Vet.Med.J.,Giza. Vol.48, No. 1. (2000): 47-55.

SOME STUDIES ON BOVINE PAPILLOMA VIRUS INFECTION IN CATTLE WITH TRIALS OF ITS TREATMENT

M. I. EISA, A.KANDEEL*, A.A. EL-SAWALHY** and M. S. ABOU EL- FETOUH***

Dept. of Animal Medicine, Fac. Vet. Med. Zagazig University.
*Dept. of Surgery, Fac. Vet. Med. Zagazig University.
**Dept. of Internal Med & Infectious and Fish Diseases. Fac. Vet. Med. El-Mansoura University.
***Dept. of Pathology, Fac. Vet. Med. Zagazig University

Received: 13.4.1999.
Accepted: 24.5.1999.

SUMMARY

172 cattle (124 freisian and 48 native breed) in Sharkia and Ismailia governorates, Egypt, suffering from skin eruptions represented by wart - like lesions on head, neck, dewlap, eye lids and occasionally legs as well as extensive generalization, had been recognized. The disease was common and severe in freisian than native breed and in youngs than adults and it occurred in some cases mixed with other infectious skin diseases as ring worm or mange.

The warts from infected animals were taken for virological and histopathological examinations. Virus was isolated on foetal bovine skin tissue culture (FBS)and on embryonated chicken eggs. Histopatholoically, hyperkeratosis and acanthosis were noticed in the epidermis of the examined warts. Virological and histopathological results revealed infection with bovine papilloma virus and suggested type II .

Spontaneous recovery had occurred in mild cases. Good results had been obtained through the treatment trials by the use of autogenous vaccine in generalized cases. Solitary large sessile warts were removed surgically. For pedunculated, medium size and small solitary warts, cryosurgical treatment was highly beneficial.

INTRODUCTION

Bovine papilloma virus (BPV) is a benign cutaneous tumor caused by infectious DNA viruuses belonging to the family papovaviridae (Wildy,

1971). At least six distinct virus types exist each producing aspecific type of lesion (Bloch et al. 1995). Most BPV strains are similar antigenically when studied in gel diffusion tests (Lee and Olson 1969).

BPV is the cause of a number of proliferative lesions in cattle (Anthony and Werner, 1995). Warts caused by bovine papilloma virus occur most frequently in calves less than 2 years old, and appear mostly on the head & neck, less commonly on other parts of the body and occasionally occur in dairy herds on the teats (Biberstein & Zee, 1990). BPV type II causes papillomatosis of the skin of the face, head, neck and dewlap, eye lids and occasionally legs of young cattle, Infection may spread either by direct contact with infected animals or indirectly by fomites e.g. from fences by trauma through minor abrasions . (Andrews et. al. 1992).

In the treatment of bovine papillomatosis, it was reported that it is ineffective in early stages, but in late stages, vaccination and surgical excision may aid in recovery (Jensen and Mackey 1979, Farris and Wood 1981, Frank 1981, Gelatt et. al. 1988, and Greenough and Johnson 1988). Autogenous vaccines for the treatment of bovine papillomatosis are effective. When large persistent growth is present, removal by cryosurgery or cold steel surgery may be attempted (Andrews et. al. 1992).

Bovine papillomatosis was described in Egypt by many authours (Soliman & Iskander, 1963, Abdel- Gaffar et. al. 1975, Youssef, 1976, Gaballah, 1993, Dawlat et. al. 1997) .

MATERIAL AND METHODS

Animals :-

During 1998, in Sharkia and Ismailia governortes, Egypt, 172 cattle (124 freisian and 48 native breed) suffering from cutaneous proliferative lesions were investigated, table (1). Full clinical examination was applied on each infected animal.

Table (1): Age and breed of infected investigated animals.

Breed	Age / Total No.	Young		Over 2 years
		Less than 1year	1-2 years	
Freisian	124	44	58	22
Native	48	19	23	6
Total	172	63	81	28
		144		

Samples:-

Warts from 64 infected animals were removed surgically and kept in:-

a) Phosphate buffered saline solution (BPS) pH 7.4 for virological examination .

b) Neutral buffered formalin solution (10%)for histopathological examination.

Virological examination :-

Each warts sample was ground then 20% suspensions were made in phosphate buffered saline and clearified by centrifugation at 3000 r. p. m. for 30 minutes. Then antibiotic (penicillin , streptomycin - Cid, Egypt) was added to the supernatant warts extract and stored at - 20C° until used (Pierre and Michel 1993).

Virus isolation :-

a) On primary foetal bovine skin (FBS) cell culture

Foetal bovine skin cell culture (FBS) tubes were prepared and 0.2 ml. of the warts extract was dropped in each tube and left for 2 hours for adsorption . The tubes were then incubated at 37C° and examined daily for any cytopathic effect (CPE). The cells were harvested every week and subjected to freezing and thawing cycles 2-3 times, and cells passage was done for 3 successive times and examined for any CPE . (Freshney 1987) .

b) In embryonated chicken eggs :-

Into the chorio - allantoic membrane (CAM) of each egg 0.2 ml of the warts extract was inoculated. Inoculated eggs were incubated at 37C° & examined daily. Embryos that died within 24 hours were excluded. While CAM with living or dead embryos within 3-7 days post inoculation were harvested. Ground harvested CAM were re-inoculated for 5 passages . (Schmidt and Emmons 1989).

Histopathological examination :-

Specimens for histopathological examination were taken from the warts, and fixed in 10% neutral buffered formalin. Paraffin sections were prepared, stained with hematoxyline & eosin and examined microscopically (carleton et. al 1967) .

Suspected other skin lesions were examined according to Kelly (1984) and Quinn et. al. (1994) .

Trials of treatment of infected cases :-

Ten animals which were mildly affected were left without any treatment and kept under observation for 8 months (time of study).

Treatment trials were applied on 30 infected cases classified into 3 groups, 10 animals in each group. The first group included animals with generalized infected lesions. They were vaccinated using an autogenous vaccine prepared from wart tissues of the affected animals as has been described by (Theilen and Madewell 1987). Finely ground

wart tissue were suspended in 0.4% formalin solution and antibiotic (penicillin , streptomycin - Cid, Egypt) was added . Each animal was injected subcutaneously twice with 5 ml with 10 day intervals.

The second group of animals which had the large sessile warts was treated surgically. The surgical interfernce was performed under the effect of a local infiltration anaesthesia using xylocine 2% (Astra, Sadertalje, Sweden) after the animal was tranquilized by xylazine (Rompan, Bayer) in a dose of 0.02 mg/kg B.W. The area of the lesion was prepared aseptically before anaesthesia, the lesion was excised with a part of the healthy skin and subcutaneous tissue. The skin was closed with non absorbable suture material (silk) that was removed 10 days post operatively.

The third group comprised animals with pedunculated, medium sized and small solitary warts. They were treated by carbon dioxide probe freezing (Spembly P.C.G. cryosystem, spembly limited, England) which was applied on the neck of the lesion or on the lesion itself in medium sized and small ones. Single sission of two cycles was used for all cases.

RESULTS

1) Clinical signs:-

Out of 172 infected animals, 144 (83.7%) were less than 2 years and 28 (16.3%) were over 2 years, table (1). The affected animals showed small hairless, firm button-like elevations projecting slightly above the skin surface (in 89 cases). Thier diameter ranged from 1mm to several centimeters. In 47 cases they developed to become coarse and cauliflower like, sessile or pedunculated and single or multiple in number. The lesions (wart-like) were commonly sited on the head, neck, dewlap & eye lids and occasionally legs. Extensive generalization with loss of appetite and loss of weight was seen in 36 calves (Fig. 1, 2, 3).

Mixed infection was observed in 5 calves (ring worm in 3 calves and mange in 2 calves) (Fig. 4).

2) Viral isolation:-

a- on primary cell culture:-

The cytopathic effect (CPE) of the virus on FBS appeared 7-10 day post inoculation in the first passage. The CPE was rough granules, varying in shape & size scattered on the surface of the culture and some cell aggregation. The second and third passages also gave similar changes within 5-7 days.

b- Culativation on chick embryo:-

The development of multiple pin-head pock lesions on CAM was observed in the first passage while further passages showed thick and oedematous membranes.

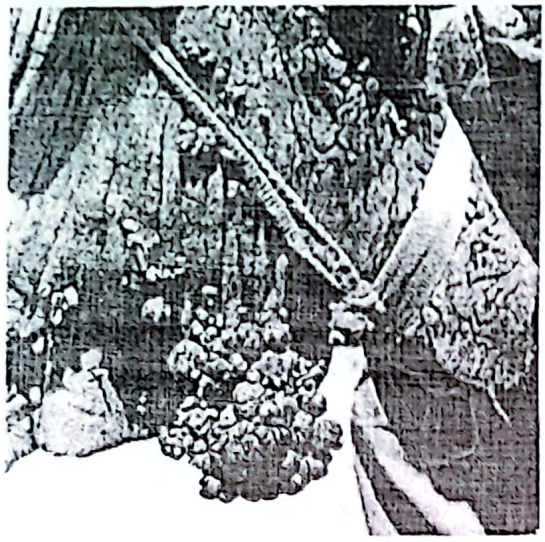

Fig. (1): Generalized bovine papilloma lesions (cauliflower-Like) in a calf.

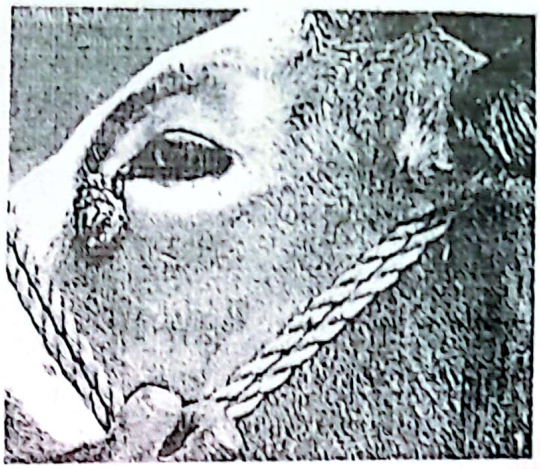

Fig. (2): Bovine papilloma lesion on the eye-lid in adult cattle.

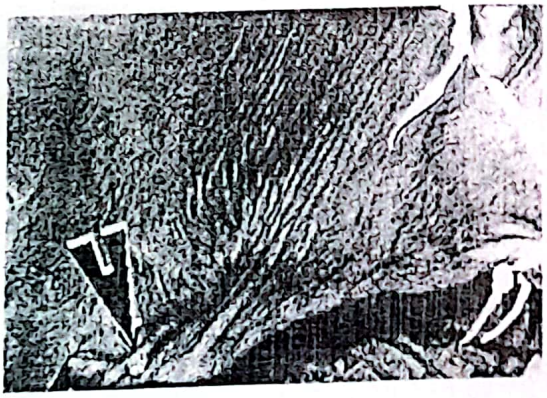

Fig. (3): Bovine papilloma lesion on dewlap in adult cattle.

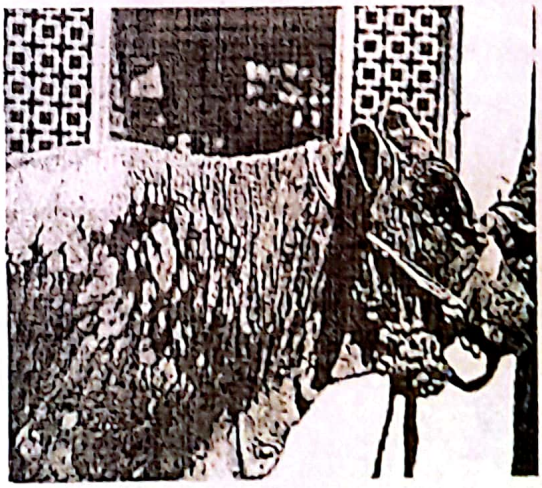

Fig. (4): Bovine papilloma lesion mixed with ring worm in a calf.

3) Histopathological examination:-

The histopathological examination revealed multiple papillary projections. These projections were finger-like and contained fibrovascular connective tissue which was covered by a well differentiated layer of heavily keratinized stratified squamous epithelium (hyperkeratosis) as well as proliferation of stratum spinosum (Acanthosis) was also noticed. (Fig. 5, 6). Leukocytic infiltration especially neutrophils and lymphocytes had been detected in the dermis.

4) Trails of treatment of infected cases:-

Spontaneous recovery had occurred in 10 mild cases within 2-8 months (the time of study).

Good results had been obtained by the use of autogenous vaccine in the generalized cases of group one. Recovery in 3-5 weeks was recorded in 7 cases (70%), while the 3 others showed poor response.

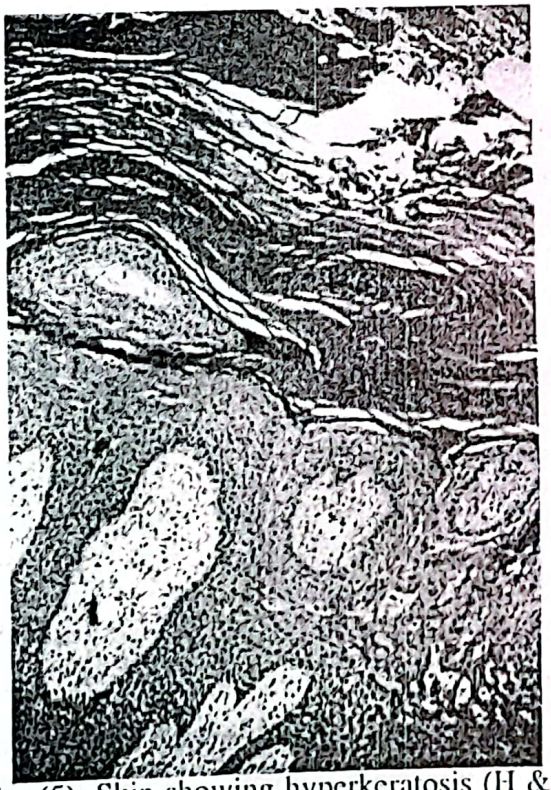

Fig. (5): Skin showing hyperkeratosis (H & E, x 400).

Fig. (6): Skin showing acanthosis (H & E, x 400).

Cases of group 2, that was treated surgically, gave good results and it was noted that a considerable degree of resolution of the remaining warts occurred several weeks postoperatively.

Cryosurgical treatment proved to be highly beneficial in gorup 3. Single session of two cycles was enough to obtain good response and sloughing of the lesions occurred through a period of 12-14 days leaving a small wound that healed rapidly leaving white hairless scar after 4 weeks (Fig. 7).

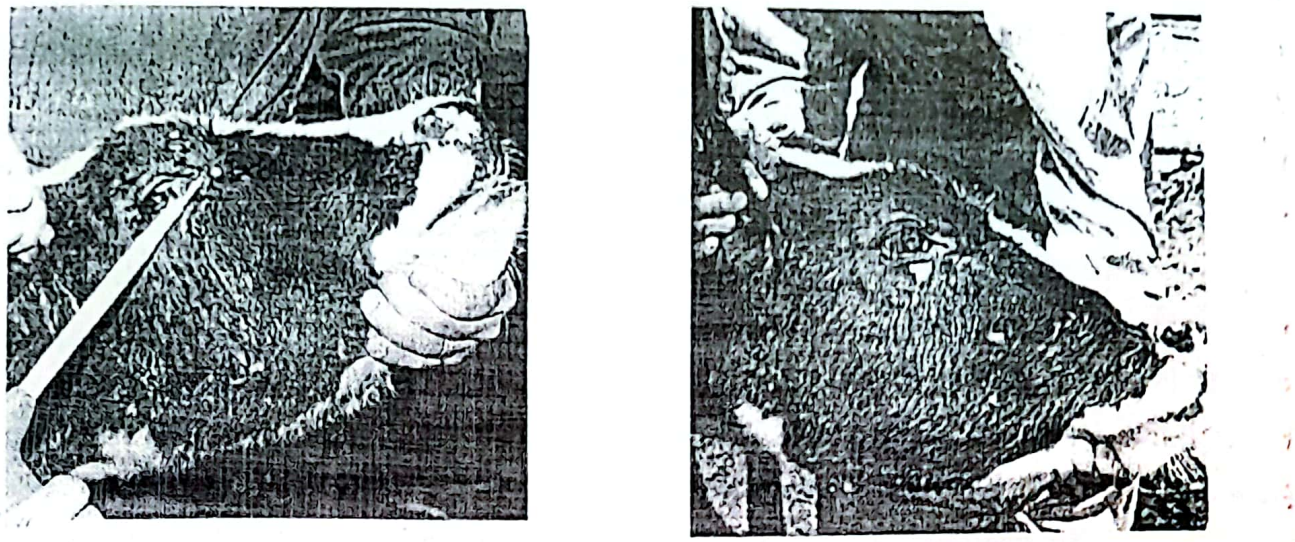

Fig. (7): Cryosurgical application for a pedunculated wart on the eye lid of a freisian calf (a) and after its sloughing (b).

DISCUSSION

Bovine papillomatosis is an infectious disease of the skin of manily young cattle and is widely distributed allover the world. In the present study, the skin lesions (warts) were scattered on the head, neck, dewlap, eye lids and occasionally legs of cattle. The warts were sessile or pedunclated and single or multiple. Extensive generalization had been occurred in young less than 2 years old which lead to loss of appetite and weight. Simialr bovine papillomas were reported by Andrews et al., 1992, and Anthony & Werner, 1995. The disease was common in youngs than in adults. Out of 172 infected investigated cattle, 144 (83.7%) up to 2 years were infected while 28 (16.3%) over 2 years were infected indicating that the disease is more common in youngs. This finding is in agreement with that of Robert, (1981) and Anthony & Werner (1995). Also the skin eruptions leions were more severe in freisian than native breed. The native breed may have some resistance to the infection with BPV.

The bovine papilloma virus was isolated on foetal bovine skin culture (FBS) and on embryonated chicken eggs. The virological and histopathological results revealed infection with bovine papilloma virus and suggested type II. These finding concides with those of Andrews et al. (1992) Jone et al. (1996), and Dawlat et al. (1997).

The bovine papilloma virus (BPV) infection may be mixed with other infectious skin disease as ring worm or mange. Out of 172 infected cattle with BPV, 3 calves were infected with ring worm and 2 calves were infested with mange. In the nature the virus enters through skin, and most successful transmissions require an injury or abrasion or by direct or indirect effects of ectoparasitism (Robert, 1981 and Andrews et al., 1992).

Spontaneous regression within 2-8 months had occurred in 10 animals suffering from mild lesions. Moreover the trials of treatment of generalized and solitary ones were successful. Treatment of generalized cases by the autogenous vaccine was effective and most cases had recovered, others gave poor response that might be due to the low virus concentration in the epithelial tissue of the warts in which the lesions in early stages and this may have some effect on the efficiency of autogenous vaccines. This results concided with Theilen & Madewell 1987, Andrews et al., 1992, Radostitis et al., 1994 and Bezerra et al., 1998.

The surgical removal is proved to be necessary and effective for larger warts, supporting the finding of (Jensen and Mackey 1979, Frank 1981 and Greenough and Johnson 1988). Cryosurgical treatment was effective in pedunculated, medium and small sized warts. The single sission of freezing with two cycles was enough to obtain good results, as has been reported by Farris and Wood (1981), Gelatt et al. (1988) and Behery (1992).

ACKNOWLEDGEMENT

The authors express their cardial thanks to **Dr. M.H. Ahmed**, Dept. of Virology, Animal Health Research Institute, Dokki, Giza for his help throught the work and the facilities he gave us at the Virology Dept.

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