Bacteriological investigation of pyometra of Black Bengal goats obtained at slaughter

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Abstract
Nine uteri of Black Bengal goats (Capra hircus) affected with pyometra were collected from three slaughterhouses at Kishoreganj district of Bangladesh. Both horns of each uterus were washed with phosphate buffered saline for isolation and identification of bacteria and its load. The bacterial loads in the uterus were high, ranging between $1 \times 10^7$ and $2.8 \times 10^7$. Six different bacterial species were identified and confirmed by Polymerase Chain Reaction (PCR). There were five Escherichia coli, six Streptococcus sp., five Staphylococcus sp, one Salmonella sp., one Pasteurella sp. and one Bacillus sp. All had mixed infections containing two or three types of bacterial pathogens. Further studies are needed for the virulence determination and antibiogram profiles. (Bangl. vet. 2019. Vol. 36, No. 1 - 2, 1 – 7)

Introduction
Genital infections in female domestic ruminants are often caused by opportunistic secondary invaders, especially Escherichia coli, which has frequently been isolated in ewes (Manes et al., 2010; Martins et al., 2009; Sargison et al., 2007), goats (Ababneh and Degefa, 2006) and cows (Sheldon et al., 2008). Under stress conditions, these opportunist bacteria may cause genital infection that can lead to reproductive failure in ruminants (Levinson and Jawetz, 1994; Shallali et al., 2001).

Uterus normally remain free from bacterial infection, but can get contaminated during mating and at parturition. Various reproductive disorders and diseases have been reported in Black Bengal goats, which often limit the reproductive performance (Ahmed, 1993; Bhuiyan et al., 1988; Rahman et al., 2010; Roy et al., 2001). Uterine infections, caused by variety of microorganisms, need more attention towards treatment and control. The present study was planned to detect bacteria in uteri of Black Bengal goats at slaughterhouse.

Materials and Methods

Sample collection and processing
A total of 256 female genitalia of Black Bengal goats were collected from three slaughterhouses at Kishoreganj district of Bangladesh during October 2016 to

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December 2018. Those with pyometra were transported to the laboratory under cool conditions. Aseptically, 2 ml of sterile phosphate buffered saline (PBS) was used to wash each horn of each uterus. The wash was collected using sterile syringe.

**Quantification of bacterial loads in the uterus**

The uterine washes were diluted 10-fold and three of such dilutions were streaked over nutrient agar plates (three plates per dilution) and incubated at 37°C for 24h. The number of colonies in the individual plates were counted to calculate the bacterial load, expressed as colony forming units (CFU)/ml.

**Isolation and identification of the bacteria**

Samples were pre-enriched in nutrient broth at 37°C for 24 hours. For pure culture, small amount of pre-enriched culture was placed on an inoculation loop and streaked across the surface of nutrient agar plates. Selected single colonies from nutrient agar plates were streaked into selective agar media: blood (HiMedia, India), MacConkey (HiMedia, India), eosin methylene blue (EMB) (HiMedia, India), *Salmonella-Shigella* (SS) (HiMedia, India), xylose lysine deoxycholate (XLD) (HiMedia, India) and incubated at 37°C for 24 - 48 hours. The colony morphology was recorded. Bacteria from selected colonies were stained with Gram’s stain. Sugar fermentation test, methyl-red (MR) test, Voges–Proskauer (VP) test, catalase test, oxidase test was done for bacterial identification as described by Cowan (1985).

**Molecular detection of bacterial species by PCR**

The molecular identity of *Escherichia coli*, *Salmonella* sp. and *Pasteurella* sp. was confirmed by PCR. Bacterial DNA was extracted using DNA extraction kit (Promega, USA) according to the manufacturer’s instructions. The list of primers used in PCR is shown in Table 1. PCR amplification was performed in a final volume of 25 µl containing 12.5 µl ready master mix (DreamTaq DNA Polymerases, Thermo Scientific, USA), 2 µl (50 ng/µl) of DNA template, 2 µl (100 mM) of each primer, and 8.5 µl nuclease-free water. The thermal profile was initial denaturation at 94°C for 5 min, followed by 35 cycles each consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 50 sec, and final extension at 72°C for 10 min. The PCR amplified products were separated by electrophoresis on 1.5% agarose gel and visualized under UV light in a gel documentation system.

**Table 1: List of primers used in the molecular detection of bacterial species by PCR**

| Pathogens   | Genes | Primer name | Sequence (5’ - 3’ | Amplicon Size (bp) | Reference          |
|-------------|-------|-------------|-------------------|--------------------|--------------------|
| *Escherichia coli* | yjaA | EC_YjaA.1  | TGAAGTGTACAGGAGACGCTG | 211                | Clermont et al., 2000 |
|             |       | EC_YjaA.2  | ATGGAGATGCGTTCCTCAAC |                    |                    |
| *Salmonella* sp. | invA | invA F     | GTGAAATTATCGCGTTCGGCAA | 284                | Rahn et al., 1992  |
|             |       | invA R     | TCATCGCACCAGTCAAGGAACC |                    |                    |
| *Pasteurella* sp. | 16S rRNA | KMT117 | GCTGTAACAGAAGACTGCCCAC | 460                | Townsend et al., 2001 |
|             |       | KMT1SRp6   | ATTCGCTATTTACCCAGTG |                    |                    |
Statistical analysis
Graphs were prepared using GraphPad Prism 5.0 software. To visualize the prevalence of co-occurrences of bacterial pathogens, an UpSetR plot was prepared using an online platform (https://gehlenborglab.shinyapps.io/upsetr/).

Results and Discussion
Detection of bacterial load
The bacterial load in the uterus of Black Bengal goats with pyometra was quantified in nine goats. The loads ranged from $1 \times 10^7$ to $2.8 \times 10^7$ (Fig. 1). The loads in the right horn of B/1, B/4, B/7, B/9, B/11, B/12 samples were $2.7 \times 10^7$, $2.4 \times 10^7$, $1 \times 10^7$, $2.7 \times 10^7$, $2.6 \times 10^7$ and $2.6 \times 10^7$, respectively while in left horns were $2.8 \times 10^7$, $2.4 \times 10^7$, $1.3 \times 10^7$, $2.2 \times 10^7$, $2.4 \times 10^7$ and $2.7 \times 10^7$, respectively. Bacterial loads in the right horn of K/20 and K/24 samples were $2.8 \times 10^7$ and $2.4 \times 10^7$, while in left horns were $2.6 \times 10^7$ and $1.7 \times 10^7$, respectively. Bacterial loads in the right horn of Bt/30 sample were $2.4 \times 10^7$, while in left horn was $2.1 \times 10^7$.

![Fig. 1: Bacterial load in uteri of Black Bengal goats collected from slaughterhouses, and affected by pyometra.](image)

Isolation and identification of bacteria
Out of 256 uteri, 56 (21.9%) showed pathological changes including nine cases (2%) of pyometra. Six bacterial species were identified based on colony characteristics, Gram’s stain and biochemical tests.

All bacterial isolates were further characterized using biochemical tests (Table 2). The *E. coli* fermented all five-basic sugars with production of both acid and gas, catalase, indole and MR positive but VP negative. Both *Staphylococcus* and *Bacillus* isolates fermented all five-basic sugar with formation of acid, catalase, indole and MR positive but VP negative. The *Pasteurella* isolate fermented dulcitol, sucrose and mannitol with production of acid and showed catalase, indole and VP positive but MR negative. The *Salmonella* isolate fermented dulcitol, maltose and mannose with the production of acid and was MR positive. The *Streptococcus* fermented all five basic sugars except mannose with acid production and was MR positive.
Table 2: Biochemical characteristics of the isolated bacteria from uterus of Black Bengal goats

| Sugar fermentation | Catalase | Indole | MR | VP | Bacterial isolates       |
|--------------------|----------|--------|----|----|--------------------------|
| D                  | ML       | S      | L  | MN |                          |
| AG                 | AG       | AG     | AG | AG | +                        |
| A                  | A        | A      | A  | A  | +                        |
| A                  | -        | A      | A  | A  | -                        |
| A                  | A        | -      | A  | -  | +                        |
| A                  | A        | A      | A  | -  | -                        |

Note: D = Dulcitol, ML = Maltose, S = Sucrose, L = Lactose, MN = Mannitol, MR = Methyl Red, VP = Voges-Proskauer, A = Acid, AG = Acid and Gas, '+' = positive, '-' = negative.

The PCR method successfully amplified the target DNA and confirmed the identity of five isolates of E. coli, one of Salmonella sp. and one of Pasteurella sp.

Prevalence of different bacteria in pyometra affected uterus of Black Bengal goats

A total of 19 bacteria belonging to six genera were identified (Table 3). There were five E. coli, six Streptococcus sp., five Staphylococcus sp., one Salmonella sp., one Pasteurella sp. and one Bacillus sp.

Table 3: Types and frequency of bacteria isolated from uteri of Black Bengal goats with pyometra

| Types of bacteria | Number | % (n = 18) |
|-------------------|--------|------------|
| Escherichia coli  | 5      | 27.8%      |
| Streptococcus sp. | 6      | 33.3%      |
| Staphylococcus sp.| 5      | 27.8%      |
| Salmonella sp.    | 1      | 5.6%       |
| Pasteurella sp.   | 1      | 5.6%       |
| Bacillus sp.      | 1      | 5.6%       |
| **Total**         | 19     |            |

Two or three bacterial pathogens were in all nine uteri (Fig. 2). E. coli and Streptococcus sp. were in three uteri. E. coli, Streptococcus sp. and Staphylococcus sp. were in one uterus.

Six different types of bacteria were in uteri with pyometra in Black Bengal goats. Vaginal bacteria get access into the uterus during the peripartum period leading to metritis and endometritis (Levinson and Jawetz, 1994). It is important to identify causal agents with a view to providing remedies.
Fig. 2: An Upsets plot showing co-presence of different bacterial species in the pyometra affected uterus of Black Bengal goats.

The development of uterine disease depends on the immune response of the animals, as well as the species and number of bacteria. Clinical signs of uterine infection vary (Azawi, 2008). A very high bacterial load and at least six bacterial species were detected. The species are well known for their pyogenic properties. Other than the ubiquitous *E. coli*, three different species of *Staphylococcus* sp., *S. aureus*, *S. intermedius* and *S. epidermidis* were reported as major pathogens in the uterus of goats in Pakistan (Rind and Shaikh, 2000). However, further characterization of the bacteria is required to confirm their species and genetic constitution.

The pathogenic organisms may lead to sterility or infertility. All the tested uteri had mixed infection with two or three pathogens. A similar study on uteri of slaughtered goats in Pakistan detected very high (80%) rate of bacterial infections and 11 bacterial species were isolated (Rind and Shaikh, 2000). The present findings corroborate the observations of Tadayon *et al.* (1980) and Talan *et al.* (1989) who detected 2 - 4 species in a single wound sample. Malik *et al.* (1987) studied 395 mucus samples from infertile cattle and recorded mixed infection. A similar trend was encountered by Tadayon *et al.* (1980) who recorded 29.1% mixed infections that contained 2 - 4 different species. Malik *et al.* (1987) detected mixed infections from 64% of uterine mucus samples of infertile cattle.
Conclusions
A high bacterial load in the uteri of Black Bengal goats were detected in slaughterhouse materials. At least six different bacterial species were involved in pyometra, which occurred as co-infection of two or three pathogens. Presence of mixed bacterial species is common in the uterine infection and deserves further study with antimicrobial susceptibility.

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