

## Pathological investigation and identification of bacteria from bovine uterus

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### ABSTRACT

The present study was carried out to determine the occurrence of bacteria as well as pathological lesions in the uterus of slaughtered cattle. A total of 20 selected samples from 30 randomly collected samples were taken from the uteri with grossly identifiable lesions such as congested, hemorrhagic, cystic and mucus filled uteri. The used methods were cultural technique, staining procedure and different types of biochemical test and histopathology. The bacteriological swab samples were collected from the lumen of the uteri in nutrient broth. The histopathological samples were collected in 10% neutral buffered formalin. The collected tissues were fixed, processed, sectioned, stained and studied with light microscope. The mostly observed gross lesions were hemorrhagic, congested and mucus filled uterus with some abnormalities on the ovary. All the gross lesions were not found in all uteri. The occurrence of bacteria 22.92% *Bacillus* spp, 12.50% *Streptococcus* spp, 25.00% *Staphylococcus* spp, 31.11% *Escherichia coli* and 10.417% *Klebsiella* spp. were recorded in 20 uteri of cattle. Microscopically, acute endometritis was characterized by thickening of the epithelial layer, infiltration of neutrophil in the submucosal layer, dilatation of endometrial glands and congestion of blood vessels. In chronic endometritis, infiltration of lymphocytes, macrophages, plasma cells in the sub mucosal layer and proliferation of fibrous connective tissue within the myometrium. In adenomyosis, dilatation of endometrial glands and presence of endometrial gland in the myometrium were recorded. The microscopic lesions were variable in uteri. The occurrence of acute endometritis was 33.33%, chronic endometritis 26.66% and adenomyosis 6.66% respectively. In acute and chronic endometritis, the isolated bacteria were *Bacillus* spp, *Streptococcus* spp, *Staphylococcus* spp, *Escherichia coli* and *Klebsiella* spp. while in adenomyosis cases the isolated bacteria were *Bacillus* spp, *Staphylococcus* spp and *Escherichia coli*. To understand the role of these bacteria in the production of pathological lesions further studies are needed.

### INTRODUCTION

The cattle are the important source of meat and milk in our country. In our country, cattle occupy the second position in livestock population. It plays an important role in the rural economic and export trades of Bangladesh. High reproductive efficiency is very much important for achieving the maximum return from this animal. Research on uterus of cattle has got paramount important from the standpoint of national development by increased production of animals. Any abnormality in reproductive system may interrupt animal production. In cattle, the infectious diseases in uterus as

metritis, endometritis etc. in long standing cases cause infertility (Roy, 2001).

Bacterial contamination of the uterine lumen is common in cattle after parturition, often leading to infection and uterine disease. Clinical disease can be diagnosed and scored by examination of the vaginal mucus, which reflects the presence of pathogenic bacteria such as *Escherichia coli* and *Arcanobacterium pyogenes*. The infertility associated with uterine disease is caused by damage to the endometrium and disruption of ovarian cyclic activity. Bacteria modulate endometrial prostaglandin secretion, and perturb ovarian follicular growth and function. Understanding the molecular basis of uterine

disease will lead to novel approaches to treat infertility (Sheldon et al. 2008).

Many authors have indicated that failure to breed is an important reason for discarding animals from herds, particularly of dairy cattle. For example, Beynon and Howe (1974) suggested a value of 35% of all discards in a representative sample survey. David et al. (1971) claimed that probably few cows remain infertile permanently, or for long periods - they had observed only 8 % of genital abnormalities visible to the naked eye in random abattoir surveys. Little information has been published about the extent of the effort made by an owner to obtain breeding of his animals before discarding them. Donaldson (1971) conducted a small trial with beef cows that were not pregnant at the end of the breeding season; 18 of the 38 cows had normal embryos after being kept with a bull for 39 days. In a study of 41 animals which had been discarded as infertile from 6 dairy farms over a 5-year period, in another study found that 12 out of 21 nulli- and primiparous cattle conceived readily, while only 2 of 20 multiparous cows did so. Twelve of the mature cows had chronic inflammation of the genital tract.

Uterine bacterial flora was studied by swabbing, culturing and identifying bacteria in 502 post-slaughter bovine uteri of which 291 (58.0%) were nonpregnant and 208 (41.4%) were pregnant. Fetal maceration was found in two (0.4%) uteri whereas fetal mummification was found in one (0.2%). *Streptococci*, coliforms, *Proteus*, *Klebsiella*, *Staphylococci*, *Bacilli* and *Micrococci* were isolated from the endometrium and fetal fluid. Coliforms formed the largest proportion (41.3%) of the uterine bacterial flora and dominated both the endometrium (38.7%) and fetal fluid (46.6%). Although pregnancy significantly ( $P < 0.05$ ) influenced bacterial isolation, the site of swabbing and follicular activity had no influence ( $P > 0.05$ ) on bacterial isolation. Organs with corpora lutea (CL) especially the nonpregnant and early pregnant (up to 90 days) had the highest frequency of bacterial isolation. Coliforms were isolated from the two organs with fetal maceration while no bacteria were isolated from the only organ with mummification. These bacteria, especially coliforms, are the major uterine bacterial flora of

indigenous cattle of Uganda (Owiny and Acon 1998).

There is no alternate of fertility, however reproductive disorders causes infertility ultimately leading to substantial economic losses through increased calving intervals, loss of milk production and culling of useful breeding animals. Specific and non-specific infectious agents during pre and postpartum periods frequently invade the uterus and produces metritis and endometritis. The isolation of microorganisms along with histopathological studies of uterus after slaughtering is known to be of paramount importance in the diagnosis and prognosis of the infection. A few studies have been performed in Bangladesh on bovine uteri of slaughtered cows (Roy, 2001), (Rahman et al. 2002), (Gani et al. 2008), (Rahman et al. 2008), (Pariza et al. 2009). Therefore, the present research has been undertaken to find out the uterine bacterial organisms associated with different types of histopathological changes in slaughtered cows.

## MATERIALS AND METHODS

### Sample collection and examination

Grossly identifiable lesions such as congested, hemorrhagic, cystic and mucus filled uteri from slaughtered cattle of different ages from kewatkhali region and district slaughter house of Mymensingh sadar during July to December, 2009 used for this investigation.

Aseptically swabs were collected from lumen of uteri in nutrient broth for bacterial isolation and identification. Isolation and identification of different organisms were performed by culturing, staining, different biochemical (carbohydrate fermentation, Methyl Red and Voges-Proskauer (MR-VP), indole test) tests and enzyme activity (catalase test) test.

For histopathology, the samples were collected and fixed in 10% buffered formalin. After fixation the samples were trimmed and again fixed for 1-2 days. Then processing of samples (processing, embedding, sectioning etc.) was done. The processed samples were collected on slides. Staining with routine H & E stain was performed

following routine procedure. Finally different pathological changes in tissue level were studied under microscope available in the Department of Pathology.

### Isolation and identification of organisms

A total of 20 selected samples were collected directly from 20 uteri of cattle (at different ages) for bacteriological isolation in nutrient broth. These samples were carried in cool box with ice to the Department of Pathology, BAU, Mymensingh. Test tubes containing samples were incubated for 72 hours at 37°C. Grossly identifiable lesions such as congestion, hemorrhage, cystic and mucus filled uteri of collected samples from Kewatkhali and district slaughter house of Mymensingh sadar were preserved for histopathology in plastic pot containing 10% neutral buffered formalin.

### Culture of organisms

From the nutrient broth 20 uteri swab samples were placed in nutrient agar plate and incubated for overnight at 37°C for the growth of the organisms. All samples were subcultured in nutrient agar, MacConkey agar, EMB agar and Blood agar. A small amount of inoculums from the nutrient agar were spread into different culture media and incubated at 37°C for overnight. The identification of the organisms were carried out by the different colony morphology, staining character and biochemical tests as described by Merchant et al. 1976, Buxton et al. 1977, Cheesbrough 1985, Granum 2001, Brooks et al. 2002, Stocki et al. 2002, Naowarat 2007. On the basis of colony, staining characters, and biochemical tests, the organisms were isolated and identified.

### Morphological characterization

Microscopic study was done for all 20 uterine swabs samples after primary culture and subculture. Gram's staining was performed as per recommendation of Hucker and Conn (1923); Merchant and Packer (1967). Leishman's staining was performed for 4 samples. With the inoculation loop, a small amount of bacterial colony was taken on clean glass slide. One drop of water was added

then it was mixed properly. Later, the slide was first air dried and then fixed with gentle heat.

### Biochemical test

Biochemical (carbohydrate fermentation, Methyl Red and Voges-Proskauer (MR-VP), indole test) tests and enzyme activity (catalase test) test was performed as described by Cowan (1985).

### Histopathology

The collected uterine samples from slaughtered cows were selected for histopathological study. The formalin fixed tissues were trimmed with 1.5 cm × 1 cm size. Then the tissues were processed, sectioned and stained following standard procedure (Luna 1968). Specific samples containing lesions from each group were used in histopathological study. Hematoxylin & Eosin staining was performed and Photomicrography was taken at the Department of Pathology using photomicrographic camera (Olympus PM-C 35 Model) fitted with Olympus microscope (Olympus, Japan).

## RESULTS

### Gross changes in the uterus of slaughtered cattle

All the 20 samples showed congestion and hemorrhage on the serosal surface. In addition to this, the most consistent gross changes were found in uterus that were mucus filled lumen of uterus and hemorrhage in the luminal surface of uterus. However gross lesions in all samples were slightly variables.

### Isolation and identification of different bacteria

#### *Bacillus* spp

According to the colony characters, staining character and biochemical test of *Bacillus* spp. the colonies were white, dull, dry and undulating on nutrient agar (Fig. 1). The organism produced haemolysis on blood agar (Fig. 2) and no growth on EMB agar (Fig. 3) *Bacillus* spp. was gram positive cylindrical rod, straight or slightly curved with rounded ends, present in singly or in chain

(Fig. 4). *Bacillus* spp. fermented dextrose, maltose, and sucrose with production of acid. The organism cannot ferment lactose and mannitol (Fig. 5); they were positive for MR and negative for VP test (Fig. 6). In catalase test the organism showed positive for *Bacillus* spp. (Fig. 10).

### ***Streptococcus* spp.**

According to colony characters, staining character, biochemical test and enzymatic activity test *Streptococcus* spp. formed small, white, hard dew drop-like colonies on nutrient agar (Fig. 7) and haemolysis on blood agar (Fig. 8). *Streptococcus* spp. was gram positive cocci remained in pairs and had short to long chains (Fig. 9) and showed catalase negative (Fig. 10).

### ***Staphylococcus* spp**

In colony the organism formed yellow cream color large, smooth, glistening and opaque colonies on nutrient agar (Fig. 11) and clear zones of hemolysis in blood agar plate (Fig. 12) indicating *Staphylococcus* spp. *Staphylococcus* spp. was gram positive cocci and arranged in grape like clusters stained with Gram's staining method of staining (Fig. 13). In biochemical tests *Staphylococcus* spp. could ferment dextrose, maltose, lactose, mannitol and sucrose (Fig. 14); they were positive for MR and negative for VP test and produced indole (Fig. 15). *Staphylococcus* spp. showed catalase positive (Fig. 16).

### ***E. coli*.**

*E. coli* produced smooth circular colonies with dark centers and metallic sheen on EMB agar (Fig. 17), pink to rose red colonies-lactose fermenting colonies on MacConkey agar (Fig. 18). In staining *E. coli* was short rod, varying from coccoid bipolar shapes to long filamentous forms. It occurred singly or short chains. It was gram negative (Fig. 19). *E. coli* fermented dextrose, lactose, maltose and mannitol with the production of acid and gas (Fig. 20). The organisms were MR positive, VP negative and produced indole (Fig. 21). *E. coli* showed catalase positive (Fig.16).

### ***Klebsiella* spp**

*Klebsiella* spp. produced purple colored colonies, no metallic sheen in EMB agar (Fig. 22). *Klebsiella* spp. were gram negative, short rod shaped bacilli in gram staining method (Fig. 23). *Klebsiella* spp. produced acid and gas in sucrose, lactose, dextrose, maltose and mannitol (Fig. 24). They were positive for VP reaction, indole negative and MR negative (Fig. 25).

### **Occurrence of different bacteria isolated from uterus**

The occurrence of bacteria in uterus (20 swabs sample) 22.92% *Bacillus* spp, 12.50% *Streptococcus* spp, 25.00% *Staphylococcus* spp, 31.11% *Escherichia coli* and 10.417% *Klebsiella* spp. (Table 1).

**Table 1:** Represents the overall occurrence of bacteria isolated from bovine uterus (n=20)

Sl. No.	Isolated Organisms	Number of positive case	% of positive case	% of isolates
1	<i>Bacillus</i> spp.	11	55	22.92
2	<i>Streptococcus</i> spp.	6	30	12.50
3	<i>Staphylococcus</i> spp.	12	60	25.00
4	<i>Escherichia coli</i>	14	70	31.11
5	<i>Klebsiella</i> spp.	5	25	10.41

### **Gross pathology**

All the 20 samples showed hemorrhage on the serosal surface (Fig. 26). In addition to this, the most consistent gross changes were found in uterus that were congestion, mucus filled in the

lumen of uterus. Some abnormalities were also found on the ovary. Small cyst like structure was found on the ovary, somewhat hemorrhagic CL was also found on the ovary.

### **Histopathological study**

All the 20 uteri showing the lesions of congestion, hemorrhage, cystic and mucus filled uteri were collected and processed for histopathology. Microscopically, there were hyperplasia of epithelial layer, somewhere distortion of the epithelial layer, glandular hyperplasia was also observed and the blood vessels were congested. A large number of endometrial glands were entered

into the muscular layer. All the lesions were not found in same uterus. The changes were categorized as acute endometritis (33.33%) (Fig. 27), chronic endometritis (26.66%) (Fig. 28,) and adenomyosis (6.66%) (Fig. 29). Some glands became more enlarged that there was disappearance of the lumen of the gland (Table 2).

**Table 2:** Shows pathological conditions of bovine uterus (n=30)

Sl. No.	Pathological conditions	Number of positive case	% of positive case
1	Acute endometritis	10	33.33
2	Chronic endometritis	8	26.66
3	Adenomyosis	2	6.66

**Table 3:** The isolates of bacteria in different pathological conditions of bovine uterus (n=30)

Sl. No.	Pathological condition	Number of positive case	% of positive case	Isolates of bacteria with %
1	Acute endometritis	10	33.33	<i>Bacillus</i> spp. (13.04 %).
<i>E. coli</i> (26.08 %)				
<i>Streptococcus</i> spp. (13.04 %)				
<i>Staphylococcus</i> spp. (30.43 %)				
2	Chronic endometritis	8	26.66	<i>Klebsiella</i> spp. (17.39 %)
				<i>Bacillus</i> spp. (30.00 %)
				<i>E. coli</i> (35.00 %)
				<i>Streptococcus</i> spp. (15.00 %)
3	Adenomyosis	2	6.66	<i>Staphylococcus</i> spp. (15.00 %)
				<i>Klebsiella</i> spp. (5.00 %)
				<i>Bacillus</i> spp. (40.00 %)
				<i>Staphylococcus</i> spp. (40.00 %)
				<i>E. coli</i> (20.00 %)



Fig. 1 *Bacillus* spp. produces white, dull and undulating colony on nutrient agar.



Fig. 2 *Bacillus* spp. Produces haemolysis on blood agar.

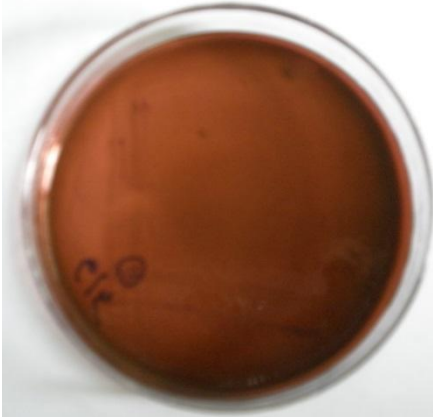


Fig. 3 *Bacillus* spp. produces no growth on EMB agar.

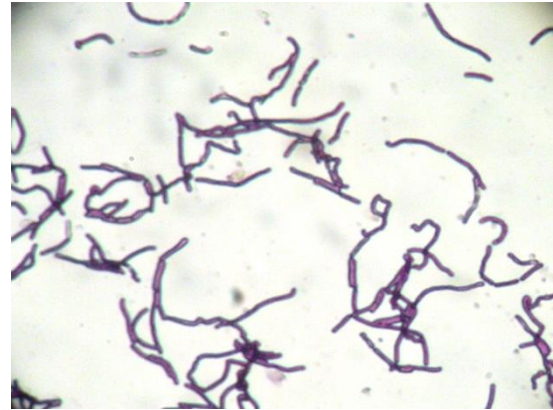


Fig. 4 *Bacillus* spp. showing gram positive, cylindrical rod arranged in single and long chain. (Modified Gram's staining,  $\times 830$ ).

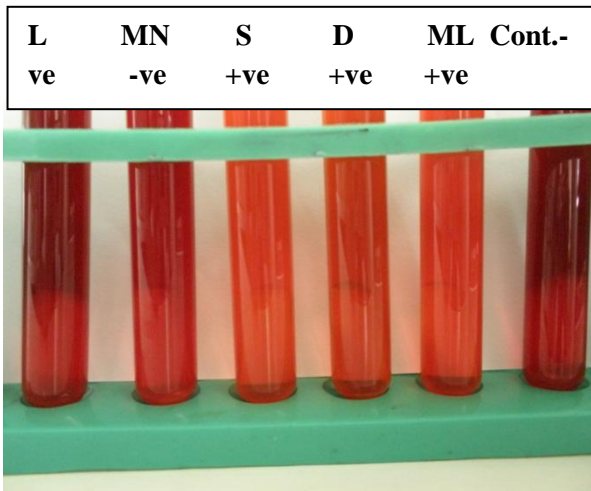


Fig. 5 Fermentation of dextrose, maltose, and sucrose with production of acid and no reaction in mannitol and lactose by *Bacillus* spp.

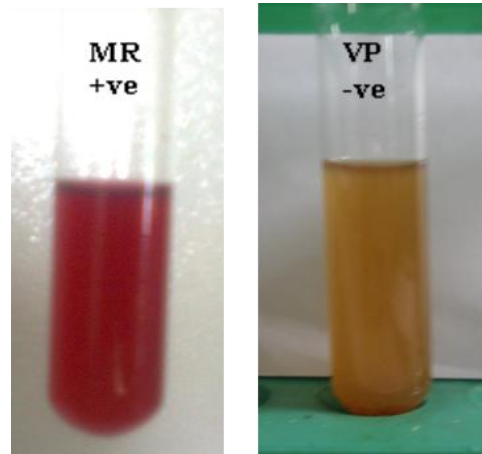


Fig. 6 *Bacillus* spp. produces bright red color in MR test and no color change in VP test indicating positive for MR and negative for VP test.

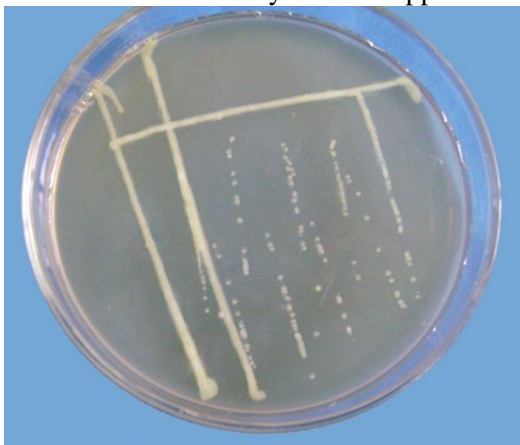


Fig. 7 Small, white, hard dew drop colonies on nutrient agar from isolated *Streptococcus* spp.



Fig. 8 Haemolysis on blood agar from isolated *Streptococcus* spp.



Fig. 9 Isolated *Streptococcus* spp. arranged in pairs and short to long chains (Modified Gram's staining, ×830).



Fig. 10 Catalase test *Bacillus* spp. produces bubbles (Upper) and *Streptococcus* spp. produces no bubbles

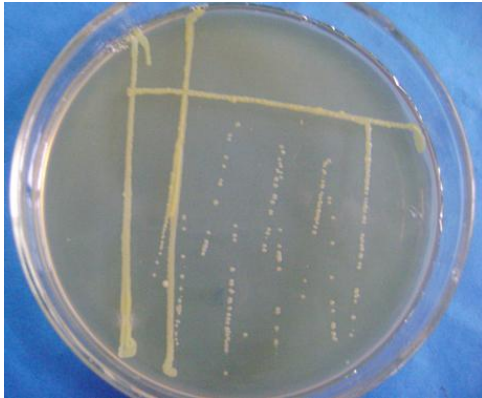


Fig. 11 Yellow cream color large, smooth, glistening and opaque colonies on nutrient agar from *Staphylococcus* spp.

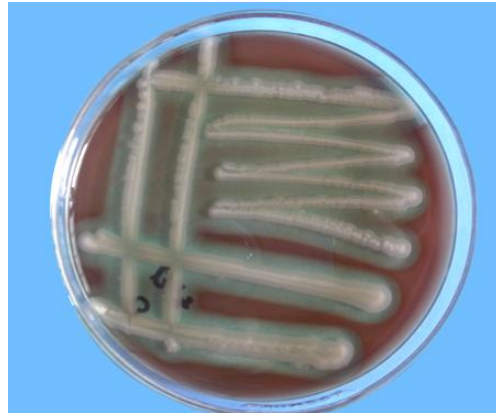


Fig. 12 Clear zones of hemolysis in blood agar plate from *Staphylococcus* spp.

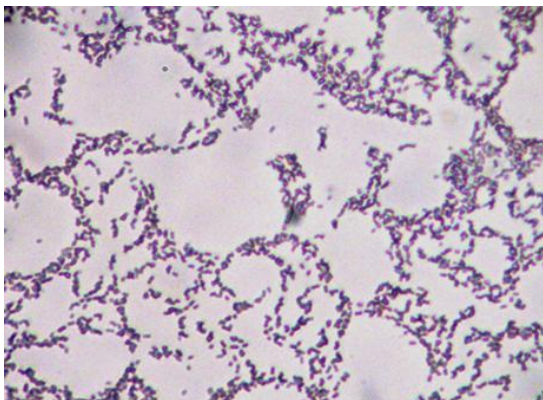


Fig. 13 Isolated *Staphylococcus* spp. cocci bacteria and arranged in grape like clusters (Modified Gram's staining, ×330).

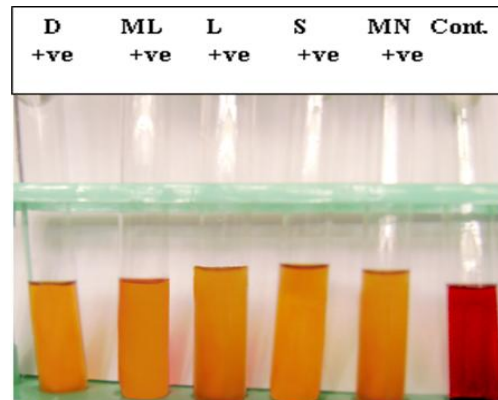


Fig. 14 Fermentation of dextrose, maltose, lactose, sucrose and mannitol by *Staphylococcus* spp.

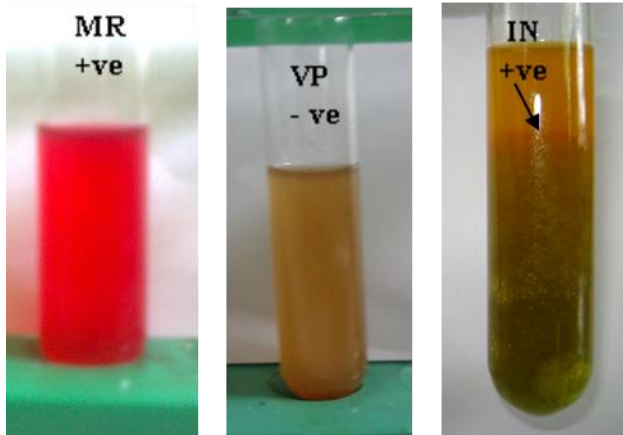


Fig. 15 *Staphylococcus* spp produces bright red color in MR test and indole test and no color change in VP test indicating positive for MR and indole test and negative for VP test.

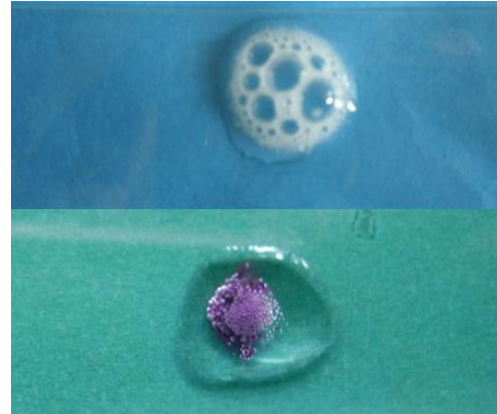


Fig. 16 Catalase positive test with production of O<sub>2</sub> from hydrogen peroxide by isolated *Staphylococcus* spp (upper). *E. coli* produces bubbles with hydrogen peroxide indicating catalase positive (lower).



Fig. 17 Smooth, circulation with dark centers and metallic sheen on EMB agar from isolated *E. coli*

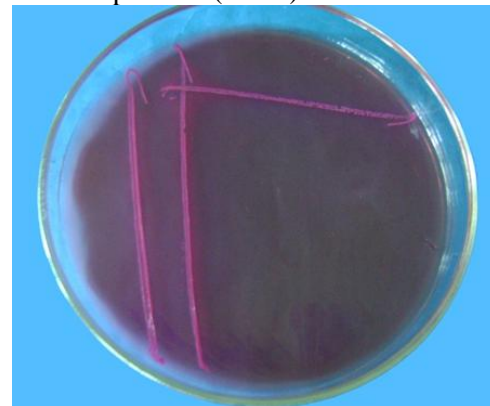


Fig. 18 *E. coli* showing pinkish colored colonies on MacConkey agar media.

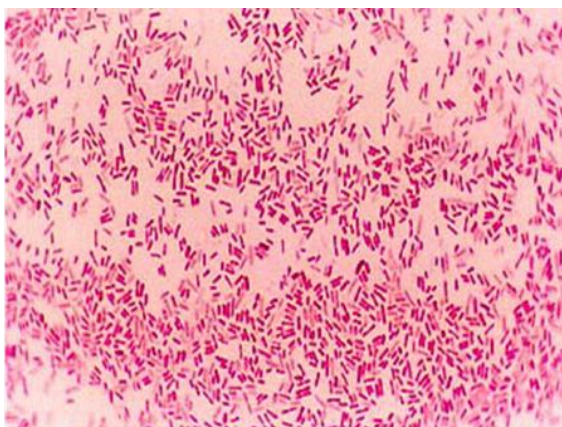


Fig. 19 *E. coli* in Gram's staining showing gram negative, short rod shaped organisms (Modified Gram's staining, ×830)

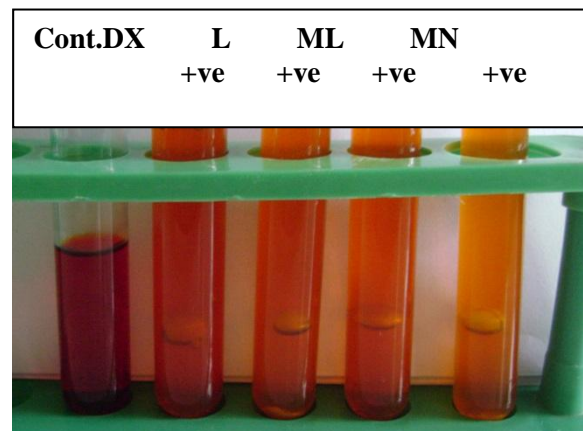


Fig. 20 Fermentation of dextrose, lactose, maltose and mannitol with production of acid and gas by isolated *E. coli*.



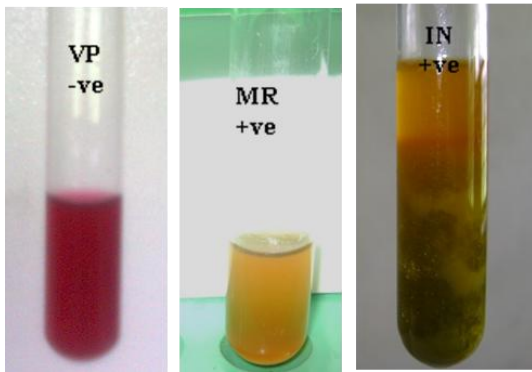


Fig. 21 *E. coli* produces red color in MR test and in indole test indicating positive test and no color change in VP test indicating negative test.

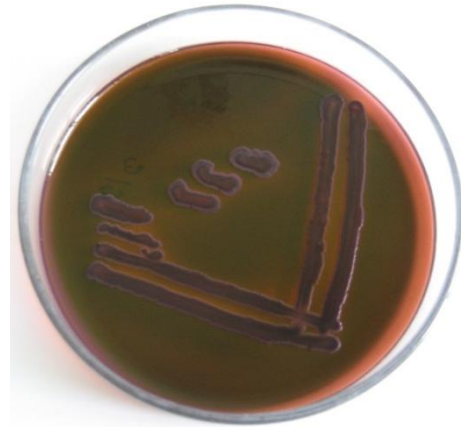


Fig. 22 *Klebsiella* spp produces purple colored colony in EMB agar media.

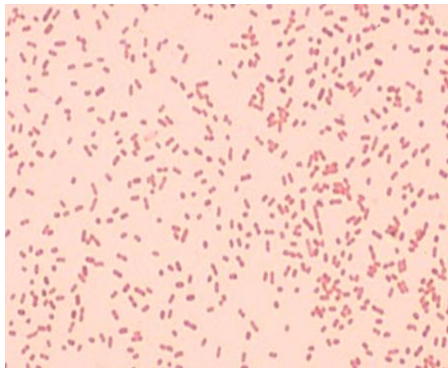


Fig. 23 *Klebsiella* spp in Gram's staining showing gram negative, coccobacillary shaped organisms (Modified Gram's staining, ×830).

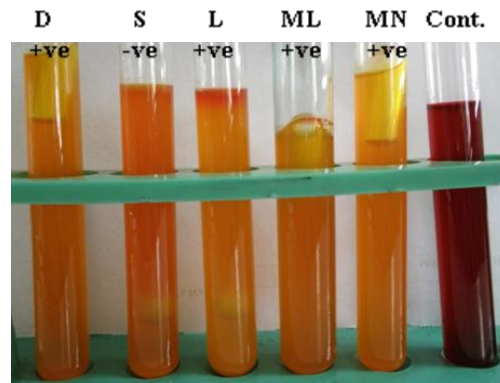


Fig. 24 Fermentation of dextrose, sucrose, lactose, maltose and mannitol with production of acid and gas by isolated *Klebsiella* spp.

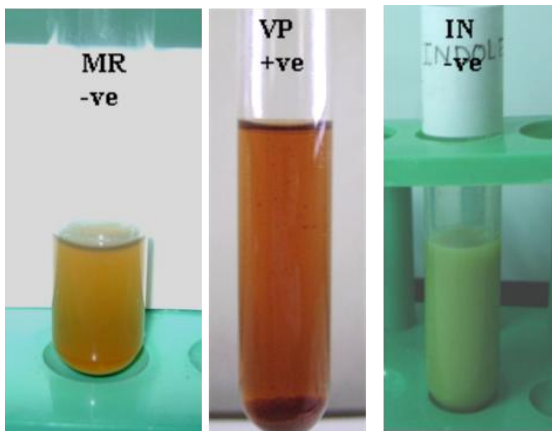


Fig. 25 *Klebsiella* spp produces no color in MR and in indole test indicating negative test and strong bright red color in VP test indicating positive test.



Fig. 26 Uterus: shows hemorrhage on the serosal surface of uterus and small cyst like structure on the ovary.

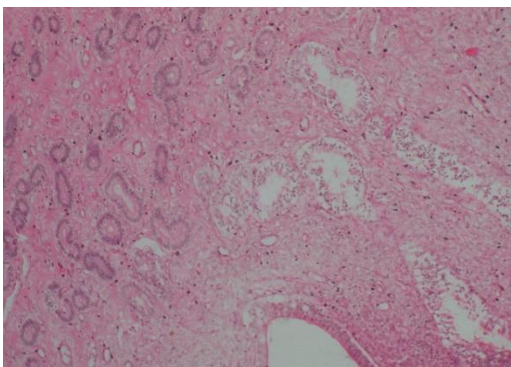


Fig. 27 Uterus: Acute endometritis: characterized by thickening of the epithelial layer, infiltration of neutrophils in the submucosal layer, dilatation of endometrial glands and slight congestion of blood vessel (H & E staining,  $\times 84$ ).

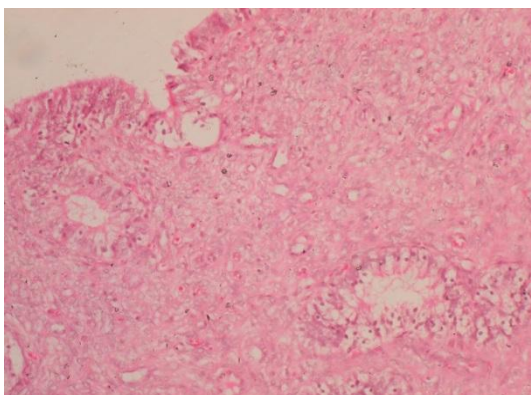


Fig. 28 Uterus: Chronic endometritis: characterized by infiltration of lymphocytes, macrophages, plasma cells in the submucosa. Proliferation of fibrous connective tissue within the myometrium (H & E staining,  $\times 334$ ).

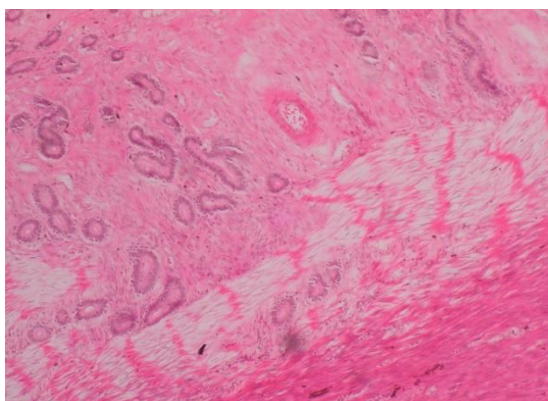


Fig. 29 Uterus: Adenomyosis: characterized by the presence of the endometrial glands within the myometrium (H & E staining,  $\times 84$ ).

## DISCUSSION

In this investigation, an attempt had been made to find out the various bacteriological agents in the uterus of cattle collected from abattoirs as well as occurrence of various pathologic affections in the uterus of cattle. These pathologic agents in severe cases may interfere in reproductive performance of cattle.

In the present study, the most consistent gross changes were found in uterus that were mucus filled lumen of uterus and hemorrhage on the luminal surface of uterus. However gross lesions in all samples were slightly variable. The gross lesions of uterus were similar to these findings of others (McEntee 1983, Jones et al. 1997, McDougall, 2005).

This study was conducted on 20 uterine swabs samples. Among these samples the occurrences of bacteria were 22.92% *Bacillus* spp, 12.50% *Streptococcus* spp, 25.00% *Staphylococcus* spp, 31.11% *Escherichia coli* and 10.417% *Klebsiella* spp. Among the isolates *Escherichia coli* was in higher percentage.

Routine methods of bacterial cultures in different media, specific colony characters, microscopic examination, different staining techniques and different types of biochemical tests were used for the isolation and identification of 5 genera of bacteria (*Bacillus* spp, *Streptococcus* spp, *Staphylococcus* spp, *Escherichia coli* and *Klebsiella* spp.) from the uterus of slaughtered cattle. The type of bacterial isolates in the present study coincided with the findings of Owiny and Acon (1998). They found *Streptococci*, coliforms, *Proteus*, *Klebsiella*, *Staphylococci*, *Bacilli* and *Micrococci* from the endometrium and fetal fluid. In this study, *Proteus*, *Micrococci* were not recorded may be because of less number of case recorded. In addition to this, occurrence of different bacteria from uterus had been reported in many countries of the world (Kaczmarowski et al. 2004, Sheldon et al. 2004, McDougall, 2005, Yavari et al. 2007).

Microscopically detectable lesions were acute endometritis, chronic endometritis and

adenomyosis, while (Sawamukai et al. 1994, Abalti et al. 2006) reported lesions into greater numbers. This variation may be due to number of samples examined, selection of samples and site examined. The occurrence of acute endometritis appeared to be 10 (33.33%), chronic endometritis was 8 (26.66%) and adenomyosis was 2 (6.66%). The occurrence of endometritis was higher than these values reported by Abalti et al. (2006) 3.9 %; LeBlanc (2008) 15-20%. The histopathological study of endometritis corresponded with the findings of other (LeBlanc et al. 2003).

The isolated bacteria from different types of pathological conditions were: in acute endometritis *E. coli* (26.08 %), *Streptococcus* spp. (13.04 %), *Bacillus* spp. (13.04 %), *Staphylococcus* spp. (30.43 %) and *Klebsiella* spp. (17.39 %); in chronic endometritis *E. coli* (35.00 %), *Streptococcus* spp. (15.00 %), *Bacillus* spp. (30.00 %), *Staphylococcus* spp. (15.00 %) and *Klebsiella* spp. (5.00 %); in adenomyosis *E. coli* (20.00 %), *Bacillus* spp. (40.00 %) and *Staphylococcus* spp. (40.00 %). Here, the same types of bacteria have been isolated from different pathological lesions. However the role of bacteria in developing different types of lesions in the uterus is needed to be clarified.

## CONCLUSION

A total of 20 selected samples were collected directly from 20 uteri of 30 randomly collected uterine samples of cattle at different ages.

Post mortem examination was performed on 20 selected uteri. The gross pathological lesions were hemorrhage, congestion, mucus filled uterine lumen etc. found almost in all cases.

The overall occurrences of bacteria in 20 uterus were 22.92% *Bacillus* spp, 12.50% *Streptococcus* spp, 25.00% *Staphylococcus* spp, 31.11% *Escherichia coli* and 10.417% *Klebsiella* spp.

Microscopically, the lesions were categorized into acute endometritis characterized by thickening of the epithelial layer, infiltration of neutrophil in the submucosal layer, dilatation of endometrial glands and slight congestion of blood vessel; chronic endometritis characterized by infiltration of

lymphocytes, macrophages, plasma cells in the submucosal layer and proliferation of fibrous connective tissue within the myometrium and adenomyosis characterized by the presence of endometrial glands within the myometrium. These microscopic lesions were variable in uteri.

Bacteria isolated from acute endometritis were *E. coli* (26.08 %), *Streptococcus* spp. (13.04 %), *Bacillus* spp. (13.04 %), *Staphylococcus* spp. (30.43 %) and *Klebsiella* spp. (17.39 %); in chronic endometritis *E. coli* (35.00 %), *Streptococcus* spp. (15.00 %), *Bacillus* spp. (30.00 %), *Staphylococcus* spp. (15.00 %) and *Klebsiella* spp. (5.00 %); in adenomyosis *E. coli* (20.00 %), *Bacillus* spp. (40.00 %) and *Staphylococcus* spp. (40.00 %). Here, the same types of bacteria have been found in different pathological conditions. However, further study would be necessary to know the role of specific bacteria in developing specific lesions in the uterus.

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