The Bangladesh Veterinarian (2019) 36(1 - 2): 1 – 7

Bacteriological investigation of pyometra of Black Bengal goats obtained at slaughter

ABMJ Uddin, M Nooruzzaman, T Akter, MAHNA Khan and MM Hossain*
Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

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 1×10^7 and 2.8×10^7. 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

*Corresponding author:- E-mail: mmhossain04@yahoo.com.au

DOI: https://doi.org/10.3329/bvet.v36i1-2.55744

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 60ºC for 30 sec, and extension at 72ºC for 50 sec, and final extension at 72ºC 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 EC_YjaA.2	TGAAGTGTCAGGAGACGCTG ATGGAGATGCGTTCCTCAAC	211	Clermont *et al.*, 2000
Salmonella sp.	*invA*	invA F invA R	GTGAAATTATCGCGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	Rahn *et. al.*, 1992
Pasteurella sp.	*16S rRNA*	KMT1T7 KMT1SRp6	GCTGTAAACGAACTCGCCAC ATCCGCTATTTACCCAGTGG	460	Townsend *et al.*, 2001

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 1×10^7 to 2.8×10^7 (Fig. 1). The loads in the right horn of B/1, B/4, B/7, B/9, B/11, B/12 samples were 2.7×10^7, 2.4×10^7, 1×10^7, 2.7×10^7, 2.6×10^7 and 2.6×10^7, respectively while in left horns were 2.8×10^7, 2.4×10^7, 1.3×10^7, 2.2×10^7, 2.4×10^7 and 2.7×10^7, respectively. Bacterial loads in the right horn of K/20 and K/24 samples were 2.8×10^7 and 2.4×10^7, while in left horns were 2.6×10^7 and 1.7×10^7, respectively. Bacterial loads in the right horn of Bt/30 sample were 2.4×10^7, while in left horn was 2.1×10^7.

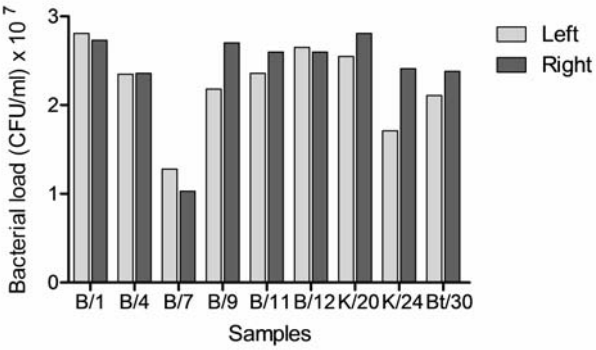

Fig. 1: Bacterial load in uteri of Black Bengal goats collected from slaughterhouses, and affected by pyometra.

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	+	+	+	-	*Escherichia coli*
A	A	A	A	A	+	+	+	-	*Staphylococcus* sp.
A	A	A	A	A	+	+	+	-	*Bacillus* sp.
A	-	A	-	A	+	+	-	+	*Pasteurella* sp.
A	A	-	-	A	-	-	+	-	*Salmonella* sp.
A	A	A	A	-	-	-	+	-	*Streptococcus* sp.

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.

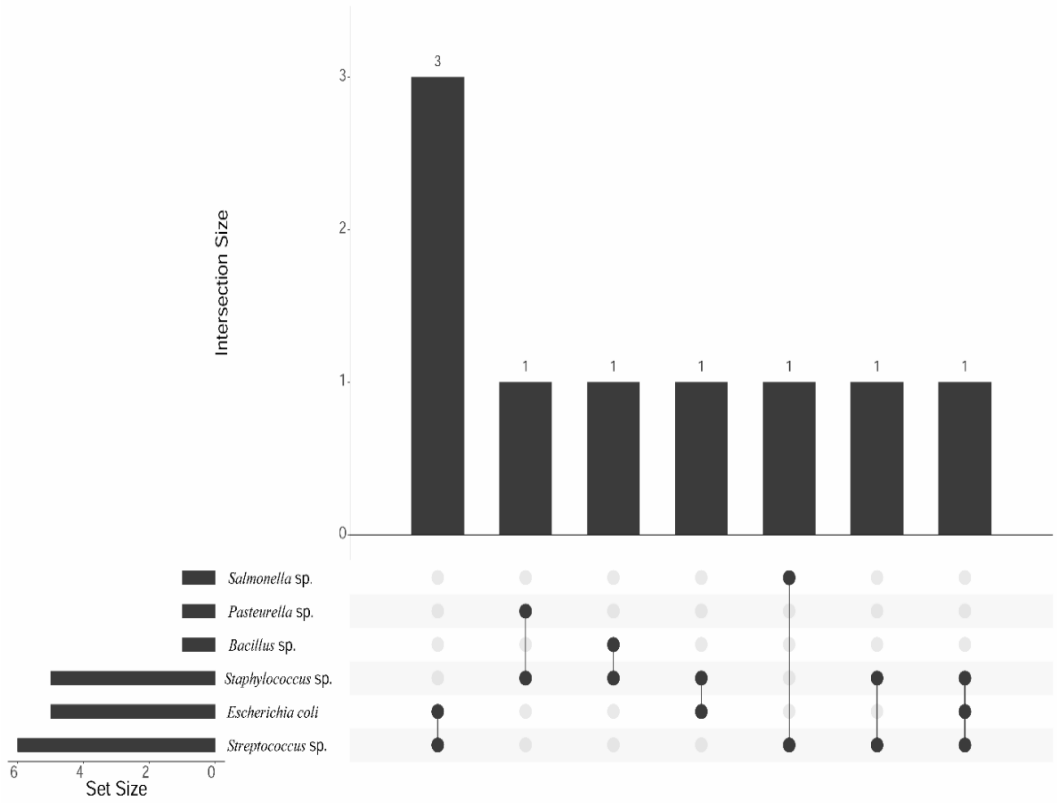

Fig. 2: An Upsets plot showing co-presence of different bacterial species in the pyometra affected uterus of Black Bengal goats.

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.

Acknowledgements

The financial support by Bangladesh Agricultural University Research System (BAURES) is appreciated.

References

Ababneh MM, Degefa T 2006: Bacteriological findings and hormonal profiles in the postpartum Balady goats. *Reproduction in Domestic Animals* **41** 12-16.

Ahmed JU 1993: Incidence of gynaeco-obstetrical disorders in Black Bengal goats. *Bangladesh Agricultural University Research Progress* **7** 499-505.

Azawi OI 2008: Postpartum uterine infection in cattle. *Animal Reproduction Science* **105** 187-208.

Bhuiyan MJ, Hossain MI, Moslehuddin, Shahasuddin M 1988: Female reproductive disorders in Black Bengal goats of Bangladesh. *Bangladesh Veterinarian* **15** 49-50.

Clermont O, Bonacorsi S, Bingen E 2000: Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and Environmental Microbiology* **66** 4555-4558.

Cowan ST 1985: Cowan and Steel's Manual for Identification of Medical Bacteria. 2nd edn, Cambridge University Press, Cambridge, London.

Levinson WE, Jawetz E 1994: Medical Microbiology and Immunology. Third edition. Prentice Hall Int. Inc., Englewood Cliffs, New Jersey, USA. pp. 20-23.

Malik SZ, Chaudhry MA, Nazir A, Rehman N 1987: Different antibiotics in the treatment of uterine infections in repeat breeding cows in Faisalabad. *Pakistan Veterinary Journal* **7** 60-62.

Manes J, Fiorentino MA, Kaiser G, Hozbor F, Alberio R, Sanchez E, Paolicchi F 2010: Changes in the aerobic vaginal flora after treatment with different intravaginal devices in ewes. *Small Ruminant Research* **94** 201-204.

Martins G, Figueira L, Penna B, Brandão F, Varges R, Vasconcelos C, Lilenbaum W 2009: Prevalence and antimicrobial susceptibility of vaginal bacteria from ewes treated with progestin-impregnated intravaginal sponges. *Small Ruminant Research* **81** 182-184.

Rahman MH, Chowdhury EH, Saha SS, Islam A, Alam MGS 2010: Abattoir study of reproductive diseases in goats. *Bangladesh Veterinarian* **25** 88-91.

Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galán JE, Ginocchio C, Curtiss III R, Gyles CL 1992: Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and Cellular Probes* **6** 271-279.

Rind R, Shaikh, SN 2000: Bacteriological studies on the uteri of slaughtered goats. *Pakistan Journal of Biological Sciences* **3** 1944-1947.

Roy BJ, Hossain MM, Chowdhury EH, Biswas PK, Hossain AKMA, Saha S 2001: Etio-pathological investigation of systemic diseases in slaughtered Black Bengal goats. *Bangladesh Veterinary Journal* **35** 67-73.

Sargison ND, Howie F, Mearns R, Penny CD, Foster G 2007: Shiga toxin-producing *Escherichia coli* as a perennial cause of abortion in a closed flock of Suffolk ewes. *Veterinary Record* **160** 875-876.

Shallali AA, Hussein AM, Salih MM, Dafalla EA 2001: A preliminary report on bacteria isolated from the female genital tract of Sudanese sheep and goats. *The Sudan Journal of Veterinary Research* **17** 55-63.

Sheldon IM, Williams EJ, Miller AN, Nash DM, Herath S 2008: Uterine diseases in cattle after parturition. *Veterinary Journal* **176** 115-121.

Tadayon RA, Cheema RH, Muhammed SI 1980: Microorganisms associated with abscesses of sheep and goats in the South of Iran. *American Journal of Veterinary Research* **41** 798-802.

Talan DA, Staatz D, Staatz A, Goldstein EJ, Singer K, Overturf GD 1989: *Staphylococcus intermedius* in canine gingiva and canine-inflicted human wound infections: laboratory characterization of a newly recognized zoonotic pathogen. *Journal of Clinical Microbiology* **27** 78-81.

Townsend KM, Boyce JD, Chung JY, Frost AJ, Adler B 2001: Genetic organization of *Pasteurella multocida* cap Loci and development of a multiplex capsular PCR typing system. *Journal of Clinical Microbiology* **39** 924-929.