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Incidence of bovine endometritis and antibiogram of isolated pathogens in West Tripura District, Tripura

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Abstract
The goal of this study were to investigate the incidence of endometritis in West Tripura district, Tripura and to determine the prevalence of bacteria and antibiotic sensitivity profile in endometritic crossbred cows. Repeat breeder cows were examined for gynaecological findings, uterine cytology and positivity to white side test to identify them as endometritic cows. Cervico vaginal mucus were examined for bacterial isolation and identification followed by antibiotic sensitivity test of isolated pathogens. The incidence of endometritis in repeat breeder crossbred cows was 38.7%. The most common pathogen isolates recovered were *E. coli* (37.5%) followed by non-lactose fermenting organism under the family Enterobacteriaceae (27.5%), *Staphylococcus aureus* (22.5%), *Streptococcus* spp. (7.5%), and *Bacillus* spp. (5%). Ciprofloxacin, amikacin and gentamicin were most sensitive antibiotics against micro-organisms causing endometritis.

Keywords: Repeat breeder, incidence, endometritis, antibiogram

1. Introduction
Endometritis is defined as an inflammation of the endometrium with the underlying glandular tissues of the uterus without any systemic signs (Udhayavel *et al*., 2013) [33].The incidence of bovine endometritis is varied from 3.4% to 40% (Gilbert *et al*., 2005) [10]. Common bacterial species associated with uterine diseases are *Escherichia coli*, *Trueperella pyogenes*, Fusobacterium spp. and Prevotella spp. (Carneiro *et al*., 2016 [7]). Factors associated with alteration of normal uterine involution after parturition are related to occurrence of endometritis (Osawa, 2021) [20]. Cervico vaginal mucus (CVM) is a potent source for bacterial investigation related to uterine infection (Adnane *et al*., 2018) [2]. Different antibiotics and hormone are used for the treatment of endometritis (*Kumar et al*., 2019) [17]. It is important to determine the causative agents and their sensitivity profile to antimicrobial drug for effective treatment of endometritis. Therefore, the objectives of present study were to evaluate the incidence of bovine endometritis and to determine the prevalence of bacteria and antibiotic sensitivity profile in endometritic crossbred cows.

2. Materials and Methods
The study was conducted between January, 2023 and December, 2023 in the Department of Livestock Farm Complex and Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Tripura, India. Repeat breeder crossbred cows (>90 days postpartum) were examined for gynaecological findings, uterine cytology and positivity to white side test to identify them as endometritic cows. CVM sample was collected as method described by Savia *et al*. (2021) [26] and immediately brought to the laboratory for further study.

2.1. pH: The pH of CVM was measured using pH indicator strips as method described by Tsiligianni *et al*., (2001) [32].

2.2. White Side Test (WST): WST was conducted as method described by Bhat *et al*. (2015) [6]. 1 ml of CVM was mixed with 1 ml of 5% sodium hydroxide and heated upto boiling point. The changes of colour after cooling were studied and graded as turbid or no colour (normal), light yellow colour (mild infection), yellow colour (moderate infection) and dark yellow colour (severe infection) (Figure 1).

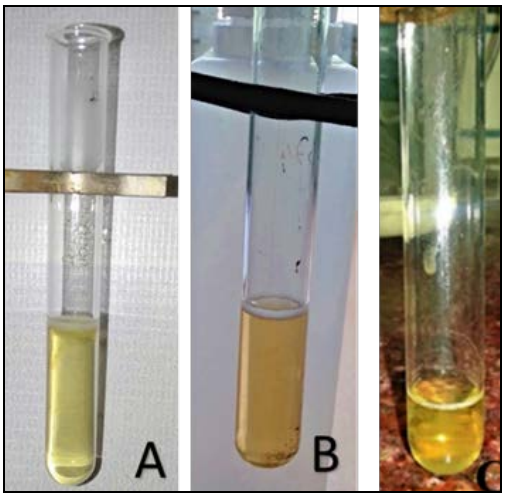

Fig 1: Intensity of colour change in white side test (A-Mild infection, B-Moderate infection, C-Severe infection)

2.3. Polymorphonuclear (PMN) cell count (%): Low volume flush technique was used to collect uterine flush (Salah *et al*., 2017) [23]. 10 ml of uterine flushing was centrifuged at 3000 rpm for 15 minutes. Cytological smears were prepared from sediment of uterine flushing on slide. The smears were fixed with methanol for 2 min and stained with giemsa stain for 25-30 min. The stained smears were observed under oil immersion at 100 x magnification, 200 cells were counted for per cent of PMN cells (Figure 2).

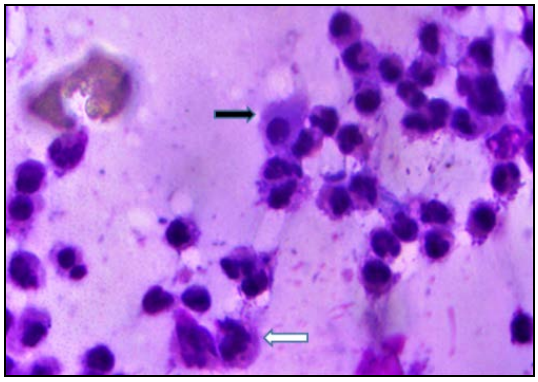

Fig 2: Endometrial cytology showing the presence of PMN cells indicated by white arrow marks and epithelial cells indicated by black arrow mark

2.4. Isolation and identification of microorganism: The CVM sample were inoculated on nutrient agar, blood agar, MacConkey agar and Mannitol salt agar. The media were incubated at 37 °C for 24 h and each distinguishable colony was transferred on to fresh nutrient agar plate for purification after gram staining. Purity of each culture was tested and used for biochemical and other characterization (Figure 3 & 4). Catalase test, oxidase tests, coagulase test, sugar utilization test, indole test, Methyl Red-Voges-Proskauer (MR-VP) test, citrate utilization test, triple sugar iron test were performed for biochemical characterization as per the procedure of Quinn *et al*. (1994) [22].

Fig 3: a) Mixed bacterial growth on nutrient agar b) Mixed bacterial growth on blood agar c) Mixed growth of *E. coli* and NLF on MacConkey agar d) Growth of *S. aureus* on Manitol salt agar (yellow colonies) e) Growth of *E. coli* (green metallic sheen) on eosin metheylne blue agar

2.5 Antibiotic Sensitivity test: Commonly used antibiotic for the treatment of endometritis in Tripura were chosen for antibiotic sensitivity test as method described by Bauer *et al*. (1966) [4]. Ciprofloxacin (5µg), ceftriaxone (30 µg), tetracycline (30 µg), amikacin (10 µg), gentamicin (10 µg), ofloxacin (5 µg) and levofloxacin (5 µg) antibiotic disc (Hi-Media Pvt. Ltd., India) were used. Organism from pure culture was inoculated in nutrient agar broth and incubated at 37^0C for 24 h and spread plated in Muller Hinton Agar (MHA) plate. Then antibiotic discs were placed on the MHA plate followed by incubation at 37^0C for 24 h. Sensitivity of antibiotics were evaluated on the basis of diameter of zone of inhibition (Figure 5).

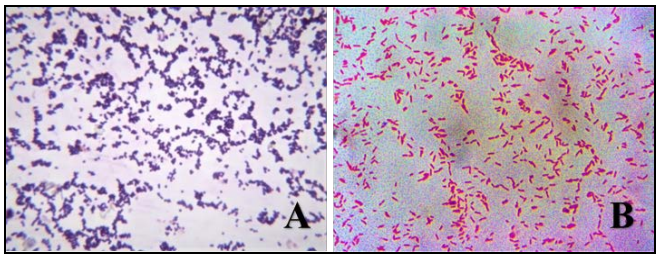

Fig 4: Gram's staining (A) Gram positive cocci (B) Gram negative rods

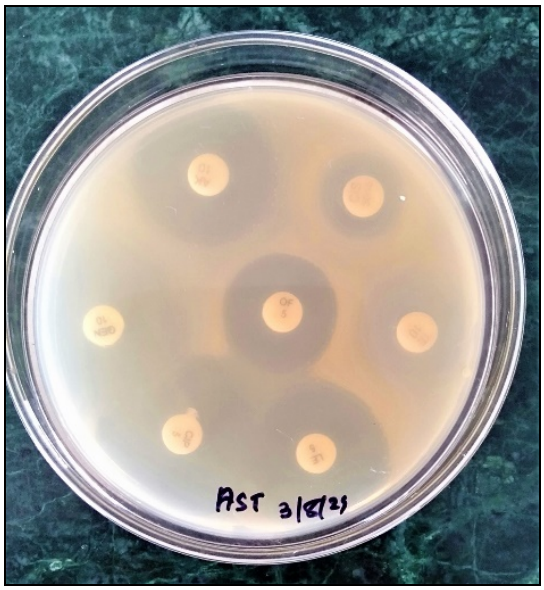

Fig 5: Antibiotic sensitivity test of isolates

3. Result and Discussion

3.1. Incidence: A total of 62 numbers of repeat breeder cows were examined during the study period and 24 cows were

diagnosed as having endometritis. Thus, the occurrence of repeat breeding due to endometritis was 38.7% in West Tripura district, Tripura. This finding was in conformity with Janowski *et al*. (2013) [13] who found 40.2% subclinical endometritis using the threshold of 10% PMN cell, whereas Bedewy and Rahawy (2019) [5] observed 16.66% subclinical endometritis in repeat breeding cows. Salasel *et al*. (2010) [24] reported 53% using 3% threshold of PMN cell for the diagnosis.

3.2. Diagnosis: The observations on the nature of the CVM revealed that all the endometritis affected cows had abnormal uterine discharges of cloudy mucus (29.16 %), mucus containing pus flakes (33.33%) and mucopurulent (37.5%) which is in close proximity reported by Hussein and Hussein (2022) [12]. Based on the result of white side test, present study showed variable grade of severity of endometritis viz. 16.66% mild infection, 37.5% moderate infection and 45.83% severe infection. This findings in the present investigation are close to the findings of Sarkar *et al*. (2018) [25]; Singh *et al*. (2017) [29] and Kumar *et al*. (2013) [16]. The value (Mean + SE) of pH and PMN cell count (%) of endometritic cows were 8.22±0.062 and 10.5±0.64 respectively. The result of the current study in mean pH of CVM was according to the findings of Krishnan *et al*. (2015) [15]. Hamana *et al*., 1976) [11] revealed normal pH of CVM varied from 6.0 to 7.8. The value of PMN cell (%) was in conformity with Mali *et al*. (2020) [18] who reported that the mean uterine cytology (PMN cell %) in normal cyclical cows was varied from 0.7±0.26 to 1.4±0.30 and with uterine infection was varied from 7.4±0.68 to 9.5±0.54.

3.3. Bacterial isolation: *E. coli* (37.5%) were most common isolates followed by non-lactose fermenting organism under family Enterobacteriaceae (27.5%), *Staphylococcus aureus* (22.5%), Streptococcus spp. (7.5%) and Bacillus spp. (5%) (Table 1). Mixed infection were detected in 70.83% cases and single isolates in 29.17% cases. The results are in corroboration with the different finding which showed *E. coli* as the most common pathogen isolated by Udhayavel *et al*., 2013[33] (36.66%), Takamtha *et al*., 2013[30] (24.07%) and Abreham *et al*., 2017[1] (42.1%). On the contrary, Barman *et al*. (2013)[3] revealed non-lactose fermenting organisms belonging to family Enterobacteriaceae were the most common isolates (25%) followed by *E. coli* (20%) and other bacterial isolates. Deori *et al*. (2004)[9] reported 75% cases of mixed infections with *E. coli* (22%) was the most common isolate followed by *Staphylococcus aureus* (20%); meanwhile, Manjhi *et al*. (2019) [19] revealed Staphylococcus spp. as the predominant pathogen.

Table 1: Bacterial isolates and antibiotic sensitivity tests for different isolates

Bacteria isolates	No of isolates (%)	Antimicrobial agents tested						
		CIP	TE	AK	Gen	OF	LE	CF
		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Staphylococcus aureus	9 (22.5)	8 (88.9)	4 (44.4)	8 (88.9)	5 (55.5)	9 (100)	9 (100)	5 (55.5)
Streptococcus spp.	3 (7.5)	3 (100)	1 (33.3)	3 (100)	1(33.3)	3 (100)	3(100)	1(33.3)
Bacillus spp.	2 (5)	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	2 (100)	1 (50)
E. coli	15 (37.5)	15 (100)	4 (26.7)	15(100)	15(100)	6 (40)	7 (46.7)	2 (13.3)
NLFs under family Enterobacteriaceae	11 (27.5)	11 (100)	3 (27.3)	10 (90.9)	11 (100)	5 (45.4)	6 (54.5)	3 (27.3)
Total	40 (100)	38 (95)	13 (32.5)	37 (92.5)	33 (82.5)	25 (62.5)	27(67.5)	12 (30)

NLF- Non-Lactose-Fermenter, CIP-Ciprofloxacin, TE-Tetracycline, AK-Amikacin, Gen-Gentamicin, OF-Ofloxacin, LE-Levofloxacin, CF-Ceftriaxone

3.4. Antibiotic sensitivity test: Antibiogram revealed that ciprofloxacin (95%), amikacin (92.5%) and gentamicin (82.5%) were the most effective drugs against different bacterial isolates. The sensitivity for tetracycline, ofloxacin, levofloxacin and ceftriaxone were 32.5%, 62.5%, 67.5% and 30% respectively (Table 1). These findings are very much in agreement with the finding of various earlier report (Abreham *et al*., 2017 [1]; Pandey *et al*., 2018 [21]; Khalil *et al*. 2022 [14]). In our study ceftriaxone showed sensitivity in minimum number (30%) of isolates. In contrary, high sensitivity to ceftriaxone were reported by Udhayavel *et al*. (2013) [33] and Manjhi *et al*. (2019) [19]. Chacko *et al*. (2020) [8] revealed highest sensitivity for ciprofloxacin (88.31%) and least sensitivity with ceftriaxone (25.97%) and cephalexin (19.48%) which support our present study. In the present investigation, it was found that *Staphylococcus aureus* was found to be 100% sensitive to levofloxacin and ofloxacin followed by amikacin (88.9%) and ciprofloxacin (88.9%), ceftriaxone (55.5%), gentamicin (55.5%) and tetracyclin (44.4%). Streptococci spp. was 100% effective to ciprofloxacin, amikacin, levofloxacin and ofloxacin and Bacillus spp. was found to be 100 % sensitive to levofloxacin and ofloxacin followed by others as shown in table 1. Sharma *et al*. (2017) [27] reported that *S. Aureu*, Streptococci spp. and *B. cereus*, were sensitive to levofloxacin (94.08%), ofloxacin (50.83%), and ciprofloxacin (53.33%). In the present study, *E. coli* was 100 % sensitive to ciprofloxacin, amikacin and gentamicin followed by levofloxacin (46.7%), ofloxacin (40%), tetracyclin (26.7%) and ceftriaxone (13.3%). NLFs organism isolates were 100% effective to ciprofloxacin and gentamicin followed by amikacin (90.9%), levofloxacin (54.5%), ofloxacin (45.4%), tetracyclin (27.3%) and ceftriaxone (27.3%). Chacko *et al*. (2020) [8] reported high sensitivity of *E. coli* to ciprofloxacin and gentamicin. Triadi *et al*. (2022) [31] also reported high sensitivity to ciprofloxacin (100%) and gentamicin (93.33%). Abreham *et al*. (2017) [1] revealed that gram negative bacteria isolated from uteri of slaughtered cows were sensitive to amikacin (100%). The variation in these results might be due to difference in the sensitivity pattern of bacterial isolates. These antibiotics are commonly used for uterine infection in Tripura, particularly during postpartum period as intra uterine therapy. To the authors' best knowledge, this is the first attempts to investigate the incidence and antibiogram of endometritic cow in Tripura. Less susceptibility of bacteria to a particular antibiotic might be due to the prolonged exposure to the same drug. Hence, a definite regime of administration of antibiotics should be adapted for the control of endometritis.

4. Conclusion

The study has demonstrated high prevalence (38.7%) of endometritis in repeat breeding cows in West Tripura district, Tripura. *E. coli*, non-lactose fermenting organisms under the family Enterobacteriaceae and *Staphylococcus aureus* were

the major etiological agent and ciprofloxacin, amikacin and gentamicin were found to be most sensitive antibiotics against microorganisms causing endometritis.

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6. References

1. Abreham S, Hailu M, Worku A, Tsegaye S. Antibiotic resistance of aerobic bacteria isolated from uteri of slaughtered cows in and around Addis Ababa, Ethiopia. Journal of Veterinary Science & Technology. 2017;8(1):406. DOI: 10.4172/2157-7579.1000406.
2. Adnane M, Meade KG, Farrelly CO. Cervico-vaginal mucus (CVM): An accessible source of immunologically informative biomolecules. Veterinary Research Communications. 2018;42:255-263.
3. Barman P, Yadav MC, Bangthai A, Kumar H. Antibiogram of bacteria isolated from bovine endometritis. Veterinary Research International. 2013;1(1):20-24.
4. Bauer AW, Kirby WMM, Sherries JC, Turck M. Antibiotic Susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1966;45:493-496.
5. Bedewy RB, Rahawy MA. Comparative study for detection of subclinical endometritis in local cows. Advances in Animal and Veterinary Sciences. 2019;7(4):289-294.
6. Bhat FA, Bhattacharyya HK, Fazili MR, Hussain SA, Khan MZ. Studies on estrual cervical mucus of repeat breeding cows with special reference to ovulatory disturbances and genital infection. Theriogenology Insight. 2015;5(2):113-123.
7. Carneiro LC, Cronin JG, Sheldon IM. Mechanisms linking bacterial infections of the bovine endometrium to disease and infertility. Reproductive Biology. 2016;16:1-7.
8. Chacko L, Promod K, Ravishankar C, Harshan HM, Abdul Azeez CP, John Martin KD. Uterine bacterial isolates of early postpartum endometritis and its antibiogram. International Journal of Current Microbiology and Applied Sciences. 2020;9(8):1590-1600.
9. Deori S, Kumar H, Yadav MC, Rawat M, Srivastava SK. Intrauterine administration of bacterial modulins: An alternative therapy for endometritis. Journal of Applied Animal Research. 2004;26(2):117-121.
10. Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 2005;64(9):1879-1888.
11. Hamana K, Oyama T, Kato T, Kano Y. The pH of the bovine cervical mucus at estrus. The Japanese Journal of Animal Reproduction. 1976;21(4):123-129.
12. Hussein EK, Hussein KA. Assess the efficiency of silver nanoparticles for treatment of endometritis in Iraqi breed cows. Egyptian Journal of Veterinary Sciences. 2022;53(2):263-271.
13. Janowski T, Baranski W, Lukasik K, Skarzynski D, Rudowska M, Zdunczyk S. Prevalence of subclinical endometritis in repeat breeding cows and mRNA expression of tumor necrosis factor α and inducible nitric oxide synthase in the endometrium of repeat breeding cows with and without subclinical endometritis. Polish Journal of Veterinary Sciences. 2013;16(4):693-699.
14. Khalil FS, Laila E, Kortam LE, Aref NE. Some bacteriological and immunological studies of endometritis in mares before and after alternative treatments using platelets rich plasma (and\or) antibiotics. Assiut Veterinary Medical Journal. 2022;68(173):16-30.
15. Krishnan BB, Kumar H, Mehrotra S, Singh SK. Effect of leukotriene B4 and oyster glycogen in resolving subclinical endometritis in repeat breeding crossbred cows. Indian Journal of Animal Research. 2015;49(2):218-222.
16. Kumar P, Purohit GN, Mehta JS. Diagnosis of endometritis in cows using metricheck, uterine cytology and ultrasonography and efficacy of different treatments. Veterinary Practitioner. 2013;14(2):351-354.
17. Kumar Satish D, Purohit GN. A discussion on risk factors, therapeutic approach of endometritis and metritis in cattle. International Journal of Current Microbiology and Applied Sciences. 2019;8(5):403-421.
18. Mali SS, Rangnekar MN, Amle MB, Khillare KP, Mal AB, Mhase PP. Efficacy of intrauterine ozone therapy in repeat breeder cows with subclinical uterine infection. Haryana Vet. 2020;59:83-86.
19. Manjhi VK, Bajaj NK, Shukla SP, Shrivastava N, Kaurav PS, Thakur RK. Bacterial isolation and antibiogram of uterine lavage from repeat breeder cows suffering from subclinical endometritis. Journal of Animal Research. 2019;9(2):293-296.
20. Osawa T. Predisposing factors, diagnostic and therapeutic aspects of persistent endometritis in postpartum cow. The Journal of Reproduction and Development. 2021;67(5):291-299.
21. Pandey NKJ, Gupta HP, Prasad S, Sheetal SK. Therapeutic management of repeat breeding due to sub-clinical endometritis in crossbred cows. International Journal of Science, Environment and Technology. 2018;7(6):1868-1874.
22. Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology. Edn 1, Mosby Publishing Company, London; c1994.
23. Salah N, Yimer N, Wahid H, Rosnina Y, Khumran A, Mahdi E, *et al*. Assessment of three different endometrial cytological sampling methods in postpartum beef cows. The Journal of Animal & Plant Sciences. 2017;27(5):1515-1521.
24. Salasel B, Mokhtari A, Taktaz T. Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. Theriogenology. 2010;74:1271-1278.
25. Sarkar P, Kumar H, Patra MK, Mandal D. Effect of administration of autologous plasma along with leucocytes on hormonal changes in relation to recovery rate and conception in endometritic cows. Journal of Animal Research. 2018;8(6):1099-1103.
26. Savia CL, Osorio JS, Rodriguez JV, Guibert EE, Rinaudo A. A simple and reliable refractometric method to determine the total solids concentration of the cervico-vaginal bovine mucus samples. Heliyon; c2021. p. 7. https://doi.org/10.1016/j.heliyon.
27. Sharma A, Singh M, Kumar P, Sharma A, Kashyap A, Bala NI, *et al*. Bacterial isolation, culture sensitivity test, endometrial cytology of postpartum cows and assessment of their reproductive performance. International Journal

of Current Microbiology and Applied Sciences. 2017;6(9):519-527.
28. Sheldon IM, Lewis GS, Leblan S, Gilbert RO. Defining postpartum uterine disease in cattle. Theriogenology. 2006;65:1516-1530.
29. Singh PP, Pande N, Bhavna, Agrawal R. Clinical and biochemical studies on endometritic repeat breeding cows following treatment with levamisole. Haryana Vet. 2017;56(1):55-57.
30. Takamtha A, Phanaratkitti V, Adirekkiet O, Panyapornwitaya V, Boonyayatra S, Kraeusukol K. Prevalence of isolated bacteria from clinical endometritis uterine and antimicrobial susceptibility in postpartum dairy cows. Chiang Mai Vet. J. 2013;11(3):237-245.
31. Triadi B, Suwarno S, Damayanti R, Estoepangesti ATS, Sugihartuti R, Sarudji S. Antibiotic sensitivity test of *Escherichia coli* and *Staphylococcus aureus* isolated from the reproductive tract of dairy cows. Journal of Animal Reproduction. 2022;11:72-80.
32. Tsiligianni T, Karagiannidis A, Brikas P, Saratsis P. Chemical properties of bovine cervical mucus during normal estrus induced by progesterone and/or PGF2α. Theriogenology. 2001;56(1):41-50.
33. Udhayavel S, Malmarugan S, Palanisamy K, Rajeswar J. Antibiogram pattern of bacteria causing endometritis in cows. Vet. World. 2013;6(2):100-102.