Indian J Anim Health (2023), 62(2): 378-382
DOI: https://doi.org/10.36062/ijah.2023.17322

Research Article

Hematological effect of antibiotic therapy in endometritis affected cows

K. Kumar[1*], K. Ray[1], A. K. Jha[2] and R. K. Yadav[3]

[1]Department of Veterinary Gynaecology and Obstetrics, West Bengal University of Animal and Fishery Sciences, Kolkata- 700 037, West Bengal, India; [2]National Avian Research Programme, Parwanipur, Bara, Nepal; [3]Department of Pharmacology, Institute of Agriculture and Animal Science, Paklihawa, TU, Nepal

Abstract

A total of 12 nos. of endometritis affected cows were selected and randomly allotted to two groups (A and B) containing six animals in each group and given piperacillin and tazobactum for 5 days. Estrus cervical mucous and blood were collected before and after antibiotic treatment. Estrus cervical mucous was collected for bacterial load, pH, white side test, antibiotic sensitive test, and blood was collected for estimation HB, PCV, TEC, TLC, lymphocyte and neutrophil estimation. The pH of cervical mucous before treatment was alkaline (more than 7.90) in each group. After treatment with antibiotic, we observed a significant reduction of pH in the treatment group, which ranged between 7.10 to 7.90. HB, PCV, TEC and lymphocyte were significantly increased, whereas the TLC and neutrophil were significantly decreased in the treatment group.

Keywords: Blood, Cervical mucous, Piperacillin, Tazobactum

Highlights

- Seven antibiotics used for zone inhibition test viz; ceftriaxone (30 mcg), gentamicin (10 mcg), levofloxacin (5 mcg), moxifloxacin (5 mcg), penicillin-G (10 mcg), piperacillin/tazobactum (100/10 mcg) and tetracycline (30 mcg).
- Blood parameters of endometritic cattle altered from healthy cattle.
- In endometritic cattle, viscous off-white purulent uterine discharge was observed.
- Endometritis is one of the major gynaecological problems affecting the reproductive efficacy and economics of milk production in dairy animals.
- Most sensitive antibiotic was selected on the basis of size of zone of inhibition test with the help of standard disc diffusion technique.

INTRODUCTION

Endometritis is defined as the inflammation of the endometrial lining of the uterus characterized by mucopurulent or purulent vaginal discharge (Baranski *et al*., 2022). Postpartum endometritis in dairy cows has been defined as inflammation of endometrium occurring 21 days or more after parturition with systemic signs of illness and viscous off-white purulent uterine discharge (Sheldon *et al.*, 2006). Signs of systemic illness include decreased milk yield, toxemia, elevated heart rate and dehydration (Sheldon *et al.*, 2008). Uterine infections such as endometritis, metritis, mucometra and pyometra are common reproductive problems in cattle. Among these, endometritis is one of the major gynaecological problems affecting the reproductive efficacy and economics of milk production in dairy animals (Kumar *et al.*, 2022). Uterine diseases such as metritis and endometritis are common disorders of dairy cows that negatively affect reproductive performance (Gilbert *et al.*, 2005). Metritis is one of the most important postpartum diseases in dairy cows (McLaughlin *et al.*, 2013). Most common bacteria present to be found during uterine infection are *Escherichia coli* or *Trueperella pyogenes* in the postpartum uterus. (Miller *et al*., 2007; Santos *et al*., 2011; Silva *et al*., 2023). Factors responsible for endometritis are hygiene of the perineum at the time of calving (Schuenemann *et al.*, 2011), peripartum metabolic status (LeBlanc, 2008; Konyves *et al.*, 2009), parity (Timran *et al.*, 2021), retention of placenta (LeBlanc *et al.*, 2002; Sheldon *et al.*, 2009), twins and dystocia (Adnane and Chapwanya, 2022). Postpartum reproductive disorders cause heavy economic losses in dairy sector. Incidence of clinical and subclinical endometritis in crossbred cows was 12% and 29.69%, respectively (Kumar *et al.*, 2018). Presently, treatment of such infertility is mainly achieved using intrauterine antibiotics, antiseptics and less commonly by hormones

*Corresponding Author, E-mail: keshavkumar14691@gmail.com

(Mahour *et al.*, 2020). Antibiotics such as penicillin, ampicillin and cloxacillin are given by the intrauterine route for the treatment of metritis (Malinowski *et al.*, 2011; Nak *et al.*, 2011). The main objective of this experiment was to analyse the effect of antibiotic therapy in endometritis affected cows on different constituents of blood.

MATERIALS AND METHODS

A total of 12 animals were selected on the basis of history and breeding records, per-rectal examination, pH of estrual mucus and white side test of cervical mucus. The animals were divided into two groups with six animals in each group, viz A and B. In group A, 30 mL normal saline was introduced via intrauterine for first two days, followed by intravaginal once daily for consecutive three days. In group B, 30 mL piperacillin and tazobactam (Cipla Ltd.) was introduced intrauterine for first two days and intravaginal for consecutive three days once daily. All the groups were treated intrauterine for first two days, followed by intravaginal lavage for next three days due to cervical closing post estrus. Estrus cervical mucous and blood were collected before and after treatment (At subsequent estrus). Estrus cervical mucous was collected for pH estimation, white side test, antibiotic sensitive test, and blood was collected for HB, PCV, TEC and TLC estimation. The generated data were analyzed using paired sample t-test.

White side test: Cervical mucus was mixed with equal volume of 5% sodium hydroxide (NaOH) solution in a test tube and boiled over a spirit lamp for few minutes. The appearance of light yellow or dark yellow colour was taken as a positive indication of subclinical endometritis (Fig. 1A). Samples showing no colour change after boiling were considered as negative, i.e., free of endometritis.

Selection of the most sensitive antibiotic: Seven standard antibiotic discs were subjected to antibacterial activity of mixed culture population obtained from 12 cervical mucus samples. Ceftriaxone (30 mcg), gentamicin (10 mcg), levofloxacin (5 mcg), moxifloxacin (5 mcg), penicillin-G (10 mcg), piperacillin/tazobactum (100/10 mcg) and tetracycline (30 mcg) were used for the selection of most sensitive one by standard disc (Hi Media Laboratories Ltd., Mumbai) diffusion technique (Fig. 1B). Broth prepared from cervical mucus samples of endometritic crossbred cows was used. Sterile swab containing bacteria was swabbed over the surface of Mueller-Hinton Agar plates under laminar airflow. Inoculated plates were dried in laminar air flow for 10 minutes. Antibiotic discs were placed over the inoculated plates at equal distances. All the plates were incubated at 37°C for 24 hours to observe and measure the zone of inhibition around the discs and on the basis of the size of the zone of inhibition. Most sensitive antibiotic was selected for the treatment group.

Specific bacteria identified in the uterus of dairy cattle with endometritis include *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, *Prevotella* spp., *Bacteroides* spp., *Fusobacterium nucleatum* and *Proteus mirabilis* (Jeon and Galvao, 2018).

Treatment protocol: A total of 12 screened out endometritic crossbred cows were then randomly allotted to two groups containing six animals in each group, and

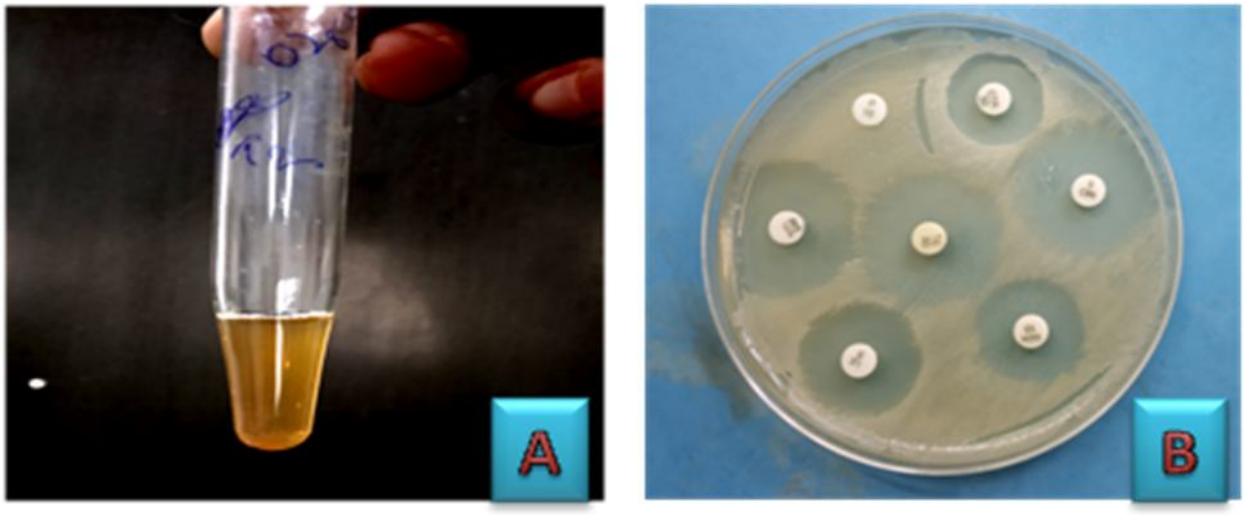

Fig. 1. A. Conducted of white side test and B. Selection of antibiotic on antibiotic sensitive test for endometritic cattle

antibiotics were given as per the following schedule.
Group A (Control): - Normal saline (NS) @ 30 mL IU/ animal once daily
Group B (Antibiotic): - Piperacillin+Tazobactum (Cipla Ltd) @ 30 mL IU/animal once daily

RESULTS

The efficacy of treatment by piperacillin and tazobactum was evaluated and found to be significantly better on recovery rate shown in Table 1.

The mean Hb values of endometritic repeat breeding crossbred cattle of all the two groups at pre- and post-treatment have been presented in Table 1. The mean haemoglobin values of animals in groups A and B before the treatment were 8.65±0.21 and 8.64±0.17, respectively. After treatment, the corresponding values recorded were 8.56±0.18 and 12.39±0.14, respectively. Haemoglobin (gm/dL) value at post-treatment subsequent estrus in treatment group B was significantly increased, whereas, in control group A, the difference between pre- and post-treatment haemoglobin value was non-significant. Haemoglobin value was found to be increase after treatment with antibiotic in endometritic repeat breeding cows.

The mean PCV values of group A and group B before the treatment were 26.91±0.18 and 27.83±0.33, whereas, after treatment, the corresponding values were 27.08±0.08 and 33.59±0.25. PCV values recorded in the present study showed a significant increase in the treatment group. But, in control group A, the difference between pre- and post-treatment values was non-significant.

The mean TEC in the blood of endometritic repeat breeding crossbred cows of all the two groups at pre- and post-treatment have been presented in Table 1. Before treatment, the mean TEC in groups A and B were 3.81±0.05 and 3.80±0.05, respectively. After treatment, the mean TEC recorded were 3.96±0.05 and 5.65±0.11. TEC value significantly increased from pre- to post-treatment in treatment group B except in control group A.

The mean TLC in the blood of endometritic repeat breeding crossbred cows at pre- and post-treatment in all the two groups have been presented in Table 1. Before treatment, the mean TLC in groups A and B were 9.58±0.09 and 9.67±0.03, respectively. After treatment, the mean TLC recorded were 9.30±0.05 and 7.39±0.04, respectively in group A and group B. TLC value in the blood was observed to be decreased post-treatment with antibiotic in treatment group B. After treatment, the mean TLC values in the treatment group were significantly low as compared to the control group, indicating recovery of the treated cows.

The mean percent neutrophils in the blood of endometritic repeat breeding crossbred cows at pre- and post-treatment in all the two groups have been depicted in Table 1. The mean percent neutrophils before treatment were recorded as 58.75±0.24 and 59.66±0.07 in groups A and B, respectively. After treatment, the mean percent neutrophils count recorded were 57.58±0.30 and 35.69±0.37 in groups A and B, respectively.

The mean lymphocyte percent counts in the blood of repeat breeding endometritic cows before treatment were 36.41±0.33 and 35.67±0.15 in group A and B, respectively. After treatment, the mean percent lymphocyte counts were 37.91±0.10 and 60.33±0.14 in respective groups.

DISCUSSION

The present finding regarding maximum reduction in bacterial load after piperacillin and tazobactum treatment (Table 1) was in accordance with the finding of Bhat *et al.* (2013), who also reported that piperacillin and tazobactum was the most effective antimicrobial drug against most of the bacteria found in cervical mucus of endometritic repeat breeding cows. The present finding of mean Hb, PCV and TEC values (Table 1) are

Table 1. Haematological parameters of endometritic cattle before and after treatment

Parameters	Group A (N=6)		Group B (N=6)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Bacterial load	225^{Aa}±5.92	216^{Ac}±3.18	213^{Aa}±1.74	1.56^{Bd}±0.10
Haemoglobin	8.65^{Aa}±0.21	8.56^{Ab}±0.18	8.64^{Aa}±0.17	12.39^{Bc}±0.14
PCV	26.91^{Aa}±0.18	27.08^{Ac}±0.08	27.83^{Ab}±0.33	33.59^{Bd}±0.25
TEC	3.81^{Aa}±0.05	3.96^{Ac}±0.05	3.80^{Aa}±0.05	5.65^{Bd}±0.11
TLC	9.58^{Aa}±0.09	9.30^{Bc}±0.05	9.67^{Aa}±0.03	7.39^{Bd}±0.04
Neutrophil	58.75^{Aa}±0.24	57.58^{Ac}±030	59.66^{Ab}±0.07	35.69^{Bd}±0.37
Lymphocyte	36.41^{Aa}±0.33	37.91^{Bc}±0.10	35.67^{Aa}±0.15	60.33^{Bd}±0.14

Means bearing different superscripts vary significantly (P<0.05) within the groups (A, B) and between the groups (a, b, c, d)

in agreement with the findings of Kekan *et al.* (2005), Sarkar *et al.* (2016) and Yogesh *et al.* (2022). The neutrophilia in the present study at pre-treatment might be due to chronic walled of inflammatory lesions. A significant decrease ($P \leq 0.05$) in the mean neutrophil count was recorded in the treatment group. Kekan *et al.* (2005) and Dash *et al.* (2019) also found neutrophilia in endometritic cows. Decreased lymphocyte observed in the present study might be a relatively reduced lymphocyte developed as a result of increased neutrophil and/or due to extravasation of lymphocyte from the circulation to the site of inflammation or infection (Benjamin, 1985). Lymphopenia was observed at pre-treatment in all the groups. Similar mean lymphocyte percent count in infections repeat breeding cow was reported by Ponraj *et al.* (2017).

After using piperacillin and tazobactum Hb, PCV, TEC and lymphocyte were significantly increased in the treatment group, whereas we observed that the TLC and neutrophil were significantly decreased in the treatment group.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: KK, AKJ: Involved in the investigation, data generation, preparing original draft; KR: Engaged in conceptualization, data corrections, supervision and final editing; RKY: Involved in statistical analyses.

ACKNOWLEDGEMENT

The authors would like to acknowledge all the technicians of WBUAFS for their support and cooperation.

REFERENCES

Adnane M and Chapwanya A, 2022. Role of genital tract bacteria in promoting endometrial health in cattle. Microorganisms, 10: 2238, doi: 10.3390/microorganisms10112238

Baranski W, Baryczka A, Zdunczyk S, Tobolski D and Janowski T, 2022. Prevalence of subclinical endometritis in dairy cows that recovered after treatment of clinical endometritis with cephapirin and PGF2α. Theriogenology, 192: 166-171, doi: 10.1016/j.theriogenology.2022.08.031

Benjamin MM, 1985. Outline of Veterinary Clinical Pathology, 3rd edn. Kalyani Publishers, New Delhi, pp 44,79-92,127-129,145-146, ISBN: 9788170966432, 8170966434

Bhat FA, Bhattacharya HK, Hussain A, Nadeem M and Wani AR, 2013 Microbial profile, antibiogram and conception rate following treatment in repeat breeder cows. Intas Polivet, 14(1): 42-48

Dash S, Basu S, Mishra PC and Ray K, 2019. Effect of immunomodulators on certain haematological parameters in ameliorating bovine endometritis. Int J Chem Stud, 7(2): 1736-1739

Gilbert RO, Shin ST, Guard CL, Erb HN and Frajblat M, 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology, 64(6): 1879-1888, doi: 10.1016/j.theriogenology.2005.04.022

Jeon SJ and Galvao KN, 2018. An advanced understanding of uterine microbial ecology associated with metritis in dairy cows. Genomics Inform, 16(4): e21, doi: 10.5808/GI.2018.16.4.e21

Kekan PM, Shirbhate R and Nimbukar MV, 2005. Haematological studies during oestrous cycle in regular and repeat breeding cows. Indian Vet J, 82: 805-806

Konyves L, Szenci O, Jurkovich V, Tegzes L, Tirián A *et al.*, 2009. Risk assessment of postpartum uterine disease and consequences of puerperal metritis for subsequent metabolic status, reproduction and milk yield in dairy cows. Acta Vet Hung, 57(1): 155-169, doi: 10.1556/AVet.57.2009.1.16

Kumar A, Gupta HP and Prasad S, 2018. Studies on the immunomodulatory and therapeutic efficacy of turmeric (*Curcuma longa*) on endometritis in repeat breeding crossbred cows. Int J Agric Sci, 10(2): 5069-5072

Kumar K, Ray K, Mandal D, Jha AK and Sarkar P, 2022. Haematological effect of garlic and turmeric in endometritic cattle. Pharm Innov J, 11(7): 1636-1639

LeBlanc SJ, 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. Vet J, 176(1): 102-114, doi: 10.1016/j.tvjl.2007.12.019

LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP *et al.*, 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J Dairy Sci, 85(9): 2223-2236, doi: 10.3168/jds.S0022-0302(02)74302-6

Mahour SS, Nema SP, Rajput N, Chhabra D and Karmore SK, 2020. Therapeutic efficacy of ethanolic herbal extracts and ciprofloxacin in metritic crossbred cows. J Entomol Zool Stud, 8(5): 1221-1224

Malinowski E, Lassa H, Markiewicz H, Kaptur M, Nadolny M *et al.*, 2011. Sensitivity to antibiotics of *Arcanobacterium pyogenes* and *Escherichia coli* from the uteri of cows with metritis/endometritis. Vet J, 187(2): 234-238, doi: 10.1016/j.tvjl.2009.12.010

McLaughlin CL, Stanisiewski EP, Risco CA, Santos JEP, Dahl GE *et al.*, 2013. Evaluation of ceftiofur crystalline free acid sterile suspension for control of metritis in high-risk lactating dairy cows. Theriogenology, 79(4): 725-734, doi: 10.1016/j.theriogenology.2012.11.029

Miller ANA, Williams EJ, Sibley K, Herath S, Lane EA *et al.*, 2007. The effects of *Arcanobacterium pyogenes* on endometrial function *in vitro*, and on uterine and ovarian function *in vivo*. Theriogenology, 68(7): 972-980, doi: 10.1016/j.theriogenology.2007.07.013

Nak Y, Dagalp SB, Cetin C, Nak D, Alkan F *et al.*, 2011. Course and severity of postpartum metritis cases following

antibiotic and PGF2α administration in postpartum metritis cows infected with BoHV-4. Transbound Emerg Dis, 58(1): 31-36, doi: 10.1111/j.1865-1682.2010.01174.x

Ponraj P, Chang S, Rajesh VN, Veeraselvam M and Rajesh KD, 2017. Prevalence of various pathological conditions in female buffaloes (*Bubalus bubalis*). Asian Pac J Reprod, 6(1): 5-12, doi: 10.12980/apjr.6.20170102

Santos TMA, Gilbert RO and Bicalho RC, 2011. Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows. J Dairy Sci, 94(1): 291-302, doi: 10.3168/jds.2010-3668

Sarkar B, Ray K and Sarkar U, 2016. Prevalence of uterine infection in relation to certain haematological and biochemical changes of blood serum in dairy cows. Indian J Anim Res, 50(4): 557-560, doi: 10.18805/ijar.9419

Schuenemann GM, Nieto I, Bas S, Galvão KN and Workman, JD, 2011. Dairy calving management: effect of perineal hygiene scores on metritis. J Dairy Sci, 94(1): 5494-5501

Sheldon IM, Lewis GS, LeBlanc S and Gilbert RO, 2006. Defining postpartum uterine disease in cattle. Theriogenology, 65(8): 1516-1530, doi: 10.1016/j.theriogenology.2005.08.021

Sheldon IM, Williams EJ, Miller ANA, Nash DM and Herath S, 2008. Uterine diseases in cattle after parturition. Vet J, 176(1): 115-121, doi: 10.1016/j.tvjl.2007.12.031

Sheldon IM, Cronin J, Goetze L, Donofrio G and Schuberth HJ, 2009. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod, 81(6): 1025-1032, doi: 10.1095/biolreprod.109.077370

Silva JCC, Siqueira LC, Rodrigues MX, Zinicola M, Wolkmer P *et al.*, 2023. Intrauterine infusion of a pathogenic bacterial cocktail is associated with the development of clinical metritis in postpartum multiparous Holstein cows. J Dairy Sci, 106(1): 607-623, doi: 10.3168/jds.2022-21954

Timran DAV, Reyes J, Gilbert RO, Lefebvre RC, Baena LGP *et al.*, 2021. Incidence, clinical patterns, and risk factors of postpartum uterine diseases in dairy cows from high-altitude tropical herds. J Dairy Sci, 104(8): 9016-9026, doi: 10.3168/jds.2020-18692

Yogesh, Kulkarni S, Reddy S, SV H, BG R *et al*., 2022. Haemato-biochemical profile of repeat breeding buffalo in relation to different phases of oestrus cycle under field conditions. Pharm Innov J, 11(10S): 801-804

Received: 26.12.2022, Accepted- 23.09.2023, Published: 28.10.2023 (Online), 01.12.2023 (Print)
Section Editor: Prof. S. K. Nandi, Associate Editor