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Studies on Pathology and Therapeutic Efficacy of Anthiomaline in Bovine Papillomatosis

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ABSTRACT

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Bovine papillomatosis is a viral disease of cattle characterized clinically by development of multiple benign tumours termed warts. The diagnosis of bovine papillomatosis was confirmed by clinical and pathological examinations of the warts. The aim of the present investigation was to study the pathology of papillomas and to evaluate the therapeutic efficacy of anthiomaline in bovine papillomatosis. Six buffaloes suspected with papillomatosis were treated with anthiomaline (lithium antimony thiomalate) at the dose rate of 15 ml deep intramuscularly on alternate days in adult animals and 7 ml deep intramuscularly on alternate days in young ones i.e. calves. Gross and histopathological examination of the tumour masses revealed five cases of papilloma characterised by similar lesions of orthokeratotic hyperkeratosis, finger-like papillae with numerous vacuolating cells with eccentrically placed nuclei and one of leiomyoma. Anthiomaline was found to be effective in all the cases as no recurrence was observed in follow up study period of one year. It may be concluded that anthiomaline can be effectively used for treating cases of papilloma in bovines.

Introduction

Bovine papillomatosis is an infectious disease caused by bovine papillomaviruses (BPVs) which are non-enveloped double stranded DNA viruses belonging to the *Papillomaviridae* family. Papillomatosis consist of hyperproliferative lesions affecting both cutaneous tissue and the mucosa. The papillomas may occur in different parts of the body. Six different types of bovine papilloma virus have been characterized (BPV-1 to BPV-6). Bovine papilloma can appear in cattle of

any age, but it is seen more commonly in young animals of less than two years old and the tumors regress spontaneously due to the animal's immune response without significant scarring (Olson *et al.*, 1992; Campo *et al.*, 1994; Smith, 1996 and Jelinek and Tachezy, 2005). The disease is usually spread by direct contact with infected animal and virus enters through skin by cutaneous abrasions. Extensive lesions lead to depreciation in both the aesthetic and economic value of the animal owing to loss of body condition, hide value, increased risk owing to trauma, consequent

wounds, haemorrhages, myiasis, necrotic dermatitis, mastitis and interference in suckling, milking and coitus. These warts may regress spontaneously or occasionally persist, and, in the presence of additional critical genetic or environmental factors, can progress to cancer. Different methods can be used to treat bovine papillomas. Keeping in view of above facts the present study was conducted to understand the pathology of bovine papillomas and to evaluate the efficacy of anthiomaline in the treatment of papillomatous tumours.

Materials and Methods

Present study was carried out on six clinical cases of buffaloes suspected of papilloma tumour conditions that were presented to the Department of Veterinary Clinical Complex, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. History and clinical examination of cases was conducted to record age and sex, any bleeding /ulceration, infection/inflammation, regional or distant metastasis and gross appearances. Tissue samples were collected from multiple sites separately in 10% buffered formalin for histopathological examination.

Histopathological studies

For pathological examination of the tumours, representative tissues pieces of 3-5mm were surgically cut and placed in 10% buffered formalin subsequently processed by paraffin embedding technique as described by Luna, 1968. The histopathological technique involved fixation, washing, dehydration, clearing, embedding, section cutting, staining and microscopical examination.

The tissue samples were washed in running tap water to remove formalin, processed in ascending grades of alcohol for dehydration and cleared in benzene, infiltrated and

embedded in paraffin. The paraffin embedded tissues were cut into 3-4 micron thick section using automated microtome and stained with Hematoxylin and Eosin as per procedure of Luna (1968).

Treatment

The treatment of the animals affected with papillomatosis was carried out by administration of anthiomaline (lithium antimony thiomalate) at the dose rate of 15 ml deep intramuscularly on alternate days in adult animals and 7 ml deep intramuscularly on alternate days in young ones i.e. calves. The above treatment was repeated for a minimum of six times or till complete recovery. Other supportive therapy included administration of injection of vitamin C and B-complex.

Results and Discussion

The details regarding age, sex, gross appearances of the cases are described in table 1. The results revealed that the age of affected animals ranged from 3 months to 8 years and out of six, three cases were present in male calves and three in adult buffaloes. Clinically, the papillomatous growths with wart/nodule like structures were present on different regions of the body (Fig. 1 and 2). The warts present were either singly or multiple on different regions of body. The nodules were firm, dry and physically hard in four cases, whereas in the other two cases, the nodules were broadly attached at the base giving it an appearance of a circular disc.

Histopathological examination

Histopathological examination revealed that five out of six cases were of papilloma and one of leiomyoma. The detailed histopathological findings of these cases are described as follows:

Bovine Papillomas

Microscopically, papillomas were characterized by orthokeratotic hyperkeratosis, finger-like papillae with numerous vacuolating cells with eccentrically placed nuclei (Fig. 3). The growths were outward papillary projections of stratified squamous epithelium. These papillary projections consisted of different layer of stratified epithelium having keratinized layer on periphery and connective tissue in centre. Most of prickle cell layer have vacuolated cytoplasm. In stratum granulosum cell layer, large number of cells appeared

degenerated and also showed vacuolated cytoplasm. Histological features of papillomas in different cases of the present study were almost similar.

Therapeutic efficacy

The efficacy of anthiomaline in the treatment of bovine papillomatosis was found to be 100% as all the five affected cases recovered after treatment and there was no report of recurrence even after one year of follow up study.

Table.1 Gross and histopathological description of all tumour cases in bovine

Case no.	Animal species	Age (Years)	Sex	Location	Histopathological Diagnosis
1	Buffalo calf	0.3	M	Brisket region	Papilloma
2	Buffalo calf	0.5	M	Thigh region	Papilloma
3	Buffalo calf	0.4	M	chest region	Papilloma
4	Buffalo	8	F	Face region	Leiomyoma
5	Buffalo	8	F	Eye area	Papilloma
6	Buffalo	6	F	Base of ear	Papilloma

Fig.1 and 2 Bovine Papilloma: Papillomatous growths with wart/nodule like structures on different regions of the body

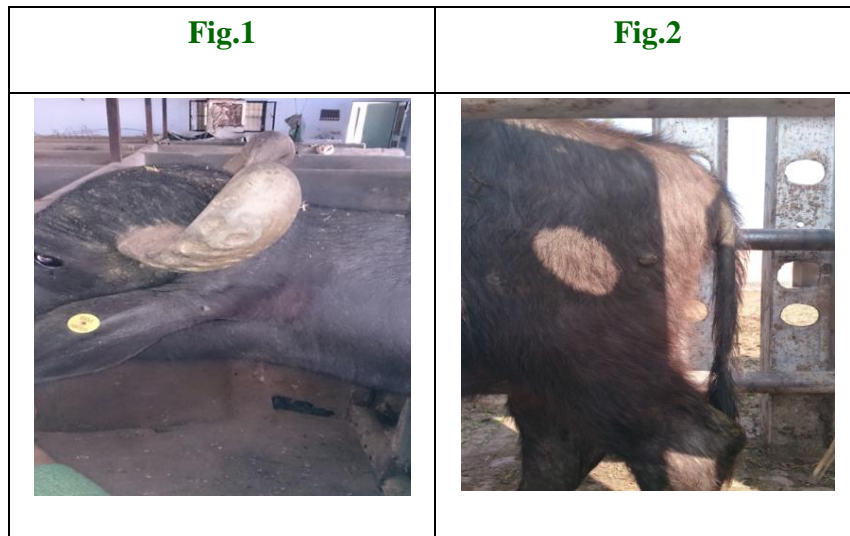
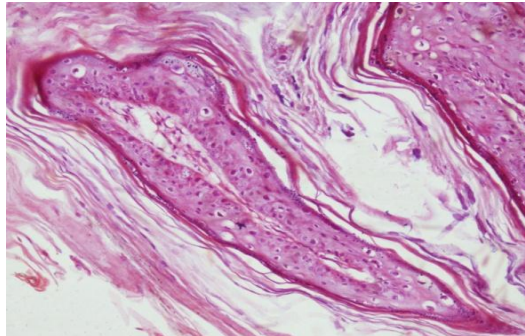


Fig.3 Bovine Papilloma: Photomicrograph of bovine papilloma characterized by orthokeratotic hyperkeratosis (black arrow), finger-like papillae with numerous vacuolating cells with eccentrically placed nuclei. (H & E stain X100).



Bovine papillomatosis is contagious in animals in which it naturally occurs and is caused by six different serotypes of epitheliotropic. Bovine papilloma viruses (BPVs) having double stranded DNA as viral genome distinguished by their restriction endonuclease cleavage. Amongst the cutaneous affections as encountered in bovine calf crops, benign cutaneous neoplasm (tumours) or warts caused by BPVs forms a most common clinical entity. It can be defined as benign nodular lesions, finger like projections or cauliflower like small growths on the skin arising from stratified squamous epithelium. It may be single (solitary) or appear in multiples (Jana and Mukherjee, 2013). The common sites for the development of cutaneous warts are head, eyelids, ears, neck, dewlap, brisket, shoulders and legs, occasionally on the back, para-genital region and along the lower line of abdomen (Miller and West, 1972; Aiello, 1988; Smith, 1996). Bovine cutaneous papillomas were observed mainly on the head and neck, but in some animals localisation in other parts of body has also been reported (Cimtay *et al.*, 2003; Atasever *et al.*, 2005). In present study the age ranges from 0.3 to 8 years and the sites for papilloma were brisket, eye, ear, back and thigh. Kavithaa *et al.*, 2014 studied 24 Jersey cows aged 6-8 years at Erode District of Tamil Nadu. The disease affects young

animals more often and more severely, but may affect cattle of all ages (Olson, 1993; Smith, 1996 and Nicholls and Stanley, 2000). Affected cattle were usually less than 2 years old and the tumours regress spontaneously within one year (Olson *et al.*, 1992; Campo *et al.*, 1994; Smith, 1996 and Jelinek and Tachezy, 2005). In present study the affected animals were in contact with each other. Although the spread of the disease was usually by direct contact, many factors such as contaminated food and equipment, castration, injections, inheritance, nutritional imbalance, hormonal imbalance, and suppressing the immune system may play roles in the spread of disease (Campo *et al.*, 1994; Dinc, 1995; Nicholls and Stanley, 2000; Otter and Leonard, 2003). Histopathologically, varying degrees of hyperplasia of the epidermis with irregular papillary projections into the dermis was common and it was seen in all animals. In the epidermis moderate to severe acanthosis, mild to severe hyperkeratosis, hydropic degeneration of keratinocytes and many koilocytes with variably sized keratohyalin granules were present. Also rare presence of intranuclear inclusion bodies was observed only in the basal cells of the epidermis. Dermis showed mild to moderate hyperplasia of the connective tissue that consisted of blood vessels, fibroblasts, focal hemorrhage

and mild infiltration of lymphocytes. The microscopic findings of the tumor that observed in the present study were similar to those described. The literature reveals that papillomas occur most commonly at sites of abrasion where papilloma virus may enter the epidermis and resulting in papillomatous growth (Goldschmidt and Handrich, 2002). Studdert *et al.*, (1998) reported papilloma of ear in calves following tattooing.

For treatment of these cases different methods have been reported like cauterisation, excision, cryotherapy, administration of local anaesthesia, autologous or heterologous vaccination, and autohaemotherapy or use of drugs for the nonspecific stimulation of the immune system (Bajric *et al.*, 1983; Amin *et al.*, 1997; Silva *et al.*, 1998). Bajric *et al.*, (1983) has reported papillomas to regress completely in the majority of cattle undergoing administered autohaemotherapy 3–4 times at intervals of 7–10 days. Biricik *et al.*, (2002) has reported following the administration of autohaemotherapy for 4 times at an interval of 3 days. Having treated bovine papillomatosis with levamisole at a dose of 2.5 mg/kg/day on days 1, 3, 5, 7, 9 and 16. Cihan *et al.*, (2004) have reported 100% recovery within 7–12 days after the completion of treatment. In the present study, animals which were given anthiomaline for six times on alternate day. All animals recovered within 12-14 days after the completion of treatment and there was no report of recurrence. It may be concluded that pathological changes in the papillomas were similar and anthiomaline can be effectively used for treating cases of papilloma in bovines.

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