## Original Article

# Isolation and Identification of Uterine Microorganisms in Postpartum Dairy Cows

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The aim of this study was to isolate and identify uterine microorganisms in postpartum cows and to observe the occurrence of uterine infection in crossbred and local cows. One hundred and nine samples, from both crossbred and local dairy cows were collected from Chittagong area. They were inseminated artificially (n=89) and naturally (n=20). Samples were collected at least 3 months after calving. Among the selected cows, 33 had different reproductive diseases and the rest were from apparently healthy cows. The crossbred dairy cows were more susceptible to reproductive diseases (19.27%) than the local ones. Uterine infection was higher (52%) in cows inseminated artificially than that of natural ones (21.21%). Nineteen different types of bacteria were isolated from both diseased and healthy animals, except *Clostridium spp* and *Nocardia spp* which were not found in diseased animal. Likewise, *Bacillus spp* and *Salmonella spp* were absent in healthy animals. Most prevalent bacteria in diseased animal were *Staphylococcus spp* and *Streptococcus spp* found in 33% and 27.3% of the cases respectively. In non-diseased cases, the highest number of samples was positive for *Bacteroied spp* (13.2%) followed by *Streptococcus spp* (10.4%). On average, the highest number of bacterial population was isolated from diseased samples and most of those bacteria are pathogenic.

Key words: Uterine infection, Identification, Postpartum, Dairy cows

## Introduction

Uterine infection is a major problem in reproductive management and inefficient estrous detection. The bovine uterus is sterile before calving and the cervix provides a barrier against intra-uterine invasion by pathogens during gestation[1]. Following calving, the uterus may become infected due to presence of infectious or non-specific microorganisms. Several bacterial, protozoan, viral and mycoplasmal infections are common in postpartum period. The most common and economically important bacteria for uterine infection are *Actinomyces spp*, *Escherichia coli, Fusobacterium spp*, *Pasteurella spp, Pseudomonas spp*, and *Staphylococcus spp*[2]. Cows with uterine infection in the postpartum period generally have lower conception rates at subsequent breeding. A high percentage of cows have bacteria present in the uterus during the first two weeks after calving may be considered as infected. However, by two months post-calving, the prevalence of uterine infection decline to less than 10 percent. Even though, pathogenic bacteria persist in some cows causing uterine disease by preventing physiological uterine involution resulting in sub-fertility in individual cows or even in the whole herds[3]. Bovine genital infections, either specific or non-specific in nature, account for large number of pregnancy failure in cows[4]. Generally, non-specific infection of the genitalia is considered to be the main cause of repeated conception failure[5,6]. Bacterial infection is the most important among the various causes of endometeritis[7]. Such a condition may cause cervicitis or endometritis of various degrees, which may lead to embryonic death and repeat breeding problems[8]. These infections affect fertility by altering the uterine environment resulting in sperm transport impairment, sperm death and hostile environment to subsequent development and maintenance of the conceptus leading to their death[9]. Incidence of uterine infection in cattle decreases with the increasing of the postpartum period, ranging from 90% in 25 days of calving to postpartum to 9% in 46-60 days of postpartum period[10].

Although, Bangladesh has a good number of cattle populations, however, there is still no treatment strategy against reproductive diseases. Most of the cattle owners in Bangladesh are not solvent economically. Therefore, any damage of their highly priced animal even after treatment of uterine diseases and sub-fertility or death is a serious burden for them. The reason behind this could be lack of skill to diagnose uterine infection properly and non-specific treatments. Identification of uterine microorganisms and selection of effective drugs could be a good reproductive management in cow. Considering the above facts, therefore, the present piece of research was conducted to know the occurrence

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of uterine infection in dairy cows and to identify the common bacterial population associated with uterine infection in cows.

**Materials and Methods**

The research was conducted in the Department of Medicine and Surgery, Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong during the period from June, 2009 to December, 2009. The animals were selected from Chittagong metropolitan area. A total of 109 (n=109) cows including both crossbred (n=66) and local (n=43) were selected which crossed a minimum period of three months after parturition. They were in between 2 and 8.5 years of age, categorized as 7-8.5, 5-6 and 2-4 years old. The studied cows were also categorized according to parity. They were in different parity from $1^{st}$ to $3^{rd}$ and few cows were more than $3^{rd}$ parity. The animal inventory is presented in the Table 1.

A questionnaire was prepared and used to record information on age at birth, breed, parity, current physiological status and reproductive performance, recent major clinical illness, last calving date, nature of calving, insemination date and nature of insemination, diseases and treatment. Information in this study was recorded by face to face interviewing the owner and from the individual animal card, routine clinical case register, breeding register maintained at the dairy farm, record book of Artificial Insemination center etc. Clinical examination was also done to identify any abnormalities of cows.

*Management*

The cows were kept under traditional management systems. The housing was open or semi-intensive for most of the local cows. Some crossbred cows were reared in the intensive farming system. Both stall and group feeding were practiced round the year. The local cows were supplied with straw, roadside grass, bran and *ad libitum* water twice or thrice a day. Approximately, 8-10 kg roughage and 1.5-2 kg concentrate were provided to crossbred cows twice daily with free access to drinking water. Hand milking was practiced twice a day

*Clinical examination*

Prior to collection of samples, each cow was examined clinically. Body conformation, posture and gate, mucous membrane, respiration rate, pulse rate, temperature and any abnormalities, if present, were taken for each cow. Finally, thorough rectal palpation was done to detect estrus and disease conditions of the cows.

*Uterine sample collection*

Before collection of transcervical uterine sample, the perineal region of the cows were washed and cleaned properly with fresh water and potassium permanganate solution. Left handed rectal palpation was performed to collect uterine sample by using AI tube. A 10 ml syringe adjusted behind the glass made AI tube with rubber pipe was introduced into the uterus through cervix guided by rectal palpation. The collected sample was transferred to the vaccuotainer tube containing phosphate buffer saline (PBS) for aerobic culture and thio-glycolate broth for anaerobic bacterial culture. Paraffin oil was poured on to the surface of anaerobic sample to create an anaerobic condition. All these samples were preserved at -20 °C until culture.

*Culture of organisms*

For the growth of aerobic bacteria the samples were plated on nutrient agar, McConkey agar and mannitol Salt agar media and incubated at 37°C for 48 hours. On the other hand for anaerobic bacterial growth, anaerobic incubator was used. In the case of anaerobic bacteria, nutrient agar and blood agar media were used. All the procedure was conducted at 37 °C temperature for 48 hours.

*Isolation and identification of bacteria*

The selected colonies of bacteria were identified on the basis of their colony characteristics, Gram stain, hemolytic properties and growth on selective media. After presumptive identification, the culture specific biochemical tests were performed.

*Statistical Analysis*

All data obtained were computerized by MS Excel and subjected to standard statistical analysis. STATA/IC - 11 were done for *p* value. Differences were considered to be significant at 5% level of significance.

**Results and Discussion**

Different types of reproductive diseases or disorders viz. endometritis, pyometra, cervicitis, abortion, vaginitis, maggot infestation in vagina and bloody discharge were recorded during sample collection. The first four types were considered as 'uterine infection' due to presence of pus. A total of 109 cows selected in

**Table 1**. *Distribution of animals according to breed, age and parity*

| Breed | Total number | Age(Year) | | | Parity | | | |
|---|---|---|---|---|---|---|---|---|
| | | Number of cows | | | Number of cows | | | |
| | | 2-4 | 5-6 | 7-8.5 | 1st | 2nd | 3rd | >3rd |
| Crossbred | 66 | 3 | 26 | 37 | 15 | 33 | 15 | 3 |
| Local | 43 | 0 | 12 | 31 | 15 | 21 | 5 | 2 |
| Total | 109 | | | | | | | |

this study, 33 had symptoms of different reproductive diseases, whereas 76 were apparently healthy. The prevalence of uterine infection was found in a total of 24 cows (Table 2). Nine others showed reproductive diseases of different types. Variation in the occurrence of uterine infection may be due to the influence of age and parity as well as breed of the sire[11,12]. The bloody discharge was not considered as uterine infection because no other sign was observed except blood in the sample. It could be due to genital injury during collection of sample. Vaginitis (3.03%) and maggot infestation (10%) were also observed in this study. Among different reproductive disorders, the endometritis were highest (33.33%) followed by pyometra (18.18%), cervicitis (15.15%) and abortion (6.06%) (Table 2).

The overall prevalence of endometritis during postpartam period in dairy cows may vary in different report[13,14]. Clinical endometritis is one of the most common disorders in dairy cows, causing decreased fertility and large economic loss[15]. Another very common uterine infection of cows is pyometra, which accounted for 18.18% of total reproductive disorder in this study. Other studies by Tadesse (1999)[16] and Oumermohammed (2003)[17] reported even higher percentage of pyometral cases in cows in their research. The prevalence of abortion in this study was 6.06% which is similar to the study of Shiferaw (1999)[18] and Kassahun (2003)[19]. On the other hand, Oumermohammed (2003)[17] and Durrani (2009)[20] observed 3.19% and 3.2% of abortion cases in cows. In this study, abortion found only in crossbred cows. It could be due to contamination of semen with Brucella organism during artificial insemination.

Table 3 shows the correlation between age and reproductive diseases. The highest percentages of reproductive diseases found in cows of 7-8.5 years old followed by the age of 5-6 years. The cows of 2-4 years old had lowest percentages of reproductive diseases. Among the diseased cows, the occurrence rate was more than 71% in crossbred and 91% in local cows of higher age group and 23.8% in crossbred and 8.3% local in cows of 5-6 years old. It is also observed that on average crossbred are most susceptible (63.63%) to reproductive diseases than the local cows (36.37%). This finding has similarities with other studies stated that crossbred cattle are more susceptible to diseases than indigenous cattle[21]. Mukasa-Mugerwa (1991)[22] conducted the study in crossbred and local cows of more than six years old and stated that significantly higher prevalence rate of reproductive problems in crossbred animals than indigenous zebu may be due to the fact that crossbred are less adapted to tropical conditions of high temperature and humidity, disease and low feed quality than zebu cattle making them more susceptible than indigenous zebu. Age and parity had significant effect on the prevalence of reproductive health problems, where increasing of age increases the susceptibility of uterine disease in cows which may be due to decreasing immunity, increasing microbial load, nutritional insufficiency, and increasing reproductive disorders[23-26]. Increasing age generally indicates the increasing number of parity. In this study it is found that increasing age increasing the prevalence of reproductive problems in cows, its may due to more parity give chance to entrance of microorganisms and more scope of contamination during and after parturition.

**Table 2**. *Incidence of reproductive diseases or disorders (n=33)*

| Diseases | Crossbred | Local | % Repr. diseases | % Uterine infection among reproductive diseases | %UI(N=109) |
|---|---|---|---|---|---|
| Endrometritis (UI) | 5 | 6 | 33.33(11) | 33.33 (11) | |
| Pyometra (UI) | 4 | 2 | 18.18(6) | 18.18(6) | |
| Cervicitis with UI | 4 | 1 | 15.15 (5) | 15.15 (5) | |
| Abortion (UI) | 2 | 0 | 6.06 (2) | 6.06 (2) | 22.02 |
| Vaginitis | 0 | 1 | 3.03 (1) | - | |
| Maggot in Vagina | 1 | 2 | 10.00 (3) | - | |
| Bloody discharge | 5 | 0 | 15.15 (5) | - | |
| Total | 21 | 12 | 100 (33) | 72.72 (24) | 22.02 |

**Table 3.** *Reproductive diseases in different age in cows*

| Breed | Age (year) % Reproductive diseases | | | % (n=33) |
|---|---|---|---|---|
| | 2-4 | 5-6 | 7-8.5 | |
| Crossbred | 4.8 (1) | 23.8 (5) | 71.4 (15) | 63.63 (21) |
| Local | - | 8.3 (1) | 91.7 (11) | 36.37 (12) |

Uterine infection was observed in 17 cows (n=89) those were inseminated artificially and in 7 cows (n=20) inseminated naturally. Among the apparently diseased cows were 52% and 21.21% infection was found in those inseminated artificially and naturally, respectively. The result is indicated that prevalence of uterine infection (15.6%) is higher in case of artificial insemination when compared to natural insemination (6.42%) (Table 4). The possible cause may be due to tendency on artificial insemination in crossbred cow and the organisms transmit during insemination.

The organisms including *Fusobacterium spp*, *Streptococcus spp*, *Pseudomonas spp Staphylococcus spp*, *Actinomyces spp*, *Haemophilus spp*, *Micrococcus spp*, *Bacteroides spp*, *Escherichia coli*, *Pasteurella spp*, *Diplococcus spp*, *Peptococcus spp*, *Klebsiella spp*, *Enterobacter spp*, *Bacillus spp*, *Nocardia spp*, *Clostridium spp*, *Salmonella spp* and *Proteus spp* were isolated from uterine sample. Table 5 shows the list of bacteria found in both diseased and non diseased samples, only *Nocardia spp* and *Clostridium spp* were absent in diseased and *Bacillus spp* and *Salmonella spp* were absent in non diseased sample. A significant number of bacteria were identified from diseased samples and they were *Fusobacterium spp* 16 (48.5%) (p=0.000), *Actinomyces spp* 11 (33.33%) (p=0.001), *E. coli* 11 (33.33%) (p=0.002), *Pseudomonas spp* 11 (33.33%) (p=0.003) and *Haemophilus spp* 11 (33.33%) (p=0.001) (Table 5). The isolated of *Pseudomonas* and gram negative minute rod shaped bacteria were obtained only from repeat breeder cows with mucopurulent uterine discharges[27]. *Actinomyces pyogenes* is considered to be a major pathogen of the reproductive tract among others and *Hemophilus spp* is well known to cause uterine infection. Bacterial causes are most important behind endometritis in cows. *Arcanobacterium*, *Staphylococcus*, *Streptococcus*, *E. coli* and *Klebsiella spp* are found to be associated with endometritis in cows[7]. Predominantly identified bacteria in this study were *Staphylococcus spp* and *Streptococcus spp* accounting 10 (33%) and 9 (27.3%), respectively. Presence of other bacteria were between the range of 1 (3.00%) to 6 (18.20%) (Table 5). In a study by Gani (2008)[27] showed that *Staphylococcus spp* was predominant (37.8%), followed by *E. coli* (29.7%), *Pseudomonas* (18.9%) while Gram negative minute rod shaped bacteria was found in 24.3% of cases in repeat breeder cows.

In non-diseased cases, the highest number of samples was positive for *Bacteroied spp*. (13.20%). *Streptococcus spp*. was found in 10.4% of the cases. The similar number of *Diplococcai spp* and *Micrococcai spp* were observed in 9.2% of the cases.

**Table 4**. *Type of insemination and uterine infection*

| Type of insemination | Uterine infection | % Diseased cows (n=33) | % All cows(N=109) |
|---|---|---|---|
| AI (n=89) | 17 | 52.00 | 15.60 |
| NS (n=20) | 7 | 21.21 | 6.42 |

**Table 5**. *Isolation and identification of microorganisms from uterine samples in cow*

| Name ofMicroorganisms | Total Number N= 109 | Group-I (Diseased)(n= 33) | Group-II(Non diseased) (n=76) | P Value |
|---|---|---|---|---|
| *Fusobacterium spp* | 18 (16.51) | 16 (48.50) | 2 (2.63) | 0.000 |
| *Actinomyces spp* | 14 (12.84) | 11 (33.33) | 3 (3.94) | 0.001 |
| *Escherichia coli* | 12 (11.00) | 11 (33.33) | 1 (1.31) | 0.002 |
| *Pseudomonas spp* | 16 (14.70) | 11 (33.33) | 5 (6.57) | 0.003 |
| *Haemophilus spp* | 14 (12.84) | 11 (33.30) | 3 (3.94) | 0.001 |
| *Staphylococcus spp* | 16 (14.70) | 10 (33.00) | 6 (7.89) | ns |
| *Streptococcus spp* | 17 (15.60) | 9 (27.30) | 8 (10.52) | ns |
| *Micrococci spp* | 14 (12.84) | 7 (21.21) | 7 (9.21) | ns |
| *Pasteurella spp* | 11( 10.10) | 6 (18.20) | 5 (6.57) | ns |
| *Enterobacter spp* | 5 (4.60) | 4 (12.12) | 1 (1.31) | ns |
| *Klebsiella spp* | 6 (5.50) | 4 (12.12) | 2 (2.63) | ns |
| *Proteus spp* | 5 (4.60) | 4 (12.12) | 1 (1.31) | ns |
| *Bacteroides spp* | 13 (11.92) | 3 (9.10) | 10 (13.15) | ns |
| *Peptococci spp* | 7 (6.42) | 3 (9.10) | 4 (5.26) | ns |
| *Salmonella spp* | 3 (2.75) | 3 (9.10) | 0 (00) | ns |
| *Diplococci spp* | 8 (7.33) | 1 (3.03) | 7 (9.21) | ns |
| *Bacillus spp* | 1 (0.90) | 1 (3.03) | 0 (00) | ns |
| *Nocardia spp* | 2 (1.83) | 0 (00) | 2 (2.63) | ns |
| *Clostredium spp* | 1 (0.90) | 0 (00) | 1 (1.31) | ns |

p= 0.000,0.001,0.002,0.003and 0.001is statistically significant. ns=not significant

However, on average, the highest numbers of organisms were found in diseased samples and most of those bacteria are pathogenic. About 60% of the identified bacteria from uterus commonly found in repeat breeders as well as from diseased animals and the frequency of the isolates were much higher than that of normal cows[26,27]. Uterine disease is commonly associated with *Escherichia coli, Arcanobacterium pyogenes, Fusobacterium necrophorum* and *Prevotella* species. Indeed, *A. pyogenes, F. necrophorum*, *Prevotella* and *Streptococcus* species have been shown to act synergistically to enhance the likelihood of uterine disease[28]. In another study by Erin (2005)[2], showed that the most common and economically important bacteria for uterine infection are *Actinomyces, Escherichia coli, Fusobacterium, Pasteurella, Pseudomonas* and *Staphylococcus*. From the above discussion it is easily understood that most of the endometrial and pyogenic cases and or uterine infections were caused by *Fusobacterium spp, Actinomyces spp, E. coli, Pseudomonas spp* and *Haemophilus spp. Staphylococcai spp, Streptococcus spp, pasteurella spp, Enterobacter spp, Klebsiella spp* and *Proteus spp.*

## Conclusion

In conclusion, the prevalence of uterine infection is higher in crossbred than local cows. Cows were inseminated artificially suffered more in uterine infection than inseminated naturally. Different pathogenic, non pathogenic, facultative, aerobic and anaerobic bacteria were identified from uterus in dairy cows. However, higher percentages of bacteria were found in diseased samples than non diseased samples. It also observed that older cows are most susceptible to different reproductive disorders and uterine infection than younger.

## Acknowledgement

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