

Pathological and Fungal Isolation study of dog skin disease in Iraq Governorates

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KEYWORDS

COMBI method,
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ABSTRACT

Dogs are susceptible to several types of infectious diseases can be worrisome for their owner because some of them are fatal if not treated soon enough. Fungal infection can be contracted through different parts of body furthermore many of them are zoonotic infection among human and dogs the signs of any disease typical depend on location of infection either local or system infection. Local infection usually involves skin surface that look like wound the objective of this study was to briefly about the most common pathogenic fungal infection in dogs by describing information about the causative agent, clinical signs and grossly appearance with histopathological change skin examination current study were done on 200 dogs collected in Iraq governorates (Baghdad, Erbil, Anbar, Sulaymaniyah) during a period 6 months (January – June) 2023. 200 examined dogs with different age, breed and sex (k9, ornamental and stray dogs) were observed fungal isolation and pathological examination with clinical signs by using diagnostic tools for skin biopsies (biopsy punch) from suspected infected skin dogs. Results showed pathogenic fungi after collected on fungal culture for detection pathogenic fungal (Dermatophyte Test Agar) D.T.M Agar Base. Fungal isolation constitutes the following pathogenic fungi (100 infected samples from 200 suspected samples). 60% *Aspergillus niger*, 30% *Trichophyton Verrucosum*, 10% *Alternaria* spp. Clinical signs and grossly lesion showed complete skin ulcer, alopecia, odema, abscessation while microscopic appearance revealed skin ulcer, hyperkeratosis, folliculitis, necrosis, apoptosis, pyogranuloma and granulation tissue with septate hyphae in skin dermis and epidermis which infected with *Aspergillus niger*.

1. Introduction

Skin disease is the noble disease which have various type of cause. it may be due to environmental pollution source (1) Or maybe industrial activities increased (2) Food pollution (3) Mineral toxins (4) Bacterial cause (5) Fungal cause (6) Parasitic cause (7) And tumor cause (8) Dermatophytosis is the most common fungal infection Affecting skin, hair and Nails and dogs and cats. It is characterized by superficial skin infection confide to keratinized epithelium. The warm and humid climate is most suitable condition for infection. this infection is a transmitted by direct contact with the infected animals or with contaminated equipment with fungus such as grooming equipment (9). The Cutaneous mycobiota associated with skin disease associated with a variety of a human skin disease include acne vulgarize (10). Atopic dermatitis (AD) (11). Fungal infection require keratin for hair growth (12). Dermatophytes are classified in to three groups paid on their habitat. zoophilic mostly found in animals occurring transmission to other animals or to humans. anthropophilic mostly found in the humans transmitted between humans and rarely to animals and geophilic (13). The affected animals usually have alopecia, Scaly, Custard, erythematous Popular lesion, especially and face and limbs was deep inflammatory and separative lesion (14). *Aspergillus niger* major may occur in the skin associated with trauma, surgery and foreign body to skin. It's mold causing necrotizing skin (15). *Trichophyton Verrucosum* is a genus onygenales infected hair, skin and Nails of dog causing infecting granuloma its development of Poth smooth walled Marco and microconidia (16;17).

Alternaria spp. Is a fungus causing disease it's an opportunistic pathogen produce mycotoxin called fumonisin B1 genes with chain of spore's formation (18).

2. Material and method

200 cases of dog (K9, or mental and stray) with different ages, sex and breed were examined during the period 6 months from (January-June) 2023. the dogs collected from different governorates in Iraq (Baghdad, Erbil, Anbar, Sulaymaniyah). Fungal isolation and identification with pathological examination were done in the department of pathology and poultry medicine veterinary college Baghdad

Method:

The 200 dogs were examined carefully for any abnormal skin lesion 100 samples suspected infected were taken from edge and center of lesion by surgical blade blunt on the skin (Biopsy 654bunch) the size about 8mm. These samples were taken for pathogenic fungal characterization cultured on (dermatophyto test agar D.T.M.) For growth and isolation, the pathogenic fungus Lactophenol cotton blue stain use it for fungal diagnosis under light microscope 40% Samples rapidly fixed in 10% neutral buffered formalin solution for microscopic examination.

Histopathological Examination

100 Suspected infected skin samples Were processed in Lee astrological technique by ascending grads of ethanol alcohol 70-80-90 and 100%. Then impeded in xylene four clearance after that section cutting by microtome about (5mm) thickened finally stained by hematoxylin and eosin stain which stained the nuclei and cytoplasm (H&E). Other stain periodic acid schifft stain (PAS) special stain for fungal. (19).

1. Results and Discussion

Fungal characterization and identification Fungal which isolated from dog skin were cultured and the growth of fungus revealed 60% *Aspergillus niger*, 30% *Trichophyton Verrucosum* and 10% *Alternaria* spp.

Fungal isolation



Fig (1) *Aspergillus niger*
Black growth

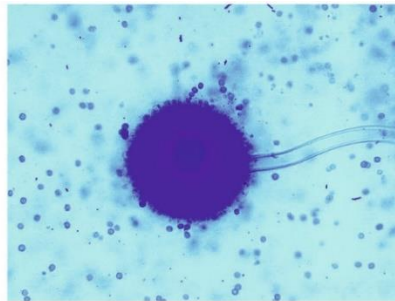


Fig (2) *Aspergillus niger*
(Lactophenol blue stain, 40x)



Fig (4) *trichophyton verrucosum*
Irregular growth

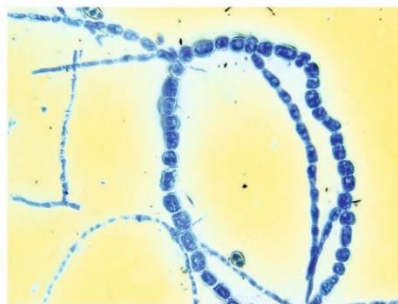


Fig (3) *trichophyton Verrucosum*
(Lactophenol blue stain, 40x)



Fig (5) *Alternaria spp.*

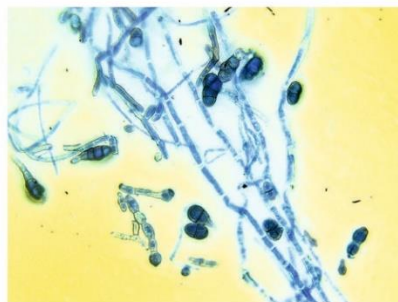


Fig (6) *Alternaria spp.*
(Lactophenol blue stain, 40x)

clinical signs and grossly appearance:

Different irregular locally and skin ulcer with alopecia where present on the neck, for-limbs and flank. Multiple elevated irregular nodules with necrotic and congested skin in some area Hyperpigmentation and elevated skin nodules.



Figure7: dog skin multiple red necrotic irregular Cauliflower-like appearance nodules pedunculate with ulcerated surface and secondary infected a raised on the fore-limb (squamous cell carcinoma with fungal *Alternaria spp.*). **Figure8:** dog skin severs alopecia with a pendulous abdomen with large skin ulcer (dermis and epidermis) with irregular large leg ulcer isolation from skin lesion (*Aspergillus niger* - *Staphylococcus pseudintermedius*). **Figure9:** dog skin Complete ulcer in the neck skin with alopecia (*Aspergillus niger* - *Staphylococcus pseudintermedius*).

Microscopic appearance:

Severe acanthosis of epidermal layer with apoptosis and mononuclear cells inflammatory cell infiltration in dermal layer (**fig10**) Vacuolar degeneration with apoptosis (**fig 11**) Severe mononuclear cells infiltrated in dermis layer (**fig12**) ulcer of epidermal layers, fibroma consist of whorls and interlacing bundles fibroblast and collagen fibers. The tumor cells have large pale elongated nuclei with variable degrees of vascularization (**fig 13**) Cystic dilation of hair follicles surrounded by mononuclear inflammatory cells and dermal lymphocyte aggregation (**fig 14**) Infected granulation tissue with mono and polymorphic inflammatory cells in dermal layer (fig 15) Aspergillus hyphae (separate) in dermis layer (**fig 16**).

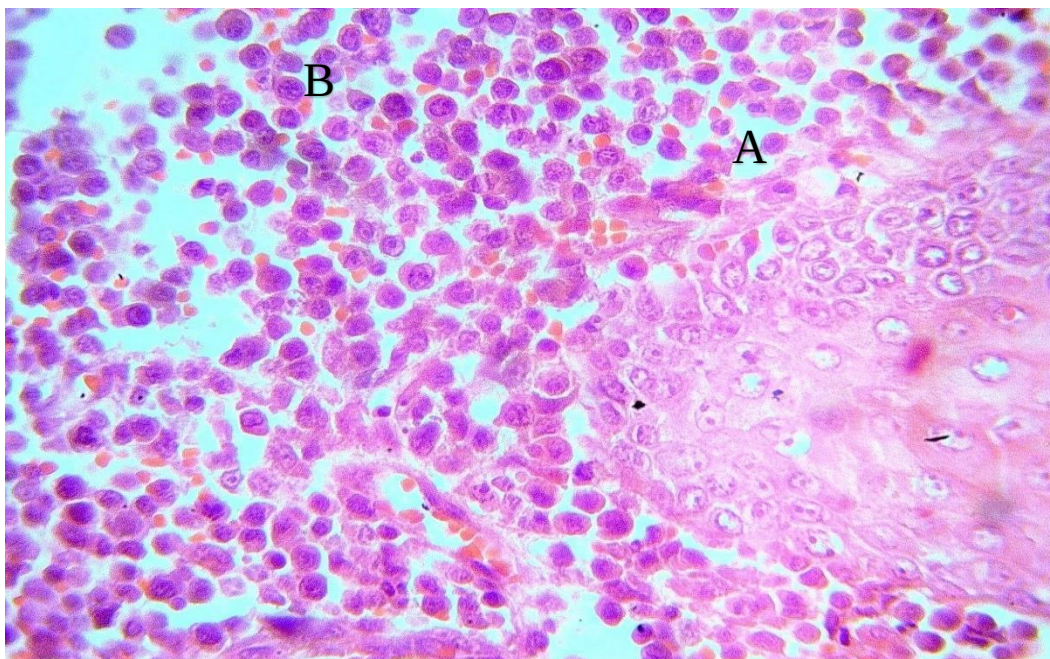


Figure 10: microscopic section in dog skin: **A:** Sever acanthosis of epidermal layer with apoptosis **B:** sever mononuclear cells infiltration in dermal layer (**H&E stain, 400×**).

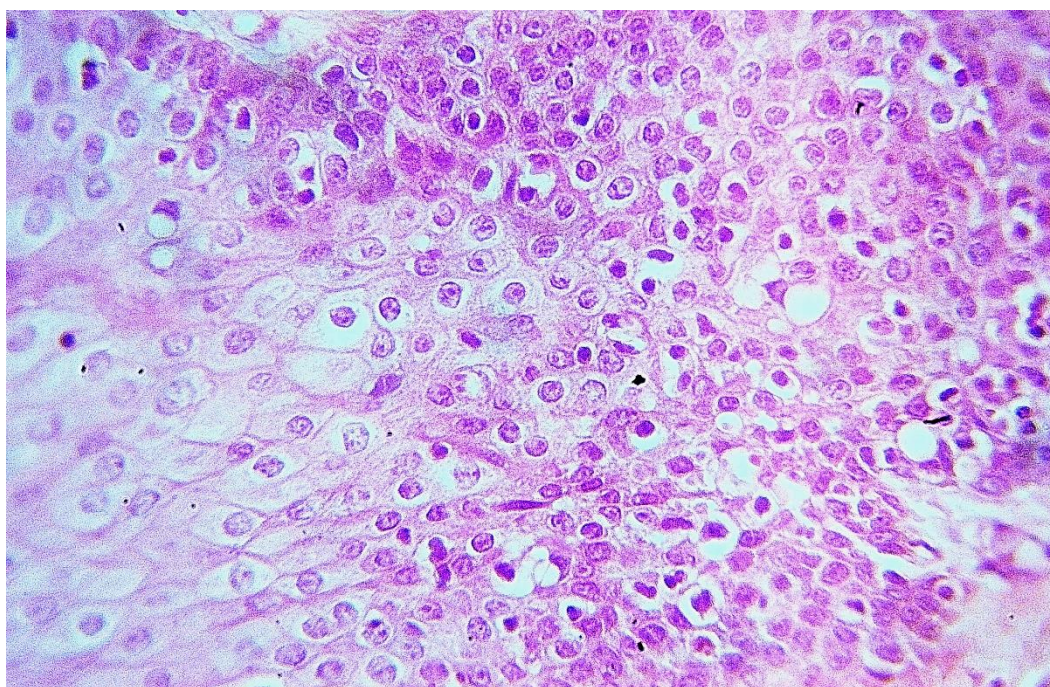


Figure 11: Microscopic section in dog skin: Acanthosis of epidermis layer with vacuolar degeneration and apoptosis star (**H&E stain, 400×**).

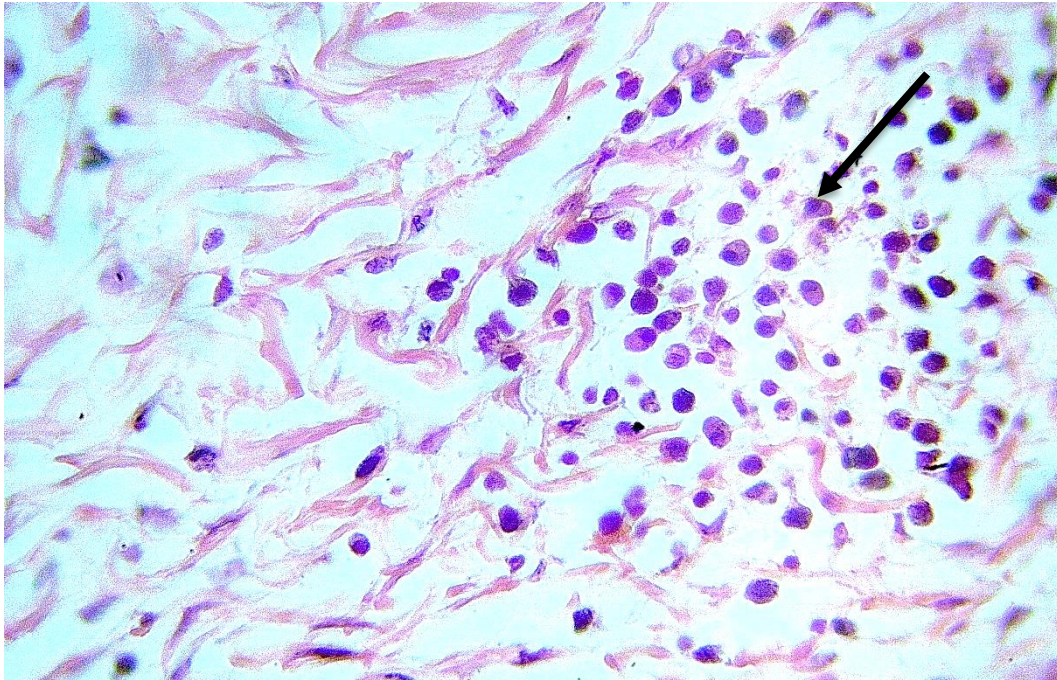


Figure 12: microscopic section in dog skin: Sever mononuclear inflammatory cell infiltrated in dermis layer (H&E stain, 400×).

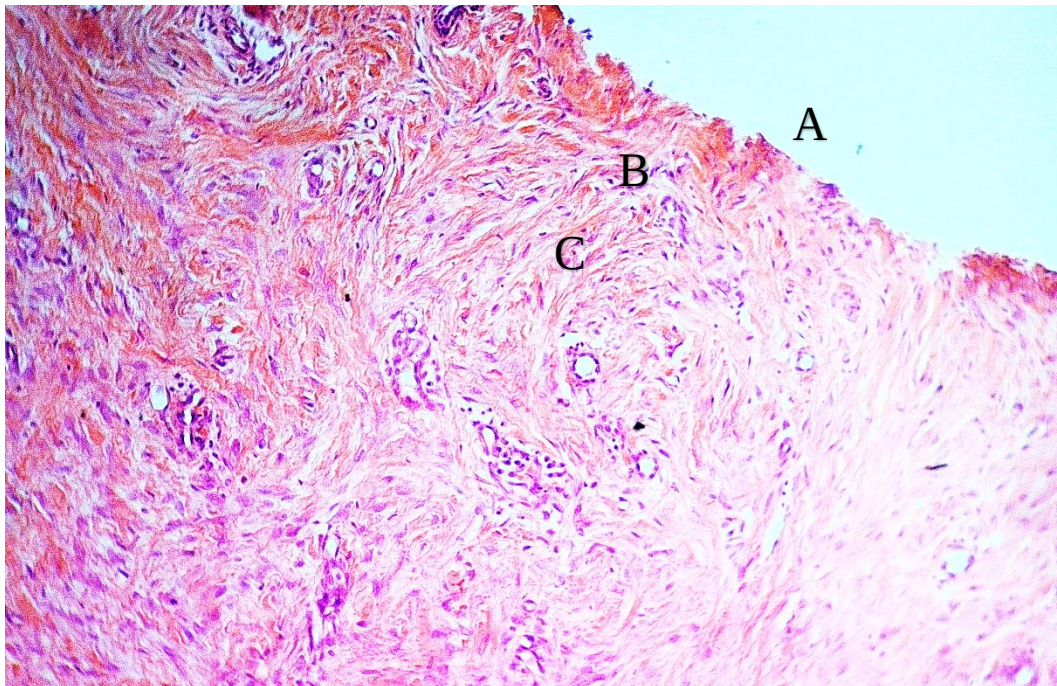


Figure 13: A: microscopic section in dog skin complete ulcer of epidermis layer B: fibroma consist whorls and interlacing bundles fibroblast and collagen fibers, the tumor cells have large pale elongated nuclei with variable degrees of vascularization C: infected fibrous tissue with mononuclear cells and newly blood vessels surrounded by mononuclear cells (H&E stain, 200×).

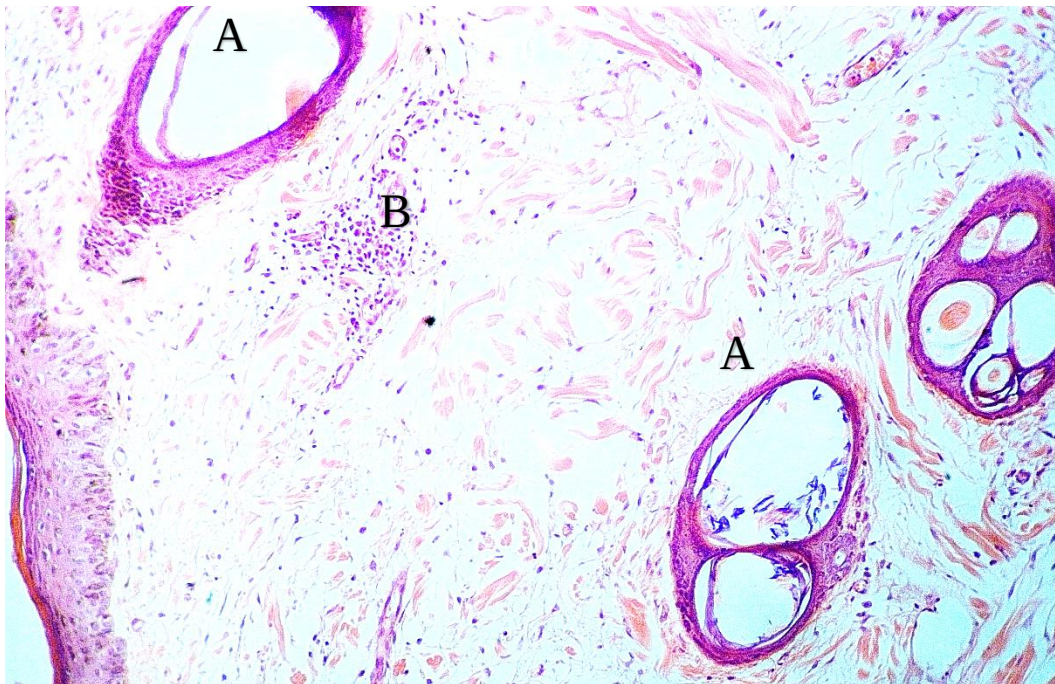


Figure 14: Microscopic section in k9 dog skin: **A:** cystic dilation of hair follicles surrounded by mononuclear inflammatory cells **B:** dermal lymphocytic aggregation (H&E stain, 200×).

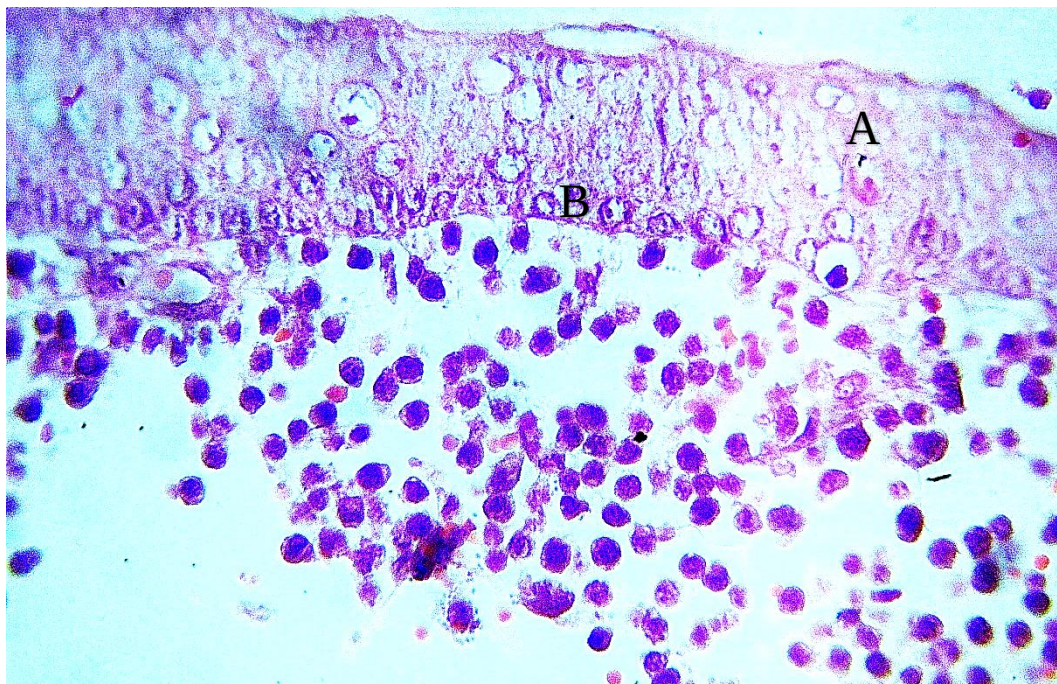


Figure 15: Microscopic section in k9 dog skin: **A:** acanthosis of epidermal layer with vacuolar degeneration **B:** apoptotic cells and mononuclear cells infiltration in dermal layer (H&E stain, 400×).

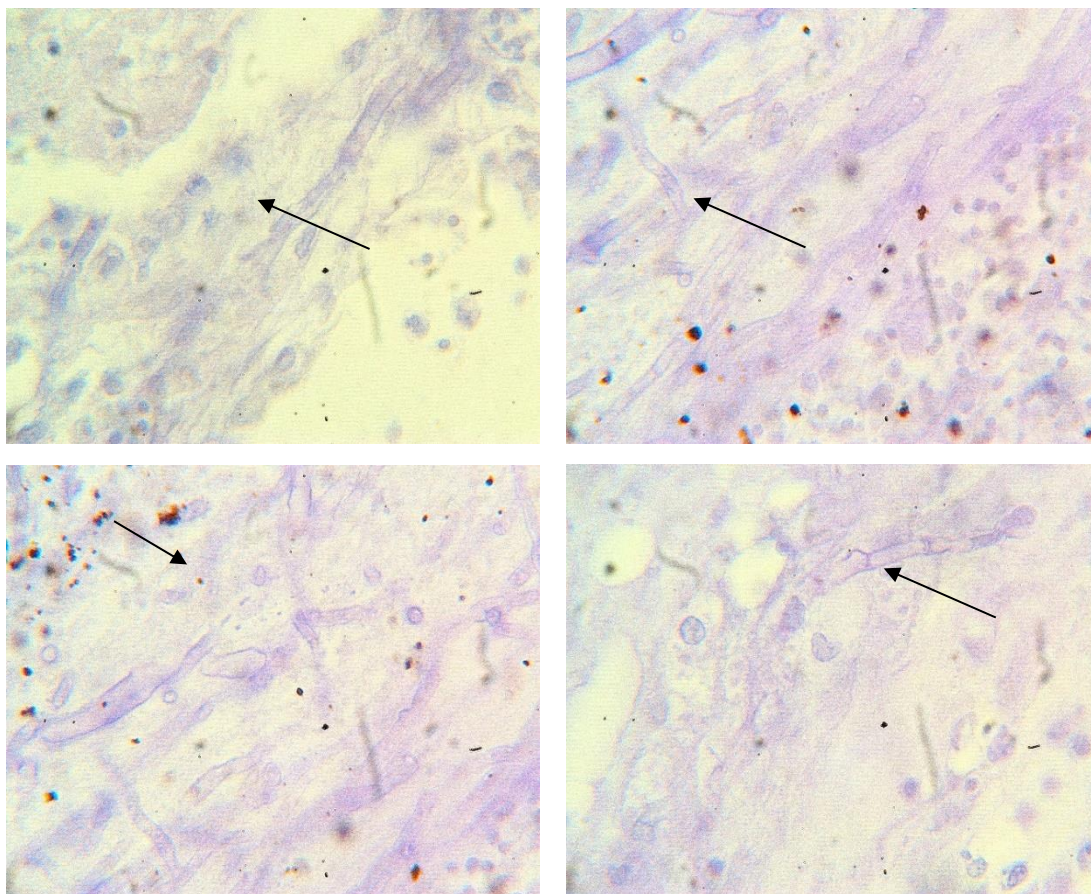


Figure 16: Microscopic section in stray dog skin: Showed *Aspergillus niger* Hyphae (septate) (PAS stain, 200×).

Discussion:

The pathogenic isolation of fungi in current study from 100 sample of infected dog skin where: 60% *Aspergillus niger*, 30% *trichophyton Verrucosum* and *Alternaria spp.* 10%. *Aspergillus niger* where isolated from dog skin they isolated as *asp. niger* from the skin agreement with (20). *Aspergillus niger* infection may occur in a skin associated with trauma, Surgery and foreign body to skin *Aspergillus niger* is a mold that is rarely reported in pneumonia distribution of the binding forces of *Aspergillus niger* spores unknown and invasive aspergillosis causing necrotizing skin PAS stain in dog skin infected with *Aspergillus niger* showed septate hyphae in dermis and epidermis layer (21;22). *Trichophyton Verrucosum* a genus onygenales that infects the skin, hair, nails, of animals and human causes Ring worm infection, especially in cattle. Development of both smooth walled Marco and microconidia and to study the *T. Verrucosum* isolation about 30% from 100 samples of infected skin dog. Infected granuloma, which present in dog skin and current study is chronic inflammation characterized by the formation of tumor like masses composed of inflammatory Granulation tissue with diffuse infiltration of macrophage, epithelial cells, local histiocytes Divide to provide some recruitment two local macrophage population These granuloma invasions by pathogenic bacteria and fungi, causing infected granuloma (23,24;25).

Alternaria spp. is fungus causing diseases it's an opportunistic pathogen infected upper inspiratory tract in compromised Immunity produce mycotoxin called fumonisin B1 genes and it has a distinct Appearance with chains of spores forming a club like shape (26). Papilloma, which appear in current study in dog skin is benign tumors of surface epithelia appear as warty or over growth and pedunculated on skin epidermis like polyps under microscope the Papilloma supplied by a core of connective tissue stroma containing blood vessels with evidence of inflammatory reaction in stroma with necrosis and ulceration its May due to chronic irritation due to bacterial or fungi above pathogen, causing skin hyper Legia and disrupt the basement membrane and the main type in current study is stratified squamous cell papilloma Causing increase thickening of the prickle cell layer (acanthosis) an increase in the amount of adherent keratin (hyperkeratosis in sometimes) Retention of the nuclei of the desquamating keratinized cells in some areas Parakeratosis (26;27). Our study revealed Detection of skin tumor, present of Dermatofibroma in some dog skin composed of spindles cells arranged Radiating from or (Mat-like) consist of fibroblast with a variable amount of collagen other cases and current study were reported squamous cell carcinoma epithelium like skin with its usually ulcerate to from a typical carcinomatous ulcer. Grossly looked as papillary with deep cleft that give cauliflower like appearance and have a base border than papilloma. Tumor cells Large and polyhedral and similar to the cells of stratum spinosum of epidermis and neoplastic cells often from concentric lamination of keratin (cancer pearls) Result in current study revealed that affected skin dog by microbio and do too chronic irritation, bacterial, toxin, or mycotoxin microbiota may cause skin tumor (5;27;28).

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