

## CUTANEOUS PAPILOMATOSIS IN HORSES: RESPONSE OF HORSES TO AUTOGENEOUS WART VACCINE

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### ABSTRACT

Cutaneous papillomatosis is usually regarded as benign epithelial tumor caused by papilloma virus. The condition is species specific and has the tendency to spread frequently. In this study, a total of 15 horses suffered from cutaneous papilloma were used to study the efficacy of the autogenous papilloma vaccine. Each horse was examined clinically and few wart lesions were removed surgically and divided into two portions. One portion was fixed in 10% buffered neutral formaline for histopathological studies. The second portion was submitted for autogenous papilloma vaccine preparation. A total of 13 horses proved to be infected with cutaneous papilloma received three doses of 0.5 ml on weekly intervals intradermally. Two other horses with cutaneous papilloma were left with no vaccine to serve as control. Results indicated that all horses received the vaccine were free from papilloma lesions 3-5 weeks following the last dose of the vaccine whereas the control groups remained infected.

**Keywords:** Horses, papillomatosis, autogenous vaccine

### INTRODUCTION

Papillomas are caused by a number of different papilloma viruses which infect and transform epithelial and mucosal tissues in animals (Campo, 1992). Equine papilloma virus is usually considered host specific and several types have been identified within each species (Huck, 1965). The equine papillomaviruses cause equine papilloma and do not infect calves, lambs, dogs, rabbits, or guinea pigs (Cook and Olson, 1951). The disease is world wide in distribution and is more common in horses younger than three years of age that are housed in close contact with each other (Mansmann *et al.*, 1985; Moulton, 1990). Different methods have been used to treat equine papilloma (Wynjones, 1979). Intratumoral cisplatin chemotherapy was found to be practical and effective treatment of sarcoid and papilloma in horses (Theon *et al.*, 1993). Intra-lesional immunotherapy by *Corynebacterium parvum* has been used to treat bovine papillomas (Hall *et al.*, 1994). A formalinized suspension of bovine warts with inactivated virus provides a vaccine for prophylactic immunization with cutaneous warts (Barthold *et al.*, 1976). Therefore, the aims of the present study were to study the occurrence of equine papilloma in Jordan and to evaluate the efficacy of autogenous formalinized vaccine to treat horses clinically infected with cutaneous papilloma.

### MATERIALS AND METHODS

#### Horses

An outbreak of cutaneous papilloma was seen in horses used for polo and race purposes. Those horses were from polo and police stables located in Zarka province, Jordan. To find the effectiveness of autogenous vaccine, a total of 15 horses of 1.5 to 3 years old were used. A routine clinical examination was undertaken. Surgical specimens that were fixed in 10% buffered formalin and stained with haematoxylin and eosin was performed. Horses were divided into 2 groups. Group one involved 13 horses suffered from warts. Each horse received 3 doses of 0.5 ml intradermally on weekly interval of the prepared vaccine in the neck area. Group two involved two horses with cutaneous warts received 0.5 ml of buffered normal saline to serve as control.

#### Preparation of the Autogenous Vaccine

Two to three wart materials were excised surgically as aseptically as possible at skin line. The cutaneous layer was put in sterile saline at 1:10 ratio, then minced with a pair of scissors and ground in a mortar and pestle. The homogenate solution was filtered through several layers of clean gauze to remove larger pieces of tissue materials. To inactivate the virus formalin was added to the final concentration of 0.5%



(Olson and Skidmore, 1959). The suspension was incubated at 37°C for 24 hours, then a loopful of the fluid was plated on blood agar to check sterility. The pH was adjusted to be 7.0, then refrigerated until used.

## RESULTS

### Clinical Signs

Cutaneous warts were diagnosed in 15 horses among a total of 164 horses kept for race purpose. The horses of 1.5 to 3 years of age and of both sexes showed lesions. Clinically, papilloma occurs most commonly on the nose and around the lips as small, elevated circumscribed horny masses of 2 to 10 mm in diameter and their number from several warts to even more than 100 warts covering the entire muzzle (Fig. 1). Few warts were also seen near the lower eyelids and on the cheek areas. The warts had a cauliflower like appearance strongly attach the dermis.



Fig. 1: A 3 year old horse with cutaneous warts on the muzzle.

### Histopathological findings

Microscopically, the papillomas consisted principally of hyperplastic epithelium supported on a vascular connective tissue framework. There was elongation of the dermal papillae. Also, there was marked acanthosis or thickening of the prickle cell layer.

### Treatment

Two to three weeks following the last dose of the vaccine the warts started to disappear gradually and within 8 weeks all warts disappeared spontaneously. However, control horses remained infected during the same period of observation. No recurrence of lesions has been observed in any treated horses during 12 months following vaccination.

## DISCUSSION

Papilloma is among the most common tumor found in horses (Sundberg *et al.*, 1977). A retrospective survey of tumors and tumor like lesions encountered in horses in a veterinary practice in south east Queensland between 1956 and 197 were conducted (Pascoe and Summers, 1981). The results showed that equine sarcoid, papilloma and squamous cell carcinoma were the most occurring tumors. In another retrospective study of neoplasms in horses at Purdue University, Animal Disease Diagnostic Laboratory from January 1970 to December, 1974, Sundberg *et al.* (1977) reported the diagnosis of 21 neoplasms from 687 equine necropsies (3.1%) and 215 from 635 biopsies (33.9%) totaling 236 neoplasms from 1322 cases (17.9%). Those neoplasms were sarcoïds (43.6%), squamous cell carcinomas (24.6%), papilomas (5.5%), nerve sheath tumors (4.2%), melanomas (3.8%), lipomas (3.0%), granulosa cell tumours (2.5%), fibromas (2.1%), cholesteatomas (1.3%) and lymphosarcomas (1.3%). Papilloma lesions most often appear on the nose and lips of horses (Junger *et al.*, 1984). The disease transmitted mainly by direct contact with infected horses (Mansmann *et al.*, 1982). According to early European records, investigator estimated that annually one-eight of all European horses suffered from warts (Radostitis *et al.*, 1994). Equine congenital cutaneous papillomatosis has been reported sporadically (Garma-Avina *et al.*, 1981). The possibility that the virus persists *in utero* and may reach the fetal skin is supported by the identification of papillomavirus in the vulvar papillomas (Junger *et al.*, 1984).

The histopathological findings were pathognomic for the diagnosis of equine viral papilloma. The presence of intranuclear inclusion bodies is of significant diagnostic value. Hamada *et al.* (1990) studied the histopathological development of equine cutaneous papillomas in 78 warts naturally occurring in Thoroughbred or Arab horses. Lesions in the natural cases were categorized into three phases: growth, development and regression. Main lesions of the growing phase were marked hyperplasia of the basal cells and mild to moderate acanthosis, hyperkeratosis with a few intranuclear inclusion bodies which were positive with antiovine papillomavirus serum

(Hamada *et al.*, 1990). In the developing phases, there was prominent acanthosis with cellular swelling and fusion. Many intranuclear inclusion bodies were also present in swollen or degenerative prickle cells and granular cells. In the regressing phase, epidermal layers were almost normal with only slight hyperplastic change (Hamada *et al.*, 1990; Hamada *et al.*, 1992).

Our results have shown that effective vaccine for horses can be produced by the formalinization of wart tissues. Such vaccines have also been used for the treatment of cutaneous papillomatosis in cattle (Olson and Skidmore, 1959; Moulton, 1990). Immunity may last up to 6 months after vaccination so that they are resistant to subsequent challenge (Rosenberger and Grunder, 1959; Lee and Olson, 1968). In more recent studies, scientists cultured the virus in standard growth system (O'Banion *et al.*, 1986) and tissue culture vaccine is available. It has long been assumed that papilloma regression is mediated by immunological mechanisms which are probably cellular in nature (Hall *et al.*, 1994). Also, Wyn-Jones (1979) reported the successful ablation of the tumor mass and preventing regrowth in 19 of 21 periocular tumor groups in horses using radioactive gold <sup>198</sup> grains..

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