“The Pathogenicity of Environmental and Human Isolates of *Candida albicans* to Rabbits"

By:

Fatima Abdelmuti Ahmed

B.V.M., Sudan University of Science & Technology

October, 2004

Supervisor:

Dr. Abdel Hafeez Hassan Nimir

A dissertation submitted to the University of Khartoum in partial fulfillment of the requirements for the degree of Master of Science in Microbiology (M.Sc. Micro.)

Department of microbiology,

Faculty of Veterinary Medicine, University of Khartoum

November, 2010
DEDICATION

To my Parents

who

always pray for me.

To my wonderful brother Abdulla

who

always supported me.

To my Sisters and brothers.

To my friends and colleagues.
ACKNOWLEDGMENTS

First of all, thanks and praise are to ALLAH, the compassionate and the most merciful for giving me strength and health during the period of the study. Thanks to my supervisor Dr. Abdel Hafeez Hassan Nimir, Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, for guidance, advice and help during the study period.

My deepest thanks to prof. Ahmed Abdelrehem Gameel and his associates, Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum, for the preparation and reading of the histopathological sections. Dr. Nuseiba Hamed and Dr. Eiman Osman and the staff and technicians of Department of Pathology, Central Veterinary Research Laboratory, Soba (CVRL) for the preparation of the Histopathological sections.

Thanks also extend to all of the staff and technicians of Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, for their valuable advice and assistance.

Finally, an honorable mention goes to my family and friends for their understanding and support.
# List of contents

<table>
<thead>
<tr>
<th>subject</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgment</td>
<td>ii</td>
</tr>
<tr>
<td>List of contents</td>
<td>iii</td>
</tr>
<tr>
<td>List of tables</td>
<td>v</td>
</tr>
<tr>
<td>List of figures</td>
<td>vi</td>
</tr>
<tr>
<td>Abstract (English)</td>
<td>vii</td>
</tr>
<tr>
<td>Abstract (Arabic)</td>
<td>viii</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>3</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>2.1. History</td>
<td>3</td>
</tr>
<tr>
<td>2.2. The genus Candida</td>
<td>3</td>
</tr>
<tr>
<td>2.3. Scientific classification</td>
<td>4</td>
</tr>
<tr>
<td>2.4. Morphology and identification of genus Candida</td>
<td>4</td>
</tr>
<tr>
<td>2.4.1. <em>Candida albicans</em></td>
<td>5</td>
</tr>
<tr>
<td>2.5. Non-<em>Candida albicans</em> species</td>
<td>5</td>
</tr>
<tr>
<td>2.5.1. <em>Candida tropicalis</em></td>
<td>6</td>
</tr>
<tr>
<td>2.5.2. <em>Candida krusei</em></td>
<td>6</td>
</tr>
<tr>
<td>2.5.3. <em>Candida glabrata</em></td>
<td>6</td>
</tr>
<tr>
<td>2.5.4 <em>Candida parapsilosis</em></td>
<td>7</td>
</tr>
<tr>
<td>2.5.5. <em>Candida guilliermondii</em></td>
<td>7</td>
</tr>
<tr>
<td>2.5.6. <em>Candida kefyr</em></td>
<td>7</td>
</tr>
<tr>
<td>2.6. Human candidosis</td>
<td>8</td>
</tr>
<tr>
<td>2.7. Candida in animals</td>
<td>8</td>
</tr>
<tr>
<td>2.7.1. Epidemiology and pathogenesis</td>
<td>9</td>
</tr>
<tr>
<td>2.8. <em>Candida albicans</em> and environment</td>
<td>10</td>
</tr>
<tr>
<td>2.9. Diagnosis</td>
<td>11</td>
</tr>
<tr>
<td>CHAPTER THREE</td>
<td>12</td>
</tr>
<tr>
<td>MATERIALS AND METHOD</td>
<td>12</td>
</tr>
<tr>
<td>3.1. Rabbits</td>
<td>12</td>
</tr>
<tr>
<td>3.2. <em>Candida albicans</em> isolates</td>
<td>12</td>
</tr>
<tr>
<td>3.3. Preparation of Inocula</td>
<td>13</td>
</tr>
<tr>
<td>3.3.1. Turbidity standard for inoculum preparation</td>
<td>13</td>
</tr>
<tr>
<td>3.4. Inoculation of rabbits</td>
<td>14</td>
</tr>
<tr>
<td>3.5. Histopathology</td>
<td>15</td>
</tr>
<tr>
<td>3.5.1. Fixation</td>
<td>15</td>
</tr>
<tr>
<td>subject</td>
<td>page</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.5.2. Tissue processing</td>
<td>15</td>
</tr>
<tr>
<td>3.5.3. Dehydration</td>
<td>15</td>
</tr>
<tr>
<td>3.5.4. Clearing</td>
<td>15</td>
</tr>
<tr>
<td>3.5.5. Wax impregnation and embedding</td>
<td>15</td>
</tr>
<tr>
<td>3.5.6. Microtomy</td>
<td>16</td>
</tr>
<tr>
<td>3.5.7. Manipulation of section</td>
<td>16</td>
</tr>
<tr>
<td>3.5.8. Staining of the section</td>
<td>16</td>
</tr>
<tr>
<td>3.5.9. Mounting</td>
<td>16</td>
</tr>
<tr>
<td>3.6. Media and reagents</td>
<td>17</td>
</tr>
<tr>
<td>3.6.1. Corn Meal Agar</td>
<td>17</td>
</tr>
<tr>
<td>3.6.2. Czapek-Dox Agar + Tween80</td>
<td>17</td>
</tr>
<tr>
<td>3.6.3. Sabouraud dextrose agar (SDA)</td>
<td>18</td>
</tr>
<tr>
<td>3.6.4. Yeast Nitrogen Base</td>
<td>18</td>
</tr>
<tr>
<td>3.6.5. Carbohydrate Assimilation (Auxanographivc) Tests</td>
<td>18</td>
</tr>
<tr>
<td>3.6.6. Fermentation Medium</td>
<td>19</td>
</tr>
<tr>
<td>3.6.6.1. Sugar Medium Broth Base (Oxoid)</td>
<td>19</td>
</tr>
<tr>
<td>3.6.6.2. Test Sugar</td>
<td>19</td>
</tr>
<tr>
<td>3.7. Identification of Yeast Isolates</td>
<td>20</td>
</tr>
<tr>
<td>3.7.1. Colony characteristics</td>
<td>20</td>
</tr>
<tr>
<td>3.7.2. Morphology of vegetative cells</td>
<td>20</td>
</tr>
<tr>
<td>3.7.3. Mycelium or Pseudo mycelium production</td>
<td>21</td>
</tr>
<tr>
<td>3.7.4. Formation of chlamydospores</td>
<td>21</td>
</tr>
<tr>
<td>3.7.5. Germ tube test</td>
<td>21</td>
</tr>
<tr>
<td>3.7.6. Auxanographic test</td>
<td>21</td>
</tr>
<tr>
<td>3.7.6.1. Assimilation of Carbohydrates</td>
<td>21</td>
</tr>
<tr>
<td>3.7.7. Sugar fermentation</td>
<td>22</td>
</tr>
<tr>
<td>3.7.8. Growth at 37ºC</td>
<td>23</td>
</tr>
<tr>
<td>CHAPTER FOUR</td>
<td>24</td>
</tr>
<tr>
<td>RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>CHAPTER FIVE</td>
<td>37</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>37</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>39</td>
</tr>
<tr>
<td>Recommendations</td>
<td>39</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>40</td>
</tr>
</tbody>
</table>
List of tables

**Table 1**: Table (1) contain impression Smears and Growth at 37°C for detection of Candida albicans in rabbits which injected by human isolates……………………………………26

**Table 2**: contains impression Smears and Growth at 37°C for detection of Candida albicans in rabbits which injected by environmental isolates………………………………..27
List of figures

Fig 1: impression smear from kidney of Rabbit No (3) infected with *Candida albicans* human isolates No (2) showing long chain of pseudohyphae. Gram stains X400.........................................................31

Fig: 2: Rabbit No (1) infected with *Candida albicans* isolate (1) human. Kidney Section showing tubular necrosis and presence of pseudohyphae. PASx400.................................................................32

Fig 3: Rabbit No (1) similar Fig No (1). Kidney Section showing tubular necrosis and dense cluster of pseudohyphae. PASx400.................................32

Fig: 4, Rabbit No (4) infected with *Candida albicans* isolates No (2) human. Kidney Section showing numerous pseudohyphae and blastospores. PASx250.................................................................33

Fig: 5, Rabbit No (4) Kidney Section showing necrosis of tubules and presences of multiple areas of pseudohyphae. PASx250.................................33

Fig: 6, Rabbit No (4) Lung section showing collapse and emphysema PASX250.................................................................34

Fig: 7, Rabbit No (6) infected with *Candida albicans* isolates No (3) environmental. Liver section showing dilated congested central veins and sinusoids vacuolar changes.PASx400.........................................................35

Fig: 8, Rabbit No (7): Heart showing Myocardial degeneration, focal areas of necrosis and infiltration of mononuclear cells ESX100.........................36

Fig: 9, Rabbit No (8): Heart showing myocardial degeneration and infiltration of mononuclear cells ESX250.........................................................36
Abstract

The objective of this study was to compare the pathogenicity of environmental (Chicken pens) and human isolates of the yeast *Candida albicans* to rabbits.

Four isolates of *Candida albicans* (2 environmental and 2 human) were injected intravenously into eight apparently healthy local breed rabbits aged 6-8 months (4 male and 4 female). Inocula were in the form of culture suspensions of the yeast (2 rabbits per isolate). All rabbits infected with the environmental isolates died within 24-48 hours postinfection, whereas those infected with the human isolates died after 4-5 days postinfection.

All rabbits were subjected to necropsy examination soon after death. Tissue specimens from liver, kidneys, lungs and heart of each animal were taken for microscopy, histopathology and culture to compare the pathogenicity of environmental and human isolates of *Candida albicans* to rabbits.

*Candida albicans* was recovered from all organs in both groups, and was demonstrated in impression smears and histopathological section of kidneys in group 1 only, which was inoculated with the human isolates.

It seems that the environmental isolates of *Candida albicans* are more pathogenic to rabbits than human isolates.
المستخلص

هدفت هذه الدراسة لمقارنة أمراضية معزولات من خميرة البيضاء من مصادر بيئية (آكلات الدواجن) وأخرى برغزية للأرانب. حقوقت أربعة أرانب بمعلق مزرعي من المعزولات البيئية (أرانب لكل معزول) وحققت أربعة أرانب أخرى بمعلق مزرعي من المعزولات البشرية (أرانب لكل معزول).

الأرانب المحقونة بمعلق المعزولات البيئية نفقت خلال 24-48 ساعة بعد الحقن في حين وان الأرانب المحقونة بالمعزولات من أصل بشرى نفقت بعد 4-5 أيام من حقنها.

أخضعت كل الأرانب فور نفوقها للتشريحة البيانية وأخذت الكبد والكليتان والرئتان والقلب من كل منها للفحص المجهري والمزرعي والنسجي المرضي. المبيضة البيضاء أعيد عزلها من كل الأعضاء في المجموعتين وقد رصدت في المسحة المضغوطة وقطاع الأنسجة المريضة لكلى فقط في المجموعة الأولى والتي تم حقنها بمعزولات بشرية.

ينبغي من نتائج هذه الدراسة ان المعزولات المبيضة البيضاء من مصدر بيئة أكثر إمراضية للأرانب من تلك المعزولة من مصدر بشرى.
CHAPTER ONE

Introduction

Fungi are eukaryotic organisms with approximately 300,000 different species. About 180 species are recognized to cause disease (mycosis) in man and animals (Greenwood et al., 2002). Although formerly considered to be plants, they are now generally assigned to their own kingdom, Fungi (Strohl et al., 2001).

The yeast Candida albicans has its known habitat in the mouth, throat, intestinal tract, and perhaps other inner body surfaces of man, certain mammals and birds, and it can readily be isolated from many healthy individuals. Under special and only partly recognized conditions (diabetes mellitus, pregnancy, prostrating diseases, and antibiotic treatment) this yeast may cause disease (Candidosis) of a variety of organs (mouth, intestine, vagina, skin, bronchi, lungs, avian crop). As C. albicans has been isolated almost exclusively from man and animals, it is generally assumed that infection with this yeast takes place from man or animal to man or animal by intimate contact. Candida albicans has until now been reported as isolated only twice from non-animal sources. According to Skinner (1947), C. ddoi (now considered a synonym of C. albicans) was isolated by Negroni and Fisher from decaying vegetables, and Menna and Parle (1954) isolated three strains of C. albicans from two soil samples in New Zealand. The most pathogenic of the Genus Candida is Candida albicans which causes a wide variety of disorders, including thrush (Grigoriu et al., 1987), Candida enteritis (Muller, 1993), vulvovaginitis and urinary tract Candidosis (David
et al., 1992), mucocutaneous Candidosis (Filler and Edwards, 1993) and invasive Candidosis (Bikandi et al., 1998).

*Candida albicans* has been categorized as an obligate saprophyte of warm-blooded animals because of its rare occurrence in host-free environments (Van Uden and Castelo Branco, 1963). Recognition of the species is facilitated by its production of characteristic chlamydospores and germ tubes (Ahearn, 1974). *C. albicans* has been found sporadically in fresh and marine waters and will survive for prolonged periods in stored waters.

**Objective of study**

To compare the pathogenicity of environmental and human isolates of *Candida albicans* to rabbits.
CHAPTER TWO
LITERATURE REVIEW

2.1. History

Infection caused by yeast-like fungi has been known in man for over 100 years. Lagenbeck (1839) first demonstrated a yeast-like fungus in the lesions of thrush. His finding was confirmed by Gruby (1842).

The genus *Candida* and species *C. albicans* were described by botanist Christine Marie Berkhout in her doctoral thesis at the University of Utrecht in 1923. Over the years, the classification of the genera and species has evolved. Obsolete names for this Genus include *Mycotorula* and *Torulopsis*. The species has also been known in the past as *Oidium albicans* (Robin, 1853). In 1890 Zopf named the fungus *Monilla albicans* and for many years the disease was called moniliasis. The current classification is nomen conservandum, which means the name is authorized for use by the International Botanical Congress (IBC).

The early studies (Redaelli, 1924; Benham, 1931) have shown that the rabbit is readily susceptible to infection which was induced by the intravenous inoculation of *Candida albicans*. Fuentes et al., (1952) studied the symptomatology, survival time, and pathology in guinea pigs, mice, rabbits, and rats injected intravenously with numbers of *C. albicans* cells varying according to the weight of the animals.

2.2. The genus *Candida*

The genus *Candida* contains more than 150 different species. Some of these species are members of the normal flora of the mucous membrane of
the oral cavity, vagina and gastro-intestinal tract in man and as many as 80% of people may show colonization of these sites in the absence of overt infection (Murray et al., 1998).

*C. albicans* is the most significant pathogenic species. Other *Candida* species pathogenic to humans include *C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. dubliniensis,* and *C. lusitaniae.*

2.3. Scientific classification

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Saccharomycotina

Class: Saccharomycetes

Order: Saccharomycetales

Family: Saccharomycetaceae

Genus: *Candida*

(Berkhout, 1923).

2.4. Morphology and identification of genus Candida

*Candida* is characterized by globose to elongate yeast-like cells or blastoconidia that reproduce by multilateral budding. Most Candida species are also characterized by the presence of well developed pseudohyphae. However this characteristic may be absent, especially in those species formally included in the genus *Torulopsis.* Arthrospores, blastospores and colony pigmentation are always absent. Within the genus Candida, fermentation, nitrate and inositol assimilation may be present or absent, and
all inositol positive strains produce pseudohyphae. (Mycology on line, 2010).

On agar media at 37°C at room temperature, Candida species produce soft, opaque, cream-colored pasty colonies with a yeasty-odour. Pseudohyphae appear as submerged growth below the agar surface. Several procedures are available for identification of Candida spp., most of which combine morphological, physiological, and biochemical tests (Lodder, 1974).

2.4.1. Candida albicans

C. albicans is a dimorphic fungus, which characteristically develops both as yeast cells and as pseudohyphae. Colonies on Sabouraud's dextrose agar at 25°C are white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to semispherical budding yeast-like cells or blastoconidia, 2.0-7.0 x 3.0-8.5 Mm in size.

On cornmeal following 72 hours incubation at 25°C, abundant branched pseudohyphae and true hyphae with blastoconidia are present. The blastoconidia are formed in grape-like clusters along the length of the hyphae. Terminal chlamydoconidia may be formed with extended incubation.

2.5. Non- Candida albicans species

Species of Candida other than C. albicans are normal flora of Cutaneous and mucocutaneous surfaces.

In a review of 1591 cases of Candida infection published in 37 reports between 1952 and 1992, it was found that species other than C. albicans
were the causative agents in 46% of systemic infections; *C. tropicalis* accounted for 25% of infections, *C. glabrata* for 8%, *C. parapsilosis* for 7% and *C. krusei* for 4% (Wingard, 1995).

An extensive review of new yeast pathogens was published by Hazen, (1995). The incidence of infection by non *C. albicans* species such as *C. parapsilosis, C. glabrata, C. krusei, C. guilliermondii, C. lipolytica* and *C. kefyr*, were on the increase.

2.5.1. *Candida tropicalis*

On Sabouraud's dextrose agar colonies are white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to semi spherical budding yeast-like cells or blastospores, 3.0-5.5 x 4.0-9.0 Mm in size.

2.5.2. *Candida krusei*

On Sabouraud's dextrose agar colonies are white to cream colored, smooth, glabrous yeast-like colonies. Microscopic morphology shows predominantly small, elongated to ovoid budding yeast-like cells or blastospores, 2.0-5.5 x 4.0-15.0 Mm in size.

2.5.3. *Candida glabrata*

On Sabouraud's dextrose agar colonies are white to cream colored, smooth and glabrous yeast-like in appearance. Microscopic morphology shows numerous ovoid, budding yeast-like cells or blastospores, 2.0-4.0 x 3.0-5.5 Mm in size. No pseudohyphae are produced.
2.5.4. *Candida parapsilosis*

On Sabouraud's dextrose agar colonies are white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows predominantly small, globose to ovoid budding yeast-like cells or blastospores, 2.0-3.5 x 3.0-4.5 Mm in size, with some larger elongated forms present.

2.5.5. *Candida guilliermondii*

On Sabouraud's dextrose agar colonies are white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to semi spherical budding yeast-like cells or blastospores, 2.0-4.0 x 3.0-6.5 Mm in size.

2.5.6. *Candida kefyr*

On Sabouraud's dextrose agar colonies are white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows numerous short-ovoid to long-ovoid, budding yeast-like cells or blastospores, 3.0-6.5 x 5.5-11.0 Mm, sometimes becoming elongate (up to 16.0 Mm) in size.
2.6. Human Candidosis

Primary or secondary mycotic infection can be caused by members of the genus Candida. The clinical manifestations may be acute, sub acute or chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in candidaemia, endocarditis and meningitis. In healthy individuals, *Candida* infections are usually due to impaired epithelial barrier functions and occur in all age groups, but are most common in the newborn and the elderly. They usually remain superficial and respond readily to treatment. Systemic Candidosis is usually seen in patients with cell-mediated immune deficiency, and those receiving cancer chemotherapy, immunosuppression, or transplantation therapy. Several species of *Candida* may be an etiological agent, most commonly, *C.albicans* and rarely *C. tropicalis, C. krusei, C. parapsilosis, C. guillermondii, C. kefyr* (*C. pseudotropicalis*) and *C. Torulopsis (glabrata)*. These are all ubiquitous and occur naturally on humans (Rippon, 1988).

2.7. Candida in animals

*Candida* species are natural in habitants of the alimentary, genital and upper respiratory tracts of mammals (Greene and Chandler, 1998). These yeast-like fungi can cause opportunistic infections in animals treated with antibiotics, corticosteroids, cytotic agents and immunosuppressive drugs (Macphail *et al.*, 2002; Tunca and Haziroglu, 2004). In the veterinary literature, occasional skin and intestinal infections caused by Candida species have been reported (Carrasco *et al.*, 1993; Ochiai *et al.*, 2000).
Of the Candida species, \textit{C. albicans} is most commonly isolated in animals (Barker \textit{et al.}, 1992).

Factors associated with candidal infections are disruption of mucosal integrity; indwelling, intravenous, or urinary catheters; prolonged administration of antibiotics; and immunosuppressive drugs or diseases. The organism most frequently infects birds, in which it involves the oral mucosa, esophagus, and crop. Superficial infections limited to the mucous membranes of the intestinal tract have been described in pigs and foals. Systemic Candidosis has also been described in cattle, calves, sheep, and foals secondary to prolonged antibiotic or corticosteroid therapy. In cats, Candidosis is rare but has been associated with oral and upper respiratory diseases, pyothorax, ocular lesions, intestinal diseases, and cystitis. Infections are rare in dogs and horses. However, \textit{Candida sp.} has been considered a cause of arthritis in horses and mastitis and abortion in cattle (Kahn, 2005).

\textbf{2.7.1. Epidemiology and pathogenesis}

\textit{C. albicans} is a minor constituent of the normal flora of the skin and mucosal surfaces of all domesticated animals. It can opportunistically increase in numbers and become invasive in animals whose normal bacterial flora has been disrupted by prolonged antibiotic therapy and abnormal nutrition. Also stresses that compromise immune defenses predispose animals towardout breaks by Candidosis.

Candidosis (thrush) in birds-chickens, pigeon, turkeys, pheasant, and grouse is common and can cause significant losses. The lesions involve the mouth, crop, proventriculus, and gizzard and consist of whitish circular areas
or elongated patches along the crests of folds in the mucosa. These areas become confluent and rather large and finally slough off, leaving superficial ulcers. Epidemics in very young birds can cause heavy mortality. Infections also occur in older birds but are less severe. Beemer et al. (1973), detected venereal Candidosis in geese.

Systemic Candidosis has been reported in feedlot cattle (McCarty, 1956). The clinical signs included dyspnea, nasal discharge, diarrhea, and wasting. In calves, following prolonged antibiotic therapy, C. albicans has caused lesions in rumen, liver, lungs, brain, and kidneys (Cross et al., 1970). Candidosis has been reported in piglets on an artificial diet (McCrea and Osborne, 1957) and in older pig (Barker and Cadman, 1963).

Affected animals exhibit white pseudo membrane on the tongue, esophagus, and stomach. Cutaneous Candidosis has been described in dogs and cats (Kral and Uscavage, 1960). Candidosis is extremely rarely reported disease in horses, oral candidosis (white, pseudomembranous plaques and ulcers on tongue) was reported in immunodeficient foals (McClure, 1985).

Candida arthritis (C. tropicalis, C. parasilosis) has also been reported in horses (Madison, 1995). Vulovoginal Candidosis was reported in six thoroughbred mares following the oral administration of altrenogest (Montes, 2001). The perivulvar and perineal skin was erythematous, pustular, eroded and variably hyper and hypo pigmented.

2.8. Candida albicans and environment

The organism has been recovered from soil, hospital environments, inanimate objects, and food. This yeast is isolated infrequently from other habitats, including aquatic environments receiving urban sewage effluents
Isolations from these sources appear to be due to recent contamination with human or animal excrement (Ahearn et al., 1968; Cook, 1970). It is also found in the environment, particularly on leaves, flowers, water, and soil.

2.9. Diagnosis

Diagnosis of yeast infections is done via Gross appearance of lesion and microscopic examination or culturing. For identification by light microscopy, a scraping or swab of the affected area is placed on a microscope slide. A single drop of 10% potassium hydroxide (KOH) solution is then added to the specimen. The KOH dissolves the skin cells but leaves the Candida cells intact, permitting visualization of pseudohyphae and budding yeast cells typical of many Candida species.

For the culturing method, a sterile swab is rubbed on the infected skin surface. The swab is then streaked on a suitable culture medium. The culture is incubated at 37°C for several days, to allow development of yeast or bacterial colonies. The characteristics (such as morphology and color) of the colonies may allow initial diagnosis of the organism.
CHAPTER THREE
MATERIALS AND METHODS

3.1. Rabbits

Four male and four female, apparently healthy, local breed rabbits, about 6-8 months old, were used as experimental animals in this study. Prior to infection, oral swabs from all rabbits were examined by culture for the presence of *Candida albicans*, and all were found to be free of the yeast. Animals were kept for a week before infection to allow them to adapt to the confinement conditions.

3.2. *Candida albicans* isolates

Four isolates of *Candida albicans* (2 environmental and 2 human isolates) were employed in this study. All four isolates were obtained from the culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum. The environmental isolates were originally isolated from aviaries (chicken pens) during a survey in Omdurman area. The human isolates were obtained from profuse pure urine cultures of woman suffering from urinary tract infection. All cultures were maintained in the laboratory by subculturing on Sabroud Dextrose Agar slants, at 3-months intervals, and kept refrigerated at 4°C.

The identity of cultures as *C.albicans* isolates was reconfirmed by germ tube formation, chlamydosporulation, sugar fermentation and auxanographic methods.
3.3. Preparation of Inocula

To prepare the inoculum, fresh cultures were grown on Sabouraud Dextrose Agar for 48h at 37ºC. Yeast suspensions were prepared by harvesting cells and washing them 3 times in sterile normal saline by centrifugation at 3,000 rpm for 5 minutes.

3.3.1. Turbidity standard for inoculum preparation

To standardize the inoculum density for rabbit's infection, a barium sulfate (BaSO₄) turbidity standard, equivalent to a 0.5 McFarland was used.

BaSO₄ 0.5 McFarland standard was prepared by adding 0.5ml of barium chloride (1.17%w/v BaCl₂ .2H₂O) to 99.5 ml of sulfuric acid (H₂SO₄) (1%w/v) with constant stirring. The suspension was thoroughly mixed to ensure that it's even. The absorbance of turbidity was measured in spectrophotometer at a wavelength of 625 nm using cuvettes with a 1cm light path and water as a blank standard. The absorbance of the standard turbidity was 0.11, then 3ml of the standard was distributed into sterile screw-cap tubes of the same size and stored for up to six months protected from light at room temperature, and then was agitated vigorously before use. (Monica, 2006).

In a good light the turbidity of the suspension was matched to the turbidity standard (mixed well immediately before use) against a card with a white background and contrasting back lines.
3.4. Inoculation of rabbits

Rabbits were infected via the ear vein by injecting 1ml of saline suspension of *Candida albicans* containing $1 \times 10^6$ cells/ml). Animals were divided into 2 groups. Group one contained 4 rabbits, each one was injected by 1 ml of *Candida albicans* isolate from human and a similar dose was given to each of group two rabbits with Inocula prepared from the environmental isolates.

After infection, rabbits were closely observed daily for signs of any abnormality.

All rabbits were subjected to necropsy examination soon after death. Tissues specimens from liver, kidneys, lung and heart of each animal were taken for microscopy, culture and histopathology.

Impression smears were prepared from all organs, stained with Gram stain and examined microscopically for the possible presence of any fungal elements.

 Cultures from rabbit organ were made by pressing part of the organ several times on Sabouraud Dextrose Agar plates then spreading with the loop to cover the agar surface. Inoculated plates were incubated at 37°C with daily examination for 7 days before being discarded as negative. Any yeast colonies yielded were subcultured on SDA plate for purification and reconfirmation.

 Histopathological sections were stained with Periodic Acid- Schiff reagent and Haemotoxylin and Eosin stains according to the methods described by (Drury and Wallington, 1980).
3.5. Histopathology

3.5.1. Fixation

About one cm³ of sample were fixed at least three days so as to preserve and fortify the tissue.

3.5.2. Tissue processing

It was performed automatically using Elloit tissue processor. The formalin fixed tissues were labeled and immersed in 70% alcohol for 15 minutes before it was introduced to the processing machine.

3.5.3. Dehydration

It is essential in the preliminary process, because paraffin did not penetrate the tissue in the presence of water. Using different ascending grads of ethyl alcohol (varying between 70% to absolute alcohol) prevent the distortion that would accompany the direct transfer of tissue from 10% formalin to absolute alcohol.

3.5.4. Clearing

Xylene and chloroform were used as clearing agents.

3.5.5. Wax impregnation and embedding

Tissues were embedded in paraffin wax of a melting point 54 to 56 moulds of suitable size then the blocks were allowed to cool and solidity.
3.5.6. Microtomy

Microtomy of embedded tissues was performed using a rotatory microtome (Baired and Tatlock Ltd, England). The sections produced were 4-6 microns in thickness.

3.5.7. Manipulation of section

The sections were fixed on a glass slide and dried at 37°C for an hour.

3.5.8. Staining of the section

Section was first, freed of wax using xylene and then rehydrated using different descending grades of alcohol ranging from absolute to 70% alcohol. The tissues were cleaned with xylene and stained with eosin and haemotoxylin. Selected sections were also stained with periodic acid-Schiff (PAS).

3.5.9. Mounting

The staining sections were covered with glass cover slips, which were fixed by Canada balsam. The sections were all allowed drying for 24 hours before they were examined.
3.6. Media and reagents

The following media were used for the isolation and maintenance of yeast species. Commercial media were prepared according to the manufacturer’s recommendations.

3.6.1. Corn Meal Agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn meal extract (Oxoid)</td>
<td>2.0 g.</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Preparation**

Seventeen gram of the dehydrated medium were suspended in a litre of distilled water, brought to the boil to dissolve, pH 6.0 and then sterilized by autoclaving at 15 lbs/sq.in. (121°C) for 15 minutes.

3.6.2. Czapek-Dox Agar + Tween80

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czapek-Dox Agar (Oxoid)</td>
<td>33.4 g.</td>
</tr>
<tr>
<td>Tween80 (poly oxyethylene sorbitan mono-oleate)</td>
<td>10 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Preparation**

The dehydrated medium 33.4 g. were dissolved in a litre of distilled water, 10 ml of Tween 80 added, pH 6.8 and the medium was sterilized by autoclaving at 10 lbs/sq.in. (115°C) for 20 minutes.
3.6.3. Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycological peptone (Oxoid)</td>
<td>10.0g.</td>
</tr>
<tr>
<td>Dextrose (D-glucose)</td>
<td>40.0g.</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0g.</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.05g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Preparation**

Sixty five grams of dehydrated medium were suspended in a litre of distilled water, dissolved by heating, chloramphenicol was added, PH 5.6 mixed and sterilized by autoclaving at 15lbs/sq.in. (121°C) for 15 minutes.

3.6.4. Yeast Nitrogen Base

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto-yeast nitrogen base (Difco)</td>
<td>67.0g.</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Preparation**

Yeast nitrogen base powder (67g.) were suspended in a litre of distilled water, 15g. agar added, dissolved by heating, pH 5.4. dispensed and sterilized by autoclaving at 10 lbs/sq.in. (115°C) for 20 minutes.

3.6.5. Carbohydrate Assimilation (Auxanographic) Tests

Saturated aqueous solution of: glucose, D-Galactose, maltose, sucrose, lactose, inositaol and trehalose, were prepared and pipetted on 6x6mm filter paper discs in petri dishes, the excess solution removed and discs dried at 56°C. These impregnated discs were used as carbohydrate Sources in the
assimilation tests.

3.6.6. Fermentation Medium

3.6.6.1. Sugar Medium Broth Base (Oxoid)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>lab-lemco beef extract (Oxoid)</td>
<td>5.0g.</td>
</tr>
<tr>
<td>Peptone (Bacto)</td>
<td>10.0g.</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.0g.</td>
</tr>
<tr>
<td>Di-sodium hydrogen phosphate</td>
<td>2.0g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

Indicator

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromothymol blue (granules)</td>
<td>0.1g.</td>
</tr>
<tr>
<td>0.1N NaOH</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>47.5ml</td>
</tr>
</tbody>
</table>

3.6.2. Test Sugar

D-glucose, D-Galactose, sucrose maltose lactose and raffinose were used in the fermentation tests.

Preparation (Lodder, 1974)

The fermentation of all sugar used was tested in 2% solutions in 75X10mm cotton-plugged test tubes containing Durham tube (15X6mm), except in the case of raffinose where a 4% solution was employed.
The sugars were dissolved in a 0.5 solution of Oxoid beef extract as the basal broth medium, incorporating bromothymolblue as an indicator.

Ingredients of the broth were dissolved by steaming, pH adjust to 7.2-7.3, indicator added (12ml/litre), and the broth sterilized by autoclaving, in 100ml volumes, at 15 lbs/sq.in. (121°C) for 15minutes.

After sterilization, the sugar powders were added to the individual bottles containing basal medium to the final concentration of 2%, except in the case of raffinose where a final concentration of 4% was used. 1.5ml aliquots of the sugar solutions were finally dispensed into sterile cotton-plugged test tubes containing inverted Durham tubes and sterilized by tyndalization, by steaming for 20minutes.each day, for 3 consecutive days.

3.7. Identification of Yeast Isolates

After primary isolation, yeast cultures were purified on SDA containing chloramphenicol, then single colonies, were selected. These pure cultures were used for the preparation of inocula and identification of organism was carried out according to the criteria defined and methods described by Lodder (1974); Barnett and Pankhurst (1974); Nimir (1980); Koneman and Roberts (1985); and Quinn et al., (2002).

3.7.1. Colony characteristics

Yeast isolates were grown on SDA plates at 28°C for 3-4 days, colonial characteristics were noticed, then the plates were left at room temperature for a week before the final observation. The surface of the growth, margin, appearance consistency, smell and pigments were all recorded.

3.7.2. Morphology of vegetative cells

Wet mounts from 3-4 days old pure cultures were examined microscopically to determine the size, shape and type of budding of the vegetative cells.
3.7.3. Mycelium or pseudo mycelium production

The ability to pseudo-or true mycelium was determined by culturing on corn meal agar. Plates were inoculated from young yeast cultures by streaking over the agar surface. A sterile coverslip was placed over a portion of the streak. Inoculated plates were incubated at 28ºC for 7 days, with daily examination for the presence of filamentation.

3.7.4. Formation of chlamydospores

Streaks from young cultures were made on the surface of Czapek-Dox agar containing Tween 80, and sterile coverslips applied. Plates were incubated at 28ºC for 7 days and examined daily for chlamydospore production.

3.7.5. Germ tube test

Half ml of sterile sheep serum was inoculated with a small inoculum from a young culture, incubated at 37ºC for 3 hrs and examined for germ tube production.

3.7.6. Auxanographic test

3.7.6.1. Assimilation of Carbohydrates

The ability of a yeast species to utilize a specific carbohydrate compound as a sole carbon source was tested by the auxanographic method using Difco yeast nitrogen base as the basal medium.

Seven carbohydrate compounds glucose, D-Galactose, maltose, sucrose, lactose, inositol, and trehalose, were used in the primary tests for every yeast isolate.

Inocula for the assimilation tests were prepared by making aqueous suspension of the yeast, from 3-4 days old cultures on SDA slants in universal bottles grown at 28ºC, by adding 3ml sterile distilled water and carefully washing the yeast grown from the agar surface. Twelve drops of
the yeast suspension were then added to 20ml of melted yeast nitrogen base agar previously cooled to 45°C in a water bath, using a sterile Pasteur pipette. The yeast suspension and agar were well mixed, poured into 2 sterile Petri dishes (10ml inoculated medium per plate) and allowed to solidify. The carbohydrate-impregnated discs were then placed on the agar surface with sterile forceps, using 7 discs per plate, one central and 6 peripheral, with the sugar numbers marked on the back of the plate.

The inoculated plates were incubated at 28°C, with daily examination, for 7 days. A heavy yeast growth around the disc was regarded as positive. In doubtful cases, the test was repeated.

3.7.7. Sugar fermentation

Six sugars: glucose, galactose, sucrose, maltose, lactose and raffinose were used in the fermentation tests with all isolates.

The same yeast inoculum, used for the assimilation tests, was used to inoculate the fermentation tubes. Two drops of the yeast suspension were added to each sugar fermentation tube. The inoculated tubes were gently swirled between the palms to mix, and were incubated at 28°C, with daily examination, for up to 2 weeks. The reactions were finally recorded as production of gas, production of acid and gas, or no apparent fermentation. A test was regarded as positive when there was acid and gas production. When raffinose was fermented, the volume of gas contained in the Durham tube was estimated, since differential fermentation of raffinose was considered to be of diagnostic value in yeast speciation (Lodder, 1974).
3.7.8. Growth at 37°C

All yeast isolate were tested for growth at 37°C on SDA plates held for 3 days. A visible growth was regarded as positive. In case of weak growth, the test was repeated.
CHAPTER FOUR

RESULTS

All four rabbits infected with the environmental isolates of *Candida albicans* died within 24 hours postinfection. They exhibited nervous signs (staggering) before death. Postmortem lesions were in the form of generalized hemorrhage, enlargement of the liver, heart, kidneys and lungs.

Two of the animals infected with human isolates died four days postinfection and the other two died after five days postinfection. Enlargement of the liver, heart, kidneys and lungs was observed in all four rabbits.

Group(1): Rabbits infected with human isolates revealed the presence of *Candida albicans* in smear impression from kidneys, in the form of long pseudohyphae as is shown in Fig (1), whereas impression smears from livers, lungs, and hearts of rabbits in the same group did not show the presence of such elements indicative of *Candida albicans*.

Cultures from all organs of rabbits in this group yielded positive growth for *C.albicans* ranging in amount from profuse growth from the kidneys to light growth in other organs, (table 1).

Histopathological sections from kidneys of all four rabbits revealed the presence of pseudohyphae and yeast cells (blastospores), whereas those from livers, lungs, hearts, of all animals were negative for such elements.

Group (2): Rabbits infected with the environmental isolates gave positive cultures for *C.albicans* from all organs ranging from moderate to scanty
growth. However, none of the impression smears from them showed any evidence \textit{C.albicans} elements, (table 2).

None of the histopathological sections from any organ of the four rabbits showed any evidence of the presence of \textit{C.albicans} elements.
Table (1) Result of impression Smears and Growth at 37°C for detection of *Candida albicans* in rabbits injected by human isolates.

<table>
<thead>
<tr>
<th>test</th>
<th>impression Smears</th>
<th>Growth at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits number organs</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
</tr>
<tr>
<td>kidneys</td>
<td>+ve   +ve   +ve   +ve</td>
<td>+ve   +ve   +ve   +ve</td>
</tr>
<tr>
<td>liver</td>
<td>-ve   -ve   -ve   -ve</td>
<td>+ve   +ve   +ve   +ve</td>
</tr>
<tr>
<td>lungs</td>
<td>-ve   -ve   -ve   -ve</td>
<td>+ve   +ve   +ve   +ve</td>
</tr>
<tr>
<td>heart</td>
<td>-ve   -ve   -ve   -ve</td>
<td>+ve   +ve   +ve   +ve</td>
</tr>
</tbody>
</table>
Table (2) Result of impression Smears and Growth at 37ºC for detection of *Candida albicans* in rabbits injected by environmental isolates.

<table>
<thead>
<tr>
<th>test</th>
<th>impression Smears</th>
<th>Growth at 37ºC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits number organs</td>
<td>5 6 7 8</td>
<td>5 6 7 8</td>
</tr>
<tr>
<td>kidneys</td>
<td>-ve -ve -ve -ve</td>
<td>+ve +ve +ve +ve</td>
</tr>
<tr>
<td>liver</td>
<td>-ve -ve -ve -ve</td>
<td>+ve +ve +ve +ve</td>
</tr>
<tr>
<td>lungs</td>
<td>-ve -ve -ve -ve</td>
<td>+ve +ve +ve +ve</td>
</tr>
<tr>
<td>heart</td>
<td>-ve -ve -ve -ve</td>
<td>+ve +ve +ve +ve</td>
</tr>
</tbody>
</table>
**Histopathology**

**Group (1)**

Rabbit No (1):

Kidney: tubular necrosis and presence of fungal structures. At places tubules were widely separated (edema). glomeruli showed dilated Bowman’s space (Fig 2 and 3).

Liver: dilated central veins and vacuolar changes in hepatocytes. No fungal structures seen but bacterial structures (cocci and bacilli) were observed.

Lung: alveoli contained homogenous pinkish material indicative of edema.

Heart: no significant observation.

Rabbit No (2):

Kidney: many areas showed tubular necrosis and infiltration by mainly neutrophils and some lymphocytes. Fungal structure and spores seen in the lesion.

Liver: slight sinusoidal congestion and cytoplasmic vaculation in hepatocytes.

Lung: congestion, areas of emphysema and collapse with presence of mononuclear cells in alveoli.

Heart: multiple areas of myonecrosis and cellular infiltration of mononuclear cells.
Rabbit No (3):

Kidney: multiple areas of necrosis and cellular infiltration mononuclear cells. Fungal structures are seen.

Liver: sinusoidal congestion.

Lung: congestion, mural thickening of blood vessels.

Heart: myocardial necrosis and infiltration of mononuclear cells.

Rabbit No (4):

Kidney: congestion especially medulary vessels. Wide areas of tubular necrosis. Presence of Fungal structure detachment of tubular lining cells from basal membrane. Glomerular capillaries were congested and Glomerular tufts lobulated. (Fig 4 and 5).

Liver: distinct lobulation, sinusoidal congestion.

Lung: alveolar edema and areas of collapse and emphysema as is Shown in Fig (6).

Heart: myocarditis (muscle necrosis and infiltration of cells).

**Group (2)**

Rabbit No (5):

Kidney: lobulated tufts and congestion.

Liver: Sinusoidal congestion and focal hepatocytes degeneration with mononuclear cells, infiltration in portal areas.

Lung: edema, congestion and areas of emphysema and collapse.
Heart: focal area of hemorrhage.

Rabbit No (6):

Kidney: focal necrotic areas characterized by loss of tubular structure and dissociation of tubular cells.

Liver: dilated veins and sinusoids vacuolar changes, cocci and coccobacilli seen. Thickening of bile ducts with vesiculated nuclei of lining epithelium (Fig 7).

Lung: areas of emphysema and collapse with thickening of interstitial tissue (pneumonitis).

Heart: multiple areas of myocardial degeneration and necrosis.

Rabbit No (7):

Kidney: lobulated tufts and congestion of Glomeruliar capillaries.

Liver: focal areas of dilated congested capillaries.

Lung: areas of emphysema, collapse, congestion, hemorrhage.

Heart: Myocardial degeneration, focal areas of necrosis and infiltration of mononuclear cells (Fig 8).

Rabbit No (8):

Kidney: tubular necrosis and cellular infiltration of mononuclear cells.

Liver: Sinusoidal congestion.

Lung: interstitial thickening with areas of collapse and emphysema. Heart: myocardial degeneration and infiltration of mononuclear cells (Fig 9).
Fig: 1, impression smear from kidney of Rabbit No (3) infected with *Candida albicans* human isolates No (2) showing long chain of pseudohyphae. Gram stains X400.
Fig: 2, Rabbit No (1) infected with *Candida albicans* isolate (1) human. Kidney Section showing tubular necrosis and presence of pseudohyphae.PASx400.

Fig 3: Rabbit No (1) similar Fig No (1).Kidney Section showing tubular necrosis and dense cluster of pseudohyphae.PASx400.
Fig: 4, Rabbit No (4) infected with *Candida albicans* isolates No (2) human. Kidney Section showing numerous pseudohyphae and blastospores. PASx250.

Fig: 5, Rabbit No (4) Kidney Section showing necrosis of tubules and presences of multiple areas of pseudohyphae. Note lobulated Glomerular tufts. PASx250.
Fig: 6, Rabbit No (4) Lung section showing collapse and emphysema PASX250.
Fig: 7, Rabbit No (6) infected with *Candida albicans* isolates No (3) environmental. Liver section showing dilated central vein and sinusoids vacular changes. PASx400.
Fig: 8, Rabbit No (7): Heart showing Myocardial degeneration, focal areas of necrosis and infiltration of mononuclear cells E SX100.

Fig: 9, Rabbit No (8): Heart showing myocardial degeneration and infiltration of mononuclear cells ESX250.
CHAPTER FIVE

DISCUSSION

Candida albicans is a yeast that has a distinct predilection for mucosal surfaces and areas of mucocutaneous junctions of warm-blooded animals where it resides as commensal. It is well recognized as being closely associated with man and animals as a normal commensal of the mucocutaneous sites, the digestive and reproductive tracts. Its presence outside the human and animal bodies are rarely reported. Its biotype in the digestive system, is present as a minor member of the microbial flora, but under particular conditions, C. albicans becomes an opportunistic pathogenic microorganism, which may produce serious local infection and/or systemic invasion of the internal organs such as the kidney, liver, lungs, and heart (Holoymoen et al., 1982; Gheorghiu et al., 1996; Kim et al., 1998; Kuwamura et al., 2006). An interesting feature of C. albicans is its ability to grow in two different ways; reproduction by budding, forming blastospores, and in a hyphal form. In addition to the intrinsic biological interest of this dimorphism, its ability to switch between the yeast and the hyphal forms of growth has been implicated in its pathogenicity (Leberer et al., 1997; Molero et al., 1998; Whiteway and Oberholzer, 2004).

Previous studies have shown that yeast cells trapped in interstitial capillaries germinate hyphal forms which penetrate into the renal tubular lumen. Here they can proliferate protected from the host cellular defenses. Penetration back into renal tissue elicits significant tissue damage and an inflammatory response (Louria et al.1963), that are in agreement with this study.
The marked appearance of pseudohyphae in the kidneys may be due to the fact that the kidneys acts as filter for the fungus from the blood which may lead to accumulation of the fungus within the kidneys.

Absence of the fungus in impression smears and tissue sections from all organs in rabbits infected with the environmental isolates may be attributed to the fact that the isolates caused severe infection (very acute) leading to the rapid death of rabbits. This state may be due to the fact that the environmental isolates were highly pathogenic to rabbits, compared to the human isolates, which caused death 4 – 5 days postinfection.
Conclusions

It appears from the results of this study that environmental isolates of C. albicans are more pathogenic to rabbits than the human isolates.

Recommendations

1-Further studies are recommended to explain the dynamics of the infectivity of environmental isolates of C. albicans to rabbits using different isolates and large groups of animals.

2-Care should be taken to raise the awareness of personnel working in animal houses (chicken pens in particular) about the possibility of infection from the animal environment.
REFERENCES


**Macphail, G.L., Taylor, G.D., Buchanan-Chell, M., Ross, C., Wilson, S. and Kureishi, A. (2002).** Epidemiology, Treatment and Outcome of
Candidemia: a five-year review at three Canadian hospitals. Mycoses. 45: 141-145.


Van Uden, N., and Castelo Branco, R. (1963). Distribution and population densities of Yeast species in Pacific water, air, animals and kelp off


**Wilson, J.W. and Plunkett, O.A. (1965).**
