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Aspergillosis in sheep

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I. Introduction:

One of the most common farm animals in Iraq are sheep. By 1999 the sheep population in Iraq was approximately 6,750 that are distributed all over the country. Numerous breeds have been recognized, mainly the Awassi, Arabi, Karadi and Hamdani and are all of the fat-tail and carpet wool types. It has been demonstrated that Arabi, Karadi and Hamdani sheep were the most production under experimental conditions. In order to improve Awassi sheep in Iraq, they were profoundly crossing with European breeds, limited numbers of Finnish landrace and Chios sheep were introduced and kept on state farms. Unfortunately, very limited data were published, by 1990 a well-planned crossbreeding program was established between 200 Turkish Awassi, 50 Assaf, and 80 D'man sheep. Aim of this step majorly was to obtain different genotypes to that can be evaluated under different production systems considering:
a) adaptation to the prevailing environment
b) prolificacy
c) improvement according to market demand (Magid, 2003).

Keywords: Aspergillosis, Sheep, Pathogenesis

Characteristics of Aspergillus

Aspergillus spp. have been described as saprophytic filamentous fungi that are commonly found in soil, where they thrive as saprophytes, with an occasional potential to infect living hosts including plants, insects, birds, and mammals (Heitman, 2011). Aspergillosis is an umbrella term coined by Hinson, Moon, and Plummer in 1952, covering a wide range of diseases from localized conditions to fatal disseminated infections in humans and various animals which caused by fungi belonging to the genus *Aspergillus*. Disease may also result from an allergic reaction to inhaled conidia (Tell, 2005). Importantly, the genus *Aspergillus* is one of the most widespread groups of fungi on Earth, comprising approximately 300-350 species assigned to various subgenera and sections. Notably, lifestyle of different species of this genus is dramatically varied. Most species are known to produce a sexual propagule (conidia) on conidial heads. In contrast, a sexual cycle is unknown in the majority of species. Interestingly, where sexual reproduction is present, species exhibit either homothallic (self-fertile) or heterothallic (obligate outcrossing) breeding systems. A parasexual cycle has also been described in some species. The sexual stages of the Aspergilli have traditionally been assigned to different genera such as *Eurotium, Neosartorya* or *Emericella*. However, according to the new rules of the Melbourne Code adopted by the 18th International Botanical Congress in 2011, only one name can be used for one fungus, and the International Commission on *Penicillium* and *Aspergillus* (ICPA) decided to use the name *Aspergillus* in 2012. As in other fungi, sexual reproduction is governed by 'mating-type' (MAT) genes which determine sexual identity and are involved with regulation of later stages of sexual development (Varga, 2012).

Previous study has reported that Colonies are usually fast growing, white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores. Conidiophores terminate in a vesicle covered with either a single palisade-like layer of phialides (uniseriate) or a layer of subtending cells (metulae) which bear small whorls of phialides (the so called biseriate structure). The vesicle, phialides, metulae (if present) and conidia form the conidial head. Conidia are one-celled, smooth or rough walled, hyaline or pigmented are produced in long dry chains which may be divergent (radiate) or aggregated in compact columns (columnar). Furthermore, some species may produce Hülle cells or sclerotia. For identification, isolates are usually inoculated at three points on Czapek Dox agar and 2% malt extract agar and incubated at 25^{o}C. Most species sporulate within 7 days. Descriptions are primarily based on colony pigmentation and morphology of the conidial head. Microscopic mounts are best made using cellotap flag or slide culture preparations mounted in lactophenol cotton blue. A drop of alcohol is needed to remove bubbles and excess conidia (Ellis *et al*., 2007), figure (1).

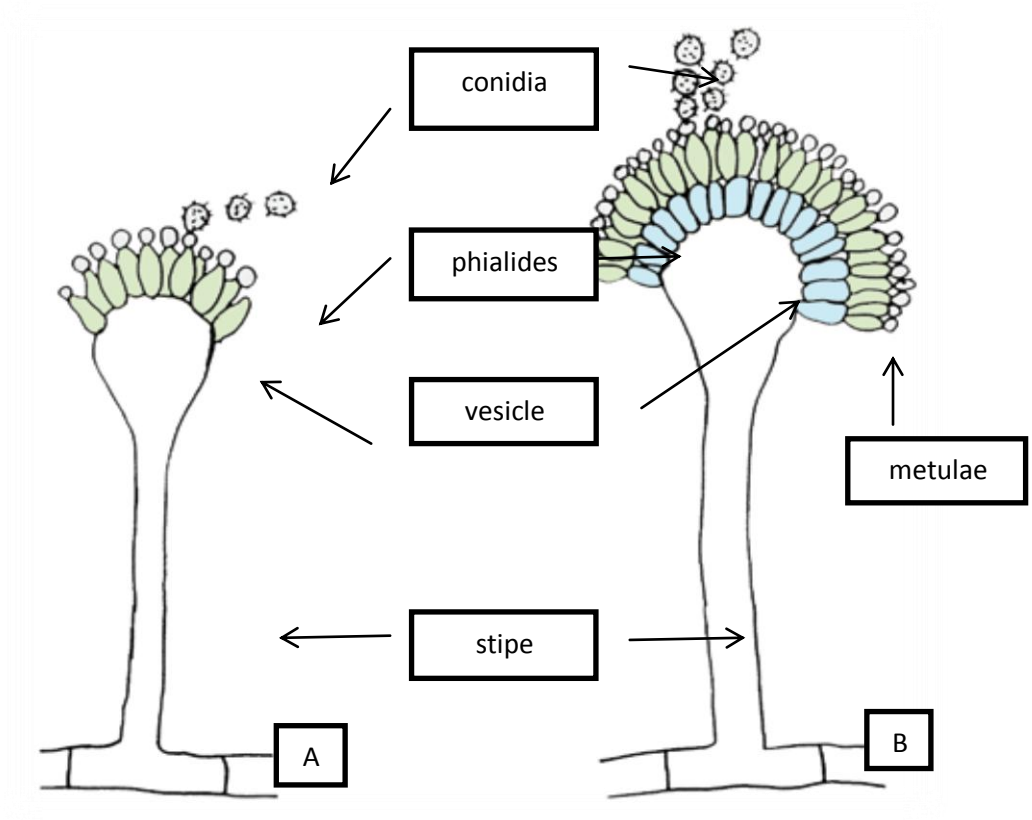

Figure (1): Conidial head morphology in *Aspergillus* **(A)** uniseriate, **(B)** biseriate.

Aspergillus flavus: Previous experiment has revealed that using Czapek Dox agar, colonies look granular, flat, with radial grooves. Starting with yellow color then quickly become bright to dark yellow-green with age. Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 µm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate), see figure (2). Conidiophore stipes are hyaline and rudely roughened, often more noticeable near the vesicle. Conidia are globose to sub-globose (3-6 µm in diameter), pale green and conspicuously echinulate. Notably, some strains produce brownish sclerotia. *A. flavus* has a world-wide distribution and normally occurs as a saprophyte in soil and on many kinds of decaying organic matter, however, it is also a recognized pathogen of humans and animals (Ellis *et al*., 2007).

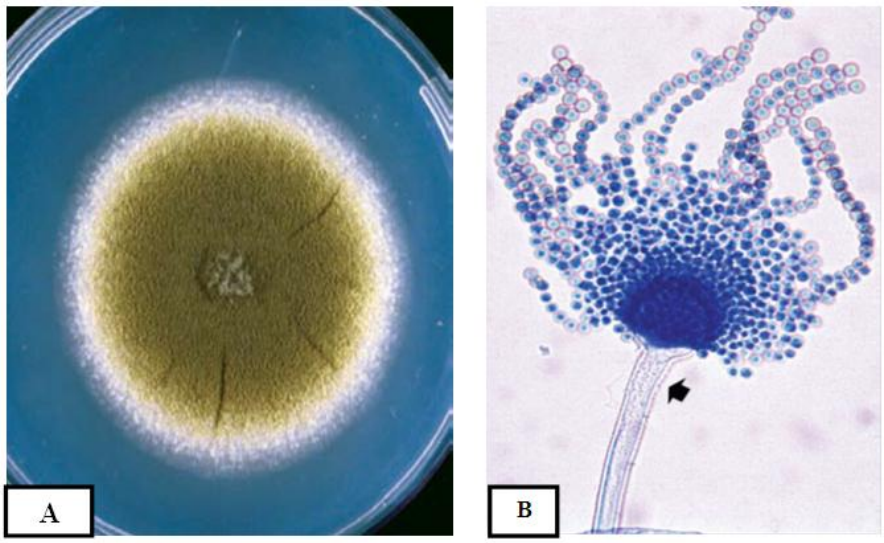

Figure (2): A- Czapek Dox agar culture of *A. flavus*, **B**- conidial head of *A. flavus.* Note: rough-walled stipe near vesicle (arrow) and that both uniseriate and biseriate conidial heads may be present.

***Aspergillus fumigatus*:** On Czapek Dox agar, colonies show typical blue-green surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores. Conidial heads are columnar (up to 400 x 50 µm) and uniseriate. Consistently, Conidiophore stipes are short, smooth walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains and are globose to sub-globose (2.5-3.0 µm in diameter), green and rough-walled to echinulate. Interestingly, this species is thermotolerant and grows at temperatures up to 55°C (Ellis *et al*., 2007).

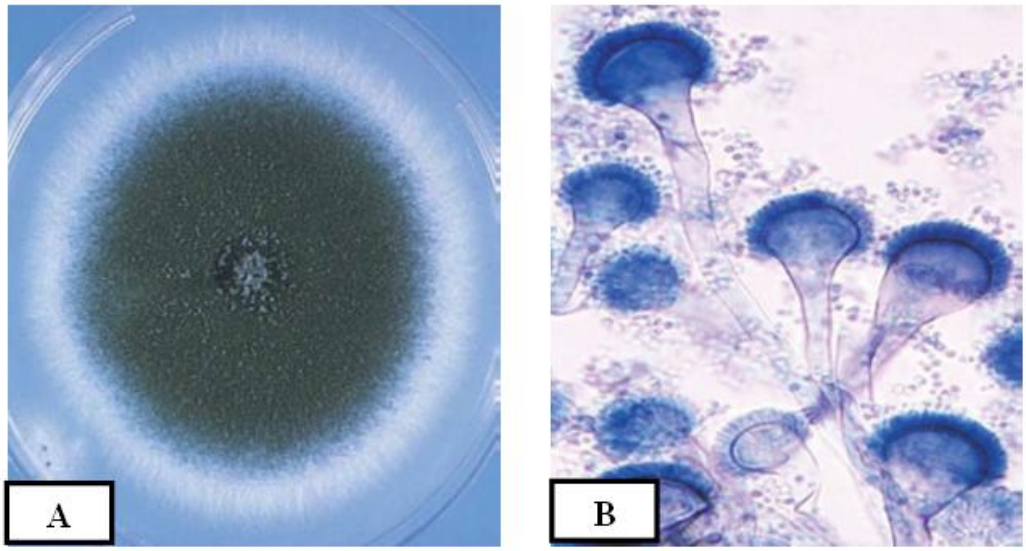

Figure (3): A- Czapek Dox agar culture of *A. fumigatus*, **B**-conidial head morphology of *A. fumigatus.* Note: uniseriate row of phialides on the upper two thirds of the vesicle**.**

***Aspergillus nidulans*: Teleomorph: *Emericella nidulans*:** On Czapek Dox agar, colonies are typically plain green in color with dark red-brown cleistothecia developing within and upon the conidial layer. Reverse may be olive to drab-grey or purple-brown. Conidial heads are short columnar (up to 70 x 30 µm in diameter) and biseriate. Conidiophore stipes are usually short, brownish and smooth walled. Conidia are globose (3-3.5 µm in diameter) and rough-walled. *A. nidulans* is a typical soil fungus with a world-wide distribution, it has also been reported causing disease in human and animals (Ellis *et al*., 2007), see figure (4).

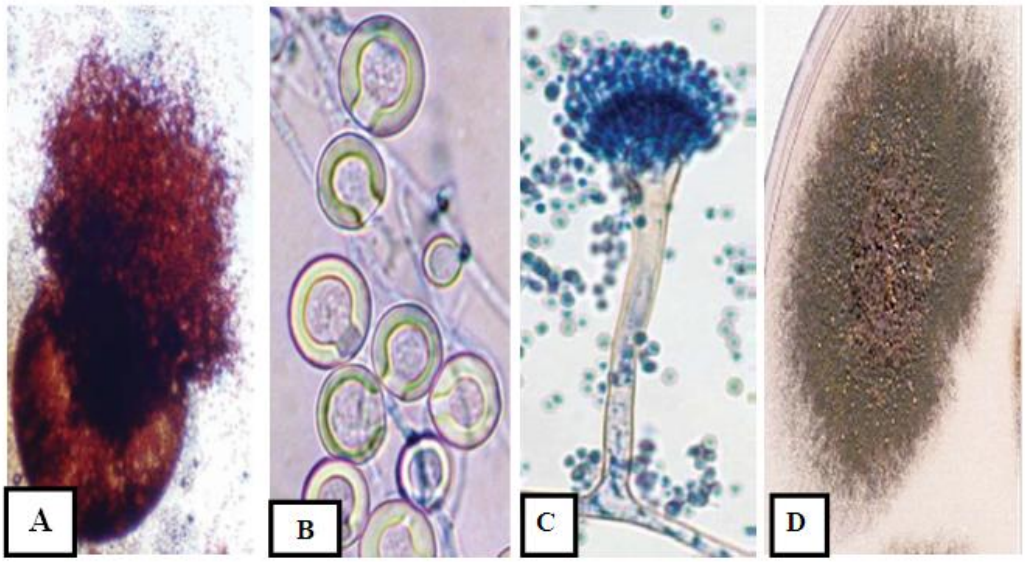

Figure (4): A- Cleistothecium of *Emericella nidulans* (anamorph *Aspergillus nidulans*) showing numerous reddish-brown ascospores and thick-walled hülle cells, **B-** cleistothecia are often surrounded by a mass of hülle cells which are up to 25 µm in diameter, **C-** conidial head and stipe and **D-** culture of *A. nidulans*.

Aspergillus niger : On Czapek Dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm by 15 to 20 µm in diameter), globose, dark brown, that becomes radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to sub-globose (3.5-5 µm in diameter), dark brown to black and rough-walled. *A. niger* is one of the most common and easily identifiable species of the genus *Aspergillus*, with its white to yellow mat later bearing black conidia. This species is very commonly found in aspergillomas and is the most frequently encountered agent of otomycosis. It is also a common laboratory contaminant (Ellis *et al*., 2007) see figure (5).

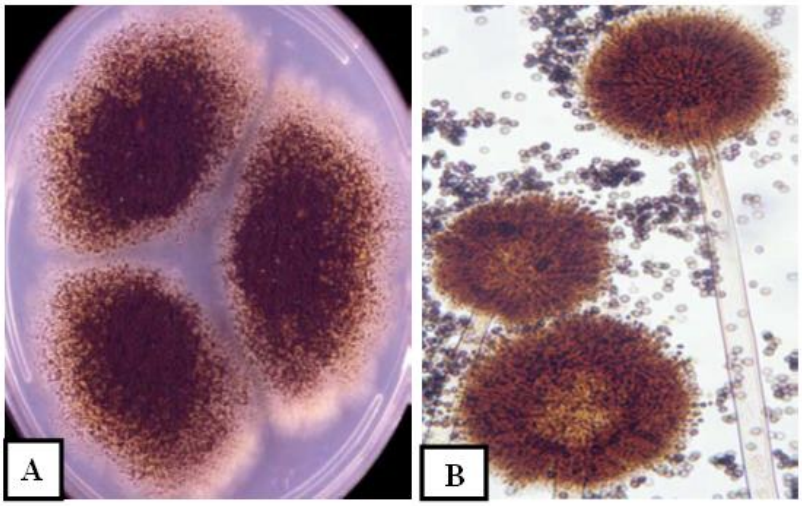

Figure (5): A- Czapek Dox agar culture**, B-**conidial head morphology of *A.niger.* Note: conidial heads are biseriate, large, globose, dark brown, becoming radiate with the phialides borne on metulae.

Aspergillus terreus*:* On Czapek Dox agar, colonies are typically suede-like and cinnamon-buff to sand brown in colour with a yellow to deep dirty brown reverse. Conidial heads are compact, columnar (up to 500 x 30-50 µm in diameter) and biseriate. Conidiophore stipes are hyaline and smooth-walled. Conidia are globose to ellipsoidal (1.5-2.5µm in diameter), hyaline to slightly yellow and smooth-walled. *A. terreus* occurs commonly in soil and is occasionally reported as a pathogen of humans and animals (Ellis *et al*., 2007), see figure (6).

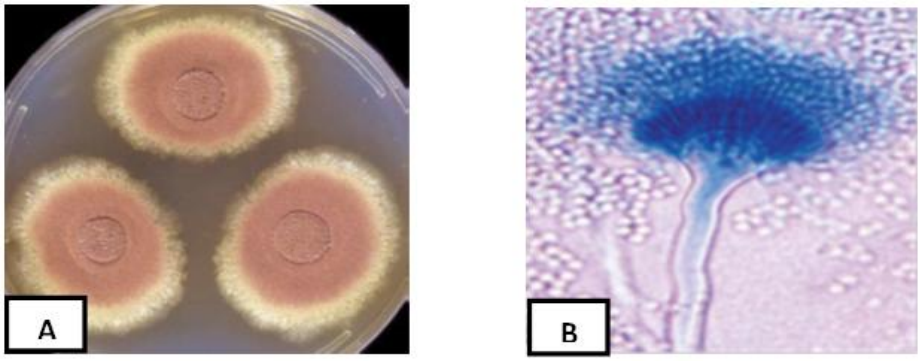

Figure (6): A- On Czapek Dox agar culture of *A. terreus***, B-** Conidial head and conidiophore of *A. terreus*. Note: conidial heads are biseriate.

Pathogenicity of Aspergillus

Numerous studies have demonstrated that Aspergillosis is primarily respiratory infection which may become generalized in animals. However, tissue predilection is highly variable among species. Notably, Aspergilloses in animals are caused by *A. fumigatus* and other few Aspergillus species. Modern classification of Aspergillus species is by polyphasic taxonomy (i.e., taking into account all available phenotypic and genotypic data and integration in a consensus classification). It has been revealed that most common forms of sheep Aspergillosis are mycotic abortion, mycotic pneumonia, mycotic mastitis, Systemic aspergillosis , nasal and

cutaneous aspergillosis. Reports by (El-Naggar *et al.*, 1997) have induced mycotic abortion experimentally in pregnant ewes. Twelve animals were inoculated i.v. with 10 ml of *Aspergillus fumigatus* spore suspension containing $2x10^7$ viable spores and 4 pregnant animals were used as control. All experimental animals aborted between 20-30- and 19-29-days post-infection in ewes and goats, respectively. Uteri, maternal and foetal placenta and foetal tissues showed thrombosis, extensive necrosis, infarctions and invasion with *Aspergillus fumigatus* hyphae. Skin lesions of aborted foetal were detected. Mycotic granulomatous inflammation was recorded in the lungs, brains, spleens and kidneys. Consistency, Pérez *et al*. (1998) diagnosed mammary Aspergillosis in four flocks of dairy sheep, comprising a total of 1,750 ewes. These animals had been treated prophylactically by intramammary infusion with cloxacillin for five months prior to lambing. Importantly, mammary Aspergillosis with concomitant spread to the regional lymph nodes was present in these flocks in a percentage ranging from 2% to 36.4% of treated sheep.

Pathologic, bacteriologic, and mycologic studies were performed in seven of the affected ewes. Outcomes of these studies have shown that some of them also had lung, kidney, and liver involvement. The pathologic reaction within lesions ranged from the acute to subacute type, dominated by necrosis and vasculitis with thrombosis, to the chronic granulomatous type, with macrophages and giant cells. The distribution of lesions and the presence of a remarkable vasculitis with fungal thrombi in the mammary gland suggested a hematogenous dissemination of the infection from this organ. Immunologic staining with monoclonal antibody MAb-WF-AF-1 together with the isolation of *Aspergillus fumigatus* in pure culture from affected tissues.

In the same line of thought, Las Heras *et al.,* (2000) carried out an intramammary *Aspergillus fumigatus* infection in dairy ewes associated with antibiotic dry therapy. Moreover, study by Garcia *et al.,* (2004) compared three techniques for the diagnosis of mammary aspergillosis in ewes: indirect ELISA to detect the level of anti-Aspergillus IgG in serum, determination of galactomannan (Platelia procedure), and detection of DNA of Aspergillus in serum by a nested PCR. Twenty sera from proven cases of Aspergillosis in ewes were positive using ELISA (100%), 80% were positive using PCR, but only 55% were positive using Platelia. All 20 control sera were negative using ELISA and PCR, whereas using Platelia methodology shows one positive and the other doubtful. The detection of antibody by ELISA in sera is therefore a reliable criterion for the diagnosis of mammary Aspergillosis in ewes. Notably, Platelia showed the same deficiencies reported in humans, with the appearance of false positives and negatives. The use of PCR was promising and might have valuable application in human medicine.

Dehkordi *et al.,* (2012) performed a study for detection of Aspergillus species (*A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus*) in aborted bovine, ovine, caprine and camel foetal by real-time PCR in Iran. After modification of real-time PCR, from the total number of 970 samples, 141 (14.53%) gave positive results for Aspergillus species. Of them, 62 (17.71%), 33 (14.04%), 27 (12.05%) and 19 (11.8%) positive specimens were detected in bovine, ovine, caprine and camel foetuses respectively. Statistical analysis showed significant differences between bovine and camel and bovine and caprine aborted foetuses. Previous experiment has demonstrated that Aspergillus abortion was the most prevalent in cattle whereas camels tended to be the most resistant. This study was the first report of direct identification of Aspergillus species by real-time PCR in aborted bovine, ovine, caprine foetuses in Iran and camel foetuses in the world. Clinical signs of fungal mastitis in dairy sheep are non-specific many filamentous fungi, including Aspergillus *spp* are saprophytic and ubiquitous in the environment, so the tend to be regarded as contaminants when isolated from milk samples (Jensen *et al*., 1996). Therefore, cases of mastitis due to Aspergillus *spp*. and other saprophytic filamentous are usually first identified following postmortem and histological examination (Jensen *et al*., 1996).

Mycological examination of aborted fetus, vaginal discharge and placenta of aborted sheep revealed the isolation of *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans, Candida krusei, Mucor* spp. *Abisidia* spp. and *Rhodotrula* spp. with incidence of (12.9%,5.7%, 2.9%, 8.6%, 2.9%, 4.3%, 2.9% and 1.4%, respectively, (Elham and Flourage (2007). Mycotic abortion caused by *Aspergillus* spp is the most important consequence of fungal infection of the genital tract. Despite fungi have been implicated occasionally in other syndromes such as vulvovaginitis or endometritis. The genera of *Aspergillus* can grow in a suitable substrate under appropriate conditions. They can produce toxins which are accounted the majority of abortion cases and can cause reproductive system infections in animals (Verma *et al*., 1999).

Another study has revealed that *A. flavus*, *A. terreus*. *A. nidulans*, *A. niger* and *A. glaucus* were detected in the genital tracts of ewes (Shokri and Yadollahi, 2017).

The saprophytic genera Aspergillus, Penicllium, and Alternaria which were commonly isolated from the environment and animal coats are well known as a main etiologic agent of pulmonary infections (Gogia, 2015). Previous work (Al-Bader, 2018) has reported that the hygienic importance of fungi colonizing in the sheep wool in Erbil/Iraq represent *Aspergillus spp* 88%.

Microbial infections of the genital tract may lead to temporary or permanent infertility in ewes as well as abortion, prenatal and neonatal loss in pregnant subjects (AL-dahash, and Fathalla 2000). Fungal infections

of the genital tract of animals have not received much attention in the past. However, with indiscriminate use of antibiotics and hormonal therapy, fungal infections are becoming more common in humans

II. Diagnosis:

Serology: Tests that performed to detect serum antibodies against *Aspergillus* species include Agar gel immunodiffusion (AGID), complement fixation, and ELISA techniques (Mohamed *et al*., 2017).

Histopathology: This technique has been done provide direct evidence of fungal hyphae, have high sensitivity, finding include mucosal ulceration and inflammation, with predominance of lymphocytes and plasma cells as well as cytologic identification of *Aspergillus* species in urine, blood, synovial fluid, lymph node, bone or intervertebral disk material (Mohamed *et al*., 2017).

Isolation of fungi: Most *Aspergillus spp*. relatively show rapid grow (typically within 48 hr) and on most microbiological media including both mycological media such as Sabouraud's dextrose agar and blood agar used for general bacteriological culture. Identification of cultures of most species Aspergillus is generally straight forward by colony and microscopic morphology (Mohamed *et al*., 2017).

Molecular identification of Aspergilla: Sequencing of genes, such as actin, calmodulin, ITS, rodlet A (rodA) and/or β-tubulin (βtub), have been applied to distinguish *A. fumigatus* from related species. Alternatively, Multilocus sequence typing can be used for the identification of those related species. It is a strategy that also involves sequencing of several gene fragments. A few other techniques, such as random amplified polymorphic DNA, restriction fragment length polymorphisms and a new proposed microsphere-based Luminex assay, may also enable molecular identification of *A. fumigatus* without sequencing (Mohamed *et al*., 2017).

Reference:

[1]. **Magid, S.A.; Al-Murrani, W.K.; Alkas, J.I. (2003).** Country report animal genetic resources. Ministry of Agriculture Baghdad: P 20.

[2]. **Heitman, J. (2011).** Microbial pathogens in the fungal kingdom *Fungal Biol Rev* 25 (1): P 48-60.

[3]. **Varga, J. (2012).** Aspergillus sex and recombination. University of Zseged – Hungary. 16th Advances Against Aspergillosis 2014 Madrid- Spain. Meliá Castilla Conference and Convention Center: P 46.

[4]. **Ellis, D.; Davis, S.; Alexiou, H.; Handke, R.; Bartley, R. (2007).** Descriptions of medical fungi, 2nd . Mycology unit women´s and chiderns hospitals, school of molecular and biochemical science university of Adelaide: P 8-14.

[5]. **Tell, L.A. (2005).** Aspergillosis in mammals and birds: impact on *veterinary medicine Med Mycol 43 Suppl 1*: P S71-S73.

[6]. **El-Naggar, A., Ahmed, Y., Ibrahim, F. and Refai, M**. **(1997).** Mycotic abortion in small ruminants induced by Aspergillus fumigatus. *J. Comp. Pathol. & Clinc. Pathol. 10*: P 59-76.

[7]. **Pérez,V.; Corpa, J.M.; García, Marín. J.F.; Adúriz, J.J.; Jensen, H.E. (1998).** Mammary and systemic aspergillosis in dairy sheep. *Vet Pathol* 35(4): P 235-40.

[8]. **Las Heras, A.; Domínguez, L.; López, I.; Payá, M.J.; Peña, L.; Mazzucchelli, F.; García, L.A.; Fernández-Garayzábal, J.F. (2000).** Intramammary Aspergillus fumigatus infection in dairy ewes associated with antibiotic dry therapy. Vet Rec. 11, 147(20): P 578-80.

[9]. **Garcia, M.E.; Duran, C.; Cruzado, M.; Andrino, M.; Blanco, J.L. (2004).** Evaluation of molecular and immunological techniques for the diagnosis of mammary aspergillosis in ewes. *Vet Microbiol*. 14, 98 (1): P17-21.

[10]. **Dehkordi, F. Safarpoor, H. Momtaz & A. Doosti, (2012).** Application of real-time PCR for detection of Aspergillus species in aborted ruminant foetuses. *Bulg. J. Vet. Med*. 15(1): P 30−36.

[11]. **Mohamed K. Refai, Ahmed L. El-Naggar, Nahed M. El-Mokhtar (2017).** Monograph on fungal diseases of sheep & goats A guide for postgraduate students in developing countries. P 71-76

[12]. **Jensen, H.E.; Espinosa de los, M.; Carrasco, L. (1996).** Caprin**e** mastitis due to aspergillosis and zygomycosis**:** a immunological and histochemicalstudy. *J. Comp. Pathol* 114: P 183-191.

[13]. **Elham, I. Atwa and Flourage, M. Rady (2007).** Bacteria and fungi associated with abortion in sheep and goat in Menoufiea Governorate. *Assiut Vet. Med. J*. 53 (113): P 326-349.

[14]. **AL-dahash, S.Y., Fathalla, M.A. (2000).** Veterinary obstetrics. 3 pages, Book House for Printing and Publishing, Al-Mousl University.

[15]. **Sobel, J.D.; Faro, S.; Force, R.W.; Foxman, B.; Ledger, W.J.; Nyirjesy, P.R. (1998).** Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *Am. J. Obstet. Gynecol*. 178 : P 203- 211.

[16]. **Verma, S., Katoch, R.C., Jand, S.K.; Nigam, P. (1999) .** Fungi associated with abortions and infertility in does and ewes. Vet. Arhiv. 69: P 1-5.

[17]. **Shokri, H and Yadollahi, M. (2017).** Isolation and identification of fungal microbiota from genital tract of ewes. *Revue Méd. Vét.*, 2 168. (4-6): P 81-86.

[18]. **Gogia, P. (2015).** Pulmonary fungal infections. Curr Med Res 5: P 221-227.

[19]. **Al-Bader, S.M. (2018).** The Hygienic Importance of Fungi Colonizing the Sheep Wool in Erbil/ Iraq. Virol Mycol 7(2): P 177.