginal bacterial isolates through improved local defense mechanism. In the present study, *E. coli*, *Staphylococcus*, *Streptococcus*, *Corynebacterium* Spp., *Bacillus* Spp., *Salmonella*, *Micrococcus* and Yeast were the commonest isolates throughout the estrous cycle of cows. However, *Proteus* and *Klebsiella* were not isolated during follicular phase, but were isolated during luteal phase of the estrous cycle. These organisms could, therefore, be considered as a part of the normal vaginal bacterial flora of the cow. The stage of the cycle did not alter the types of bacteria isolated, but increased numbers were present in luteal phases of the estrous cycle and pregnancy.

During estrous cycle, the dominance of the estrogen during the follicular phase of estrous cycle, increases the rate of migration of leucocyte into the uterine lumen and thus increases the bactericidal activity. El-Jakee *et al.,* (2008) isolated 22.47 per cent bacteria during follicular phase and 77.53 per cent during luteal phase of normal estrous cycle. However, Vlcek and Svobodova (1985) reported comparable findings of 40.90 per cent samples with bacterial isolates during follicular phase and 35.36 per cent during luteal phase of normal cycle. In the present study, bacteria were recovered from all (100%) of the 24 samples of cyclic crossbred cows (Table 1). Similar results were also obtained by El-Jakee *et al.,* (2008), who reported bacterial culture from 100 per cent of vaginal samples during normal estrous cycle. However, these findings are in contrary to the previous reports of 53.9 (Ocando *et al.,* 2010; Zambrano-Nava *et al.,* 2011) and 54.5 (Ahuja *et al.,* 2017) per cent cultural positive samples from normal fertile cows.

Bacterial isolates from vagina of acyclic and endometritic cows

The vaginal aspirates of acyclic cows were positive for *Streptococcus* Spp. (22.22%), *Bacillus* Spp. and *Staphylococcus* Spp. (16.67% each), *Corynebacterium* Spp., *E. coli*, and *Micrococcus* (11.11% each), and *Salmonella* and *Klebsiella* (5.56% each), constituting 10.59 per cent of the total bacterial isolates, whereas the vaginal discharges of endometritis cows, showed *Staphylococcus* Spp. (17.39%), *Bacillus* Spp., *Streptococcus* Spp., *E. coli*, *Salmonella* and *Klebsiella* (13.04% each), and *Corynebacterium* Spp. and *Proteus* (8.70% each), constituting 13.53 per cent of the total bacterial isolates. This higher frequency of bacterial isolates from cases of endometritis is justified due to apparent genital infection.

Table.1 Cultural isolates from vaginal discharges/aspirates of crossbred cows during different reproductive physio-pathological status (n=6 each)

Stage			Isolated organisms											Total no. of isolates
			Bacillus Spp.	*Corynebact-erium* Spp.	*E.coli*	*Klebsiella*	*Pseudo-monas*	*Staphylo-coccus*	*Salmonella*	*Strepto-coccus*	*Micro-coccus*	Yeast	*Proteus*	
Follicular Phase	Proestrus	No.	4	3	2	0	0	3	1	3	0	0	0	16
		%	25.00	18.75	12.50	0.00	0.00	18.75	6.25	18.75	0.00	0.00	0.00	9.41
	Estrus	No.	3	3	0	0	0	3	1	3	1	1	0	15
		%	20.00	20.00	0.00	0.00	0.00	20.00	6.67	20.00	6.67	6.67	0.00	8.82
Luteal Phase	Metestrus	No.	3	4	2	0	0	4	2	3	2	0	0	20
		%	15.00	20.00	10.00	0.00	0.00	20.00	10.00	15.00	10.00	0.00	0.00	11.76
	Diestrus	No.	2	2	3	2	0	4	1	2	2	2	2	22
		%	9.09	9.09	13.64	9.09	0.00	18.18	4.55	9.09	9.09	9.09	9.09	12.94
	Acyclic	No.	3	2	2	1	0	3	1	4	2	0	0	18
		%	16.67	11.11	11.11	5.56	0.00	16.67	5.56	22.22	11.11	0.00	0.00	10.59
	Endometritic	No.	3	2	3	3	0	4	3	3	0	0	2	23
		%	13.04	8.70	13.04	13.04	0.00	17.39	13.04	13.04	0.00	0.00	8.70	13.53
Pregnant	3 Months	No.	4	0	3	3	2	4	2	3	0	0	1	22
		%	18.18	0.00	13.64	13.64	9.09	18.18	9.09	13.64	0.00	0.00	4.55	12.94
	6 Months	No.	3	2	0	2	2	3	2	3	0	0	2	19
		%	15.79	10.53	0.00	10.53	10.53	15.79	10.53	15.79	0.00	0.00	10.53	11.18
	9 Months	No.	2	0	2	2	1	4	0	2	1	0	1	15
		%	13.33	0.00	13.33	13.33	6.67	26.67	0.00	13.33	6.67	0.00	6.67	8.82
	Total	**No.**	**27**	**18**	**17**	**13**	**5**	**32**	**13**	**26**	**8**	**3**	**8**	**170**
		%	**15.88**	**10.59**	**10.00**	**7.65**	**2.94**	**18.82**	**7.65**	**15.29**	**4.71**	**1.76**	**4.71**	**100.00**

The bacterial species isolated in the present study from acyclic cows (100%) agreed with previous reports of El-Jakee *et al.,* (2008) and Wagener *et al.,* (2015). Similarly, the results of endometritis samples are in agreement with the findings of El-Kader and Shehata (2001) and Barman *et al.,* (2013). However, these findings are in contrary to Patel *et al.,* (2009), Moges *et al.,* (2013) and Udhayavel *et al.,* (2013). They reported only 55 to 92 per cent samples showing bacterial growth in endometritic crossbred cows. Some researchers (Cohen *et al.,* 1996; Petit *et al.,* 2009) however isolated *Arcanobacterium pyogenes* and *Actinomyces pyogenes* as the most predominant bacteria. In a recent study on crossbred cows with puerperal endometritis (25±2 days, n=30) and repeat breeding (n=40), only 63.3 and 70 per cent cows, respectively, yielded mixed bacterial isolates, whereas the rest of the samples were sterile (Raval *et al.,* 2018). From the above discussion it is easily understood that most of the endometritic and pyogenic cases and/or uterine infections are caused by *E. coli*, *Staphylococcus* Spp., *Streptococcus* Spp., *Klebsiella* Spp. and *Proteus* Spp.

Bacterial isolates from vagina of pregnant cows

The isolated bacteria from vaginal aspirates of 3 months pregnant cows were *Bacillus* Spp. and *Staphylococcus* Spp. (18.18% each), *Streptococcus* Spp., *E. coli* and *Klebsiella* (13.64% each), *Pseudomonas* and *Salmonella* (9.09% each), and *Proteus* (4.55%), comprising 12.94 per cent of the total bacterial isolates. Another 11.18 per cent of the bacteria isolated were from six months pregnant cows with frequency of *Bacillus* Spp., *Staphylococcus* Spp. and *Streptococcus* Spp. as 15.79 per cent each, and *Corynebacterium* Spp., *Pseudomonas*, *Klebsiella*, *Salmonella* and *Proteus* 10.53 per cent each. Moreover, the vagina of nine months pregnant cows revealed *Staphylococcus* Spp. (26.67%), *Bacillus* Spp., *Streptococcus* Spp., *E. coli* and *Klebsiella* (13.33% each), and *Micrococcus*, *Pseudomonas* and *Proteus* (6.67% each, Table 1), constituting 8.82 per cent of the total bacterial isolates.

In comparison to 100 per cent of vaginal samples of pregnant cows showing bacterial growth in present study, El-Kader and Shehata (2001) and Jadon *et al.,* (2005) reported 92.30 and 89.90 per cent bacterial positive samples in pregnant cows. There was also decline in the frequency of vaginal isolates with advancing gestation as reported by El-Jakee *et al.,* (2008), who found the most predominant isolates as *E. coli* followed by *Micrococcus* Spp. El-Kader and Shehata (2001) also isolated family *Enterobacteriaceae* as the most predominant bacterial isolates followed by untypable *E. coli*, *Enterobacter aerogenes* and *Klebsiella oxytoca* from the genital tract of pregnant cows. Jadon *et al.,* (2005) isolated *E. coli* (17.7%), *Klebsiella Spp.* (5.81%), *Staphylococcus* (12.79%), and *Bacillus Spp.* (9.30%) from the genital tract of pregnant buffaloes (n=40). Further they also reported that more number of isolates was found in early stage (42.08%) than in last stage (15.83%) of gestation.

It is thus concluded that the vaginal cavity of healthy cyclic and pregnant as well as acyclic and endometritic cows shows a dynamics of bacterial isolates according to ovarian/ endocrine status, signifying its role in physio-pathology of reproduction in crossbred cattle.

Acknowledgement

We are grateful to the Dean of the Veterinary Faculty and University authorities for the facilities provided and Research Scientist & Head, LRS, AAU, Anand for cooperation while samples collection for this work.

References

Ahuja, A.K., Cheema, R.S., Narang, D. and Dhindsa, S.S. 2017. Bacterial pathogens and antibiotic susceptibility patterns of cervico-vaginal discharges in crossbred repeat breeding cows. *Int. J. Curr. Microbiol. App. Sci.*, 6(6), 1769-1775.

Barman, P., Yadav, M.C., Bangthai, A. and Kumar, H. 2013. Antibiogram of bacteria isolated from bovine endometritis. *Vet. Res. International*, 1, 20-24.

Cohen, R.O., Colodner, R., Ziv, G. and Keness, J. 1996. Isolation and antimicrobial susceptibility of obligate anaerobic bacteria recovered from the uteri of dairy cows with retained fetal membranes and post-parturient endometritis. *Zoonoses Public Health*, 43(1-10), 193-199.

Cruickshank, R. 1965. *Medical Microbiology*. 11th edn. The English Language Book Society and E & S Livingstone Ltd., Great Britain.

El-Jakee, J.A., Ahmed, W.M., El-Seedy, F.R. and El-Moez, S.A. 2008. Bacterial profile of the genital tract in female buffaloes during different reproductive stages. *Global Vet.*, 2(1), 7-14.

El-Kader, H.A. and Shehata, S.H. 2001. Bacteriological evaluation of vaginal discharges in cows with endometritis and clinically healthy heifers in Assiut governorate. *Assiut Univ. Bull. Environ. Res*, 4(2), 45-53.

Fernández, M.A., Silveira, P., Enrique, A., López, R. and Omar, F. 2006. Uterine infections in bovine female. *Revista Electrónica de Veterinaria Redvet®*, 7, 1695-7504. http://www.veterinaria.org /revistas/redvet/n101006.html.

Jadon, R. S., Dhaliwal, G. S. and Jand, S. K. (2005). Prevalence of aerobic and anaerobic uterine bacteria during peripartum period in normal and dystocia-affected buffaloes. *Anim. Reprod. Sci.*, 88(3-4): 215-224.

LeBlanc, S.J., Duffield, T.F., Leslie, K.E., Bateman, K.G., Keefe, G.P., Walton, J.S. and Johnson, W.H. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.*, 85(9), 2223-2236.

Mahmoudzadeh, A.R., Tarahomi, M. and Fotoohi, H. 2001. Effect of abnormal vaginal discharge at estrus on conception rate after artificial insemination in cows. *Anim. Sci.*, 72(3), 535-538.

Moges, N., Regassa, F., Yilma, T. and Unakal, C.G. 2013. Isolation and antimicrobial susceptibility of bacteria from dairy cows with clinical endometritis. *J. Reprod. Fert.*, 4(1), 04-08.

Nebel, R.L. and Jobst, S.M. 1998. Evaluation of systematic breeding programs for lactating dairy cows: A review. *J. Dairy Sci.*, 81(4), 1169-1174.

Ocando, J.B., Nava, S.Z., Nava, J. and Martinez, G.P. 2010. Profile of vaginal bacterial flora: A potential risk for reproduction in Criollo Limonero cows. *Revista Científica.*, 20(3), 227-234.

Otero, C., Saavedra, L., Silva de Ruiz, C., Wilde, O., Holgado, A.R. and Nader-Macías, M.E. 2000. Vaginal bacterial microflora modifications during the growth of healthy cows. *Letters in Appl. Microbiol.*, 31(3), 251-254.

Panangala, V.S., Fish, N.A. and Barnum, D.A. 1978. Microflora of the cervico-vaginal mucus of repeat breeder cows. *The Canadian Vet. J.*, 19(4), 83.

Patel, C.I. 2018. Study on reproductive microbiota in cyclic, acyclic and endometritic crossbred cattle. M.V.Sc.

Thesis, Anand Agricultural University, Anand, Gujarat, India.

Patel, P.P., Panchal, M.T., Kalyani, I.H. and Kavani, F.S. 2009. Antibiotic sensitivity spectrum of bacterial isolates from cervico-vaginal mucus of postpartum rural buffaloes. *Intas Polivet*, 10(1), 29-31.

Petit, T., Spergser, J., Rosengarten, R. and Aurich, J. 2009. Prevalence of potentially pathogenic bacteria as genital pathogens in dairy cattle. *Reprod. Dom. Anim*., 44, 88-91.

Raval, S.R., Panchal, M.T., Dhami, A.J., Hadiya, K.K. and Bhanderi, B.B. 2018. Studies on bacterial isolates and their antibiogram from genital discharges of puerperal and repeat breeding crossbred cows. *Indian J. Anim. Reprod*., 39(2), 58-60.

Sheldon, I., Noakes, D. and Rycroft, A. 2002. The vagina on uterine bacterial contamination. *Vet. Rec.*, 151, 531-534.

Udhayavel, S., Malmarugan, S., Palanisamy, K. and Rajeswar, J. 2013. Antibiogram pattern of bacteria causing endometritis in cows, *Vet. World*, 6(2), 100-102.

Vlček, Z, and Svobodová, R. 1985. Occurrence and antibiotic sensitivity of bacteria present in the cervical mucus of cows in late puerperium and postpuerpertal period. *Acta Vet. Brno*, 54(1-2), 91-97.

Wagener, K., Prunner, I., Pothmann, H., Drillich, M. and Ehling-Schulz, M. 2015. Diversity and health status specific fluctuations of intrauterine microbial communities in postpartum dairy cows. *Vet. Microbiol.*, 175(2-4), 286-293.

Zambrano-Nava, S., Boscán-Ocando, J. and Nava, J. 2011. Normal bacterial flora from vagina of Criollo Limonero cows. *Trop. Anim. Health Prod.*, 43(2), 291-294.

How to cite this article:

Patel, C.I., M.T. Panchal, A.J. Dhami, B.B. Bhanderi and Mathakiya, R.A. 2019. Isolation of Bacteria from the Vaginal Aspirates of Cyclic, Acyclic, Endometritic and Pregnant Crossbred Cows. *Int.J.Curr.Microbiol.App.Sci.* 8(03): 536-542.
doi: https://doi.org/10.20546/ijcmas.2019.803.065