Investigation into gyneco-pathological disorders and identification of associated bacteria from the genital organs of cows in Dinajpur, Bangladesh

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ABSTRACT

The present study was aimed to investigate the gynaeco-pathological disorders by post-mortem and histopathological examination, and to identify the associated bacteria. A total of 310 genital tracts of cows were collected from slaughter house of Dinajpur Sadar Upazilla during April 2009 to March 2010. Among the 310 samples, 31.29% (n=97/310) were affected with endrometritis. Similarly, 8.37% (n=26/310) cystic ovary, 6.77% (n=21/310) ovary hyperplasia, 4.84% (n=15/310) pyometra, 4.84% (n=15/310) parovarian cyst, 4.52% (n=14/310) hydrometra, 4.84% (n=15/310) ovary hypoplasia, 3.55%(n=11/310) ovaro-bursal adhesion, 1.29% (n=4/310) vaginal cyst and 0.66% (n=2/310)) hemorrhagic uterine horn were detected by post-mortem examination, the cases were reconfirmed by histopathological studies. Uterine fluid (n=50) samples were collected, and were subjected for conventional bacteriological culture and biochemical analysis. *Escherichia coli* and *Salmonella* sp. could be identified from 30% (n=15/50) and 8% (n=4/50) samples, respectively. In conclusion, various pathological disorders in the female reproductive system of cows are prevalent, that may cause reduction of calf production.

INTRODUCTION

Livestock is an integral part of agriculture, and cattle provides important source of animal protein. Nowadays, most of the people in Bangladesh rears crossbred (local breed x foreign breed) cows for both milk and meat purposes. Sometimes the cross breed, local breed and foreign breed cows become infertile due to defective breeding program, inadequate knowledge of rearing system, and faulty ration formulation. Inadequate nutrition is the major cause of ovarian atrophy and subsequent anestrus and bacterial infection. According to Hossain et al. (1986) and Farooq (2000), gyneco-pathological disorders and found mostly in cross breed animals as compared to local cows. Infertility disorder in cows of Bangladesh is commonly seen because of poor nourishment, and using of low quality semen.

Following calving, the uterus of cow 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.,

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Pseudomonas spp., and *Staphylococcus* spp. (Erin et al., 2005).

Clinical endometritis is associated with delayed uterine involution and poor fertility. Bacterial pathogens are the potential cause when cows fail to conceive on one or more cycles in the same season. Nibret et al. (2013) isolated and identified *S. pyogenes, E. coli* and *Streptococcus* spp. as most frequently associated bacteria for fertility problem. Sulake et al. (2013) isolated and identified microflora species at different parts of the female genital tract (cervix, body of uterus, horns of uterus and oviducts) of ewes and indicated that there were different types of bacteria distributed throughout genital system as *E. coli* (37.83%), *Salmonella* (21.62%), *Klebsiella* (10.81%), *Staphlococcus aureus* (6.75%), *Strptococcus* spp. (6.75%), *Proteus* (6.75%), *Micrococcus* (5.4%) and finally others such as *S. epidermid* and *Pseudomonus* spp. (4.05%). Rahman et al. (1993) carried out a study on the incidence of reproductive disorders with 2280 cows and heifers in six Artificial Insemination (AI) pocket areas namely Hazirhat (Rangpur), Puthia (Rajshahi), Avoynagar (Jessore), Comilla, Manikganj and Moshurikhola (savar) during the period from July 1990 to June 1991. However, to the best of our knowledge, there is no report on the study of reproductive health problem in Dinajpur area of Bangladesh. Therefore, the present study was designed to investigate the gyneco-pathological disorders of cows and identification of the associated bacteria with genital organs of cows in Dinajpur, Bangladesh.

MATERIALS AND METHODS

Experimental area and animals: Mostly slaughtered animals in slaughter house in Dinajpur Sadar Upazilla were used for collection of samples. The animals aged between 6-8 years. Most of the slaughtered animals were of ill health. Their weight varied from 70-100 kg.

Sample collection: A total of 310 cattle were examined and female genital tract samples such as uterus, vagina, cervix and ovary were collected from the different abattoirs houses of Dinajpur Sadar Upazilla, Bangladesh, and the samples were transferred to the Laboratory at the Department of Microbiology, and the Department of Pathology and Parasitology of Hajee Mohammad Danesh Science and Technology (HSTU) for necropsy, histo-pathological examination, and isolation and identification of the associated bacteria.

Post-mortem examination: The gross examinations of the female reproductive tract followed by post-mortem examinations of all the samples were performed. All

the reproductive tracts were examined carefully following the method of Mc Enttee (1983). The ovaries were examined for their functional activity as per the method described by Ahmed (1989).

Histo-pathological examination: During necropsy, various organs having gross lesions were collected, and were fixed in 10% buffered neutral formalin for histo-pathological studies. The formalin fixed tissue samples were then processed and stained as per standard method (Luna, 1968).

Isolation and identification of bacteria: Culture media used for isolation and purification of bacteria included: EMB agar, MacConkey agar, SS agar and nutrient agar (Oxoid, England). Inoculated media were incubated aerobically at 37°C for 24 h. The bacteria were then identified by studying their morphology, Gram staining characters, and biochemical tests.

Biochemical test: The bacterial isolates were identified by standard biochemical tests such as MIU test (Motility, indol and Urease test), TSI (triple sugar iron), Voges Proskauer (VP) test, as per the procedure of Forbes et al. (2007).

RESULTS AND DISCUSSION

Detailed post-mortem examination was performed on 310 freshly collected female genitalia of cows. On post-mortem examination, 31.29% (n=97/310) cases were detected as endometritis followed by 8.37% (n=26/310) cystic ovaries, 6.77% (n=21/310) ovarian hyperplasia, 4.84% (n=15/310) pyometra, 4.84% (n=15/310) parovarian cyst, 4.52% (n=14/310) hydrometra, 4.84% (n=15/310) ovarian hypoplasia, 3.55% (n=11/310) ovarobursal adhesions, 1.29% (n=4/310) vaginal cyst, and 0.65% (n=2/310) hemorrhage in ovary (**Table 1** and **2**); these findings were in agreement with the findings of Alam and Rahman (1979) who reported that 90% animals showed abnormalities in Dhaka slaughter house in which the most common abnormalities were recorded as ovarian cysts and pyometra. On the other hand, Shamsuddin et al. (1988) and Mollah et al. (1989) reported that about 8 to 27% cows suffered from endometritis among the principal causes of reproductive failure in cows. Borsberry and Dobson (1989) recorded 14.80% incidence of endometritis alone or in combination with other peripaturient disorders, which were lower than the present study. This variation might be due to improved health management and nutritional status of the animals. Other gross abnormalities detected in uterus

Table 1: Pathological disorders encountered at post-mortem examination of Uterus of cows.

No. of uterus observation	Endometritis	Pyometra	Hydrometra	Vaginal cyst	Hemorrhage in uterine horn
310	97	15	14	4	2
(%)	31.29	4.84	4.52	1.29	0.65

Table 2: Pathological disorders encountered at post-mortem examination of ovary of cows

No. of ovary	Ovarian cyst	Parovarian cyst	Ovarian hyperplasia	Ovarian hypoplasia	Ovarobursal adhesion	Hemorrhage in ovary
310	26	15	21	15	11	2
(%)	8.37	4.84	6.77	4.84	3.55	0.65
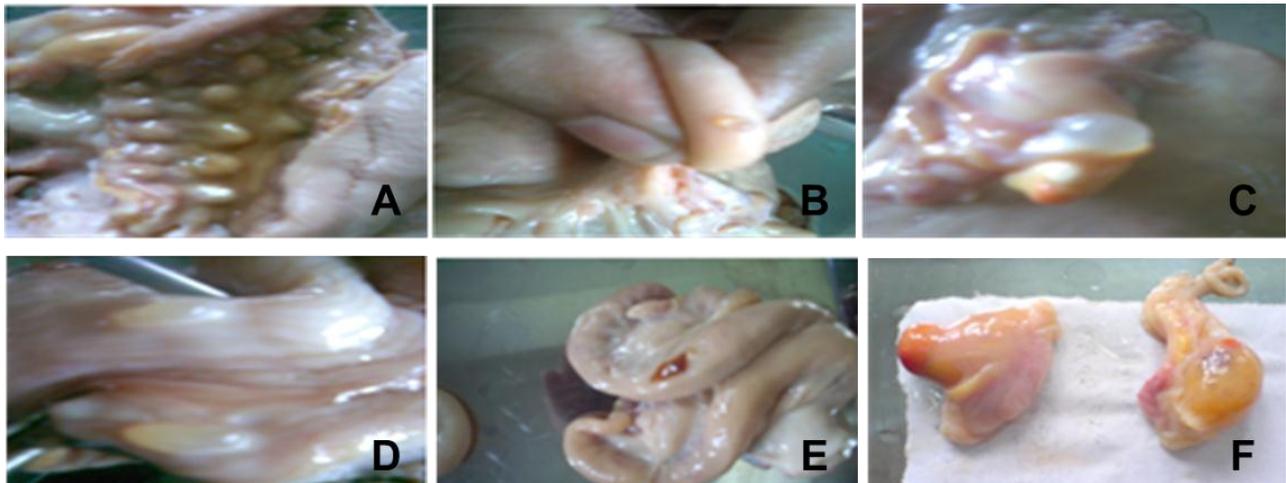
Figure 1: (A) Pyometra. (B) Hydrometra (D) Parovarian Cyst in ovary (E) Hemorrhage in uterine horn (F) Hyperplastic ovary.
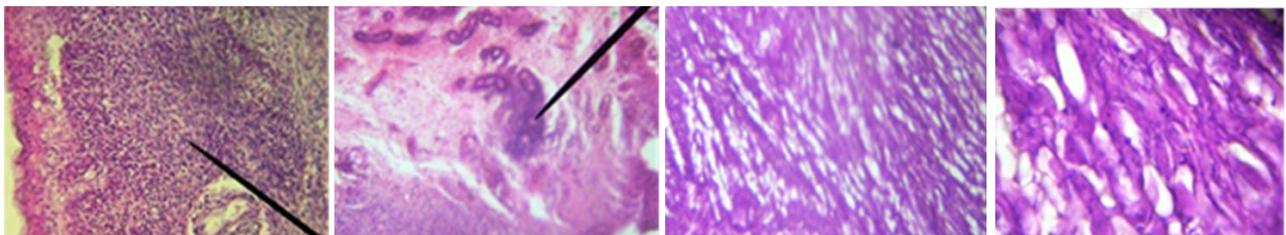
Figure 2: (A) A case of pyometra exhibiting presence of huge reactive cell infiltration (H&E; x10). (B) A case of chronic endometritis exhibiting infiltration of inflammatory cells and moderate fibrosis (H&E; x10). (C) Hyperplastic ovary characterized proliferation of fibrous tissue (H&E; x10). (D) Higher magnification of hypoplastic ovary (H&E; x10).

Table 3: Isolation and identification microorganism from female genital tracts (n=50) of cattle.

Organisms	No. of affected uterus	% of infection
Escherichia coli	15/50	30
Salmonella spp.	4/50	8

were pyometra (4.84%; n=15/310), hydrometra (4.52%; n=14/310), vaginal cyst (1.29%; n=4/310), and hemorrhage in uterine horn (0.65%; n=2/310) and in ovary, ovarian cyst (8.37%; n=26/310), parovarian cyst (4.84%; n=15/310), ovarian hyperplasia (6.77%; n=21/310) ovarian hypoplasia (4.84%; n=15/310), ovarobursal adhesions (3.55%; n=11/310) and hemorrhage in ovary (0.65%; n=2/310) (**Figure 1**). The results obtained in our study were likewise higher as compared to those of formerly documented data reprted by Chaudhari et al. (2000), in which the gross abnormalities of the genitalia included ovarian cyst (3.35%), parovarian cyst (0.26%), ovarian hypoplasia

Table 4: Cultural and biochemical characteristics of isolated organisms

EMB agar	SS agar	Staining character	Biochemical Test						Organism
			MIU			TSI			
			M	I	U	Butt	Slant	H$_2$S	
Green metallic sheen	No	Gram negative							*E. coli*
			+	+	-	Yellow	Yellow	+	
No	Small, moist, circular	Gram negative, pink color, small rod shaped, Appearance arranged in single or paired.	+	-	-	Yellow	Pink/ Black	-	*Salmonella* spp.

Legends: MIU: Motility Indole Urea; **TSI:** Triple Sugar Iron; **EMB:** Eosin Methylene Blue; **SS:** Salmonella-shigella; **(+):** Positive; **(-):** Negative.

(2.2%), ovarobursal adhesion (2.9%), and endometrocervicitis (1.7%), pyometra (0.48%), hypoplastic uterus (0.24%) and uterine cyst (0.08%). However, Ali et al. (2006) found the similar abnormalities in the reproductive tracts of descriptive cows.

In this study, massive infiltration of lymphocytes, macrophages, monocytes and plasma cells in the endometrial mucosa, stroma and utetine glands was found (**Figure 2**). On the other hand, massive infiltration of neutrophils was found in the cases of pyometra which was supported by Rahman et al. (2002)**,** Farooq (2000) and Manda et al. (2007). According to Kotowski (2001), the pathological changes more commonly found in the uterus as compared to those of ovaries**.**

The microbiological examination performed on 50 freshly female genitalia collected from slaughter house revealed that 30% (n=15/50) of genital organs were affected with *E. coli*, and 8% (n=4/50) were affected with *Salmonella* spp. (**Table 3** and **4**). These findings were in support of the findings of Nibret et al. (2013), Sulake et al. (2013) and Erin et al. (2005).

CONCLUSION

Various pathological disorders in the female reproductive system may affect the reproduction performance. Further, it may be pointed out that various pathological disorders in female reproductive system are increasing menacingly along with introduction of cross breeding program through artificial insemination. The problem may be aggravated due to inadequate veterinary coverage and gynecological knowledge, which needs more attention.

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