Research article

Ecology Journal

ISSN (Print): 2957-4471, ISSN (Online): 2708-6364, Website: https://journal.esbangladesh.org
Ecol. J. (2022) 4 (1) : 19-28

PATHOLOGICAL INVESTIGATION OF REPRODUCTIVE DISEASES OF DOE IN GAZIPUR DISTRICT

Sharmin Alam[1], Md. Taimur Islam[1], Ziban Chandra Das[2], Md. Iqbal Hossain[1], Md. Golbar Hossain[3] and Md. Golam Haider[1*]

[1]Department of Pathobiology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. [2]Department of Gynecology, Obstetrics and Reproductive Health, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. [3]Department of Veterinary Medicine and Animal Science, Rajshahi University, Rajshahi.

***Corresponding e-mail:** ghaider@bsmrau.edu.bd

Received: 30 January 2022, Revised: 21 April 2022, Accepted: 11 May 2022.

ABSTRACT

Goats are considered as one of the most promising animal resources in Bangladesh. Reproductive disorders of does are the major causes of reduced fertility as because estrous, pregnancy and parturition are prerequisites for reproduction. This experiment was carried out to isolate and identify non-specific bacteria causing infections in the reproductive system of does along with investigating the gross and microscopic lesions, and to study the prevalence of reproductive diseases of doe in Gazipur district. The study was performed at Bangabandhu Sheikh Mujibur Rahman Agricultural University from the time period of September 2018 to August 2019. A total of 30 female genitalia was examined for pathological conditions (macroscopic and microscopic lesions). Standard microbiological protocols were used to isolate and identify the bacteria e.g. bacteriological media preparation, different staining techniques, different biochemical tests, sugar fermentation tests, etc. *Staphylococcus aureus*, *Staphylococcus epididermis, Escherichia coli* and *Klebsiella pneumoniae* were isolated from the examined samples. The highest cultural prevalence was found in *Escherichia coli* and *Staphylococcus aureus* assorted infection (93.33%). The most frequently found cases were cystic ovarian diseases and metritis. *E. coli* was frequently isolated bacteria from the case of metritis. The highest lesions was observed in the ovary (83.34%) followed by uterus (66.67%), cervix (43.34%), vagina (26.67%) and fallopian tube (16.67%).

Keywords Diseases, goats, prevalence, bacteria, protocols

Introduction

In Bangladesh the contribution of the livestock sector to the gross domestic product (GDP) is 1.47 % (DLS 2019), while this sub-sector contributes 13% of total foreign currency earning of the country (Ali *et al*. 2011). Goats are economically important and promising animal resources in the developing countries, including Bangladesh. The total goat population in Bangladesh is 30-33 million which are dispersed throughout the country (DLS 2020). In Gazipur district, the number of goat population was 30,848 (BBS 2013). Goat keeping is related to the rural poor and landless people specially women (Chowdhury *et al*. 2002). Each year goat production provides 1,27,000 metric ton (MT) meat, which accounts for 25% of total red meat production in Bangladesh (FAO 2007).

The Black Bengal goats occupy about 60% of the farm households (Chowdhury *et al.* 2015). Goats are multipurpose animals, producing meat, milk, skin, hair and contribute greatly to food, rural employment, and gross domestic product. Genital abnormalities play an important role in animal breeding either by causing infertility or sterility and thus inflict heavy economic losses (Azawi *et al*. 2010). Reproduction of goats is the immediate importance to the goat keepers because of their high fertility rate and prolificacy where they produce much young's in a year (Karim *et al*. 2017). Thus, abnormalities of goat reproductive organs have a significant effect on its fertility rate (Al-Baggal *et al*. 1993). A great deal of reproductive disturbances may accrue from various structural abnormalities of the reproductive organs. Lesions affecting the uterus and ovary greatly contribute

to infertility or sterility in goats, and hence compromise enterprise profitability (Ogunbodede *et al*. 2014). Infectious reproductive diseases of goats are considered as one of the major herd health problems which are manifested through infertility, irregular cycling, abortions, fetal mummification and stillborn or weak offspring. The major reproductive problems include abortions, stillbirths, low or no milk production, uterine infections, delivery problems and kid mortality. Reproductive tract lesions reduce the feed conversion efficacy and thereby reduce milk production and pregnancy rate and forcing the owners to cull the animal (Beena *et al*. 2017).

The female goats suffer from genital pathologies which are predominated by diseases of uterus followed by ovary, cervix, fallopian tubes, and vagina. The pathological lesions of the genital tracts result into infertility or sterility in the does (Beena *et al*. 2015). Considering these constraints the present study was carried out to isolate and identify the bacteria, investigate the gross and microscopic lesions, and the prevalence of reproductive diseases of doe in Gazipur district.

Materials and Methods

The reproductive tracts of does were collected from different slaughter houses of Gazipur city corporation (GCC), Gazipur from the period of September 2018 to August 2019.

Collection of samples: The samples were collected from reproductive tracts of does after slaughter. Total thirty samples were collected for the pathological investigation which includes isolation and identification of non-specific bacteria along-with gross and histopathological study. Swabs were taken from different parts of the genital system of doe (uterine body, right and left uterine horns, ovary, cervix, vagina, fallopian tube) for isolation and identification of bacteria. Gross lesions were observed through dissections and by visual appraisal, palpation and incision. The malformatios of different parts were observed and recorded (Beena *et al*. 2015). Histopathological investigations and bacterial identification were performed in the central laboratory, Veterinary Medicine and Animal Science Faculty, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

Collection of bacteriological samples: Cotton tipped swabs were used after sterilization by autoclaving for the collection of swabs. Swab samples were taken aseptically from different parts of genitalia e.g. uterus, ovary, cervix etc. and kept in screw capped test tubes containing 10 ml of nutrient broth for bacteriological study.

Preservation of samples for histopathology: Ten Percent formalin was used for the preservation of samples after collection in plastic containers for histopathological study.

Media used for bacterial culture: Nutrient agar, Eosin Methylene Blue (EMB) agar, MacConkey agar, Mannitol salt agar, and *Salmonella Shigella (*SS) Agar were used for bacterial culture.

Morphological characterization: The presumptive colonies of different bacteria in various media were characterized microscopically using Gram's stain.

Carbohydrate fermentation test and biochemical test: Five basic sugars such as glucose, sucrose, lactose, mannitol and maltose were used for sugar fermentation tests (Haider *et al*. 2003). Catalase test was performed followed by standard procedure (Haider *et al*. 2003).

Processing of tissues: processing of tissues: Tissue samples collected from different parts of genital organs were collected and fixed in 10% neutral buffered formalin and further processed for histopathological examination.

Photomicrography: Photomicrography was taken using photomicrographic camera (ZEISS AxioCam ERc5s) facilities facilitated by Department of Gynecology, Obstetrics and Reproductive Health of BSMRAU.

Results and Discussion

In the present study, isolation and identification of non-specific bacteria, gross and histopathology were done from different parts of the genital system of doe.

Bacterial species isolated from genital system of does: Four bacterial species were isolated from genital system of does e.g. *Staphylococcus aureus*, *Staphylococcus epididermis*, *Escherichia coli* and *Klebsiella pneumoniae*

(Table 1) The highest cultural prevalence (%) was found in *S. aureus* and *E. coli* mixed infection (93.3%) (Table 1). The cultural prevalence of *K. pneumoniae* was 66.7%. These bacteria were isolated from the case of vaginitis. During this study some mixed bacterial infections were also found in some uteri such as *E. coli* mixed with *S. aureus* and *E. coli* mixed with *S. epididermis*. The cultural prevalence of *E. coli, S. aureus* mixed with *E. coli*, *S. epididermis* mixed with *E. coli*, *K. pneumonia* were 83.3%, 93.3%, 86.7% and 66.7%, respectively. The genital tract of animals normally harbors non-specific bacteria. Under stressful condition such as trauma or other infections they can cause diseases. The prevalence rate was highest in case of *E. coli* infection mixed with *S. aureus* and it was 93.3%

Colony characteristics of isolated bacteria: The isolated *S. aureus* organisms produced gray white colonies on nutrient agar media (Figure 1) and yellow colonies on mannitol salt agar media (Figure 2). The isolated *S. epididermis* organisms produced white colonies on nutrient agar (Figure 3) and pink colonies on mannitol salt agar (Figure 4). *E. coli* produced circular with a convex elevation and a smooth surface on nutrient agar; green metallic sheen on EMB agar (Figure 5); pink colonies on SS agar; pink colonies on MacConkey agar. *K. pneumonia* produced pink colonies on Macconkey agar (Figure 6) but there was no growth on mannitol salt agar. *E. coli* produced green metallic sheen on EMB agar and bright pink colonies on MacConkey agar, which is similar to the findings of Marjan *et al*. (2012). *S. aureus* produced yellowish colonies on Mannitol salt agar and gray white colonies on nutrient agar which resembles to the findings of Brown (1919). *S. epididermis* produced white colonies on nutrient agar, pink colonies on Mannitol salt agar.



Figure 1. Colonies of *Staphylococcus aureus* on nutrient agar.

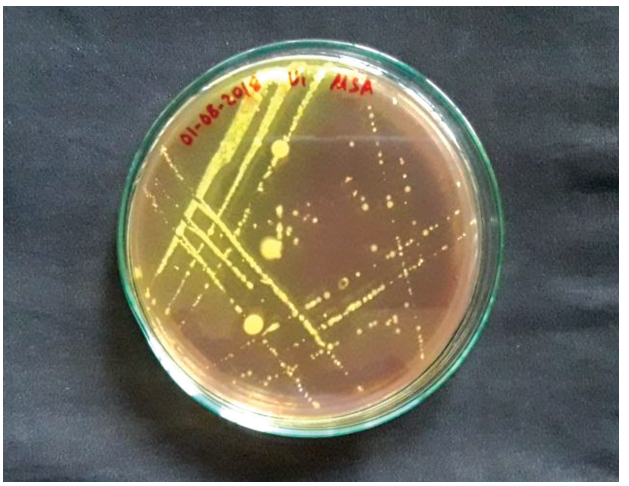


Figure 2. Colonies of *Staphylococcus aureus* on Mannitol salt agar.

Table 1. Cultural prevalence (%) of bacteria in genital system of doe

Sl. No.	Bacteria	Total Samples	Positive	Prevalence (%)
01	*Staphylococcus aureus*+ *E. coli* (mixed)	30	28	93.33
02	*E. coli*		25	83.34
03	*Staphylococcus epididermis*+ *E. coli* (mixed)		26	86.67
04	*Klebsiella pneumoniae*		20	66.67

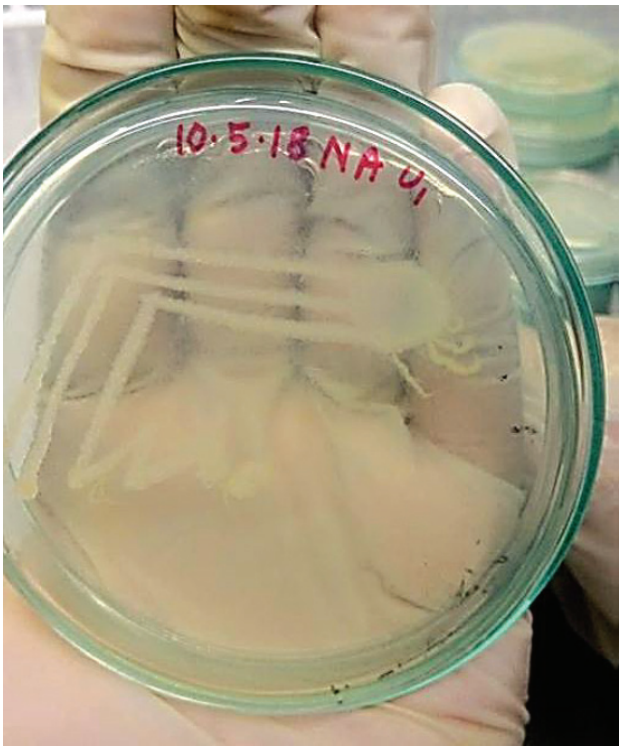


Figure 3. Colonies of *Staphylococcus epididermis* on nutrient agar

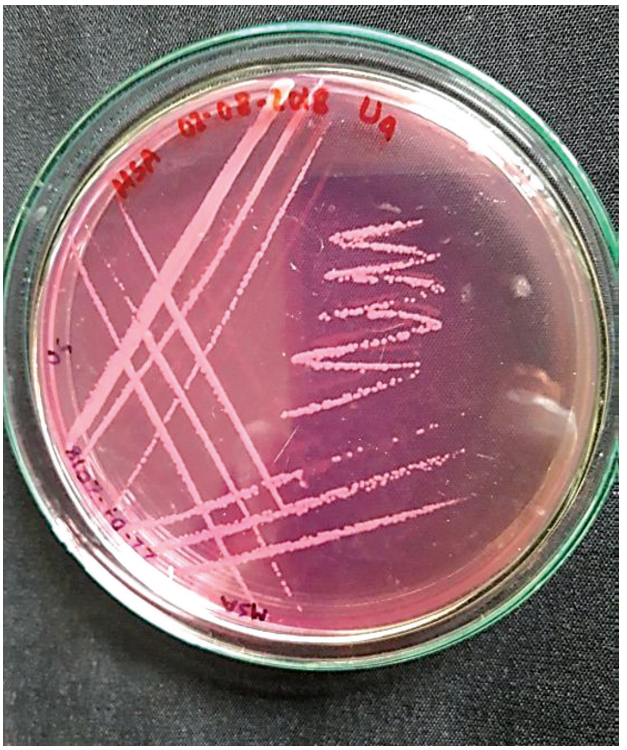


Figure 4. Colonies of *Staphylococcus epididermis* on Mannitol salt agar.

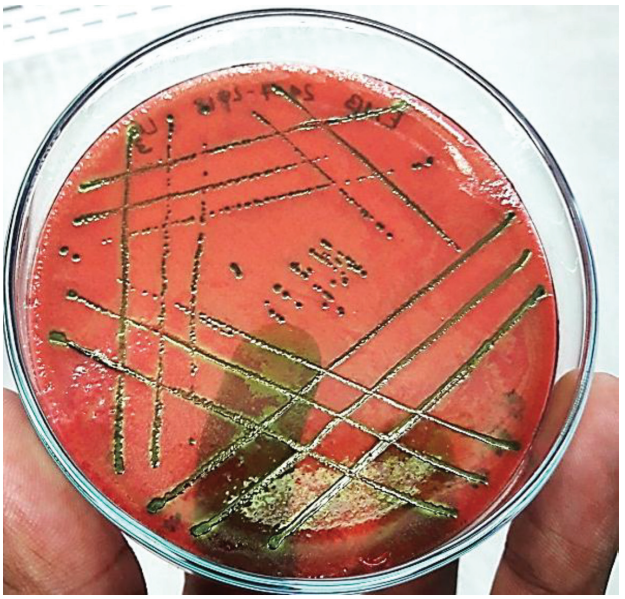


Figure 5. Colonies of isolated *E. coli* on EMB agar.



Figure 6. Colonies of isolated *Klebsiella pneumoniae* subsp. *Pneumonia* on MacConkey Agar. on Macconkey agar.

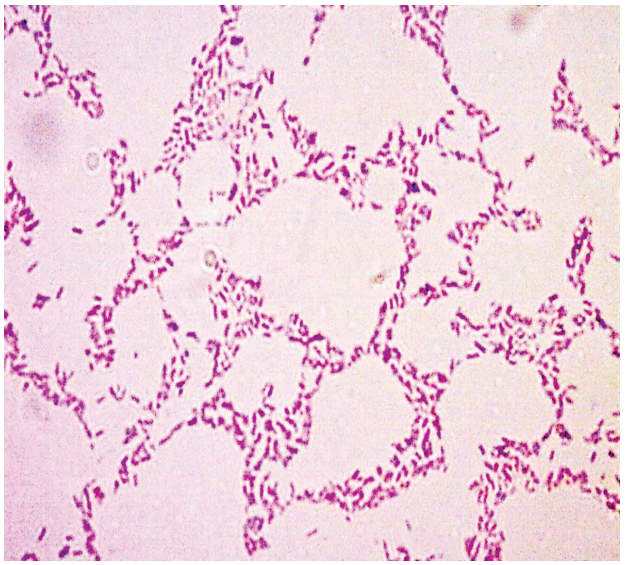


Figure 7. Isolated *Staphylococcus aureus* showed positively stained cocci shaped bacteria(Gram's stain, X100)

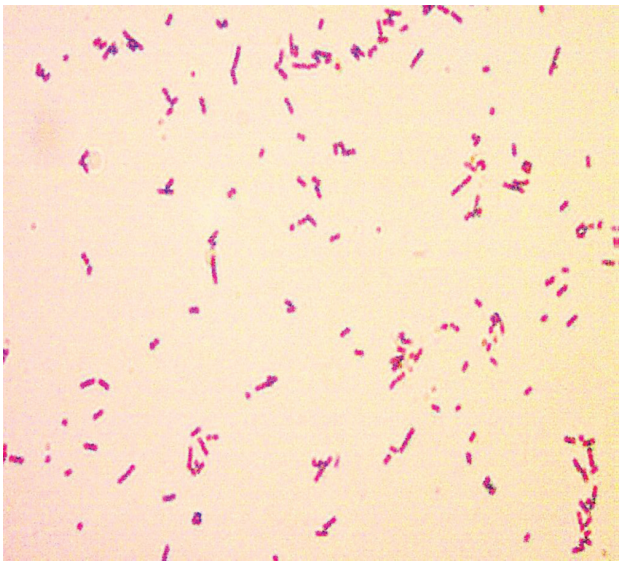


Figure 8. Isolated *E. coli* showed negatively stained bacilli shaped bacteria (Gram's stain, X100)

Morphology of isolated bacteria: *Staphylococcus aureus* organisms were cocci shaped bacteria with clusters in arrangements and Gram's positive. *S. epididermis* were cocci shaped organisms, paired in arrangements and Gram's positive in reaction (Figure 7). *E. coli* were bacilli shaped organisms; single and paired in arrangements and Gram's negative in reaction with Gram's staining (Figure 8). *K. pneumoniae* were rod shaped bacteria which appeared single, in short chains and Gram's negative (Figure 9). The morphology of isolated *E. coli* was rod shaped and Gram's negative which corresponds to the findings of Zinnah *et al*. 2007. *Staphylococcus* was cocci shaped with clusters in arrangements and Gram's positive that was similar to the finding of the others (Thaker *et al.* (2013). Chesbrough, M. (2002) reported the similar morphology of *K. pneumoniae*.

Biochemical characteristics of isolated bacteria: The isolated *Staphylococcus aureus* organisms were catalase positive. The isolated *S. epididermis* organisms were catalase positive (Figure 10). The isolated *S. aureus* and *S. epididermis* organisms were catalase positive. They were catalase positive and fermented mannitol, maltose, and sucrose but did not ferment arabinose. This result was similar with the findings of Thaker HC *et al*. (2013).

Sugar fermentation tests of isolated bacteria: *Staphylococcus aureus* fermented mannitol, maltose and sucrose but did not ferment arabinose. *S. epididermis* fermented maltose and sucrose but did not ferment mannitol and arabinose. *E. coli* fermented mannitol, maltose, sucrose, dextrose and lactose and produced gas. *K. pneumoniae* fermented D-glucose, maltose, sucrose, L-arabinose, lactose and D-mannitol (Figure 12). *E. coli* fermented five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol that was indicated by acid and gas production and this result was similar to that of Cheesbrough (2000). *S. epididermis* were catalase positive and fermented maltose and sucrose but did not ferment mannitol and arabinose, which corresponds with the findings of Al-Sariy (2014). The similar results of *K. pneumoniae* were reported by others (Cheesbrough 2000)

Prevalence (%) of overall lesions in different parts of doe genitalia: In the present study the gross and histopathological lesions in the reproductive organs of doe were recorded. A total of 30 samples were collected. Among them the prevalence of ovarian lesions was 83.3%. The predominant lesions in the ovary detected were follicular cysts followed by excessive growths on the surface of ovary, haemorrhages. Prevalence of uterine lesions was 66.7%. Metritis cases were predominant. Caruncular haemorrhages were found with intrauterine

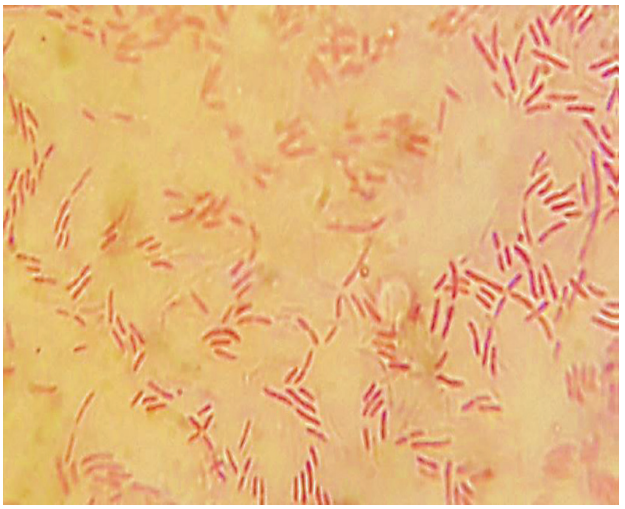


Figure 9. *Klebsiella pneumoniae* subsp. *pneumonia* showed rod shaped negatively stained bacteria arranged in short chains (Gram's stain, X100).

foetal death. Prevalence of cervical lesions was 43.3%. Prevalence of lesions in the vagina and fallopian tubes were 26.7% and 16.7%, respectively.

E. coli is a bacterium which lives in the intestine normally. However certain strains of *E. coli* are pathogenic which can cause diseases. It can invade the genitalia of doe easily through fecal contamination and can cause urogenital infections (UTI). This corresponded to Ababneh *et al*. (2006). *K. pneumoniae* is a bacterium that lives as a normal flora and does not cause any infection but if they can get entrance into other areas like genital system it can cause infections.

Gross lesions: Follicular cysts were found in the ovaries which were measured larger than 1.2 cm in diameter. A number of the uteri were found filled with pus. Excessive growths were found on the surface of ovary in few genitalia of doe among the examined samples (Figure 13). Haemorrhages were found on the cervix (Figure 14). The most frequent gross lesions observed were cysts in the ovary such as follicular cystic ovary. This was similar to other findings (Karim *et al*. 2017, Beena *et al*. 2015). Excessive growths were observed on the surface of some ovaries. Haemorrhages were found in the cervix and vagina. Some uteri were found pus filled that corresponded to Rind *et al*. (2000). The study revealed several significant pathological conditions in the reproductive organs of does.



Figure 10. *Staphylococcus aureus* showed catalase positive reaction.

The predominant lesions observed in the ovary (83.3%) followed by uterus (66.7%), cervix (43.3%), vagina (26.7%) and fallopian tube (16.7%). Follicular cysts were the most prevalent lesions observed in the ovary. Cystic ovarian disease is considered as an important cause of reproductive failure in the female goats. In the present study, few cases of metritis were found from where frequently isolated bacteria were *E. coli* and in some cases *E. coli* were isolated mixed with *S. aureus*. The findings of this study were almost similar with that of Msheila *et al*. (2014) whereas the authors found *E. coli* mixed with *S. aureus* as a cause of endometritis (Haider *et al.* 2003).

Histopathological lesions: In the section of follicular cystic ovary fibrous connective tissue and haemorrhages were found surround the cysts (Figure 15). Nucleus fragmentations were found on the ovarian section where excessive growths were observed on the ovary grossly (Figure 16). Fibrin accumulation and inflammatory cells were found in the section of uterus in the case of metritis (Figure 17). Glandular degeneration and haemorrhages were found in the section of uterus. Inflammatory cell accumulation and haemorrhages were found in the serosa of uterus (Figure 18). In the section of cervix there was

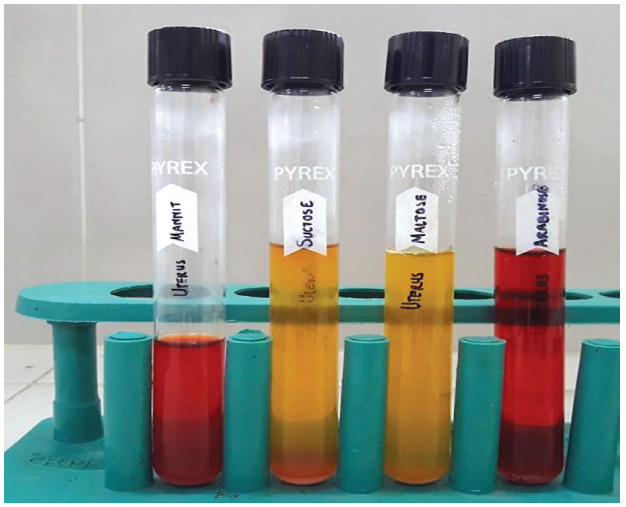


Figure 11. *Staphylococcus epididermis* fermented Sucrose and Maltose but did not ferment Mannitol and Arabinose

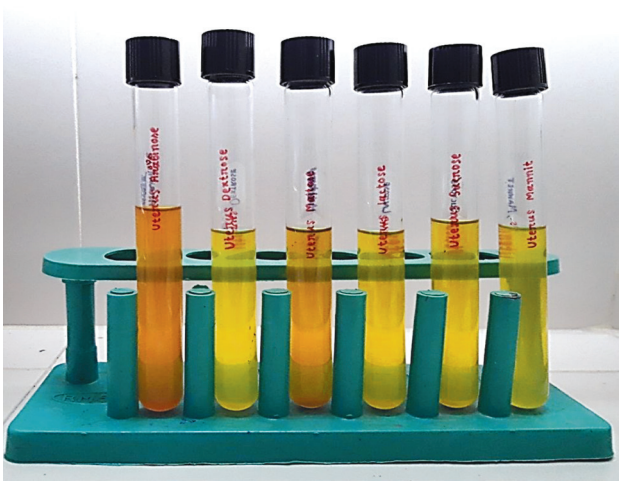


Figure 12. *Klebsiella pneumoniae* subsp. *Pneumonia* fermented Arabinose, Glucose, Lactose, Maltose, Mannitol and Sucrose.



Figure 13. Ovary with large follicles (follicular cyst).



Figure 14. Pus filled uterus.



Figure 15. Excessive growth on the ovary indicated by arrow marks

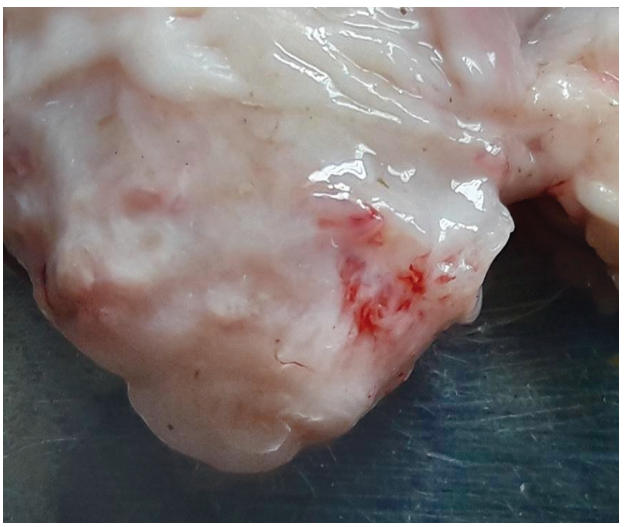


Figure 16. Cervical haemorrhages.

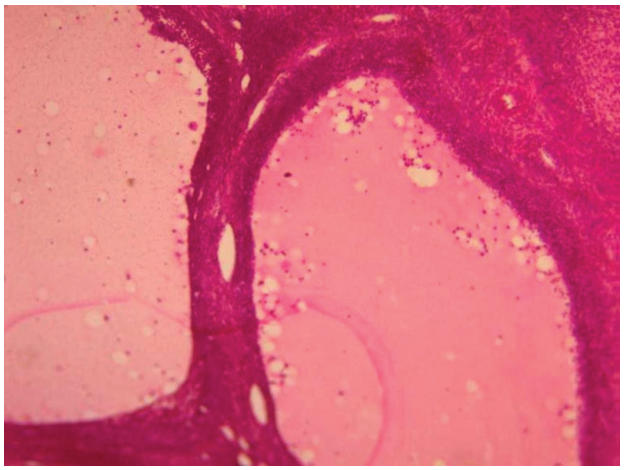


Figure 17. Accumulation of fibrous connective tissue and haemorrhages surround the follicularcyst in the section of ovary (H and E, X100).

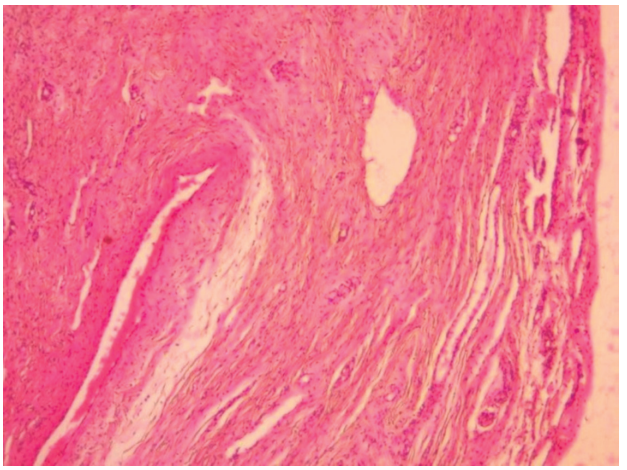


Figure 18. Fibrin deposition and inflammatory cell accumulation in the section of uterus (H and E, X100).

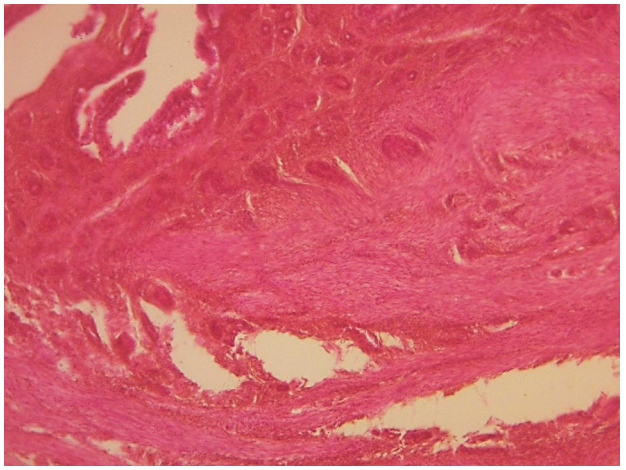


Figure 19. Proliferation of fibrous connective tissue and infiltration of inflammatory cells in the section of cervix (H and E, X 100)

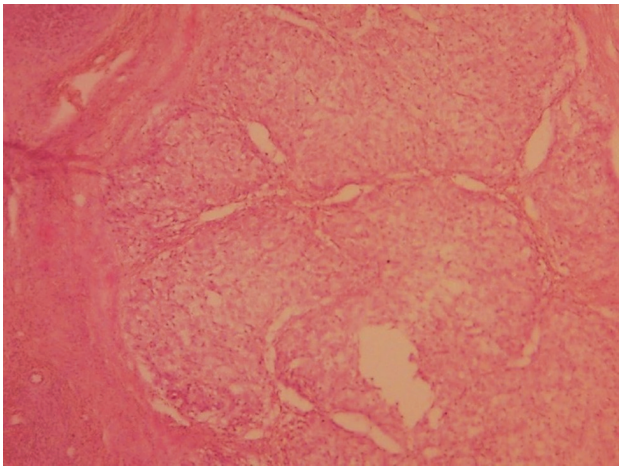


Figure 20. Infiltration of inflammatory cells and fibrosis in the section of vagina (H and E, X 100)

proliferation of fibrous connective tissue and infiltration of inflammatory cells. Glandular epithelium degeneration and infiltration of inflammatory cells were also found in the section of cervix. Infiltration of inflammatory cells and fibrosis was observed in the section of vagina (Figure 19). Haemorrhages were found in the section of vagina. In a pregnant uterus caruncular haemarrhages were observed with huge accumulation of neutrophils in the section of uterus (Figure 20). Glandular degeneration and infiltration of neutrophils were observed in the section of fallopian tube.

In the section of follicular cystic ovary the cysts were surrounded by fibrous connective tissues and haemorrhages which resemble to the findings of Karim *et al*. (2017). Fragmentations of nucleus were found in the ovarian section where excessive growths were observed grossly. Accumulation of fibrin and inflammatory cells were found in the section of uterus in the case of metritis and this finding resembles to Beena *et al*. (2015). Glandular degeneration and haemorrhages were found in the section of uterus which was corresponded to the finding of another author Mutebi (2009) in the metritis cases. In the section of uterus inflammatory cell accumulation and haemorrhages were found in the serosa. Proliferation of fibrous connective tissue and infiltration of inflammatory cells were found in the section of cervix in case of cervicitis and the finding is similar to Beena *et al*. (2015). Glandular epithelium degeneration and infiltration of inflammatory

cells were found in the section of cervix. Infiltration of inflammatory cells, fibrosis and haemorrhages were found in the section of vagina and that was the case of vaginitis. Some gravid uteri were found during the investigation. In the histopathological section of embryo caruncular haemarrhages were found with huge accumulation of. So, it can be concluded that there was infection in the embryo or any diseased condition in the doe during pregnancy. Glandular degeneration and infiltration of neutrophils in the section of the fallopian tube were found.

Conclusion

Escherichia coli, *Staphylococcus aureus*, *Staphylococcus epididermis* and *Kleibsiella pneumoniae* were involved in causing various pathological conditions of female reproductive organs of goats. Haemorrhages were observed in the different parts of genitalia. Fibrous connective tissue formation, haemorrhages, follicular cystic ovary were found most commonly. Fibrin accumulation and inflammatory cells were found in the section of uterus in case of metritis. Glandular degeneration and infiltration of neutrophils were detected in the section of fallopian tube in some cases. Caruncular haemarrhages were observed with accumulation of large number of neutrophils in the section of an embryo. The predominant reproductive diseases of doe were cystic ovarian diseases. There are several reasons behind the cystic ovarian diseases such as nutritional conditions, hormonal imbalance, environmental factors, etc. Reproductive diseases were most prevalent in the ovaries (83.34%) of doe genitalia compared to the other parts. Pathological lesions and bacterial infections in the genital tract result into repeat breed, infertility and sterility among female goats. Reproductive diseases hinder the normal fertility rates of doe. So, regular monitoring and sampling will pave the way for successful goat farming by taking essential preventive measures to cope with those diseases and will be helpful for proper diagnosis and treatment of those conditions.

References

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

Al-Baggal HAR, Al-Dahash SYA and Alwan AF. 1993. Macroscopic study of the female genital system in Iraqi goats. Small Ruminant Research, *9*: 341-346.

Ali MH, Bhuiyan MKJ and Alam MM. 2011. Retrospective epidemiologic study of diseases in ruminants in Khagrachari district of Bangladesh. Bangladesh Journal of Veterinary Medicine, *9*: 145-153.

Al-Sariy SM. 2014. Isolation and identification of some aerobic bacterial flora from female genitalia in goats in Babylon city. Journal of Agriculture and Veterinary Science, *7*: 19-22.

Azawi OI, Al-Abidy HF and Ali AJ. 2010. Pathological and bacteriological studies of hydrosalpinx in buffaloes. Reproduction in Domestic Animals, *45*: 416-420.

Beena V, Pawaiya RVS, Shivasharanappa N, Gururaj K, Gupta VK, Gangwar NK and Kumar A. 2015. Occurrence of pathological conditions in the female genitalia of goats. Indian Journal of Veterinary Pathology, *39*, 197.

Beena V, Pawaiya RVS, Gururaj K, Singh DD, Mishra AK, Gangwar NK, Gupta VK, Singh R, Sharma AK, Karikalan M, Kumar A. 2017. Molecular etiopathology of naturally occurring reproductive diseases in female goats, Veterinary World, *10*: 964-972.

Brown JH. 1919. The use of blood agar for the study of streptococci. Rockefeller Institute for Medical Research. Pp. 9-10.

Cheesbrough M. 2000. District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press. UK. Pp 64-65.

Chowdhury SA, Bhuiyan MSA and Faruk S. 2002. Rearing Black Bengal goat under semi-intensive management physiological and reproductive performances. Asian-Australasian Journal of Animal Sciences, *15*: 477-484.

DLS (Department of Livestock Services). 2020. Annual Report 2020. http://dls.portal.gov.bd/sites/default.

FAO 2007. FAO production Year Book, Food and Agricultural organization of the USA, Rome, Italy. Vol 51.

Haider MG, Chowdhury EH, Khan MAHNA, Hossain MT and Rahman MS. 2008. Experimental pathogenesis of pullorum disease with the local isolate of *Salmonella enterica* serovar. *enterica* subspecies pullorum in pullets in Bangladesh. Korean Journal of Poultry Science, 35: 341-350.

Karim MR, Faraidoon A and Muhammad SG. 2017. Histopathological study of the genitalia in goats: 1. Ovaries. Journal of Veterinary Science & Medicine, *5*: 4.

Marjan S, Kanta DK, Kishore MS and Noor R. 2014. Drug-resistant bacterial pathogens in milk and some milk products. Nutrition & Food Science, *44*: 241-248.

Mshelia GD, Okpaje G, Voltaire YAC and Egwu GO. 2014. Comparative studies on genital infections and antimicrobial susceptibility patterns of isolates from camels (*Camelus dromedarius*) and cows (*Bos indicus*) in Maiduguri, north-eastern Nigeria. Springer Plus, *3:* P. 91.

Mutebi F. 2009. Caprine ovarian and uterine lesions: An abattoir survey Doctoral dissertation, Makerere University, Kampala, Uganda.

Ogunbodede MA, Oladele GM, Ode OJ and Ubah SA. 2014. Survey of gross abnormalities and microbial load on the female reproductive tract of Maradi goats slaughtered at Bodija Abattoir, Nigeria. Advanced Journal of Agricultural Research*, 2*: 1-7.

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

Thaker HC, Brahmbhatt MN and Nayak JB. 2013. Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat, Veterinary World, *6*:10-13.

Haider MG, Hossain MG, Hossain MS, Chowdhury EH, Das PM and Hossain MM. 2003. Isolation and characterization of enterobacteria associated with health and disease in sonali chickens. Bangladesh Journal of Veterinary Medicine, *2*: 15-21.

Zinnah MA, Bari MR, Islam MT, Hossain MT, Rahman MT, Haque MH, Babu SAM, Ruma RP and Islam MA. 2007. Characterization of *Escherichia coli* isolated from samples of different biological and environment sources. Bangladesh Journal of Veterinary Medicine, 5: 25-32.