Trakia Journal of Sciences, Vol. 13, Suppl. 2, pp 274-279, 2015

Available online at:
http://www.uni-sz.bg
ISSN 1313-7050 (print) **doi:10.15547/tjs.2015.s.02.061**
ISSN 1313-3551 (online)

PAPILLOMATOSIS IN HEIFERS – COMPARATIVE STUDIES ON SURGICAL EXCISION AND AUTOGENOUS VACCINE THERAPIES

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ABSTRACT
Papillomatosis is a viral disease manifested with benign cutaneous growths (skin epithelium hyperplasia) in different body parts (abdominal and thoracic wall, udder, vulva, head, neck etc.) which is encountered in cattle, goats, dogs, rabbits, horses, rodents and men.
The purpose of the present study was to compare the efficacy of two methods for treatment of bovine cutaneous papillomatosis – surgically and by application of autogenous vaccine, and to monitor the effect of applied treatments.
The present study was performed in 14 heifers divided into two groups with clinical signs of papillomatosis from a private farm, reared and fed uniformly. The main localisation of papillomas was ventrally on the abdominal wall, along linea alba, but growths were also found on the udder, vulva and eyelids of some animals. Their size varied from small wart-like formations (1-5 cm diameter) to orange-sized growths either with a wide basis or a thin petiole. Some of them formed rosary-like conglomerates situated on the long abdominal wall axis. Material for histological examination was obtained from removed growths and for preparation of autogenous vaccine.
The results from surgical excision in the first group were good – there were no recurrences in operated heifers. After application of the autovaccine in the second group, satisfactory results were obtained, although the results on papillomas of larger size (< 3 cm diameter) were disappointing.

Key words: papillomatosis, cutaneous form, autovaccine, excision

INTRODUCTION

In cattle, papillomatosis is caused by bovine papillomaviruses (BPV), belonging to genus *Papillomavirus*, family Parovaviridae. This genus also includes viral agents causing papillomatosis in other animal species (goats, dogs, rabbits, horses, rodents) and men. The virion has an icosahedral symmetry, size of 55 nm and contains a single two-stranded DNA molecule 7-8 kb of length (1). Twelve BPV serotypes are known – BPV 1-12, causing diseases with various clinical manifestations. Bovine papillomatosis is caused by BPV types from 1 to 10 (2), but BPV-1 and BPV-2 are outlined as main agents of fibropapillomatosis in cattle due to their affinity to epithelial tissue and skin (3, 4). Under appropriate conditions, BPV-1 and BVP-2 could also cause fibroplastic papillomas in horses (5), as well as cutaneous sarcoid and non-regressing neoplasm in this species (6). BPV infection results from the replication of the virus in basal cells and subsequent formation of wart-like growths, most of which are benign and do not proliferate infinitely.

The localisation of growths is different: abdominal and thoracic wall, udder, vulva, head, neck etc. Young animals < 2 years of age are most commonly affected, although papillomatosis is also encountered in adult cattle. It is prevalent in both genders and all breeds. Heifers are more frequently affected than steers – in the latter, the disease is seen as papillomatosis of the penis (7). The incidence of the disease is the highest among Hereford, Angus and Shorthorn cattle (8). The cattle carrying the virus are reservoir and source of

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the infection. Intermediate sources of infection could be contaminated fences, electric fences, beds, cubicles, feeders, surgical instruments, fixation devices, ropes etc. The risk of transmission increases if contaminated objects have sharp edges that could cause injury to the skin. Sharma et al. (9) reported higher incidence of the disease in late lactation and the winter (37.78%), followed by autumn (33.33%), summer (20.00%) and spring (8.89%).

The diagnosis is posed on the basis of clinical signs and histopathological examination of tumour growths. Other methods to confirm the diagnosis are electron microscopy, gene expression of papillomaviruses in peripheral blood and semen through PCR (10) and immunohistochemistry (11).

Papillomavirus infection in cattle could lead to weight loss and stunted growth. Skin lesions are often located on the udder and impede milking, hence milk yields are reduced. On the other hand, papillomatosis of the excretory system in cattle could provoke a continuous and persistent haematuria, which could result in anaemia. The diseases of the urinary bladder and excretory ducts are most commonly caused by BPV 1 and 2 (12, 13). The presence of BPV in the urinary bladder is possibly due to secondary infection following genital infection and/or haematogenous spread (14, 15). The clinical manifestation of these disorders could incur serious economic losses, if not detected and treated at due time.

The purpose of the present study was to compare the efficacy of two methods for treatment of bovine cutaneous papillomatosis – surgically and by application of autogenous vaccine, and to monitor the effect of applied treatments.

MATERIAL AND METHODS

Animals

The present study was performed in 14 heifers with clinical signs of papillomatosis from a private farm housing about 50 heifers aged 7 to 13 months, reared and fed uniformly. The heifers were divided into 2 groups of 7 animals each. In the first group, the growths were totally excised surgically applying thermocauterization. The cows from the second group were treated with autogenous vaccine prepared from papillomatous growths excised from the first group.

In all affected heifers, the growths were localised ventrally on the abdominal wall, along linea alba **(Figure 1).** In two animals, growths were also found on the udder and teats, in another 2 – skin neoplasms were observed around the vulva **(Figure 2)** and one heifer exhibited wart-like disseminated growths on and around the eyelids' skin **(Figure 3).** There were no formations on the neck or thoracic wall. The papillomas had a cauliflower shape, pea- to orange-sized, non-painful, not warm, with solid-elastic consistency . The major part of growths were mobile with respect to underlying tissues, but some of them (mainly on eyelids and the vulva) were immobile. The growths have been observed 2 months ago.

Figure 1. Multiple cutaneous growths on the ventral abdominal wall of a cow.

Figure 2. Perivulvar cutaneous growths in a cow.

No previous treatment has been applied. Seven heifers (group 1) were submitted to operative treatment, and the other 7 (group 2) were treated with autogenous vaccine prepared from the excision material from the first group.

Figure 3. Cutaneous growths on the upper eyelid in a cow.

Operative technique

Before the operative excision, each heifer was fixed with safety belts in standing position and sedated with xylazine hydrochloride 2% (Xylazine, Alfasan International, Netherlands) at a dose of 0.015 mg/kg IV. The affected areas were washed with warm water and soap, then treated with 1% potassium permanganate solution for ultimate removal of dirt, followed by drying and dipping with 3% iodine. The base of each formation was infiltrated with 1% novocaine and 10-15 min later it was excised with a scalpel and pincers, at the boundary between skin and growth base **(Figure 4).** The haemostasis was performed via thermocauterization. In the region of the vulva and eyelids, haemostasis was done via tamponade with a gauze tampon soaked in iodine tincture.

Figure 4. Incision at the base of the papillomatous growth using a scalpel.

Preparation and application of the autogenous vaccine

Immediately after excision, the material was placed in 10% neutral buffered formalin. The autogenous vaccine was prepared under laboratory conditions after cleaning and selection of the material. After cutting into 0.3×0.3 cm cubes, the material was put into 500 mL saline and homogenised in an electric homogenizer at 1000 rpm for 30 min. Then it was filtered twice (through a gauze and filter paper). To prevent the development of microorganisms, 5 mL lincospectin (Lincomycin-Spectinomycin 5/10, Alfasan International B. V., Netherlands). were added to the homogenate. The mixture was placed in a thermostat for 48 h. After another filtration through filter paper, 2.5 ml 10% neutral buffered formalin were added.

The heifers from the second group (n=7) were treated with 15 mL dose of the thus prepared vaccine applied subcutaneously, followed by revaccination after 14 days. The result was detected two weeks after the revaccination.

Histopathological examination

After the gross examination of all heifers with skin growths in different parts of the body, material for histopathological examination was collected by incisional biopsy. Specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, cut on a microtome at 4 μm thickness and stained with haematoxylin-eosin. The preparations were observed under a light microscopy for final diagnostics.

RESULTS

The present study was performed in 14 Black-and-White heifers from a private farm located in southeastern Bulgaria aged 7 to 13 months. The gross examination of papillomatous skin formations showed that they were most numerous on the ventral abdominal wall, the size of walnut, some of them – the size of an orange, and the shapes were various. Some growths were attached to the ventral abdominal wall skin either on a thin petiole, being highly mobile on palpation and non-painful. Other growth were densely arranged and formed clusters, solidly attached on a wide base to the skin surface. Apart on the ventral abdominal wall, they were observed on the surface of teats, vulvar labia (in 2 heifers) and around the eyelids (in 1 heifer).

Microscopically, typical *Papilloma molle* lesions were found in studied tissue samples **(Figure 5)**, and the growth originating from the epithelial tissue was benign.

Histopathological changes of specimens showed a proliferation of connective tissue. Histologically, completely differentiated fibroblasts and fibrocytes located amongst numerous collagen fibres were seen. Cell nuclei were stained in violet-blue, had an elongated fusiform shape, with scanty cytoplasm. The microscopy demonstrated clearly differentiated tumour parenchyma and stroma. The tumour parenchyma was composed by stratified squamous non-cornified epithelium which covered the stroma. The latter was built of fibrous connective tissue and blood vessels penetrating the tumour stroma in a pattern resembling the palm of a hand. The histology provided proof that the animals were affected by papillomatosis.

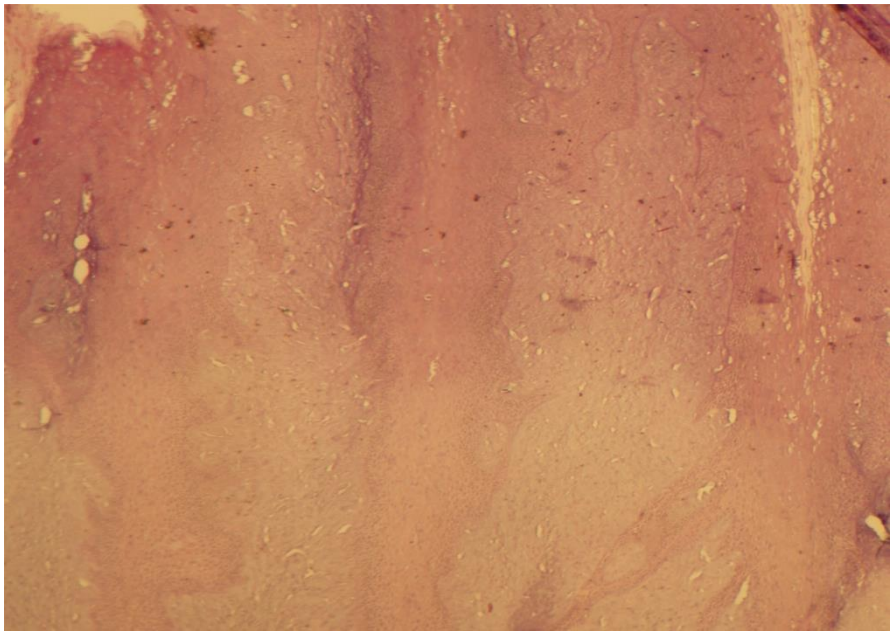

Figure 5. Papilloma molle - stratified squamous non-cornified epithelium covering the stroma, H&E, x300.

The operated animals from group 1 were monitored until the 7th day post operation in order to correct possible complications. The animals from the second group were examined weekly after treatment with autogenous vaccine (by post vaccination days 7, 14, 21, 30).

Operated heifers exhibited a milk aseptic inflammation around the excision and thermocauterized areas, manifested with milk oedema and warmth. The reaction resolved completely around the 10th-12th day. Only in one heifer, a post operation complication was observed on the first day, expressed with low-degree late haemorrhage which was overcome by another thermocauterization. Three months after the surgery, heifers did not exhibited clinical signs of papillomatosis, were completely healed, without recurrencies.

The treatment of heifers with clinical skin papillomatosis with autogenous vaccine was satisfactory only for a part of small skin growths (diameter up to 1-2 cm) and had an insignificant effect on large formations. One weeks after the first application of the vaccine, the shape of small growths was altered and their size – reduced. After another week, most of small papillomas fell whereas larger ones were still attached to the skin, but of rather smaller size. There was no effect on papillomas with diameter > 3 cm.

Two weeks after the revaccination, all small and medium-sized papillomas disappeared, but the effect on those of a size > 3 cm was unsatisfactory.

Two months after the application of the autogenous vaccine, there was no trace from small and medium-sized skin growths in the second group, but big skin formations were not influenced by the therapy. Nevertheless, no recurrencies were found out.

DISCUSSION

Papillomatosis is most prevalent among claves and young cattle under 2 years of age, but adults could be also infected. In cattle, cutaneous form of the disease could be found in almost all body parts. Some papillomas are characterised with strictly specific location depending on antigen reaction and DNA composition of agents. That is why a vaccine providing protection against a given strain is

neither effective against other strains (16), nor protects from infection with a different virus type, as not all papillomaviruses are immunologically related (17). Resistance to the BPV is built after repeated inoculations, but on the other hand, spontaneously infected cattle could express reinfection (18).

Histologically, cattle neoplasms differ from infection skin papillomas in other animal species which represent a hyperplasia of skin epithelium. Cheville and Olson (19) believe that the virus provokes a tissue response and cutaneous fibroplasia. Fibropapillomas of the reproductive system are more evident and in lesions after intradermal inoculation. In cases of atypical persisting lesions there are less visible or absent (20). It is affirmed that immune reactions are involved in the rejection of warts, but mechanisms are different from those preventing reinfection (18). Duncan et al. (21) affirm that generalised persistent papillomatosis is manifested in weak cell-mediated immune system component.

The commercial vaccines against papillomatosis or autogenous vaccines obtained from affected animals within the same herds could have a prophylactic but not a therapeutic effect (22). Jarret et al., (23) investigated the immunity against bovine papillomaviruses and found out that preventive immunisation is type-specific for most papillomaviruses. In the belief of the authors, viral structural proteins but not tumour-specific antigens elicit the immune response. Commercial vaccines are useful for partly prevention of disease if injected several times prior to the infection.

Although the cutaneous form of papillomatosis is usually benign, the form affecting the alimentary tract could become malignant. The successful treatment of papillomatosis is important and a challenge for practicing veterinarians. To this end, several methods could be used, but commercial vaccines are less effective than autogenous vaccines as in most instances, papillomatosis is provoked by strain specific for a given site or farm.

Kavithaa et al. (24) reported 92% efficacy of treating papillomatosis through autohaemotherapy consisting in four intramuscular applications of autogenous blood at one-week intervals. The same team reported 70% efficacy from the oral treatment with thuja extract, 58% efficacy from local ointment application with thuja oil and 81% cured animals with the preparation Anthiomaline™.

According to reported data, the treatment of cattle with autogenous vaccine prepared from sterile homogenised papillomatous tissue is 93.5% effective as it removed the skin and mucosal neoplasms, preventing recurrencies and new cases of papillomatosis in treated animals (25).

The surgical intervention could not be possible in some cases, when numerous animals are affected, when papillomas have spread on a large body area, when their shape and size do not allow excision or would worsen the state of the patient or threatens its life. In such instances, alternative methods of treatment are appropriate, among which is the preparation and utilisation of an autogenous vaccine. In our study, surgical excision was the more appropriate solution as the shape, size and location of papillomas permitted their easy operative removal. Furthermore, the disagreement of our results with other published reports could be due namely to the different size of growths between the studies. Yet, the use of the autogenous vaccine yielded good results but only for small papillomatosis (up to 1-2 cm diameter) whereas the outcome in larger growths was not satisfactory.

CONCLUSION

On the basis of our results, the cure rate of cutaneous papillomatous growths through surgical excision was higher than after application of autogenous vaccine. The therapeutic effect was more rapid and definitive after surgery. This allowed recommending this method for treatment of cutaneous papillomatosis but only in cases where the specific conditions, available options and clinical signs (size, shape, location) were appropriate for total excision. When the localisation, the number and size of growths are not eligible for surgery, the use of autogenous vaccine is another treatment option but possibly, it could be applied over a prolonged period of time. This could be a subject of future research.

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