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HISTOPATHOLOGICAL STUDY OF PAPILLOMATOSIS OF CATTLE SKIN IN BASRAH

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Abstract:

This research aimed to study and determine the kind of skin tumors of 25 cattle in Basrah city, after surgical removal of this tumors by using hematoxylin, eosin, and special stain. Papilloma was a proliferative neoplasm which benign typically that affecting nearly all animal species. The samples were taken from the skin of 25 infected cows and surgically removed at the College of Veterinary Medicine/Surgical Laboratory, then histopathological section prepared from this samples at Pathology and Poultry Disease Department where diagnosed. The histopathological section revealed vacuolation and degeneration of epidermis and dermis cell layers, hyperkeratosis, hyperplasia of stratum spinosum and Basale, vacuolation of dermal cells, and hyperplasia of hair follicles. Fibro papilloma in dermal layer represented by hyperplasia of fibroblast, and hair follicles in dermis. The tissue section appears positive results to special stain. The Masson stain sections revealed proliferation of connective tissues (fibro papilloma). The PAS stain section showed large amount of collagen into dermal layer.

Keywords: *Papilloma, Skin Tumors, Cattle.*

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Introduction:

The neoplasms that almost found in all types of animals was Papilloma tumors or warts. The neck was the location of warts in young cattle. Papillomatosis is a benign type of neoplasm, for example, skin papilloma, fibro papilloma and also malignancy, for example, bladder cancer in cattle which causes considerable economic losses [1, 2]. Warts also commonly known as Bovine viral papilloma, are caused by the bovine papilloma virus (BPV) that leads to the proliferation of the skin and formed verruciform lesions [3, 4]. BPV is primarily self-limiting, but warts may be removed either for cosmetic reasons or if it is irritating the animal (e.g., near the eyes). In ruminant the virus formed benign and malignant tumors, that causes economic losses significantly such as benign fibroplasia, cutaneous papilloma, esophageal, and urinary bladder cancer [5, 4]. Bovine papilloma are benign tumors of the cutaneous and mucosal epithelia and are commonly found in cattle. They are characterized in general found in mucosa of by the upper alimentary tract, skin, and localization on teats in multi-sized warts. They cause depravation of the appearance of the skin and the economic depreciation of animals [6]. Under the influence of environmental co-factors, bovine papilloma viruses (BPVs) induce lesions in cattle and may progress to cancer [7]. Bovine fibro-papillomatosis was caused by Bovine papilloma virus (BPV)which Like other papillomaviruses, BPVs are small non-enveloped viruses with an icosahedral capsid around 50–60 nm in diameter. The capsid is formed of the L1 and L2 structural proteins, with the L1 C-terminus exposed. (8,9,10), species-specific disease, and an infectious, found worldwide. It mainly affects young cattle and is associated with different predisposing factors such as immunosuppression conditions, animal age, nutritional status, parasitic infestation, bad management, stress and drugs that effect immunity, among others [11]. Fibromas, papilloma or fibropapillomas are benign proliferative neoplasms. They can be external or internal, single or multiple, partially delimited, plaque-like and papillary. Their appearance can vary from that of a grain of rice to the texture of cauliflower, and their texture can be dry or firm. They can become necrotic and detach, and may exhibit secondary bacterial contamination [12,13]. Papilloma microscopically shows squamous epithelium papillary projections, supported by fibro-vascular stroma. These epithelial projections exhibit marked hyperplasia and hyperkeratosis. In some papilloma, keratinocytes, mainly those of the stratum spinosum, shows characteristic features as kilobytes (cells with cytopathic changes), abundant clear cytoplasm or a perinuclear halo and pyknotic nuclei. Conditions present in certain regressing papilloma include reduction of epidermal hyperplasia, increased fibroblast proliferation, collagen deposits, and lymphocyte infiltration. Fibropapillomas have two components: a lining epithelium which alternates with fibrous tissue arranged in short interlocking bundles, and reactive fibroblasts. The lining epithelium does not exhibit cytopathological changes but does have marked hyperplasia and plexiform acanthosis (1,14). In large lesions, the epithelium may erode and come to resemble fibroids, in which proliferation of fibroblasts with dense collagen deposits has been observed (15). Histopathological view, varying degrees of hyperplasia of the epidermis with

irregular papillary into the dermis, low to severe acanthosis in the epidermis, low to severe hyperkeratosis, hydropic degeneration, and rarely intranuclear inclusion bodies are observed only in basal cells of the epidermis [16].

The aim of my research is histopathological study of the papilloma type and find the causes relationship with increased number of it in the late years especially in cattle.

Material and Method

1. Sample Collection

Tumor tissue biopsy collected from 1 breed of 25 cattle and the cattle aged 1 year each. Before the biopsy, the surrounding tissue was under anesthesia with local anesthetic (Lidocaine HCL). Furthermore, the sample was fixed in 10% neutral buffered formalin (NBF) solution and then made histopathological preparations.

2. Histopathological Preparations

Histopathological preparations were processed regarding the modification of the method of Kiernan [17]. Tumor samples were fixed in a 10% neutral buffer formalin (NBF) solution, then cut to a size of 1x1x1 cm, dehydrated in an ethanol series with increasing concentrations (70%, 80%, 96%, absolute ethanol 2 parts) for 2 hours each. Then clearing with xylene 2 parts for 1.5 hours, then infiltrated with paraffin infiltration parts for 2 hours each and then implanted in a paraffin block using a paraffin embedding set. The paraffin blocks were then cut to a thickness of 5 µm using a microtome. The best cut results are floated in a water bath with a temperature of 45°C. The preparation is then lifted and placed on the object-glass, then dried on a slide warmer for at least 2 hours.

The tissue slides were deparaffinized using xylene 2 parts for 5 minutes. Then rehydrated with absolute ethanol 2 parts, 96% ethanol 2 parts, 90% ethanol 2 parts each for 3 minutes, then soaking in distilled water for 1 minute. Then stained by soaking in a solution of hematoxylin for 5 minutes, then rinsing with distilled water. After that, once to acid alcohol and then put in the eosin solution for 5 minutes. Then rinsed again with distilled water, then the dehydration process with 96% ethanol 2 parts, absolute ethanol 2 parts each dipping twice. After that, cleared with xylene for 3 minutes, then mounted with adhesive material Entellan slides. The observations were made with an Olympus light microscope and documented with a micrograph photo.

3. Special Stains

1/Periodic Acid Schiff Reaction

The periodic acid Schiff Reaction Stain, often called the PAS stain, is a way to examine structures containing high amounts of carbohydrate molecules, such as the intestinal brush border, renal tubular cells, mucus, and reticular fibers of connective tissue. [4] The glycogen, glycoprotein, glycolipids, and mucins stain red or magenta color when the stain is complete. The periodic acid, a highly oxidized iodine, oxidizes the hydroxyl groups of

adjacent sugar molecules to produce aldehydes. After this step, the Schiff reagent attaches to the aldehyde and forms a red magenta color for visualization [18].

2/Masson stain

PROCEDURE:

Mordant in Bouin's solution, microwave 1 minute, allow to stand 15

Minutes, Wash in running tap water to remove the picric acid, 5 minutes, Weigert's working hematoxylin, 10 minutes Blue in running tap water for 5 minutes, rinse in distilled water, Biebrich scarlet for 5 minutes, Rinse in distilled water, phosphotungstic/phosphomolybdic acid for 10 minutes, discard solution, Transfer directly into Aniline blue for 5 minutes, Rinse in distilled water, 1% Acetic acid for 1 minute, discard solution, rinse in distilled water, Dehydrate, clear, and coverslip.

Results

All skin biopsies exhibited common pathological characteristics such as irregular hyperplasia and marked hyperkeratosis of the epidermis, presences of some hear follicles, erosions, and ulcers. The dermis shows dense collagen and ballooning degeneration of the epithelium (Fig. 1). The epidermis shows some hear follicles the basal cell layer revealed hyperplasia with the presence of hyperchromatic nuclei and moderate mitotic activity (Fig. 2). Proliferation of hear follicles and mature fibrous connective tissue was observed in the dermis, interspersed with reactive fibroblasts, dense collagen, and multiple aggregates of lymphocytes, plasma cells and macrophages (Fig. 3).

The histopathological section of skin shows positive to Masson’s stain that revealed hyperplasia of fibers in epidermis and mature connective tissues with fibroblasts in dermis (Fig. 5). While the (Fig. 4) the histopathological section positive skin to PAS stain show hyperplasia in basal cells layer, hyperchromatic nuclei and mature connective tissue in dermis. The pathological section appears positive reaction to PAS stain that revealed hyperplasia in basal cell, degeneration of epidermis cells, hyperkeratosis, and mature connective tissue in dermis (Fig.6), hyperplasia of basal cells in epidermis. Thick bundles of mature connective tissue, and fibroblast in dermis (Fig. 7).

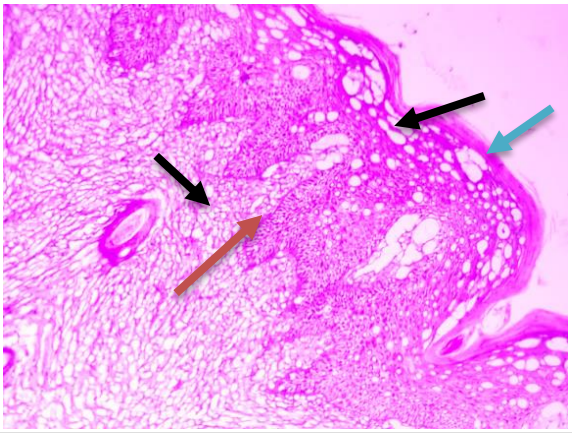

Figure 1: the histopathological section of skin show degeneration of epidermis and dermis(black arrow), irregular hyperplasia(red arrow), and hyperkeratosis(blue arrow), The basal cell layer show mitotic activity . H&E stain 100X.

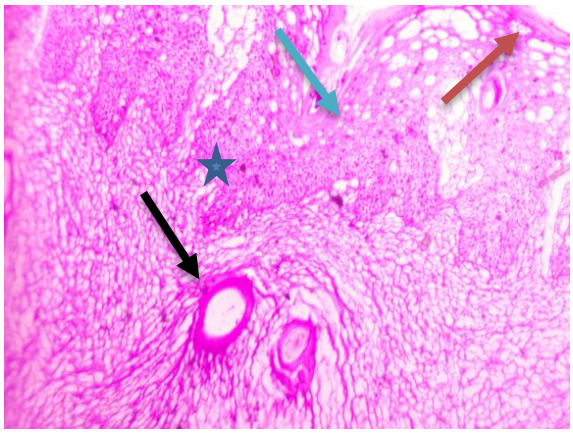

Figure 2: the histopathological section of skin show hair follicles(black arrow) and hyperkeratosis in epidermis and dermis(red arrow), irregular hyperplasia(blue arrow), The basal cell layer show mitotic activity(star) H&E stain 100X.

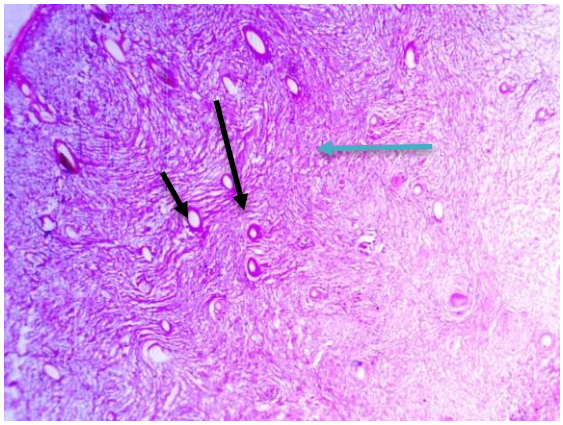

Figure 3: the histopathological section of skin show hyperplasia in hair follicles(black arrow) and mature connective tissue in dermis(blue arrow). H&E stain 100X.

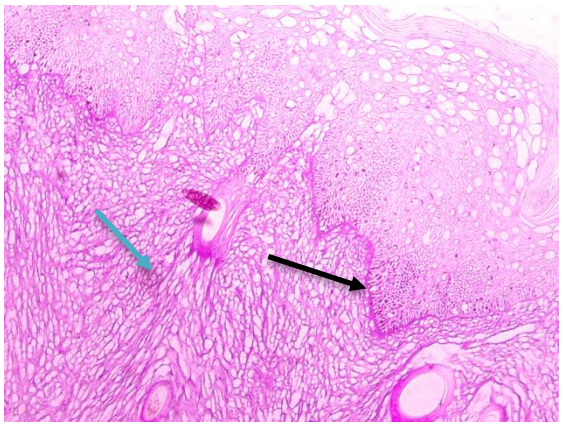

Figure 4: the histopathological section of skin shows hyperplasia in basal cells layer with hyperchromatic nuclei(black arrow) and mature connective tissue in dermis(blue arrow). PAS stain 100X.

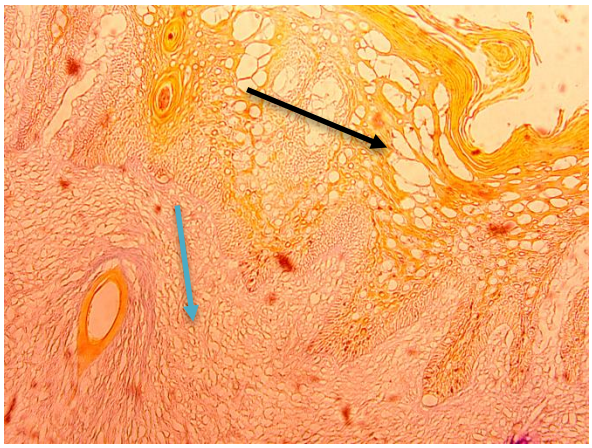

Figure 5: the histopathological section of skin shows hyperplasia in fibers of epidermis (black arrow) and mature connective tissue in dermis (blue arrow). Masson stain 100X.

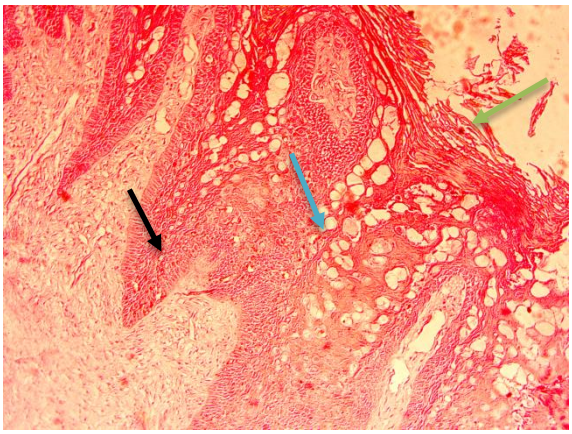

Figure 6: the histopathological section of skin shows hyperplasia in basal cell (black arrow), degeneration of epidermis cells (blue arrow), hyperkeratosis (green arrow), and mature connective tissue in dermis. PAS stain 100X.

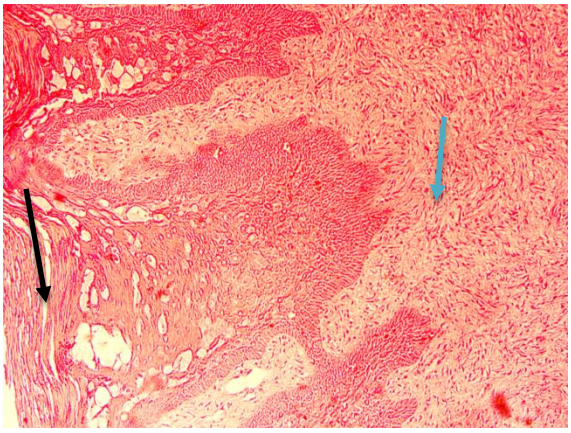

Figure 7: the histopathological section of skin shows hyperplasia of basal cells in epidermis (black arrow). Thick bundles of mature connective tissue and fibroblast in dermis (blue arrow). PAS stain 400X.

Discussion

Papillomatosis is the tumors of skin either benign (skin papilloma, fibro-papilloma) or malignant (bladder cancer) in cattle (1,2) or associated with malignant tumors in the bladder and upper digestive tract (19, 20, 21). The Bovine Papilloma Virus was the meager types that causes Bovine Papillomatosis (warts) on the skin and mucosa, and the most commonly subtypes that isolated (3, 4, 5, 6 and 22).

they are highly contagious and may affect the entire herd, since transmission occurs easily either by direct contact, or indirectly through fomites, insects, and pastures (23). For dairy cattle, the milking process also plays an important role in transmission, namely through milking equipment and hand milking.

In the present study observed presence of hyperplasia, degeneration, and hyperkeratosis in epidermis layer cells that compatible with most previous research who found the most skin alteration might be caused by mitogenic role of BPVs. (24) detected those connective tissues and epithelium affected by BPV directly. However, (25) identified

hyperplasia in epidermis and papillary projection in dermis to different degree in animals. Many forms of pathological hyperplasia result from excessive hormonal stimulation of target cells (26), the formation of intimal hyperplasia is often linked with vascular cell activation. Numerous factors promote the formation of intimal hyperplasia such as vascular wall injury, aging and inflammation. While other revealed there was no connection between the histopathological finding and involved viral types (27). In the current study histochemical results in the skin of BPV infected animal shows hyperplasia in basal cells layer, hyperchromatic nuclei and mature connective tissue in dermis. The pathological section appear positive reaction to PAS stain that revealed hyperplasia in basal cell, degeneration of epidermis cells, hyperkeratosis, and mature connective tissue in dermis that revealed to collagen deposition in the center of the lesion , surrounded by a thick capsular structure of keratin layer , because of over production and keratinized epithelial protein in the epidermis , that which agreed with(28) which was noted proliferation of cutaneous fibrous connective tissues and well developed finger like projections with connective tissue cores were showed.

Conclusion there are many cases of papillomatosis which were different in size and located in all regions of the body and appear as hyperkeratosis, hyperplasia in basal cell layers, in connective tissue and in hair follical.

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