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# Isolation and Molecular Characterization Of Aspergillus fumigatus From Hunting Dogs With Special Emphasis To Age And Gender As A Risk Factors In Diyala Province –Iraq

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## Abstract

**Aim:** The current study aims to isolation and molecular characterization of A. fumigatus from hunting dogs and evaluation of relationship between A. fumigatus infection and possible risk factors mainly age and gender

**Methods:** Ninety nine swabs from mouth, nose and ear of hunting dogs with respiratory signs were cultured on Sabouraud Dextrose Agar. A. fumigatus was identified according to morphology and PCR technique

**Results:** A.fumigatus was isolated frequently from mouth of hunting dogs at the age group (5.4 -6.2) years followed by (0.6-1.5) years and (2.5-3.3)years,(6.06%) . A.fumigatus was isolated equally from nose of hunting dogs at the age group (0.6-1.5)years ,(1.6-2.4 ) years , ( 2.5-3.3 )years,( 4.3-5.3) years and (5.4-6.2) years, (3.03%). A.fumigatus was isolated frequently from ear of hunting dogs at the age group (5.4-6.2)years;(9.09%).

No significant difference was reported between age groups of hunting dogs infected with A.fumigatus . Significant correlation was reported between older age group of hunting dogs and A.fumigatus infection for ear. Current study revealed that (63.64%) of hunting dogs were males while female represent (36.36%).Both genders were equally infected with A.fumigatus isolated from mouth and nose, (15.15%) and (6.06%) respectively .Females were infected more than males with A.fumigatus isolated from ear,(6.06%)

Neither significant difference nor correlation were reported between genders of hunting dogs infected with A.fumigatus. Males appear to be at risk of getting A.fumigatus infection at( 2.286) time than females.

**Conclusions:** A. fumigatus infection represent serious problem for hunting dogs . Mouth, nose and ear respectively exposed.

**Keywords:**

*A. fumigatus* • Hunting dogs • Risk factors• Iraq

## Introduction

E In recent years, aspergillosis opportunistic infections have been recognized as an important cause of morbidity and mortality in developing as well as developed nations. Aspergillosis is reported with increasing frequency in humans and animals from many regions of the world. There are about 600 species of Aspergillus, of which about 27 species of Aspergillus are found to be associated in various clinical disorders of humans and animals. Disease is primarily caused by *A.fumigatus*, opportunistic filament forming moulds.

These fungi are widely prevalent in environment and are recovered from soil, air, water, plant substrates.

Most cases of animal aspergillosis are sporadic. Immunosuppression, either pathologic or iatrogenic, that has been the main predisposing factor to human mycoses in general and

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aspergillosis in particular, is much rarer in animals, and thus is not considered as a significant risk factor. Infection source may vary but is mostly environmental. Heavy fungal loads may be the source of massive poultry infections whereas trauma is usually assumed to cause ophthalmic infections. Disseminated or rhino-nasal/rhino-orbital aspergillosis seem to be associated with breed predisposition related to immune deficiencies the former and skull conformation the latter.

Disseminated canine aspergillosis (DCA) affects primarily German Shepherd breed dogs, with fe-males being over-represented. It is caused most frequently by Aspergillus terre-us .Other Aspergillus spp. that may cause disseminated canine mycoses include A. deflectus, A. fumigatus, A. niger, A. flavus, A. flavipes, A. versicolor, or unspecified Aspergillus spp. Clinical signs are non-specific and include lethargy, weight loss, central nervous system signs, and ataxia due to musculoskeletal lesions . Abnormal clinical pathological test results may vary and result from the inflammatory process and/or dysfunctions of the affected organ.

The current study aims to isolate A. fumigatus from hunting dogs in Diyala province –Iraq, identification of A. fumigatus by phenotypic characterization technique , evaluation of relationship between A. fumigatus infection and possible risk factors mainly age and gender.

## Materials and Methods

This study was performed in Baqubah the capital of Diyala province, Iraq from 1 of Sep-tember 2018 to 31 of January 2019 after ethical approval from college of veterinary medicine, Diyala University ,Iraq . The study included 33 hunting dogs, with minimum age 7 months and maximum 72 months ,mean age (38.18±3.46 months). Ninety nine swabs were taken from mouth ,nose and ear. Specimen was placed on a microscopic slide, a cover slip added and warmed over a small flame just before boiling. The slide was examined under the low power and high dry objec-tives to detect fungi and their septate hyphae. For microscopic characteristics slides were stained with Lactophenol cotton blue with using adhesive tape preparation in which a small piece of transparent-adhesive tape was touched to the surface of the suspected colony, and then adhered to the surface of a microscopic slide. Photographs were taken with Digital microscopical camera [1].

A morphological examination of species was first made with naked eye and at low magnification power of microscope after that detailed examination was done according to by measuring the dimensions of the microscopic structures, photographing the micro-scopic structures and using relevant literature.

Swabs were streaked into Sabouraud's dextrose agar .The media were incubated at 37°C for 1 weeks . After seven days of incubation, plate was observed for macroscop-ic characteristics such as colony diameter, exudates, colony reverse and the isolates were identified to the species level on the basis of microscopic characteristics including conidio-phore, vesicle, metulae, phialides and conidia.

### DNA Extraction

DNA was extracted from A. fumigatus. By using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the protocol stated by the kit manufacturer.

### Concentration and purity of DNA

DNA was extracted from a hundred isolated of A. fumigatus and were concentrated in one tube. The concentration and the purity of the DNA samples were determined by Quan-tus Fluorometer at (9.9 ng/µl and 57 ng/µl) was used to detect the concentration of ex-tracted DNA to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5min incubation at room temper-ature, DNA concentration values were detected, according to the protocol stated by the kit manufacturer (Promega, U.S.A) [2].

### Primers selection and preparation

Universal primers ITS1 (5`-TCCGTAGGTGAACC TGCGG-3`) and ITS4 (5` TCCTCCGCTTATT GATATGC-3`). The 5.8S rDNA and the its 2 region, amplified from type, neotype, reference and clinical isolates of A. fumigatus by using the ITS 1 and 4 primers (Gaitanis, G et al., 2002). We're synthesized by (QIAGEN, Germany).

### PCR working solution

Optimization of PCR was accomplished after several trials. Thus the following mixture was adopted amplification reactions were produced in the 25µl final volume containing 12.5 µl Go Taq® master mixes (PR omega, USA), 2µl of the primers and 2µl DNA template and com-plete the volume by 8.2 ul nuclease-free water.

### Programmable thermal controller

Program for amplifying the 5.8S rDNA and the it's 2 region, amplified from the type of ITS1 and ITS4 for A. fumigatus. For identification of A. fumigatus .,an initial denaturation step at 95°C for five minutes was followed by thirty cycles of denaturation at 95°C for thirty se-conds, annealing at 55°C for thirty seconds, and extension at 72°C for thirty seconds, with a final extension step of 72°C for seven minutes

### Agarose gel electrophoresis

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria, according to the protocol stated by the kit manufacturer (Promega, U.S.A) [3].

## Results

Table (1) and figures (1&2) show the identification of A.fumigatus isolated from hunting dogs according to morphological features on SDA and conventional PCR .The total number of A.fumigatus isolated from hunting dogs was 17/99, (17.17%) .A.fumigatus was isolated from 10/33 (30.30%) mouth swabs ; 4/33,( 12.12%) nasal swabs and 7/33, (9.09%) ear swabs.

| Source of sample for hunting Dogs | Isolation status on SDA | | Total No. of swabs |
|---|---|---|---|
| | No growth | A.fumigatus | |
| Mouth | 23(69.7%) | 10(30.3%) | 33(100%) |
| Nose | 29(87.9%) | 4(12 .12%) | 33(100%) |

| | | | |
|---|---|---|---|
| Ear | 30(90.9%) | 3(9.09%) | 33(100%) |
| Total | 82( 82.83%) | 17(17.17%) | 99(100%) |

**Table 1:** Morphological Identification Of A.fumigatus Isolated From Hunting Dogs.

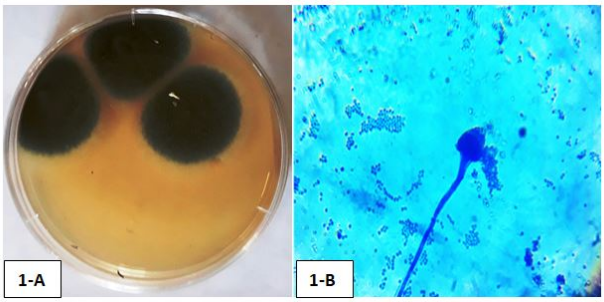


**Figure1:** A: Photomicrograph show the pure culture of A. fumigatus isolated from mouth of hunting dogs on sda; B. characteristic feature of a.fumigatus conidiophore ( 100x).

Age as a possible Risk Factor Associated With A.fumigatus Infection among Hunting dogs

As shown in table (2), A.fumigatus was isolated more frequently from mouth of hunting dogs at the age group (5.4 -6.2)years followed by (0.6-1.5)years and (2.5-3.3)years,(6.06%) . A.fumigatus was isolated more equally from nose of hunting dogs at the age group (0.6-1.5)years , (1.6-2.4 ) years ,( 2.5-3.3 )years,( 4.3-5.3) years and (5.4-6.2) years, (3.03%). A.fumigatus was isolated more frequently from ear of hunting dogs at the age group (5.4-6.2)years;(9.09%).

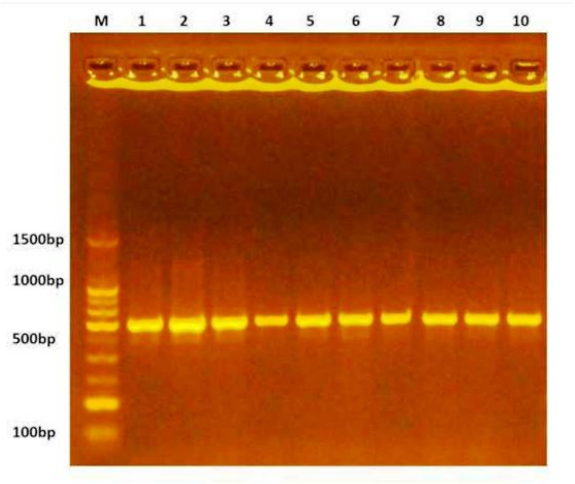


**Figure 2:** DNA products of A. fumigatus generated through ITS1 (TCCGTAGGTGAACCTGCGG), and ITS2 (TCCTCCGCTTATTGATATGC) primers, stained with Ethidium bromide. M : Molecular marker (100bp); lanes 1-10 (517bp) , A. fumigatus.

No significant difference was reported between age groups of hunting dogs infected with A.fumigatus . Significant correlation was reported between older age group of hunting dogs and A.fumigatus infection for ear.

| Age group | A.fumigatus Isolated From Hunting Mouth dogs | | A.fumigatus Isolated From Hunting dogs Nose | | A.fumigatus Isolated From Hunting dogs Ear | |
|---|---|---|---|---|---|---|
| (years) | No growth | A.fumigatus | No growth | A.fumigatus | No growth | A.fumigatus |
| 0.6-1.5 | 5 (15.15%) | 2 (6.06%) | 6(18.18%) | 1(3.03%) | 7(21.21%) | 0(0%) |
| 1.6-2.4 | 4(12.12%) | 1( 3.03%) | 4( 12.12%) | 1(3.03%) | 5 (15.15%) | 0(0%) |
| 2.5-3.3 | 4(12.12%) | 2 (6.06%) | 10(30.30%) | 1(3.03%) | 6(18.18%) | 0(0%) |
| 3.4-4.2 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 4.3-5.3 | 5 (15.15%) | 1( 3.03%) | 5 (15.15%) | 1(3.03%) | 6(18.18%) | 0(0%) |
| 5.4-6.2 | 4(12.12%) | 3( 9.09%) | 6( 18.18%) | 1(3.03%) | 4(12.12%) | 3(9.09%) |
| 6.3-7.1 | 1( 3.03%) | 1( 3.03%) | 2 (6.06%) | 0(0%) | 2 (6.06%) | 0(0%) |
| $\chi^2$ | 3.735 | | 2.579 | | 12.257 | |
| P value | 0.88 | | 0.958 | | 0.14 | |
| R | 0.128 | | -0.041 | | 0.352 | |
| P value | 0.477 | | 0.82 | | 0.045 | |

**Table 2:** Age as a possible risk factor associated with a.fumigatus infection among hunting dogs.

Gender as a possible Risk Factor Associated With A.fumigatus Infection among Hunting dogs.

Current study revealed that 21/33 of hunting dogs were males (63.64%),while female represent 12/33 (36.36%).Both genders were equally infected with A.fumigatus isolated from mouth and nose of hunting dogs , (15.15%) and (6.06%) respectively .Females were infected more than males with A.fumigatus isolated from ear2/33 , (6.06%) As shown in table (4.11).

No significant difference nor correlation were reported between genders of hunting dogs infected with A.fumigatus . Males appear to be at risk of getting A.fumigatus infection at( 2.286) time than females.

| Gender | A.fumigatus Isolated From Hunting dogs Mouth | | A.fumigatus Isolated From Hunting dogs Nose | | A.fumigatus Isolated From Hunting dogs Ear | |
|---|---|---|---|---|---|---|
| | No growth | A.fumigatus | No growth | A.fumigatus | No growth | A.fumigatus |
| Male | 16(48.48%) | 5(15.15%) | 19(57.58%) | 2(6.06%) | 20(60.60%) | 1(3.03%) |
| Female | 7( 21.21%) | 5(15.15%) | 10(30.30%) | 2(6.06%) | 10(30.30%) | 2(6.06%) |
| $\chi^2$ | 1.153 | | 0.366 | 1.31 | | |
| P value | 0.283 | | 0.545 | 0.252 | | |
| R | 0.187 | | 0.105 | 0.199 | | |

| | | | | |
|---|---|---|---|---|
| P value | 0.298 | | 0.56 | 0.266 |
| Risk Estimate | 95% Interval | Confidence | 95% Confidence Interval | 95% Confidence Interval |
| Odds Ratio for gender | Value | | Value | Value |
| (male / female) | 2.286 | | 1.9 | 4 |

**Table 3:** Gender as a possible risk factor associated with a.fumigatus infection among hunting dogs.

## Discussion and Conclusion

Current study proved that A.fumigatus was isolated from (17.17%) of hunting dogs according to according to morphological features on SDA . A.fumigatus was isolated from (30.30%) mouth swabs ; (12.12%) nasal swabs (9.09%)from ear swabs. These results considered very low compared with that reported by, they stated that A.fumigatus was recovered by classical culture tech-nique from (96.7%) of dogs with respiratory signs of sino-nasal infections. Also stated that "A. fumigatus is the most common etiological agent of canine sino-nasal aspergillosis". Current result come in accordance with that reported by stated that A. fumigatus was isolated from 6.66% of otomycosis in dogs of Sulaimania province, Iraq. Current isolation rate was very low com-pared with that reported by, stated that A. fumigatus causing sino nasal aspergillosis was recovered from 7-34 % of dogs with nasal disorders and is the second most common cause of chronic nasal discharge.

In current study ,there was no significant correlation between A.fumigatus isolation from mouth of hunting dogs and the age group of dogs. Although infections was reported mainly at the age group (5.4 -6.2)years followed by (0.6-1.5)years and (2.5-3.3)years, (6.06%) . similar scenario was reported in sion nasal aspergillosis where the A.fumigatus was isolated equally from nose of hunting dogs at younger ,middle and older age . A.fumigatus was recovered equally from the age group (0.6-1.5)years , (1.6-2.4 ) years ,( 2.5-3.3 )years,( 4.3-5.3) years and (5.4-6.2) years, (3.03%). A.fumigatus was isolated frequently from ear of hunting dogs at the age group (5.4-6.2)years; (9.09%). This can be attributed to the fact that dogs in small ages little use in professional hunting operations or may be accompanied to practice hunting alongside older dogs and therefore a few of the fungal infections .On the other hand canine sion-nasal aspergillosis may affect younger to middle age groups of dogs although others stated that there was no specific age predisposition [4].

The present study revealed significant correlation between older age group of hunting dogs and A.fumigatus infection for ear. This may attributed to dependence on the oldest dogs in the hunting operations and therefore exposure to Aspergillus, which is characterized by its abundance in nature and inhalation is very easy considering the small size of the conidial spores. In addition to the shape of the ear and the nature of anatomy, which makes a little ventilation and a lot of humidity resulted from swimming and bathing is a factor for the growth of fungi and the incidence of infection.

Current study revealed that (63.64%) of hunting dogs were males, while female represent (36.36%) .Both genders were equally infected with A.fumigatus isolated from mouth and nose of hunting dogs , (15.15%) and (6.06%) respectively .Females were infected more than males with A.fumigatus isolated from ear, ,(6.06%). No significant difference nor correlation were reported between genders of hunting dogs infected with A.fumigatus which come in accordance with. Males appear to be at risk of getting A.fumigatus infection at ( 2.286) time than females which may attributed to the fact that males were preferred by hunters due to the fact that male dogs are dedicated to hunting is characterized by a combination of agility and strength, in addition to the possibility of taking advantage of it work for entire year, since female dogs can be prevented from hunting because they are pregnant [5].

In conclusions, A. fumigatus infection represent serious problem for hunting dogs . Mouth, nose and ear respectively were exposed.

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