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## Uterine microbial isolates and Antibiogram in Endometric crossbred cows

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### Abstract

Infectious infertility in crossbred cows is known as endometritis. It is getting worse every day as a result of indiscriminate antibiotic usage in the field without proper diagnosis, the emergence of drug-resistant microbes, lack of knowledge, and poor sanitation practices. The current study examines the prevalence of endometritis, the microbiological composition of uterine discharge, the antibiogram of various bacterial isolates, the efficacy of therapeutic approaches, and the rate of conception. The antimicrobial susceptibility pattern of a microorganism implicated in endometritis is influenced by a number of factors. The presence of anatomical, pathological, and physiological barriers, the cow's immune capability, and the antimicrobial drug's pharmacologic properties, such as dispersion in the uterine cavity, stability in uterine secretions, and distribution at effective concentrations into microbial localization sites the use of focused therapy and the adoption of management techniques can help to prevent cases of escalating endometritis.

**Keywords:** Crossbred cows, microbial isolates, Antibiogram, Endometric

### Introduction

In crossbred cows, endometritis is considered one of the infectious types of sterility. In crossbred cows, abnormal parturition is a prevalent cause of endometritis. To prevent uterine infections, dairy crossbred cows should be managed with proper hygiene and sanitation practises during parturition. Microorganisms in the uterus and other reproductive parts of the cow must be considered during the examination, and they cannot be overlooked because they can lead to a slew of other reproductive problems, all of which result in significant losses in terms of animal health as well as economic consequences due to decreased conception rates and milk yield. For efficient treatment, an *in vitro* antibiogram analysis of uterine discharge samples from cows suffering from endometritis should be performed in order to save time and money. Kusum and Roy (2012) <sup>[37]</sup> regard endometritis to be one of the most contagious forms of infertility in crossbred cows. Endometritis is one of the most common reproductive disorders in postpartum dairy cows, resulting in significant economic losses due to increased open days, calving intervals, and number of services per conception (Salah and Yimer 2017) <sup>[58]</sup>. The study on endometritis was aimed to know the incidence of endometritis, bacterial and fungal content of uterine discharge and the antibiogram of different bacterial isolates (Sayyari *et al.*, 2012 <sup>[62]</sup>; Moges *et al.*, 2013) <sup>[45]</sup>; Yadav and Jadhav 2018) <sup>[77]</sup>. Bhadaniya *et al.* (2019) <sup>[6]</sup> also took therapy measures with the conception rate to determine the efficacy of each treatment. Metritis, mastitis, repeat breeding, miscarriage in the last trimester of pregnancy, retention of the placenta and reduced milk supply in females and in males orchitis, sterility and joint pains are all caused by Brucellosis (Mishra *et al.*, 2022) <sup>[52]</sup> in males. Brucellosis is caused by bacteria belonging to the genus *Brucella*.

### Incidence

The incidence rate of endometritis in examined crossbred cows was Francos and Mayer (1988) <sup>[19]</sup> who found 16% and 14% endometritis in southern Israel and northern Israel cows respectively. Fivaz and Swanepoel (1978) reported the 14% incidence of endometritis in cow. Singh (1997) <sup>[63b]</sup> and Rehman *et al.* (1990) <sup>[55]</sup> was reported incidence of endometritis as 15.40% in crossbred cow and 16% in cattle respectively. While Khanna and Sharma (1993) <sup>[26]</sup> reported 10.84% endometritis in cows. Study was indicated that the incidence of endometritis in crossbred cow was significantly more in first partum 39.68% which subsequently went on decreasing in the second partum 19.05% third partum 15.87% and fourth and onward partum

up to 12.69%. According to Sreeramulu (1995) [69] endometritis constituted 37.2%, 2.66% and 1.4% among first calver, second calver and pluriparous cows respectively. It is obviously due to the fact that wear and tear of genitalia during first parturition. The uterus is more prone to exposure of infections resulting in endometritis, while during second, third and onward partum uterus develops immunity against infections to some extent. In heifer, incidence of endometritis is only 12.69% that is very less as compared to first partum which is due to fact that the heifer was less exposed to metritis with age. Martinaz and Thibier (1984) [43] also reported that parity had only marginal effect on the epidemiological pattern of reproductive disorders including metritis. Sinha (1978) [67] was observed that the incidence of endometritis in crossbreds increased with parity in the clinics of Ranchi Veterinary College, but he did not find any particular trend in the cows at Namkom Military Dairy Farm. However, he observed a lower incidence in heifers (17.22%) than that in cows (27.10%), obviously due to fact that the cows were exposed to more risk of infection than heifers. Where as a higher incidence of endometritis as Slimane *et al.* (1994) [68] and Rao *et al.* (2001) [54] reported as 17.2%, 18%, 29.38%, 30.77% and 32.81% respectively. Sinha (1978) [67] also reported higher incidence of 28.06% and 25.23% in Friesian and jersey crossbred respectively. The variation in incidence of endometritis may be due to difference in agroclimatic condition, types of the animals, nutritional status, managemental practices and methods of diagnosis. Singh *et al.* (1981) [64] reported the incidence of endometritis 9.61% among the cows. However, Kaikini *et al.* (1981) [23] reported incidence of 8.76% and Naidu and Rao (1981) [47] reported 6.27% in crossbred cattle while Lqrens (1995) [41] observed that Ayrshire and Holstein cows from four herds in south Africa in which 7.5%, 3.6%, 4.6% and 2.8% the animal suffered from metritis in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> herds respectively. Their observation was lower than the present findings, this discrepancy of the results is due to better husbandry in herds and study was on animals in the field condition. A very much lower incidence of endometritis was reported by Francos and Mayer (1988) [19] 3.4% and Kumar *et al.* (1986) [36] 1.2% in cattle. This study was conducted on well maintained heads. Whereas study was of cattle wealth kept on always maintenance in adjoining areas, carried out and data analyzed from the same. Investigation it was found that the incidence of endometritis was more in H-F crossbred than the Jersey crossbreds cows 31.74% and 68.25% belonged to H-F crossbreds. Study H-F suffered more than Jersey from endometritis as Holstein calves lacking the CD18 glycoprotein on neutrophil cell membranes were unable to deal with normally easily stopped bacterial infections because the neutrophils were unable to migrate along blood vessels. They were thus unable to undergo diapodesis and migrate to the site of a bacterial infection. This syndrome called bovine leukocyte adhesion deficiency (BLAD) can be detected by use of a polymerase chain reaction (PCR) to detect the presence of the defective gene (Kehrli *et al.*, 1990) [24]. Rong *et al.* (1997) [56] found 44% post-partum metritis in high producing cows and 32% in medium producing Holstein cows. Singh (1997) [63] also observed higher incidence of endometritis in H-F crossbred (68.54%) and lower (31.46%) in jersey crossbred which is approximately the same observation. Prasad (1998) [51] observed endometritis (80.88%) in H-F crossbred and lower value (19.11%) in Jersey crossbreds. Rao and Kotayya (1976) [53] reported to the frequency of

occurrence of endometritis was not significant among the different crossbreds. While incidence among indigenous cows was found far lower than the crossbreds. Because of the fact crossbred especially H-F is high yielding cow. So much nutrients are required to maintain the health of crossbred where as indigenous cows are low yielding cows, not subjected to nearly body exhaustion. Chronic uterine inflammation subclinical endometritis affects approximately 37- 74% of parturient dairy cows (Gilbert *et al.* 2005; Lincke *et al.* 2007; Behera *et al.*, 2015 and Knudsen *et al.* 2016) [20, 40, 5, 27] 15–20% of cows develop clinical endometritis, and 30% develop subclinical endometritis beyond 3 weeks postpartum Cheong *et al.* (2011) [12]. Khan *et al.* (2016) [25] reported was 4.61% endometritis in dairy cattle under semi-intensive system of rearing in North-Eastern India. Jeon and Galvao (2018) [22] were found in 20% endometritis in lactating dairy cows is an acute inflammatory disease in the uterus.

### Bacteriological and fungal studies

Uterine samples of crossbred cows suffering from endometritis were taken for isolation and identification of various microorganisms. Prasad (1998) [51] and Rao *et al.* (2001) [54] where single and mixed types of isolates were found it was evident from the result that the highest percentage of isolates (42.86%) were of *E. coli*. The other organism obtained was *S. aureus*, *S. pyogenes*, *P. vulgaris*, *C. pyogenes*, *P. aeruginosa* and *Candida albicans*. The occurrence of higher percentage of *E. coli* and *Streptococcus* may be invasion of uterus through open cervix. This is possible because *E. coli* is the normal inhabitant of G.I. Tract having invasive properties (Laing, 1961). The bacterial invasion coincides with the increasing amount of lochia which together with the intrauterine temperature and pH, create suitable environment for bacterial multiplication. Highest incidence of *Esch coli* was finding of Rose (1987) [57] and Salimane *et al.* (1994) [68] in 30.16%. However, Jacob *et al.* (1995) [21] reported *S. aureus* a major isolates from the cases of endometritis in crossbred cows. Kumar (1997) [35] and Prasad (1998) [51] isolated *S. aureus* from 26.9%, 30.34%, 32.30% 32.30% and 29.14% of the uterine samples was present findings. *Streptpyogenes* was present in 14.28% of the total samples. It was supported by various workers such as Kumar (1997) [35] *C. pyogens* and *P. aeruginosa* were present in 6.35% each, of the total samples was less than the finding of Kudryavtsev *et al.* (1991) [34] and Singh(1997) [63]. 4.76% samples were found negative for bacterial growth but yielded *Candida albicans*. This observation was similar to the finding to Deka *et al.* (1985) [15] and Prasad (1998) [51] who isolated *Candida albicans* from 4.35% and 4.41% of the uterine samples. Various scientists (Saxena, 1976 and Singh *et al.*, 2001) [61, 65] also reported *Candida albicans* in different percentage from the uterine samples irrational use of antibiotics and corticosteroids lead to accelerated infection by fungi. It may be the cause of non-response of antimicrobials in the treatment of few cases of endometritis where many fungi have invaded (Kremlev and Banakova, 1997) [32]. The difference in the finding of percentage of different organisms may be due to difference in health status of animal, rate of prevalence of organisms in particular area and individual susceptibility of the animals. In the study was an attempt has been made to compare the breed wise incidence of the organisms in endometritis. In both crossbred Jersey and J-F cows, *E. coli* was isolated in highest number followed by *S. aureus* and *S. pyogenes* in Jersey cross cows, *C. pyogenes* was

found to be 10% while in H-F cross cows it was absent while these were isolated from H-F crossbreds, *P. aeruginosa* was isolated from cows 4.65% in H-F crossbred Studer and Morrow (1978) [71] 36% of *E. coli* from endometritis in H-F pure bred cows in the findings. Cohen *et al.* (1996) [14] noted 70% *C. pyogenes* in Israeli-Holstein and H-F crossbred cow which shows comparatively lower incidence of this organism as compared to the study. Apart from these organisms Cohen *et al.* (1995) [13] isolate only *Streptococcus* spp. as 36% in Israeli-Holstein cow while Vitek *et al.* (1989) [76] isolated almost all the bacteria to a varying degree in H-F crossbred as noted in the study. They also isolated different fungi while only candida albicans was isolated in this study. Bonnett *et al.* (1991) [9] also isolated different bacteria namely *E. coli*, *Streptococcus* spp. and *C. pyogenes* in H-F cows. In respect of *Staphylococcus* isolates was supports the observations recorded by a number of workers both in India and abroad in past Behera *et al.* (2015) [5] who registered *Staphylococcus* infection in major number of isolation studies in maximum number of cases in their own study. The prevalence of *E. coli* infection in present study is also well compared with *Staphylococcus* uterine infection was showed agreement with the findings of a number of workers. Some previous studies also registered higher prevalence of *E. coli* in their respective studies (Savov and Dimitrov (1973) [60], El-Sakkar *et al.*, 2008 [17]; Yilmaz *et al.*, 2012 [78]; Udhayavel *et al.*, 2013 [74]; Barman *et al.*, 2013 [4]; Takamtha *et al.*, 2013 [73]; Mshelia *et al.*, 2014 [46]. In contrast, higher percentage of *A. pyogenes* (Moges *et al.*, 2013; Sayyari *et al.*, 2012; Bicalho *et al.*, 2017 and Bhadaniya *et al.* 2019) [45, 62, 7, 6].

#### **In-vitro antimicrobial assay**

The antibiogram of various microbial isolates was studied in order to carry out an effective as well as uncontrolled rational therapy that leads to medication resistance. Koleff *et al.* (1973) [28] reported on the treatment of bovine endometritis with antibiotics based on *in-vitro* sensitivity and found that recovery was faster and required fewer treatments than with empirical therapy. In this study, different bacterial isolates from uterine endometritis samples were exposed to *in-vitro* sensitivity testing against various antimicrobial drugs in order to see if these agents may be used to treat endometritic cases. Depending on the sensitivity pattern, six different species of bacteria were extracted *in vitro*. Enrofloxacin showed maximum sensitivity (75.36%) followed by Gentamicin (65.21%) and Cephalexin (52.17%) Enrofloxacin showed maximum sensitivity against *E. coli* (85.18%), *S. pyogenes* (77.78%) and *S. aureus* (68.42%). Ziv *et al.* (1996) showed 90% *E. coli* and *Streptococci* were sensitive with MIC less than 0.25 µg/ml and 5 µg/ml of Enrofloxacin against *E. coli* in uterine samples. *P. vulgaris* and *C. pyogenes* have equal sensitivity against Enrofloxacin 66.67% and *P. pyogenes* showed sensitivity as 74.07%, 68.42% and 55.55% respectively. While against Cephalexin is 55.56%, 52.63% and 44.44% respectively. *P. vulgaris* both Cephalexin and Gentamicin Anjaneyulue *et al.* (1999) [3] reported that Enrofloxacin was 93% effective in cases of endometritis. The above noted observations with the antimicrobials are in agreement with Dholakia *et al.* (1985) [16], Sudhakar *et al.* (1986), Anetzhofer (1989) [2]. Pal and Dwivedi (1992) [49] and Singh (1997) [63] who showed that Gentamicin was most sensitive antibiotic. Percentage sensitivity to Cephalexin (52.17%) noted in present study was in agreement with the findings of Singh (1997) [63], Kumar (1997) [35], and Prasad

(1998) [51] who noted sensitivity to Cephalexin to be 48.04%, 63.95% and 53.33% in uterine samples. On the other hand Zahid (2004) [79] noted that highest number of their isolates were sensitive to neomycin and doxycycline. El-Sakkar *et al.* (2008) [17] reported that isolated microorganisms were highly sensitive to florafincol. The highest number of isolates were sensitive to Ceftriaxone least sensitive to Chloramphenicol as reported by Udhayavel *et al.* (2013) [74]. In recent studies Moges *et al.* (2013) [45] reported that all the isolates of *S. aureus* were resistant to ampicillin, oxacillin and vancomycin and *E. coli* showed resistance to polymixin, tetracycline and cefoxitin. The antimicrobial susceptibility of *E. coli* was highest against Pefloxacin, Ofloxacin, Amoxicillin-clavulanate, Ciprofloxacin, Gentamicin and Streptomycin, whereas antibiotic sensitivity of *S. aureus* showed the highest susceptibility against Ampicillin (Mshelia *et al.*, 2014) [46].

#### **Therapeutic studies**

The study was therapeutic measures adopted were based on *in vitro* sensitivity test. The beneficial effects on the use of specific antibiotic after drug sensitivity test have been reported by various workers (Oxender and Sequin. 1976; Sinha *et al.* 1977; Steffan *et al.* 1984; Bohme *et al.*, 1986 Korudziński *et al.* 1988, Pateria *et al.*, 1992. Slimane *et al.*, 1994; Kumar *et al.* 1997; Singh *et al.* 1997 Prasad, 1998) [48, 66, 70, 8, 34, 50, 68, 35, 51] and Kusum *et al.* (2008) [38]. The present study was *in-vivo* drug trial was conducted through intrauterine route which was in accordance with the recommendations of Chauhan and Takkar (1983) [11] and pateria *et al.* (1992) [50]. It was also in accordance with pattern of treatment of endometritis adopted by Kornel and Chhabra (1988) [29], Ambrose and Pattabiraman (1993) [1] and Slimane *et al.* (1994) [68]. Luna *et al.* (1991) [42] used Enrofloxacin in chronic endometritis which showed rapid absorption through uterine mucosa. Rong *et al.* (1997) [56] used Enrofloxacin on ovary in endometritis cases with 90-100% efficacy. The antimicrobial results and antibiogram were reported earlier in the study, and treatment was carried out. The treatment's efficacy was determined by the elimination of microorganisms and the rate of conception. The treated animals enhanced the clarity of their discharge, but they were unable to eliminate all of the pathogens present in the uterine discharge, as demonstrated by culture. The aggregate results of treated cows showed that 59.64 percent of samples were clear of microorganisms, while the remaining 40.35 percent of bacterial positive cases similarly responded to treatment. This could be owing to the presence of bacteria with lower pathogenicity. Similar observations were reported by Ambrose and Pittabiraman (1993) [1] and Singh (1997) [66] They reported complete recovery in 68.0% and 59.04% respectively, However, Sinha *et al.* (1977) [66] reported higher (87.80%) recovery rates. The composition of uterine fluid, the severity of the infection, and the condition of various host defence mechanisms can all affect the efficiency of treatment for uterine infections. The rate of efficacy of antimicrobial medications against diverse microorganisms was recorded as 59.64 percent in a study on the efficacy of treatment with antimicrobials in cows suffering from endometritis. After three successive artificial inseminations (A.I.) in cows treated with various antimicrobial drugs, the efficacy of treatment leading to the clearing of the bacterial infection in endometritis was detected, with a conception rate of 61.40 percent. There were 43.86 percent, 41.67 percent, and 55.55 percent of animals conceived after 1, 2, and 3 A.I.,



respectively. Kornel and Chhabra (1998) [29] noted the increased percentage of conception and insemination as compared to 2 insemination and the values were noted to be 51.06 Vs 21.27% after treatment with antimicrobial agents. Chaffaux *et al.* (1991) [10] showed interval between calving and conception is longer in late treated than early treated endometritis cases.

Venkateswarlu *et al.* (1983) [75] overall conception rate 71.93% which is slightly higher than in the study was Sudhakar (1986) [72] reported an overall CR of 59.43% which is more or less similar to the study was Samad *et al.* (1994) [59] showed 70% CR on first A.I. in animals suffering from the genital abnormalities including endometritis. Silmane *et al.* (1994) [68] showed 70% CR in cows with endometritis after treatment with antibiotics which is slightly higher than the study. Laurens (1995) reported that 2.06 insemination per conception were needed for metritic cases. Koujan *et al.* (1996) Observed a better CR after use of intrauterine betadine in endometritic cows. Treatment with various antimicrobial agent leading to conception in cows suffering from endometritis Enrofloxacin showed the highest overall percentage of conception (78.57%) with conception rate 57.14%, 66.67% and 50% after 1,2, and 3 A.I in cows. The overall conception rate using Cephalexin, Gentamycin and Lugol's solution was 66%, 55% and 33.33 found respectively Mishra *et al.* (1999) [44] observed conception rate (CR) in buffalo with Enrofloxacin as 83.33%. On the other hand, Kumar (1997) [35] noted conception rate with Cephalexin (69.23%) followed by Gentamicin (63.15%) From the study it can be assumed that the treatment with Enrofloxacin, Cephalexin and Gentamicin may be the choice of drug and accordingly may be used in treating endometritis cases so that the animals may conceive. The antibiotic sensitivity pattern of the isolates of bacteria revealed that resistance to antibiotics is on increase. The multiple drug resistance is of alarming in nature, development of resistant pathogenic strains of bacteria pose considerable threat to public health.

### Conclusion

Uterine Microbial Isolates and Antibigram in Endometritic Crossbreds is the subject of this review study. Cows with endometritis should not be overlooked because it causes a slew of reproductive issues, including low conception rates and reduced milk outputs. For efficient treatment, uterine samples from cows with endometritis should be cultured and sensitivity testing should be performed. So that we can spare farmers and our country from massive animal losses, output losses, and economic losses.

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