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Department of Microbiology

Immunoregulatory Effect and Molecular Detection of *Candida albicans* From Human and Pigeon in Diyala Province

A Thesis

Submitted to the Council of the College of Veterinary Medicine / University of Diyala in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Medicine / Veterinary Microbiology

By

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B . V . M . S (2015)

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(يا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي
الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ ۖ وَإِذَا قِيلَ انْشُزُوا
فَانْشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا
الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾)

صدق الله العظيم

سورة المجادلة – اية (١١)

Supervisors Certification

I certify that the thesis entitled (**Immunoregulatory Effect and Molecular Detection of *Candida albicans* From Human and Pigeon in Diyala Province**) was prepared by **(Mohammed Abdul hameed Hassan**) under our supervision at the department of microbiology , college of veterinary medicine , University of Diyala , as a partial fulfillment of the requirement for the Master Degree of Science in Veterinary Medicine / veterinary Microbiology .

supervisor

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/ /2022

In view of the available recommendations , I forward this thesis to debate by the examination committee.

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/ /2022

Examination committee certification

We, the examination committee , certify that the entitled thesis **"Immunoregulatory Effect and Molecular Detection of *Candida albicans* From Human and Pigeon in Diyala Province''** by **(Mohammed Abdul Hameed Hassan)** has been examined and read through all of its contents and related topics . The committee recommends that the student passed and awarded the degree of master of science in veterinary medicine (veterinary microbiology).

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Dedication

To those who lavish me with unending affection... My dearest

Mother and father.

To my loving brother and sister.....who are always there for me.

To my friends.....who always come through for me

I offer my little contribution and heartfelt thanks.

Mohammed abdul hameed Hassan..

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In The Name Of the GOD Most Gracious, The Most Merciful

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Prof. Dr Amer khazeal

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Declaration From

I here by declare that this thesis entitled (**Immunoregulatory Effect and Molecular Detection of *Candida albicans* From Human and Pigeon in Diyala Province) presented at the College of Veterinary Medicine / University of Diyala in 2022,** is my original work, except for quotations and citations which have been duly acknowledged .I also declare that it has not been submitted previously or concurrently, for any other degree at the University of Diyala or other Universities.

Mohammed Abdul Hameed Hassan

Date: / / 2020

ABSTRACT

Candidiasis is the most frequent fungal infection of the oral cavity in pigeons and human. As a result, they have the potential to trigger an opportunistic infection known as oral candidiasis. With or without signs or symptoms of Candidiasis, a high percentage of healthy persons have commensals Candida in the oral cavity.

Candida overgrowth is enhanced by local and systemic predisposing factors such as immunologic imbalances, heredity, and malignant illnesses. This study aimed to isolate and molecular detection of gene mutation in *Candida albicans* isolated from human and pigeon with the study of immunoregulatory effect. *Candida albicans* was isolated from oral mucosa of infected pigeon and pigeons breeders, while control from human and pigeon clinically don't have any sign of candidiasis and identification by using candida elective agar. The isolates were applied for gene sequence. Blood sample were collected from all above groups to apply for detection the level of IL-3, GM-CSF, and IL-25 by ELISA technique . The finding of isolated Candida spp. On Sabouraud dextrose was appeared white colored, smooth, and yeast-like appearance, Candida colonies on SDA are large, white to creamy, smooth and rounded while the colonies of *Candida* spp. isolated from pigeon on SDA are small , white , rough , and rounded .

The *C. albicans* colonies were growth on elective agar appeared as a small colonies with dark brown to black color. The following results were presented in this study based on the diagnosis of Candida spp. among study groups: control group (male) was diagnosed 59.1% (13) isolates of 100% (22), Pigeon owners was diagnosed 52%(26 isolates of 50) , control (pigeon) was diagnosed 31.8% (7 isolates of 22), and 56%

(28 isolates of 50) for infected pigeons. *Candida albicans* in this study, diagnosed among the study groups was as follows: the control (male) was diagnosed with 45.5% (10) sample isolate from 22 samples, the pigeon owner was diagnosed with 48% (24) sample isolate from 50 samples, the control (pigeon) was diagnosed with 22.7% (5) sample isolate from 22 samples, and the infected pigeon was diagnosed with 52% (26) sample isolate from 50 samples.

The study examined the level of IL-3, GM-CSF, and IL-25 in blood samples. The level of IL-3 in a group of pigeons breeders decreased significantly ($P < 0.05$) as compared to the control (male) group (28.23 ± 20.97 pg/ml) , while IL-3 levels were significantly higher in all pigeon infected groups ($P < 0.05$) than in the control (pigeon) group (14.91 ± 0.57 pg/ml). The GM-CSF levels in all pigeons breeders groups were significantly higher those in the control (male) group (13.77 ± 3.43pg/ml) , while the results of GM-CSF showed a significant decreasing at ($P < 0.05$) in all pigeon infected group compared with control (pigeon) group (53.66±40.81 pg/ml). The results of IL-25 showed a significant decreasing in all pigeons breeders group compared with control (male) group (28.15 ± 19.57 pg/ml) ,also the results of IL-25 showed a significant decreasing at ($P < 0.05$) in all pigeon infected group compared with control (pigeon) group (9.32 ±6.05 pg/ml) .

In this study, Molecular detection of *C.albicans* gene (chitin synthase) confirmed the isolatation of *C. albicans* among the study group. The control group (male) was diagnosed with 45.5% (10 confirmed *C.albicans* from 22 samples) , the pigeon owner was diagnosed with 48% (24 confirmed *C.albicans* from 50 samples), the control pigeon was diagnosed with 22.7% (5 confirmed *C.albicans* from 22 samples), and the infected was diagnosed with 52% (26 confirmed

C.albicans from 50 samples). The sequence of *C. albicans* isolated was applied for strains from humans and pigeons. The result shown that three strains of *C. albicans* isolated from a human source and one from a pigeon source have mutations in the gene sequence compared with the reference gene sequence. The frequency of mutant allel and genotype was detected as, an allel *T* was has highly mutant frequency (10 time), while allel *A* and *G* have 4 time and 1 time for allel *C*.

In conclusion: *Candida albicans* that caused the infection of pigeon and pigeon breeder has the same gene sequence and some isolates appeared with mutations that lead to the raised virulence of *C. albicans* resulting in the immunoregulatory effects.

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List of abbreviations

Abbreviate	definition
A	Adenine
AIDS	Acquired immunodeficiency syndrome
℃	Celsius
CD4	Cluster of differtentiation 4
D.W.	Distal water
DNA	Deoxyribonucleic acid
EB	Ethidium bromide
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme linked immune sorbent assay
G	Guanine
GM-CSF	Granulocyte macrophage colony stimulating factor
GSH	Glutathione s- transferase
GSSG	Glutathione disulfide
HAART	highly active antiretroviral treatment
HIV	Human immunodeficiency virus
Ho1	Heme oxygenase
HRP	Horseradish peroxidase
IFN	Interferon
IL-25	Interleukin 25
IL-3	Interleukin 3
MDA	Malondialdehyde
MHC1	Major histocompatibility complex 1
mRNA	Messenger ribonucleic acid
NAC	Non-albicans Candida
NCBI	National center for biotechnology information
NK	Natural killer
Nm	Nanometer
NQO-1	Nad quinine oxidoreductase
NRF2	Nuclear factor erythroid 2-related factor 2
Nrf2	nuclear factor- erythroid factor 2
OC	Oral candidiasis
OD	Optical density
OPC	Outpatient commitment
PAMP	Pathogen associated molecular pattern
PCR	Polymerase chain reaction

R.P.M.	Round per minute
R.T.	Room temperature
SD	Standard deviation
SDA	Sabouraud dextrose agar
SNP	Single nucleotide polymorphisms
SOD	Superoxide dismutase
T	Thymine
T.cell	T helper 1 cell
TH1	Thymus helper 1
TLRs	Toll like receptors
TMB	tetramethylbenzdine

1. Introduction

Human is sensitive to fungal diseases, which can range from mild to severe , frequent from minor surface infections to potentially fatal invasive diseases. Most fungal pathogens are opportunistic and infect immunocompromised persons, such as those suffering from HIV positive infection, tumors, radiation, transplants, or who use immuno suppression medicines . To prevent the majority of deaths, the most important priority remains effective diagnosis and treatment of high-risk populations. Candida, Aspergillus, and Cryptococcus species cause the most majority of invasive infections. The absence of effective vaccinations, standard diagnostic techniques, effective antifungal drugs, and also the development of antibiotic resistant species variants pose a worldwide threat that must be handled invasion of fungal diseases (Pathakumari *et al*., 2020).

The immunoglobulin structural genes and the products of at least two main gene clusters, and the major histocompatibility complex genes are expressed as active and passive recognition structures on immune system cells and at least some of their released products. Macrophages are essential in the regulation of immunological effector cells, regulatory subsets of thymus-derived lymphocytes interact with macrophages and with each other in the start of immunological responses (Yanping Wu *et al*., 2022) . Cell interactions need that these regulatory cells identify gene products of the major histocompatibility complex at every stage of the immune response (McDevitt, 2000).

Candida albicans (*C. albicans*) is one of the most significant pathogenic fungus in humans and pigeon. It is present in the gastrointestinal tract as a commensal. *Candida albicans* mucosal

infections are generally caused by either defects in host cell - mediated immunity, especially that caused by both primary and secondary immunodeficiency, or by antibiotic-induced changes in the intestinal flora. *C. albicans* causes systemic infections with high mortality rates less frequently. *Candida albicans* diseases may be enhanced through overexpression of the fungus's pathogenicity characteristics, in additional to host factors. *C. albicans* genetic alterations which result in phenotype diversity inside the fungal have also been found to impact its virulence at mucosal surface and throughout the body. The mannans, glucans and chitin found in the cell wall of *Candida albicans*, an opportunistic pathogenic yeast of humans, play a significant role in regulating the host immune response during candidaemia (Mora-Montes *et al.*,2011). During infection, the fungal polysaccharides are released into the bloodstream, enabling for the early recognition of infection by invasive fungi. The glucans molecular structure, which includes the polymer length and degree of branching, solubility, and impact on the activation or inhibition of leukocytes, is crucial for determining their immunological characteristics. receptors. It has been demonstrated that yeast-derived soluble -glucans can inhibit receptors such Dectin-1 and M2 inhibit multivalent binding, which is required for robust leukocyte triggering. (Jawhara *et al.*, 2020).

1.2 Aim of the study

This study was aimed to isolate and molecular identify of *C. albicans* iolated from Pigeon and Pigeons breeder with study of immunoregulatory effect .

The objectives to achieve the aim of the study are:

1. Isolation *C. albicans* from oral cavity for infected pigeon and breeder pigeon with control non infected pigeon and not pigeon breeder.
2. Preliminary differentiating of *C .albicans* according to NICKERSON agar.
3. Evaluation the status of IL-3,GM-CSF,IL-25 in the serum of pigeon breeder , pigeon and control groups .
4. Determination the mutation of *C. albicans* gene (chitin synthase) in the isolate from all groups in this study.