

Efficacy of Ceftiofur and Marbofloxacin in the treatment of staphylococcal endometritis in cross-bred cows

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Reproductive performance plays a pivotal role in the success of any dairy enterprise. Endometritis and sub-clinical endometritis are some of the key factors for conception failure and repeat breeding conditions. Intra-uterine antibiotics infusion is generally considered the choice of treatment for endometritis, however, since there is a narrow range of commercially available intra-uterine antibiotics to choose from and there is a growing emergence of resistance to these antibiotics due to its overuse, a higher generation of antibiotics needs to be tested. Ceftiofur is the antibiotic of choice for the treatment of uterine infection in bovines as it has excellent deposition in the endometrium and reaches the concentrations above minimum inhibitory concentrations (MICs) of most common pathogens of the uterus (Jeon *et al.* 2021). Marbofloxacin has excellent activity against the bacteria *Staphylococcus aureus* and has been used successfully in the treatment of bovine mastitis (Kaczorek-Lukowska *et al.* 2022). This work has therefore been performed to test the efficacy of ceftiofur sodium and marbofloxacin in the treatment of endometritis caused by *Staphylococcus* sp. in cross-bred cows.

The study was conducted on cross-bred cows from Instructional livestock farm, Bihar Veterinary College (BVC), Patna; clinical cases presented at veterinary Clinical Complex, BVC, Patna and dairy farms located in and around Patna district. HF Cross-bred cows were examined for endometritis on their first post-partum estrous in farms around Patna, Bihar. Muco-purulent flakes in the discharge of cows at estrus confirmed clinical endometritis. Uterine lavage was performed by introduction of a sterile insemination sheath, infusion of 50 mL of sterile normal saline and withdrawal of the uterine contents into a 50 mL disposable syringe. After proper mixing, two drops of uterine lavage contents were transferred into Brain Heart Infusion (BHI) broth and cultured at 37°C for 24 h. After proper mixing, broth culture was transferred from the

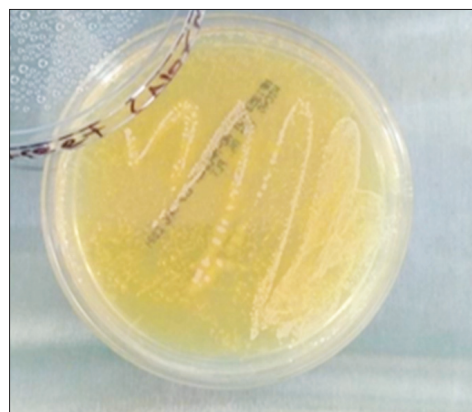


Fig. 1. *Staphylococcus* sp. colonies on UTI agar.

BHI broth into UTI agar (Himedia, India; MP1353) and cultured for 24 h at 37°C (Fig. 1). Bacterial colonies from the agar plate were confirmed to be *Staphylococcus* sp. by Gram's staining (Fig. 2) and catalase test.

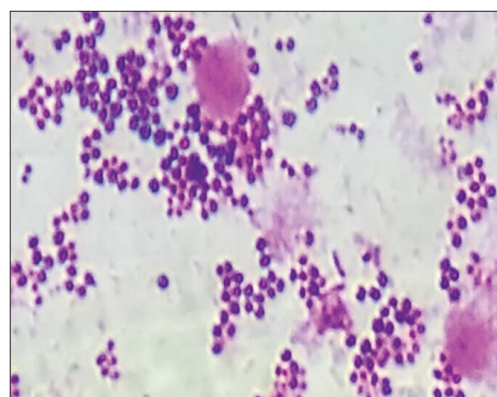


Fig. 2. Identification of *Staphylococcus* sp. after gram's staining; gram positive cocci arranged in bunches (1000× digitally zoomed).

One hundred cows found positive for *Staphylococcus* sp. from culture of uterine contents were grouped into two groups of fifty cows each. Group CEF (n=50) was treated with 1 g ceftiofur sodium (Xceft®, Alembic, India) intramuscularly (IM) once daily for five days and Group MARB

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(n=50) was treated with 10% marbofloxacin (Marbomet®, Intas, India) at the dose rate of 2 mg/kg body weight IM once daily for 5 days.

The cows treated with antibiotics were administered 500 µg cloprostenol sodium (Pragma®, Intas, India) IM, 14 days after the start of the antibiotic treatment. PGF_{2α} was administered to bring the animals at estrus within a specific period to enable timely estrus detection and artificial insemination. Cows were examined for estrus for 3 to 5 days from the day of cloprostenol administration. As the cows came to heat, the mucus discharge was collected in a sterile glass tube and observed for any muco-purulent flakes. Further, the uterus was lavaged with normal saline and bacteria was cultured by the procedure discussed above. Poly-Morphonuclear Neutrophil (PMN) count was performed following the method described by Kasimanickam *et al.* (2005), wherein thin smears were made in clean microscopic glass slides from the sediments of uterine lavage contents after centrifugation and staining the smears with Giemsa stain. All cows of the MARB and CEF groups were inseminated on the next estrus (12 h after the onset of estrus symptoms) and the adjusted conception rate was compared with the Control (CONT) group (n=50) those consisting of cross-bred cows (n=50) in second or third post-partum estrus with clean vulvar discharge and inseminated 12 h after the onset of estrus. Data were analyzed by chi-square test using SPSS software version 16.

Colonies from UTI agar plate (Fig. 1) were gram positive cocci (Fig. 2) and catalase test positive. The recovery rate of cows treated with ceftiofur sodium (CEF) and marbofloxacin (MARB) is presented in Table 1. As the cows came to estrus following antibiotics treatment, 36% of cows were found positive for endometritis (muco-purulent discharge) in the CEF group (18 out of 50) compared to 8% of cows in the MARB group, the difference being highly significant as revealed by chi-square test. With regards to cytological endometritis (PMN counts greater than 5% in uterine cytology, Duboc *et al.* 2010), 100% of cows were positive in the CEF group (32 out of 32) compared to only 26% in the MARB group (12 out of 46), the difference being highly significant (Table 1).

Table 1. Number of cows having muco-purulent flakes (clinical endometritis) or cytological endometritis (sub-clinical endometritis) following treatment

Group	Muco-purulent flakes [#]	Cytological endometritis [§] (PMN count > 10%)
CEF	18/50 (36%)	32/32 (100%)
MARB	4/50 (8%)	12/46 (26%)
Significance*	P<0.01	P<0.01

*, chi-square; #, Muco-purulent flakes in the discharge of cows at estrus is considered to be positive for endometritis, hence in this case these cows showed no clinical recovery; §, Cows showing clinical recovery (clear watery discharge), but having cytological endometritis (PMN count > 5%) indicative of sub-clinical endometritis. Note: Cows were evaluated after treatment on induced estrus with prostaglandin on 14th day of cycle.

Bacterial culture of uterine lavage content of cows after antibiotics treatment in CEF and MARB groups revealed that 66.7% of cows in the CEF group with clinical endometritis had *Staphylococcus* sp. in their culture as compared to 0% in the MARB group (Table 2). However, 62.5% of cows with sub-clinical endometritis in the CEF group had a recovery of *Staphylococcus* sp. from uterine lavage as compared to 10.9% in the MARB group, the difference being highly significant (Table 2).

The CEF and MARB group was inseminated 12 h after observed estrus and was compared with CONT group which consisted of cows in second or third post-partum estrus with clean uterine discharge. Non-return to estrus on day 21 (NR21) did not differ significantly amongst CEF, MARB and CONT groups, however, the pregnancy on day 60 differed significantly. Pregnancy was confirmed by rectal palpation on day 60, being 60% and 52% in the CONT and MARB group respectively and 32% in the CEF group (Table 3).

The endometrium is the innermost lining of the uterus and is composed of two layers, viz. stratum spongiosum abutting the myometrium and stratum compactum lining the uterine cavity. Histologically, endometritis is the inflammation of the uterine layer, no deeper than the stratum spongiosum involving hyperaemia, and infiltration of macrophages and neutrophils (Polymorphonuclear Neutrophils; PMN). Endometritis is a physiological post-partum necessary condition for uterine involution, however, its persistence beyond 21 days' post-partum is pathological and may be associated with or without purulent discharge. Clinical endometritis (CE) is therefore endometrial inflammation after 21 days' post-partum associated with purulent to muco-purulent vaginal discharge (PVD) with PMN count greater than 5% in uterine cytology (Dubuc *et al.* 2010). In contrast, sub-clinical endometritis (SCE) is not associated with PVD, however, has a PMN count greater than 5% in uterine cytology. Endometritis should be diagnosed when the cows are at estrus and with copious mucus discharge.

Staphylococcus sp. is one of the most common isolates from healthy bovine endometrium as well as cows with clinical and sub-clinical endometritis (Pajano *et al.* 2022).

Table 2. Number of cows found positive for *Staphylococcus* sp. in their endometrial flushing following treatment with Ceftiofur sodium or Marbofloxacin

Group	Clinical endometritis [#]	Sub-clinical endometritis [§] (PMN count > 10%)	Total
CEF	12/18 (66.67%)	20/32 (62.5%)	32/50
MARB	0/4 (0)	5/46 (10.9%)	5/50
Significance*	P<0.05	P<0.01	P<0.05

*, chi-square; #, Muco-purulent flakes in the discharge of cows at estrus is considered to be positive for endometritis, hence in this case these cows showed no clinical recovery; §, Cows showing clinical recovery (clear watery discharge), but having cytological endometritis (PMN count > 5%) indicative of sub-clinical endometritis

Table 3. Adjusted conception rate of cross-bred cows following treatment of endometritis with Ceftiofur sodium or Marbofloxacin

Group	NR21 [#]	CON60 [§]
CONT	41/50 (82%)	30/50 (60%)
CEF	32/50 (64%)	16/50 (32%)
MARB	35/50 (70%)	26/50 (52%)
Significance*	P>0.05	P<0.05

[#], Non-return to heat on day 21; [§], Conceived on day 60.

Though ceftiofur is the drug of choice for the treatment of uterine infection in cattle as it reaches a concentration greater than MIC ($\leq 2 \mu\text{g/ml}$; CLSI 2008) for most pathogens (Jeon *et al.* 2021) and it has been reported to be effective against *Staphylococcus* sp. (Ster *et al.* 2017), complete clinical recovery was not observed following its treatment. Contradictory results in this study might be due to increasing resistance of the organism against ceftiofur caused by its indiscriminate use.

Marbofloxacin is a second generation fluoroquinolone having excellent activity against Gram positive cocci like *Staphylococcus* sp. (Kaczorek-Lukowska *et al.* 2022). The pharmacokinetics of marbofloxacin in cattle revealed that the concentration in the blood required to be effective to kill major pathogens ($\text{MIC} \leq 1 \mu\text{g/ml}$; Serrano-Rodriguez *et al.* 2023) can be maintained with a daily dose of 2 mg/kg body weight and this dose has been found to be effective in the treatment of mastitis in cows (Schneider *et al.* 2004). An admirable recovery observed in this study with marbofloxacin proved the fact that this antibiotic is effective against Staphylococcal endometritis and this is consistent with the findings of Kohne *et al.* (2023) who reported excellent activity of marbofloxacin in the treatment of endometritis in mares.

Lietaer *et al.* (2023) in their study on post-partum cows reported that though cows with CE and SCE had greater neutrophil count ($>5\%$) in the endometrium, cows with CE had a greater percentage of viable and phagocytic neutrophils rather than just being an aggregation of apoptotic and necrotic neutrophils as in cases of SCE. As phagocytic neutrophils release large quantities of reactive oxygen species (ROS) which are highly toxic to pre-implantation embryos (Deluao *et al.* 2022), there is a decrease in conception rate in cows having clinical endometritis. The lack of phagocytic activity of neutrophils in SCE probably explains no significant decline in reproductive performance in cows reported with SCE (Barrio *et al.* 2015) with respect to the number of services per conception and calving to conception interval. Thus, the lower conception rate in cows treated with ceftiofur sodium in this study can be explained by the fact that it failed to kill *Staphylococcus* sp. in the uterus resulting in increased activity of neutrophils and production of ROS and damage to the embryos.

Following treatment of endometritis in Holstein Friesian cross-bred cows with marbofloxacin and ceftiofur sodium, 8% and 36% of cows did not show clinical recovery, respectively and amongst cows that showed

clinical recovery, 26% and 100% of cows had sub-clinical endometritis, respectively. Animals from MARB and CEF groups were inseminated on the next observed estrus and the adjusted conception rate was 52% and 32% compared to 60% in the healthy control group, the difference being highly significant. Thus, from this study, it could be concluded that the antibiotic ceftiofur sodium is losing its efficacy in the treatment of bovine endometritis caused by *Staphylococcus* sp. and marbofloxacin can be an excellent alternate choice here.

SUMMARY

Endometritis and sub-clinical endometritis are considered important factors for conception failure and repeat breeding conditions. Intra-uterine antibiotics infusion is generally recommended as the choice of treatment for endometritis. This work was performed to test the efficacy of ceftiofur sodium and marbofloxacin in the treatment of endometritis in cross-bred cows caused by *Staphylococcus* sp. One hundred cows found positive for *Staphylococcus* sp. from culture of uterine contents were grouped into two groups (CEF and MARB) of fifty cows each. Group CEF was treated with 1 g ceftiofur sodium (Xceft[®], Alembic, India) intra-muscularly (IM) for five days and Group MARB was treated with 10% marbofloxacin (Marbomet[®], Intas, India) at the dose rate of 2 mg/kg body weight IM once daily for 5 days. Animal from MARB and CEF groups were inseminated on next observed estrus after treatment and the conception rate was 52% and 32% compared to 60% in healthy control group, the difference being highly significant. The study reported antibiotic marbofloxacin as better alternative choice for the treatment of endometritis caused by *Staphylococcus* sp. in cross-bred cows.

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