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Pathological conditions in the reproductive tract of does at slaughter in Mymensingh district

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Abstract

The aim of this study was to investigate the pathological conditions in ovary and reproductive tract of does at slaughter. A total of 51 female genital tract samples together with ovaries from does > 1 and half year old were randomly collected from the slaughtered goats at Mymensingh district abattoir from August 2004 to March 2005. After collection, representative tissue samples were preserved in 10% neutral buffered formalin and swabs were collected in sterilized tubes containing nutrient broth. Tissues were processed, embedded in paraffin, sectioned and stained using standard histopathological techniques. Swabs were subjected for routine bacteriological examinations. Immunohistochemical examination was also performed on paraffin sections using a genus specific monoclonal anti-chlamydial antibody and DAKO TechMate immunperoxidase kit. Among these 51 does, 21 did not show lesion in the ovaries and in the uterine tract, uterus of 3 does contained early embryos and 27 does showed lesions either in ovaries or somewhere in the reproductive tract. The highest pathological conditions occurred in the uterus 37.7 % (endometritis: 28%, endometritis plus salpingitis: 3.9%, endometritid plus cevicitis: 3.9% and adenomyosis: 1.9%) followed by the ovaries (Follicular cyst: 3.9%, par ovarian cyst: 3.9%, ophoritis: 1.9%, granulosa theca cell tumor: 5.88%). Staphylococcus, Bacillus and Diplococcus were isolated from the uterine swab of 25 goats. Chlamydia could not be detected in any of the sections. Results showed that a large number of female goats slaughtered at Mymensingh district abattoir affected with reproductive abnormalities. Granulosa theca cell tumor is the first report in the ovary of Black Bengal goats.

Keyword: Goat, Pathological conditions, Reproductive tract

Introduction

Goat is one of the important livestock in Bangladesh and according to FAO reports (1999), total goat population in Bangladesh is 33.5 million. The government of the People's Republic of Bangladesh has given emphasis on goat and has already started a project on goat rearing. In addition to the government, number of NGOs is helping distress women, landless and marginal farmers to rear a few goats through micro-credit program aimed at poverty alleviation. A large number of female goats are slaughtered everyday in Bangladesh (personal observation). Majority of these slaughtered female goats are at the age of production (1 – 2 years). This early slaughtering of female goats is certainly hampering the goat production and hindering the goal of poverty alleviation. The main reasons of this early slaughtering of female goats are not known and not studied so far. Infertility or low productivity may guide the owner for the early slaughtering of these female goats. Various infectious and non-infectious reproductive disorders cause infertility in goats (Bhuiyan *et al.*, 1988). But there is no detail investigation on reproductive disorders and infectious agents that cause infertility in goats in Bangladesh.

The present study was carried out to investigate the reproductive disorders in does at slaughter using gross and histopathological techniques. Investigations were also attempted to identify the infectious agents with special reference to chlamydiae.

Materials and Methods

Collection of samples

A total of 51 female genital tract samples together with ovaries from does > one and half year old were randomly collected from the slaughtered goats at Mymensingh district abattoirs from August 2004 to March 2005. The age was determined by using dentation chart.

Before collecting the samples all instruments were sterilized. For bacteriology uterine swabs were collected and were kept in nutrient broth. This nutrient broth was incubated at 37^0C for 24 hours, and then stored at 4^0C. Tissue samples from ovary, oviduct, uterus and cervix were collected for histopathology. These samples were preserved in 10% neutral buffered formalin.

Macroscopic examination

The stage of the estrous cycle was assessed from the macroscopic appearance of the ovaries (Leiser *et al.,* 1988). Other gross pathological changes in the ovaries, oviducts, uterus and cervix were recorded.

Histopathological examination

Fixed sections of the ovary, oviduct, uterus and cervix were processed, paraffin-embedded, sectioned and routinely stained with Hematoxylin and Eosin (H & E) as per standard procedure (Luna, 1968). In the histopathological interpretation, considerations were given to the stage of the estrus cycle.

Bacteriological examination

Bacteriological examination of uterine swabs was performed routinely, using conventional bacteriological methods.

Immunoperoxidase staining technique to identify Chlamydiae

The paraffin sections from uterine and oviduct tissues were used to detect chlamydial lipopolysacharide (LPS) using a genus specific chlamydial anti-LPS monoclonal antibody (Clone AC –1, Progen, Heidelberg, Germany) and DAKO TechMate™ detection kit (S 2023, DAKO, Netherlands) according to Chowdhury et al. (1999). The protocol in brief was: sections were deparaffinized in zylene, rehydrated in water, incubated with .1% trypsin for 8 minutes at 37°C, treated with hydrogen peroxide for seven minutes, incubated with monoclonal antibody (1/100 dilution) for one hour at room temperature, incubated with biotinylated anti-mouse and anti-rabbit IgG for ten minutes, incubated with peroxidase conjugated streptavidin for 10 minutes and finally the reaction was visualized by using 3-amino-9-ethylcarbazole (AEC) for 7 minutes. Sections were washed with PBS after every application. A parallel positive and negative control was run every time. Paraffin embedded positive sections were a kind gift from Prof. Dr. Andreas Pospischil, Director, Institute of Veterinary Pathology, University of Zurich, Switzerland.

Results and Discussion

In this investigation an attempt had been made to find out the incidence of various pathologic affections in female reproductive tracts of goats slaughtered at abattoirs. Although information on reproductive disorders in goat is not available or scanty in Bangladesh, (Roy *et al.*, 2001) reports are available from other countries (Nair and Raja, 1972; Das *et al.*, 1979; Srivastava *et al.*, 1985; Kadu and Kaikini, 1988; Sattar and Khan, 1988). In the present study, fifty-one (51) female reproductive tracts were randomly collected from freshly slaughtered does at abattoirs in Mymensingh district. Among 51 does, 21 did not have any lesion in the ovaries or in the uterine tract, uterus of 3 does contained early embryos and 27 does showed lesions at least either in the ovaries or in the uterine tract. Therefore it appears that a large number of goats (>50%) suffered from reproductive disorders before slaughter. The incidence of different pathological conditions of ovary and female reproductive tract of goats are shown in Table 1.

Table 1. Occurrence of different pathological conditions of ovary and female reproductive tract of does at slaughter

Organ and pathological lesions	No of goat affected	Goat affected (%, n = 51)
Ovary		
Follicular cyst	2	3.9
Par ovarian cyst	2	3.9
Ophoritis	1	1.9
Granulosa-theca cell tumor	3	5.8
Uterus		
Endometritis	14	28
Endometeritis plus cervicitis	2	3.9
Endometritis plus salpingitis	2	3.9
Adenomyosis	1	1.9
Normal embryo	3	5.8

A total of 37.25% uterus showed endometritis either macroscopically or microscopically. Macroscopically, 6 out of the 51 uterus samples showed varying amount of purulent exudates in the uterine lumen (pyometra) (Fig. 1), of which 2 had fowl smell. Uterine mucosa of the two samples was extremely hemorrhagic (Fig.2). Few samples contained mucopurulent exudates in the lumen, while some were deep brown colored and some were fibrotic. Histologically, the lesions were grouped into 3: (i) acute endometritis- numerous neutrophils were present in the surface epithelia and in the lamina propria. In few cases the inflammation extended to endometrial glands and surrounding tissue became necrosed and infiltrated with lymphocytes and macrophages. (Fig. 3). (ii) chronic supporative endometritis- the endometrium was severely infiltrated with neutrophils and small abscesses were found in the lamina propria (Fig.4). There was cystic hyperplasia of the endometrial glands some of which contained neutrophils (Fig. 5). There were peri-glandular foci of lymphocytes, plasma cells and in some cases the peri-glandular area was extremely fibrotic. (iii) chronic active granulomatous endometritis- mucosal epitheilum was found almost damaged with the formation of granuloma that protruded from the surface (Fig. 6), lamina propria was severely infiltrated with large mononuclear cells with few neutrophils, hemosiderosis and vasculitis were also evident in one case. In this investigation, the incidence of uterine discrders were higher than the values obtained by Nair and Raja, 1972 (0.11%); Das *et al.*, 1979 (1.65%); Srivastava *et al.*, 1985 (0.34%); Ahmed, 1993 (1.8%). However, Bhuiyan *et al.* (1988) reported about similar incidence (33.33%) of endometritis in India as found in the present study.

In the present study incidence of salpingitis was seen concomitantly with an overshadowing endometritis as 3.92% that was higher than the values 0.9% and 0.05% obtained by Ahmed (1993) and Nair and Raja (1972), respectively. But almost similar to the value (4.85%) detected by Sattar *et al.* (1988). Cervicitis is seen concomitantly with an overshadowing endometritis or vaginitis (Jones *et al.,* 1997). In this study, cevicitis was also found along with endometritis and was thought to be of infectious origin as bacteria were isolated from each of the uterus. The incidence of cervicitis was 3.9% (N= 51). This finding is little larger than the report of Ahmed (1993). The microscopic study corresponded with the findings of others (Jones *et al.,* 1997).

A total of 16% ovaries showed pathological lesions of which 3.9% follicular cyst, 3.9% par ovarian cyst, oophoritis in 1.9% and granulose-theca cell tumor was found in 5.8% ovaries. Grossly, the cystic structures were filled with watery fluid and found protruding from the surface of the ovary. Microscopically single or multiple layers of granulosa cells and partially luteinized cells lined the cysts (Fig. 7). The cyst did not contain an ovum. The par ovarian cysts were found on the surface of the ovary. Grossly, these appeared as large round cysts filled with watery fluid attached with the ovarian surfaces (Fig. 8). In this study the incidence of follicular cyst is little higher than the values (0.24%) obtained by Satter and Khan (1983) and Virmani *et al.,* (1997) as 1.14%. Chand and Chauhan (1979) stated that cystic abnormalities were most frequent in goats. The histological features of follicular cyst corresponded with the findings of others (Virmani *et al.,* 1997).

Granulosa-theca cell tumor was characterized by proliferation of neoplastic cells resembling normal granulosa cells, being small, polyhedral with scant amount of eosinophilic cytoplasm, round to ovoid hyperchromatic nuclei (Fig. 9). Within the tumor fusiform spindle shaped cells were intermixed with the polyhedral shaped cells (Fig. 10). So far, no literature was found about granulosa theca cell tumor in goats. This is the first report of granulosa-theca cell tumor in Black Bengal goat in Bangladesh and in the goats as a whole. These are the most common ovarian neoplasm of cow and mares and occurred in older bitches. This type of tumor is derived from either alveolar follicles in the cortex or from the medullary tubules near the hilum of the ovary. These are usually unilateral and benign (Jones *et al.,* 1997). It may result in a variety of sex hormone imbalances characterized by elevated plasma levels of progesterone, estradiol or testosterone or all three. Clinically this may be manifested as signs of nymphomania, musculinization or no obvious clinical abnormalities. However, we did not have the opportunity to observe the clinical signs of these affected goats.

Three types of aerobic bacteria were isolated. These included *Staphylococcus spp.,* Diplococci and *Bacillus spp.* The organisms were isolated from the uterus, where inflammatory lesions were prominent. These bacteria mostly gain entrance into the uterus during estrous cycle following artificial insemination or coitus and complicated the endometritis. The results correspond to data from other studies (Butchaiah and Khera, 1982; Sattar *et al.,* 1988). Chlamydiae were not detected in any of the samples. Since chlamydiae show a culture or immunohistochemistry negative state in chronic persistent infection (Beatty *et al.* 1994), its possible role in infertility cannot be ruled out before more samples are analyzed by PCR.

In conclusion, results showed that a large number of female goats (> 50%) slaughtered at Mymensingh district abattoir were affected with reproductive abnormalities. Therefore, it can be assumed that poor fertility was the reason behind the early slaughtering of the female goats. These findings will definitely help the clinicians for the better management of reproductive health in Black Bengal goats. Presence of granulose-theca cell tumor in goat in this study is a new finding with immense academic interest.

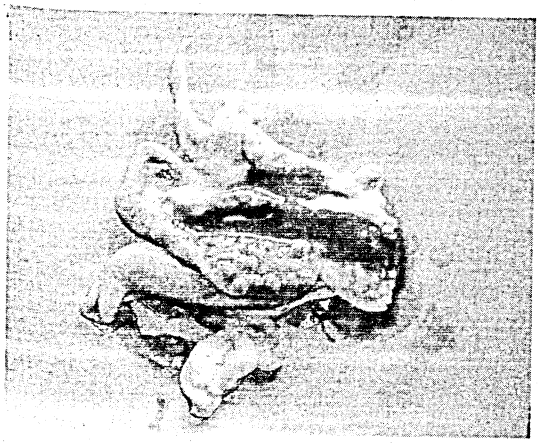

Fig. 1. Uterus showing large amount of purulent exudates in the uterine lumen (Pyometra)

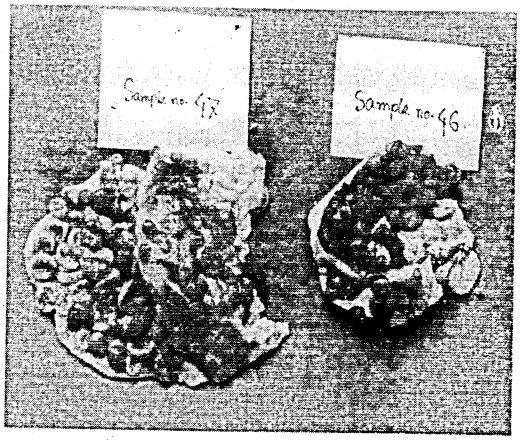

Fig. 2. Extensive hemorrhagic uterine mucosa

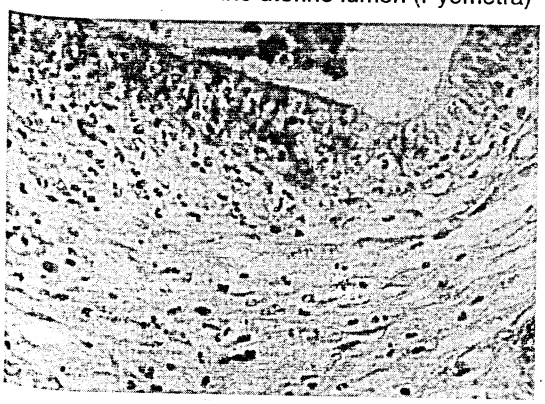

Fig. 3. Figure showing infiltration of neutrophils in the surface epithelia, lamina propria and exudates in the uterine lumen (acute endometritis) (H & E, X330)

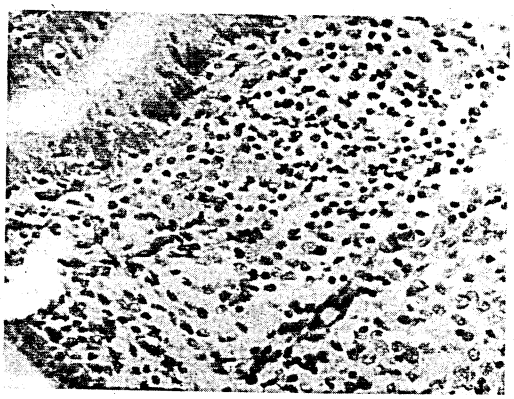

Fig. 4. Figure showing infiltration of large mononuclear cells and neutrophils in the lamina propria (chronic active endometritis) (H & E, X 330)

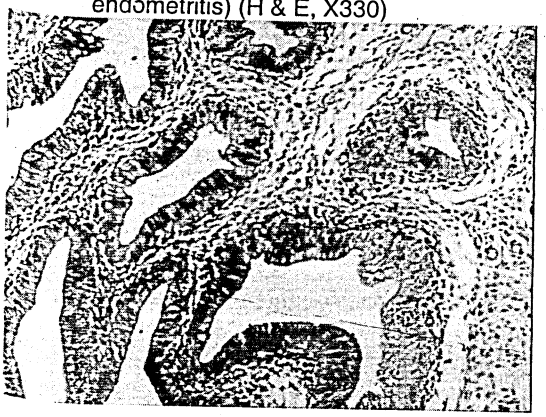

Fig. 5. Section of uterine wall showing cystic glandular hyperplasia of the endometrium (H & E, X 330)

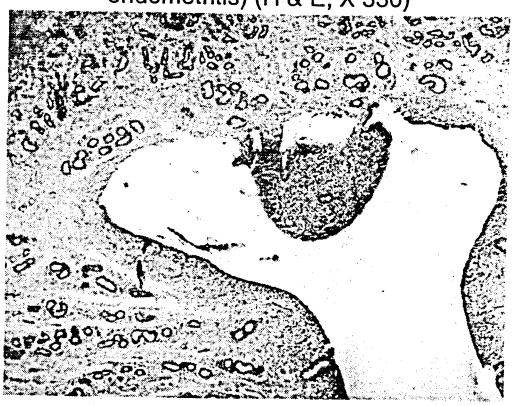

Fig. 6. Chronic active endometritis with damaged epithelium and formation of granuloma (H & E, X 82.5)

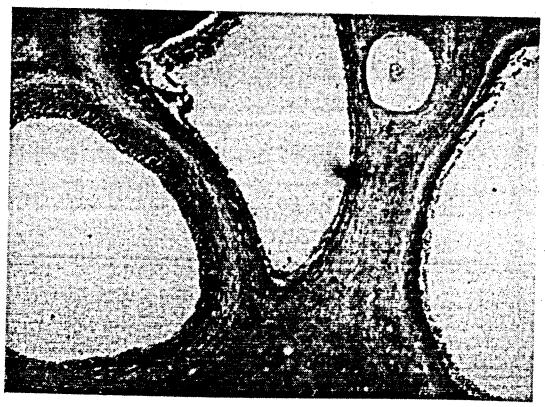

Fig. 7. Ovarian section showing multiple follicular cysts (H & E, X 82.5)

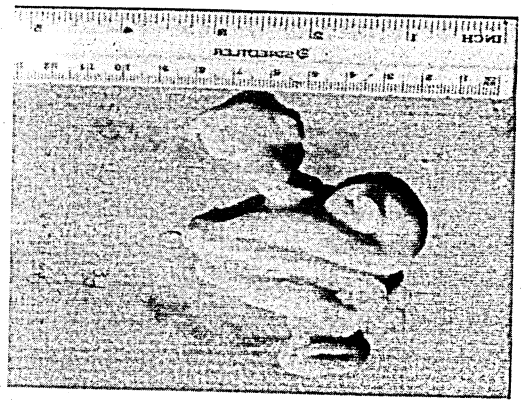

Fig. 8. Parovarin cyst in the surface of the ovary

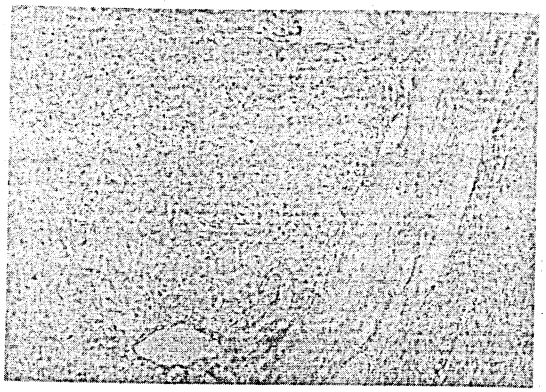

Fig. 9. Granulosa-theca cell tumor characterized by fusiform spindle shaped cells intermixed with the polyhedral shaped cells (H & E, X 82.5)

Fig. 10. Closure view of figure 9. (H & E, X 330)

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