

## **IN VITRO ANTIMICROBIAL SENSITIVITY TEST AND ITS EFFICACY IN ABANDONED COWS SUFFERING FROM ENDOMETRITIS**

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### **ABSTRACT**

The objectives of this study were to find out the *in vitro* sensitivity of antimicrobials and their *in-vivo* efficacy in 78 abandoned cows diagnosed with endometritis. Cervico-vaginal discharge collected from endometritic cows was tested against 7 antimicrobial agents. Ciprofloxacin (63.50%) and Enrofloxacin (60.32%) were highly sensitive whereas penicillin (83.64%) was found to be highly resistant against bacterial isolates. On testing their efficacy *in vivo*, combination of enrofloxacin and cephapirin benzathine yielded better results in terms of conception (31.25%). In conclusion, endometritis has been found to be one of the cause for abandoning of cows, although, 23.08% conception after treatment showed that abandoning can be avoided on choosing a susceptible antimicrobial agent for therapeutic purpose.

**Key words:** Abandoned cows, Endometritis, Goshalas, *In vivo* efficacy, *In vitro* sensitivity

Many etiologies have been associated with abandoning a cow of which endometritis is the most common one (Singh *et al.*, 2017). Specific and non-specific infectious agents during pre and postpartum period frequently invade the genital tract. These agents play a significant role in the failure of conception as a result of inflammation, denudation of uterine mucosa and change in the pH of the uterine and vaginal secretions. Uterine bacterial infections are important because they disrupt not only the function of the uterus, but also the ovary and the overreaching higher control centres in the hypothalamus and pituitary (Sheldon and Dobson, 2004). The inflammatory and immune response to uterine bacterial infection compromises animal welfare as well as causes subfertility and infertility (Singh *et al.*, 2018). Thus, for veterinarians, the diagnosis and treatment of uterine disease is a key component of fertility control programmes.

Various researchers (Moges *et al.*, 2013; Brodzki *et al.*, 2014; Sharma *et al.*, 2017) have carried out studies on the bovine genital tract with endometritis and identified a wide array of bacteria and their sensitivity to different antibiotics. Due to different types of infectious agents involved in uterine infections, a set recommendation of drugs is impossible. Ideally, identification and *in vitro* sensitivity against antimicrobial agent should be done. Subsequent treatment with an approved drug is the most practical, economical and efficient approach (Sharma *et al.*, 2017; Kumar *et al.*, 2018). In the present study, an attempt was made to study the *in vitro* sensitivity of antimicrobials and *in vivo* efficacy in abandoned cows suffering from endometritis.

### **MATERIALS AND METHODS**

The present study was carried out in 78 abandoned cows kept in Goshalas of Himachal Pradesh. After proper restraining of cows, cervico-vaginal discharge was collected and cows diagnosed to be suffering from

endometritis were selected for research purpose. The perineal area was washed with 0.01 percent potassium permanganate solution prior to sample collection followed by swabbing with ethyl alcohol. The discharge was collected using artificial insemination gun and then transferred into a sterile screw capped vial containing sterile swabs. The discharge samples collected were placed upright in the cool box with ice and were transferred to the microbiological laboratory for immediate processing for *in vitro* antimicrobial susceptibility test.

The isolates were tested for their sensitivity to various chemotherapeutic agents by disc diffusion method (Bauer *et al.*, 1966; Jorgensen and Ferraro, 2009). The test was performed using Mueller Hinton agar (Hi-media) by employing 14 antibiotic diffusion discs (Hi-media) *viz.* Ciprofloxacin (5 mcg/disc), Gentamicin (10 mcg/disc), Enrofloxacin (10 mcg/disc), Penicillin (10 units/disc), Ofloxacin (5 mcg/disc), Ceftriaxone (30 mcg/disc) and Tetracycline (30 mcg/disc). Now, the zones of growth inhibition around each of the antibiotic discs were measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium (Jorgensen and Ferraro, 2009).

After *in vitro* sensitivity test, five treatment groups were designed to study the *in vivo* efficacy of different antimicrobial agents i.e. Enrofloxacin (Fortivir, Virbac India) @ 7.5 mg/kg body weight (i/m), Enrofloxacin, i/m + Cephapirin Benzathine (500 mg)i/u (Metricf, MSD India), Oxytetracycline (Oxy LA, Zydus Animal Health Ltd.) @ 12 mg/kg body weight (i/m), Oxytetracycline (i/m) + Ciprofloxacin 125mg and Tinidazole 150mg/5ml suspension i/u (C-FloxTz, Intas Pharma Ltd.) and Oxytetracycline (i/m) + Cephapirin Benzathine (500 mg)i/u (Metricf, MSD India). Conception rate was noted following insemination in cows having clear cervico-vaginal discharge after treatment.

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## RESULTS AND DISCUSSION

### *In vitro* antimicrobial sensitivity test

Perusal of Table 1 indicates that Ciprofloxacin was highly sensitive against 63.50 per cent isolates whereas Enrofloxacin exhibited high sensitivity against 60.32 percent isolates only. However, total sensitivity was highest for Gentamicin and Ofloxacin i.e. 98.41 per cent. Highest resistance was found for Penicillin (83.64%), Ceftriaxone (10.17 %) and Tetracycline (7.94%). In earlier studies, Sharma *et al.* (2017) and Kumar *et al.* (2018) reported highest sensitivity for fluoroquinolones i.e. Ciprofloxacin, Enrofloxacin and Ofloxacin. Similarly, percent sensitivity was found to be highest for Enrofloxacin and Ciprofloxacin i.e. 92 and 96 per cent, respectively (Takamtha *et al.*, 2013; Mshelia *et al.*, 2014). Sharma *et al.* (2009) stated that Ciprofloxacin could be effective in

use of commonly used antibiotics and subsequently, development of resistant strains due to bacterial mutation (Barman *et al.*, 2013; Sharma *et al.*, 2017; Kumar *et al.*, 2018). Therefore, constant monitoring of the resistance patterns of etiological agents resulting in bovine uterine inflammation is needed as the use of proper antibiogram can be helpful in avoiding unnecessary resistance to antimicrobial agents (Malinowski *et al.*, 2011). Also, therapeutic trials should be carried out to find out the most effective antimicrobial agent before its use in diseased animals (Udhayavel *et al.*, 2013).

### *In vivo* efficacy of antimicrobial agents

In present study, conception rate was better in cows treated with Enrofloxacin and Cephapirin benzathine combination followed by Enrofloxacin and Oxytetracycline. However, Oxytetracycline in combination with Cephapirin benzathine or Ciprofloxacin and Tinidazole suspension yielded poor conception in cows treated against endometritis.

Due to different types of infectious agents involved in uterine infections, a set recommendation of drugs is impossible. A common treatment of acute endometritis is the administration of antibiotics into the uterus and a systemic antibiotic treatment (Smith *et al.* 1998). In concurrence to our study, McDougall (2003) and LeBlanc *et al.* (2002) found intra-uterine treatment with cephalapirin benzathine effective in cows suffering from endometritis. However, Singh *et al.* (2018) reported that administration of Ciprofloxacin and Tinidazole combination into the uterus lead to conception in 48 per cent endometritic cows. Also, conception rate was higher following systemic administration of Ciprofloxacin (Bala 2017; Singh *et al.*, 2018). In disagreement to our study, 80.0 per cent conception rate has been recorded following parenteral administration of Enrofloxacin (Kumar *et al.* 2004).

In our study, *in vitro* antimicrobial sensitivity was comparatively much higher as compared to *in vivo* efficacy of these agents in treatment of endometritic abandoned cows which may be due to animal's immune response to infection (Bala, 2017). Factors important in the selection of antimicrobials for the treatment of endometritis are the

**Table 1:**

Sensitivity pattern of various antimicrobials against bacteria isolated from cervico-vaginal discharge of abandoned cows suffering from endometritis

Antimicrobial agents	Degree of sensitivity (%)		
	HS	MS	R
Ciprofloxacin	63.50	33.33	3.17
Enrofloxacin	60.32	36.51	3.17
Gentamicin	9.52	88.89	1.59
Ofloxacin	50.79	47.62	1.59
Ceftriaxone	23.73	66.10	10.17
Tetracycline	52.38	39.68	7.94
Penicillin	3.64	12.72	83.64

HS- Highly sensitive, MS- Moderately sensitive, R- Resistant

treating uterine infection probably due to its least use in the field practice. In accordance to our study, moderate sensitivity to Gentamicin and Ceftriaxone was present in a study conducted by Kumar *et al.* (2018).

In concurrence to our study, Penicillin was reported to be highly resistant in studies of Barman *et al.* (2013) and Sharma *et al.* (2017). Contrarily, Sadig *et al.* (2010) found high sensitivity to Penicillin i.e. 90 per cent. However, high resistance to penicillin can be attributed to indiscriminate

**Table 2:**

Comparative efficacy of different antibiotics in abandoned cows treated for endometritis in various Goshalas

S. No.	Antibiotic(s) used	Treated	Responded	Conception (%)
1	Enrofloxacin (100 mg/mL) i/m	21	6	28.57
2	Enrofloxacin (100 mg/mL) i/m+ Cephapirin benzathine (500 mg)i/u	16	5	31.25
3	Oxytetracycline (200mg/ml) i/m	23	5	21.73
4	Oxytetracycline (200mg/ml) i/m + Ciprofloxacin 125mg and Tinidazole 150 mg/5ml suspension i/u	10	1	10.00
5	Oxytetracycline (200mg/ml) i/m+ Cephapirin benzathine (500 mg)i/u	8	1	12.50
Overall		78	18	23.08

uterine environment, the pathogen, the minimum inhibitory concentration (MIC) of the pathogen, route of administration of the antimicrobial, severity of irritation caused by the antimicrobial agent or vehicle, the antimicrobial and/or carrier agent used for the intrauterine therapy, treatment economics and therapy outcome (Singh *et al.*, 2018).

Farmers associated with marginal farming system cannot waste their precious money on repeatedly repeating cows, as it negatively affects their economic sufficiency. Thus, abandoning them becomes easier choice rather than inappropriate treatment(s) following numerous failed inseminations. Therefore, timely diagnosis and treatment with the most susceptible antimicrobial agent can be fruitful to both cow and the farmer.

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