MAMMARY GLAND NEOPLASIA IN A BUFFALO BULL- HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

Putchakayala Ramesh[1], Chitturi Sudha Rani Chowdary[2,*] and Mullinti Raghunath[3]

ABSTRACT

There are no reports of mammary gland tumors in buffalo bulls. The current communication describes an unusual occurrence of papillary cystic adenocarcinoma in a developed right fore quarter of udder in a three years old buffalo bull. The bull was presented with a well developed scrotum, descended testes and enlarged right fore quarter of udder. Histopathology of the excisional biopsy revealed papillary cystic adenocarcinoma. Immunohistochemistry for estrogen receptor alpha and progesterone receptor revealed moderate and mild positivity respectively in the glandular epithelium. In general, mammary gland does not develop in bulls except in rare cases of gynecomastia. In the present case, a quarter of the udder has developed and undergone neoplasia probably under the influence of estrogen and progesterone hormones.

Keywords: *Bubalus bubalis*, buffaloes, buffalo bull, neoplasia, mammary gland, estrogen receptor alpha, progesterone receptor

INTRODUCTION

Among domestic animals, mammary gland neoplasms are most common in dogs followed by cats. In bovines, the occurrence of mammary neoplasia is rare and the reported cases are more in cattle (Mihevc and Dovc, 2013). In cattle, fibroadenomas (Mina *et al*., 1994; Thilabault *et al*., 1997), fibrosarcomas (Povey *et al*., 1969; Ford *et al*., 1989) and carcinomas (Beamer and Simona, 1983; Murphy, 1992; Ohfuji, 2012) were reported in female animals. Occurrence of mammary gland neoplasia in buffaloes is rare. Reports in female buffaloes included mammary intraductal carcinoma (Mandal and Iyer, 1969), fibropapilloma (Joshi *et al*., 1994), fibroadenomatoid hyperplasia (de Sant'Ana *et al*., 2014) and fibroadenoma (Raval *et al*., 2015). Male mammary gland neoplasms are rare in domestic animals, though few reports exist in dogs (Saba *et al*., 2007) and a buck (Wooldridge *et al*., 1999). The present communication puts on record a very rare case of mammary gland neoplasia in a buffalo bull, its histopathology and immunohistochemistry for steroid receptors, estrogen receptor alpha and progesterone receptor.

[1]Department of Veterinary Clinical Complex, NTR College of Veterinary Science, Andhra Pradesh, India

[2]Department of Veterinary Pathology, NTR College of Veterinary Science, Andhra Pradesh, India, *E-mail: drsudha84@gmail.com

[3]Department of Veterinary Clinical Complex, NTR College of Veterinary Science, Andhra Pradesh, India

CASE HISTORY AND CLINICAL OBSERVATIONS

A three years old buffalo bull was presented with a developed and enlarged right forequarter of udder almost to the size of scrotum (Figure 1). The other three quarters of the udder remained rudimentary and the animal had fully developed and descended testes in the scrotum. Owner of the animal reported that the animal showed swelling of the quarter for the past two months. Upon gross examination, the udder was 11 to 12 centimetres in diameter with a very soft consistency ventrally and a firm consistency dorsally. Surgical resection of the quarter was advised.

Surgical procedure

The animal was positioned in lateral recumbency and the area was desensitized with linear infiltration anaesthesia using 2% lignocaine hydrochloride. After aseptic preparation of the site, an elliptical incision was given around the quarter and by blunt dissection, the quarter was resected. The subcutaneous and skin wounds were closed in routine. The animal was administered post operative antibiotics and analgesics with regular dressing of the wound. The sutures dried by 12th post operative day and the sutures were removed.

Gross pathology, histopathology and immunohistochemistry

Cut section of the quarter revealed two distinct areas, a cystic area with necrotic debris and large amount of serosanguinous fluid and a grayish white fleshy area. Tissue samples were collected in 10% neutral buffered formalin for histopathological examination by routine paraffin embedding technique and microtomy. Five micron thick sections were cut and stained by routine Hematoxylin and Eosin staining method. Four micron thick parallel sections were subjected to immunohiochemistry (IHC) for estrogen receptor alpha (ER alpha) and progesterone receptor (PR). The slides were deparaffinised and rehydrated through grades of alcohol to water. Antigen retrieval was performed at 95ºC in citrate buffer (pH:6) for 20 minutes using an antigen retriever (EZ retriever, Biogenex). The ready to use rabbit monoclonal antibody to ER-alpha and mouse monoclonal antibody to PR with a Polymer HRP detection system (Biogenex, USA) were used. IHC procedure was carried out according to the protocol of the kit with DAB as chromogen and Harris hematoxylin as counter stain. Canine uterus was used as positive control and for negative control, primary antibodies were replaced with Tris buffered saline.

Histopathology revealed papillary cystic adenocarcinoma characterized by the presence of papillae lined by 3 to 4 cells thick proliferating neoplastic epithelial cells supported by fine vascular fibrous connective tissue extending into cystic and dilated tubular luminae (Figure 2). The cytoplasm was basophilic with a single vesicular nucleus containing 1 to 2 nucleoli and moderate degree of mitotic activity. Necrosis, severe infiltration of neutrophils and mononuclear cells interspersed with fibrin were observed in the cystic area. Focal areas of calcification were also noticed in the necrotic area.

Immunohistochemistry for estrogen receptor alpha revealed moderate positivity in the nuclei of epithelial cells and myoepithelial cells (Figure 3) and that of progesterone receptor revealed mild positivity in the nuclei of epithelial cells (Figure 4).

Based on histomorphological and immunohistochemical findings, an ER alpha

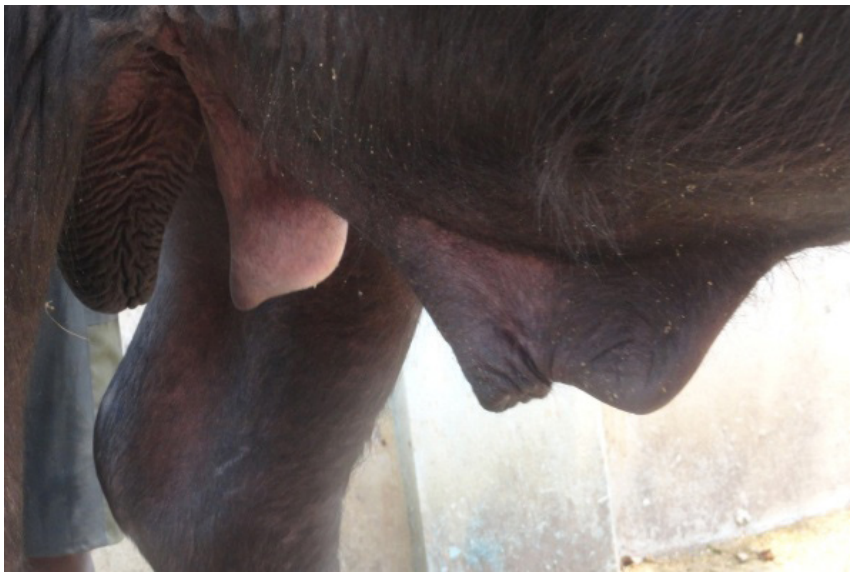

Figure 1. Enlarged right forequarter of udder almost to the size of scrotum (lateral view).

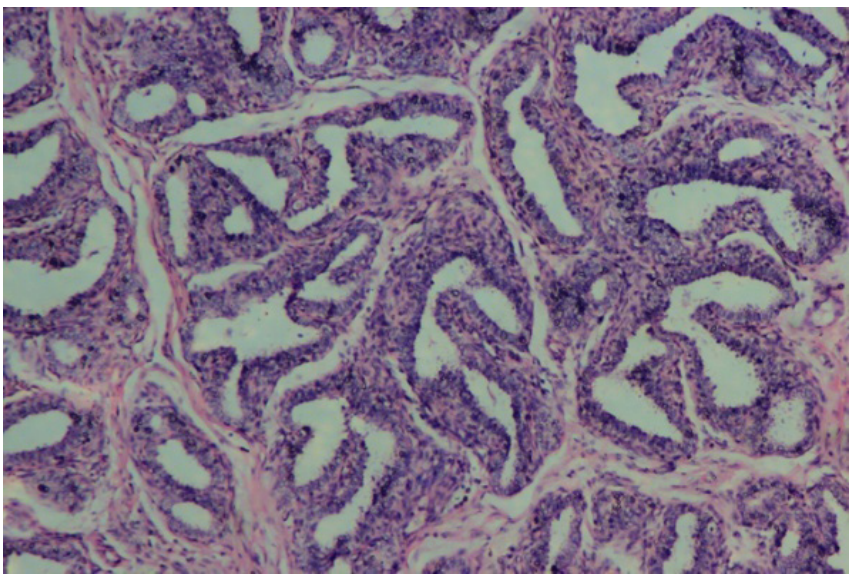

Figure 2. Photomicrograph of mammary gland showing papillae lined by neoplastic epithelium extending into the dilated tubular luminae. HE, 100x.

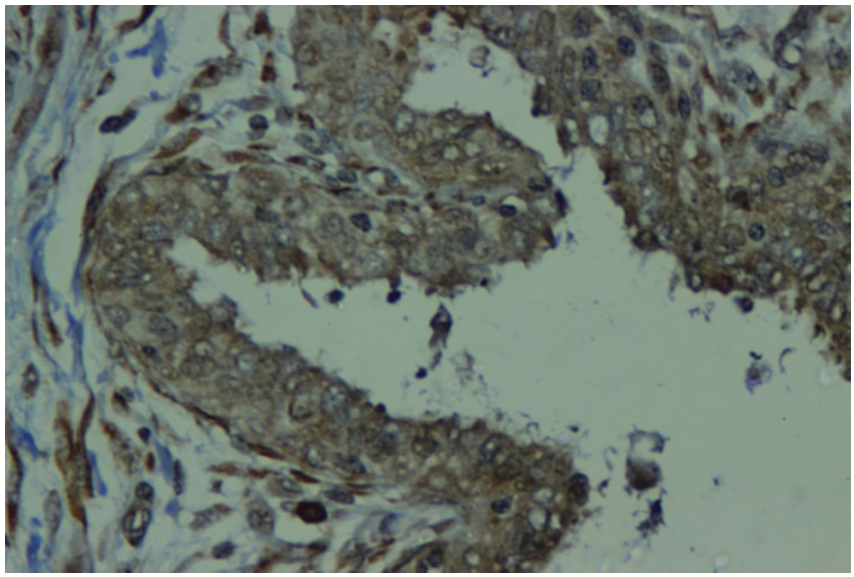

Figure 3. Papillary adenocarcinoma showing expression of ER alpha in the nuclei of epithelial and myoepithelial cells. Immunoperoxidase/ DAB substrate/ Harris haematoxylin counter stain x400.

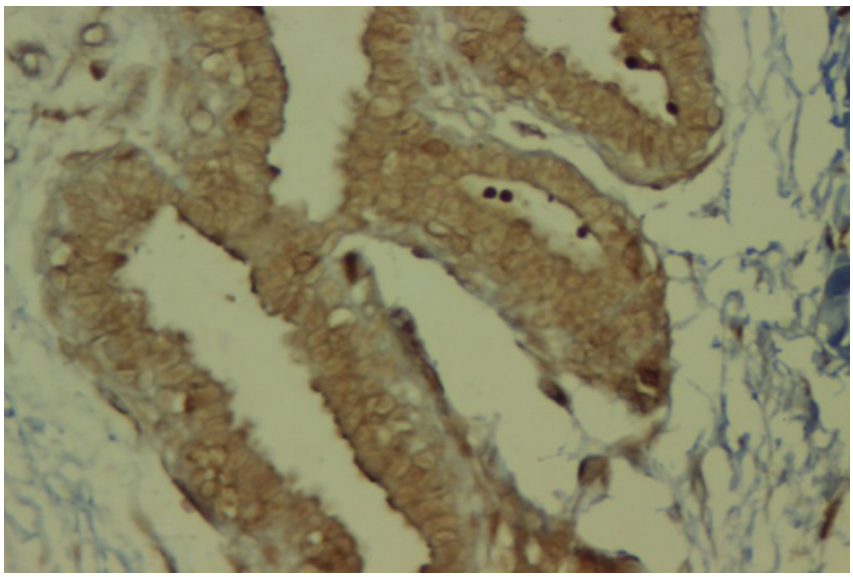

Figure 4. Photomicrograph showing expression of PR in the nuclei of epithelial cells. Immunoperoxidase/ DAB substrate/ Mayer's haematoxylin counter stain x400.

positive and PR positive mammary papillary cystic adenocarcinoma was diagnosed.

DISCUSSION

The present communication is first of its kind in buffalo bulls to the best of our knowledge. Male mammary tumors are rare in animals. There are few reports of male mammary tumors in human beings, dogs and goats. Reports of male mammary gland tumors in other species were restricted to older age group. In contrast, the bull in the present case was only three years old. In male dogs, cystadenomas, simple adenomas, complex adenomas and benign mixed mammary tumors were reported (Saba *et al*., 2007). Mammary gland adenocarcinoma was reported in a six years old Nubian buck which had been a potential breeder. Gynecomastism has been attributed as the cause of mammary gland development in males. Causes of gynecomastia in fertile male include chromosomal abnormalities, familial predisposition and hormonal effects (Wooldridge *et al*., 1999). In the present case, immunohistochemical findings revealed moderate positivity to ER alpha and mild positivity to PR which indicates a probable role of these hormones in the development of neoplasm. Previous reports on canine mammary neoplasms also revealed a similar pattern of immunohistochemical findings (Saba *et al*., 2007). However, familial predisposition and chromosomal abnormalities were not ruled out in this case. Unlike in buck where there was complete glandular involvement (Wooldridge *et al*., 1999), this case showed neoplasia of a single quarter, the reason for which has to be investigated. Follow up of present case revealed that the bull has recovered well without recurrence of tumour after surgical excision during the period of two years. Further, the bull was being used for natural insemination by the farmer and reported that the animal sired progeny during breeding season.

In summary, the present study reports an unusual occurrence of mammary gland adenocarcinoma in a bull positive for estrogen receptor alpha and progesterone receptors.

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