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Yeast Flora in the Nasal Cavity of apparently normalCamels

By:-

Hana Ali Ismail Mohamed Ahmed

Sudan University of Science & Technology

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Supervisor

Prof. Mohammed Taha. A. Shigidi

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Department of Microbiology

Faculty of Veterinary Medicine

University of Khartoum

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بسم الله الرحمن الرحيم

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Dedication

Dedication to my parents who always prays

for me

To Dr. Nimir

To all those who supported me in this

Study

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AKNOWOLEDMENT

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ABSTRACT

The objective of this study was to identify yeasts in thenasal cavities of apparently healthy camels. Hundred apparently healthy camels of different ages,40 fromthe Camel Research Centre,Faculty of Veterinary Medicine,University of Khartoum at Shambat,25 fromthe Camels inthe Research Station at Tamboul and 35 fromcamels brought for slaughter to Tamboul marketabout 150kilometers south east of Khartoum.

Sampling was done during the period fromJanuary to March(2011). Nasal swabs were streaked on SabouraudsDextrose Agar supplemented with 0.5%chloramphenicol.Isolates were identified and classified according to their microscopic appearance and biochemical reactions. The results were confirmedby Vitek2 Compact System.

Out of the 100 samples, yeasts were isolated in pure cultures from 44 samples. Thirty five of theisolates belonged to genus*Cryptococcus* and were grouped into 3species; *Cryptococcus albidus*26(59.1%),*Cryptococcus laurentii*6(13.6%)and*Cryptococcus terreus*3(6.8%) andNine(20.5%) of the isolates wereunidentified black yeasts.

*Cryptococcus albidus*and *Cryptococcus laurentii*arepotential pathogens and may cause systemic diseases in man and animal, but *Cryptococcus terreus*is not known to be a pathogen.

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الملخص

الهدف من هذه الدراسة تحديد الخمائر الموجودة طبيعيابتجويف الأنف في الإبل السليمة ظاهريا.
أخذت العينات من مائة رأس من الإبل السليمة ظاهريافي مختلف الأعمار: 40 عينةمن مركزأبحاث الإبل كلية الطب البيطري في جامعة الخرطوم بشمبات، و25 عينة من الإبلالموجودة في محطة أبحاث الإبل بتمبول و35 عينة من الإبل التي جلبت للذبيح في سوق تمبول، التي تقع على بعد 150 كيلومتر جنوب شرق الخرطوم.
جمعتالعينات خلال الفترة من يناير إلى مارس 2011 وكانت العينات عبارة عن مسحات أنفية تم زرعها في أجار السابرودكستروز المضاف إليه مضاد حيوي كلورامفينيكول 5,%،ثم التحقق من العزلات وصنفت وفقا لمظهرها المجهري ونتائج الإختباراتالبيوكيمائيةألتي أجريت عليها،وتأكيد النتائج وفقا لنظام Vitek2.

من بين 100عينة عزلت الخمائر بصورة نقية عبارة 44، تنتمي خمس وثلاثون منها إلي جنسالمستخفية وصنفت إلي ثلاثة انواع: 26 Cryptococcusalbidus(%59,1)و6 Cryptococcus laurentii(%13,6) و3Cryptococcus terreus(%6.8) و9 (%20,5) عزلات سوداء غير معرفة (Cryptococcus albidus و Cryptococcus laurentii) هي ممرضة وقد تتسبب في أمراض جهازية للإنسان والحيوان،ولكن المستخفية.Cterreusليسمن المعروف عنها أنها ممرضة.

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CHAPTER ONE

INTRODUCTION

Normal flora are bacteria, viruses, protozoa and fungi which dwell in different anatomicalregions of the body supporting a tight relationship with the animal and human homeostasis (Ingraham andIngraham, 2000; Sorumand Sunde, 2001;Tlaskalova*et al.,* 2004). These microorganisms are adapted to live within the host without causingdisease or damage.Generically they are all referred to as "flora", they are all living together in synergy when their environment is healthy(Tlaskalova*et al.,* 2004).

The density of the microbial flora of the respiratory tract varies among animals and human, within the respiratory tract itself. Resident flora are limited to the nasal cavity and pharynx where highly diverse flora can be found.Transient flora are comprised of microorganisms that enter during the breathing process and, therefore, reflect the environment in which the animal is maintained. Environmental factors, such as dry,dust ,poorventilation, increase the microbial load and types of transient flora an animal is exposed to(Dwigh,*et al*.2004).

Yeasts are organisms that exist in tropical regions. They are found in aquatic environments ,inhabiting soil , plants , atmosphere , fresh and salted water , insects and vertebrates (Carom-Sousa, 1969;Lodder ; 1970 and Ahearn, 1973).Yeasts are known to exist as commensals on or in the animal body and have been isolated from domesticated, zoo and wild animals such as

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sheep, goats, swine and horses(VanUden*et al.*1958; Van Uden ,Caromo-Sousa,1962),camels(Saez and Rinjard, 1973) byNimir,1980.

Yeasts such as *Candida, Rhodotorula*and *Cryptococcus*, have been isolated from natural substrates like leaves, flowers,sweet fruits,grains, fleshy fungi, insects, dung, soil ,humansand animals(Spencer and Spencer,1997). They play their role in the soil, plants, animals and water (RoseandHarrison ,1993).The importantspecies isolated were:*C.albicans,C. parapsilosis, C. Iusitaniae, Cr.neoforman, Cr.laurentii ,Cr.albidusand Malasseziapachydermatis.*

Camel (C*amelusDomedarius*) is a domestic animal species uniquely adapted to hot and arid environment. It is an important animal in Africa because of its adaptation to adverse climatic conditions and shortage of forage and water. It is also an indicator of social prestige and wealth (Bekele, 1999).

Little information is available about normal yeast flora in the nasal cavity of apparently healthy camel.

The objective of this study was to isolate and identify yeastsflora which may part of the normal flora of the nasal cavity of apparently healthy camels.

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CHAPTER TWO
LITERATURE REVIEW

2.1. Anatomy and physiology of the nasal cavity of camels:

In camels, the nostrils are slit-like openings in the face and they are positioned laterally. The rostal portion of the vestibulumnasi is lined with skin and fine hairs before abruptly going over into smooth mucous membrane. The camel nasal passages are protected by large muscular nostrils that can open and close at will. They filter inhaled air and prevent foreign materials such as sand from entering the respiratory tract. When a camel twitches its nose, it is cooling the incoming air and condensing moisture from its outgoing breath. Each nasal passage is divided by the major conchae into dorsal, middle, and ventral meatuses. The middle portion of the nasal cavity contains the nasal conchae while the ethmoidalconche are situated caudally. The olfactory mucosa occupies a small area of the caudal part of the ethmoidal conchae. Also, the vomeronasal organ is 150-180mm long and opens into the rostal part of the ventral measures. It is closely related to olfaction. The ventral meatus is relatively spacious and leads directly to the choanae. Two openings are located in the mucous membrane of the floor of the nostril (Al-ani,2004).

Opening of the lateral nasal diverticulum located at the junction of the skin and mucosa lies on the lateral surface of the maxilla.

Opening of the nasolacrimal duct lies medially to the opening of the lateral nasal diverticulum.

Extensive venous plexuses are found underneath the epithelial lining of the nasal cavity. The later consists of respiratory epithelium with numerous

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goblet cells for mucous secretion. Ciliated cells in the mucous membrane of the sacs produce currents that move the secretions toward the nasal cavities where they moisten the incoming dry air. The olfactory mucosa occupies a small area of the caudal part of the ethmoidal conchae. The turbinates have a total surface area of approximately 0.1 square meters in which air from the lungs is cooled (Al-ani,2004).

2.1.1. Nasal sinuses:

The maxillary sinus occupies a small part of the maxilla and rostra part of the zygomatic bone. It communicates dorsally with the lachrymal sinus. The maxillary sinus contains the well-developed lateral nasal gland which produces mucus. The frontal sinus comprises several small compartments that may communicate. The larger compartments that communicate with the fundus nasi through relatively small openings. The sphenoid sinus occurs in the pre-sphenoid bone (Al-ani, 2004).

2.2. Normal Microbial Flora of respiratory tract:-

Normal microorganisms can be transitory or permanent; transitory microorganisms colonize for short periods and originate from the environmental microbial flora. Long-lasting microorganisms are persist for long periods of time, and are similar among individuals of the same species (Ingraham, 2000; Hernandez *et al*., 2004).

Microorganisms are ubiquitous in the environment and are continuously in contact with animals and humans, this interaction arises from birth through ingestion or inhalation and elapses through the animals life-course. Each anatomical region creates its own selective environment where few microorganisms are favored above others (Tlaskalova*et al*,2004). Therefore, microorganisms from internal and external surfaces such as oral cavity, gastrointestinal, respiratory and genitourinary tracts, conjunctiva and skin of

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healthy animals and human are considered normal microorganisms (Ingraham , 2000; Sorum and Sunde, 2001; Tlaskalova*et al*, 2004). Some of these microorganisms are non-pathogenic and are dwellers of a specific region, but they can turn to be pathogens if they are moved to another region.

The respiratory tract has a normal flora as any other body system that is in contact with the external environment. Various normal flora can be isolated from a sterile swab passed deep into the nasal cavity of any healthy animal. In the upper respiratory tract (nasal cavity, the pharynx and larynx) microorganisms live in areas bathed with the secretions of the mucous membranes. The lower respiratory tract, (bronchioles and alveoli) has no resident microflora, despite the large numbers of organisms potentially able to reach this region during breathing.The upper respiratory tract is populated with a large variety and number of microorganisms, but the lower respiratory tract has relatively few microorganism inhabitants, unless there is an ongoing active infection (Williams, 1993).

The first observation of yeasts microscopically was by Van Leuwenhoek, in 1680, he sent descriptions and drawings of yeast cells to the Royal Society in London, some four years after he had made public his observations on bacteria. It was not until the first half of 19th century that significant progress was made towards an understanding of the biology of yeasts and through this to an appreciation of their physiology and biochemistry. Pasteur, (1876) proved that fermentation was due to living cells and pointed out the role of oxygen in alcoholic fermentation . After Pasteur, there followed a period of intense activity during which yeast taxonomy and morphology flourished . Jorgensen, (1886) wrote on yeast

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fermentations. The first treatise devoted entirely to yeasts was published by (Guilliermond,1912).

Over the years, more and more yeast genera and species have been described and Lodder, (1970) listed 341 species in 39 genera.

2.3. Yeast flora isolated in camels :-

Microbiological study on the nasal cavity of apparently healthy camels was carried out by Al-bashanand Al-banki (1999) in Syria. A total of 134 nasal cavity swabs were collected. Mycological examination resulted in the isolation of 6 species of fungi and one species of yeast; *C.albicans*. Study of yeast flora in other organs of camels were carried out by several author's. Abdel Sammad, (1995) studied the yeast flora of the digestive tract of camels in Sudan. From 280 samples of the camels stomach contents and small and large intestines from open Tamboul slaughter house, a total of 40 yeastspecis were isolated which belonged to two genera,*Candida* and *Torulopsis.* One of the isolates was from a rumen, 8 from reticulums ,12 from omasums ,9 from small intestines and 6from large intestines. Yeast flora of the genital tract of healthy female camels werestudied in Iran by Shokri,*et al.*,(2010). A total of 450 were obtained, The samples were taken from different parts of the genital tract including vestibules, vaginas, cervices, uterine bodies, and uterine horns of 50 camels using sterile cotton swabs. The yeast isolates belonged to 8 genera: *Candida* 73.1%, *Trichosporon* 10.1%, *Geotrichum* 7.5%, *Kluyveromyces* 3.5%, *Rhodotorula* 2.4%, *Aureobasidium* 1.4%, *Cryptococcus* 1.1% and *Prototheca*0.8%. Among different *Candida* species, *C. zeylanoides* was the most commonly isolated species, representing significant difference with other *Candida* species. The mean number of yeasts found in the vestibules (46%) was significantly higher than the results obtained from other parts. In addition,

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the mean value of CFUs from unmated females (71.1%) was significantly higher than that of mated females. The results showed that *C. zeylanoides* was a common component of healthy camel females' genital microflora and the number of yeasts varied between mated and unmated females.

2.4. Yeast flora isolated from other species:-

Yeast flora in oropharyngeal and rectal mucous membranes of healthy and critically ill neonatal foals was studied. A total of 240 swabs were collected from 21 healthy (group A) and 39 sick (group B) foals. In 14 of the 60 foals, yeast was isolated in at least one sample (23.3%). Three of the 21 foals (14.3%) were positive in group A and 11 of 39 foals (28.2%) were positive in group B. Yeasts were not isolated from rectal swabs obtained from healthy foals, whereas 5 of the 39 sick foals were positive; however, this difference was not statistically significant. No significant difference was also detected regarding oropharyngeal swabs between healthy (3/21) and sick (10/39) foals (Pirone,*et al.* 2011). In another study described the first case of*Cryptococcus*infection in Massachusetts, isolates were*Cr. neoformans*from a myxoma-like from the lung of a horse which had a persistent nasal discharge of a year's duration (Frothingham, 1902). In 1913, Meyer isolated , from a myxoma-like nasal growth of a horse in Pennsylvania, a yeast-like fungus which was designated by him, and later by Harrison, (1928), as *Torulanasalis.*

In a study of the normal flora of the nose ,throat and lower intestines of the dogs in, new Mexico,Clapper and Meade, (1962)isolated from throat and lower intestines *Candida albicans.* In another study in Scotland, samples from nasal turbinates of 100 dogs, and 75 cats undergoing routine postmortem were examined by Nimir, (1980) for yeast growth. He isolated yeasts from 38% of the specimens, 28 of them (73.7%) yielded one yeast

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species each, 9 (23.7%) yielded 2 species and 1 (2.7%) specimen yielded 4 species. The 50 yeast isolates belonged to 24 yeast species in 9 genera, namely: *Candida, Cryptococcus, Hansenula, Rhodotorula, Torulopsis, Pichia, Pityrosporum, Saccharomyces* and*Trichosporon.* Fifteen (20%) of the specimens from 75 cats yielded yeast growth. Nineteen yeast species were isolated; 11 (73.3%) of the positive specimens yielded one yeast species each, and 4 (26.7%) specimens gave 2 yeast species each. Not more than 2 yeast species were obtained from any of the positive feline turbinates. The 19 feline isolates belonged to12 species in the genera: *Candida, Cryptococcus, Hansenula, Rhodotorula*and *Torulopsis.*

Yeasts in human, animals and soil samples in Egypt were studied. Samples taken from animals were nasal and vaginal swabs. Animals examined were cattle, water buffalo, sheep and goat. Yeast isolated from 179 of the nasal swabs from animals were *R. rubra*(17.8 %), *C. albicans*(16.2 %), *Candida spp.* other than *C. albicans*(11.7 %) and *Cr. neoformans*(1.67 %), (Hala*et al.*, 2011).

Van Uden, Carmo- Sousa and Farinha, (1958) isolated yeast from the caeca of horses, sheeps, goats and swines. According to their results, the swines were regarded as the main animal reservoir for yeasts (88% of the samples were positive), followed by horses 52.4%, cattle 46.8%, sheep 6.8% and goats 6.4%. Out of the 1507 samples cultured, 486 isolates belonged to the 10 genera, 28 species and one variety. The most frequent occurrence for a single species were *Candida Sloofii*in swine (48.4%), *Trichosporomcutaneum*(*T. beigelii*)in horses (21.8%) and *Saccharomyces telluris*(14%). They obtained one *Cr.neoformans* isolate from a healthy horses suggesting that horses might contribute to the spread of this pathogenic yeast (Albert, 1991). Another study was carried out in different

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animals. Yeasts in the alimentary canal of 169 animals has been investigated by Parle, (1957). Representatives of 6 genera and17 species were isolated, but only 4 species; *T.pintolopesii, S.guttulata, C.albicans,* and *T . glabrata*were considered to be true intestinal types. *C. parapsilosis* and *C. krusei*were considered as intermediate and the remainder as transient flora. The validity of the species *T . pintolopesii*is considered doubtful

2.5. Yeasts in the nasal cavity of camels and other species :-

2.5.1.Cryptococcus:-

Cryptococcus is encapsulated yeast that is found in soil contaminated with pigeon droppings or eucalyptus trees and decaying wood. The genus *Cryptococcus* includes 37 species. Among these, *Cr.neoformans*is the only species that is pathogenic to human and animals. Other species are *Cr.albidus, Cr.laurentii, Cr.terreus, Cr.uniguttulatus, Cr.luteolus*and *Cr.gastricus*. *Cr.neoformans*has four serotypes (A to D); *Cr.neoformans* serotype A is responsible for the majority of human infections. Some strains of serotypes A and D can mate with strains of serotypes B and C. *Cryptococcus* species other than *Cr. neoformans*are, with rare exceptions, considered to be saprophytic and nonpathogenic. Infection *Cr. neoformans*is considered as an opportunistic pathogen as it affects mainly immunosuppressed individuals.

*Cryptococcus*is aglobose yeast seen in most clinical materials, such as cerebro spinal fluid and pulmonary tissue mounted in 10% KOH. A capsule may or may not be present. The capsule is best visible in India ink preparations. Aspirates and tissues are usually cultured onto Sabourauds dextrose agar. Colonies of *Cryptococcus* are fast growing, soft, glistening, smooth, usually mucoid, and cream to yellowish brown in color. It usually

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takes 48 to 72 hours to grow and well at 25 °C to 37 °C. *Cryptococcus* differs from Candida by hydrolysing urea and not forming pseudohyphae(Sridhar Rao,2009).

The respiratory tract is generally accepted as the main portal of entry into the body of organisms causing the major systemic mycosis. *Cr.albidus*is a saprophytic, encapsulated yeast usually found in air, both outdoor and indoor, and sometimes on human skin. It is not usually considered to be a primary pathogen. Most cryptococcal infections of humans and animals are caused by *Cr.neoformans*. Several cases of *Cr. albidus*infection have been reported in humans over the past 20 years. In the veterinary literature, 2 equine cases have been described: a genital infection and mycotic keratitis and we*Cr. albidus*are a potential pathogen (Olivia *et al.*, 2005).

Due to isolation of saprophytic cryptococci from canine and feline nasal cavities it is reasonable to expect their presence in the upper respiratory tract of animals as a result of inhalation of dust. *Cr.albidus* has been isolated from air by Saito (1922) and from soil by Sneller and Swatek (1974). Phaff and fell (1974) reported that all17 strains of this species received by them from investigators in various parts of the world had originated from terrestrial sources. *Cr.laurentii*was isolated from soil by Phaff and Spencer (lodder,1974) and by Sneller and Swatek (1974).

*Cr.gattii*was reported for the first time by Rodríguez, *et al.,*(2006) from 5 epidemic outbreaks of cryptococcosis in goats grazing freely in west Spain grasslands. In all outbreaks, mycological studies were possible from samples obtained on necropsy of some animals dead during the epidemic. The animals belonged to various milking breeds and were grazing with variable

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status of health and husbandry. Goats affected withcryptococcosis showed similar respiratory symptoms, consisting of mucopurulent nasal discharge, cough, dyspnea and progressive cachexia, causing death in a period of 2 to 4 weeks. In three outbreaks many animals also showed ataxia, midriasis, blindness and progressive paralysis. Clinical prevalence varied from 2 to 12% in the different outbreaks. It is evident that in spite of the great amplitude of geographical distribution observed for *Cr. gattii*, this species has a limited presence, possibly restricted to determined habitats, as that of infection of goats flocks in Spain. Veterinarians must be concerned about cryptococcosis in grazing animals.

2.5.1.1. Clinical Significanceof *Cryptococcus*:-

*Cryptococcus neoformans*is regarded as the principle pathogenic species, *Cr. albidus*and *Cr.laurentii*have also been implicated in human infections. *Cr. albidus*is cosmopolitan, found on plants and in water and on skin of animals and humans. Although infections with *Cr. albidus* are rare, it should be considered as a potential cause of ocular and systemic disease in immunoincompetent patients. Literature reports include: cutaneous infection, scleral ulceration of a 16-year-old girl with AIDS, eyes and blood of lymphoma patients, leukemia patients and in a rare case of mucormycosis empyema. *Cr.laurentii*has been reported as a rare cause of pulmonary and cutaneous infection but it was cause peritonitis in humans. It may also be occasionally recovered as a saprophyte from skin (Garelick, 2004). *Cr.terreus*is not a known pathogen; it is occasionally isolated as a contaminant in clinical laboratories. There was isolated*Cr. terreus* from bone marrow transplant patients (Alangaden*et al.,*1994).

2.5.2. Candida:-

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Candida are almost universal on normal adult skin (Schiefer, 1997) and *C. albicans* is part of the normal flora of the mucous membranes of the respiratory, gastrointestinal, and female genital tracts which cause no disease. But overgrowth of several species including C.albicans can cause superficial infections such as oropharyngealcandidiasis (thrush) and vulvovaginal candidiasis (vaginal candidiasis). Oral candidiasis is common in elderly denture wearers (Akpan, and Morgan, 2002).candidiasis may become a systemic disease producing abscesses, thrombophlebitis, endocarditis, or infections of the eyes or other organs. The genus *Candida* includes about 150 different species; however, only a few are known to cause human and animal infections. *C. albicans* is the most significant pathogenic species. Other Candida species pathogenic in mammal include *C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. dubliniensis, and C. lusitaniae*(Schiefer, 1997 and Fidel, 2002).

2.5.2.1.Clinical significance ofCandida:-

C.albicans occurs naturally as a commensal of mucous membranes and in the digestive tract of humans and animals. It accounts for up to 70% of *Candida* species isolated from sites of infection and has been reported as a causative agent of all types of candidiasis. Environmental isolations are usually from sources contaminated by human or animal excretion, such as polluted water, soil, air and plants (Schiefer, 1997 and Fidel, 2002).

2.5.3. Rhodotorula:-

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is a pigmented yeast, part of the Basidiomycota phylum, quite easily identifiable by distinctive orange or red colonies when grown on SDA (Sabouraud's Dextrose Agar). This distinctive colour is the result of pigments that the yeast creates to block out certain wavelengths of light that would otherwise be damaging to the cell. Colony colour can vary from being cream coloured to orange, red, pink and yellow. *Rhodotorula* is a common environmental inhabitant, it can be cultured from soil, water, and air samples. It is able to scavenge nitrogenous compounds from its environment remarkably well, growing even in air which has been carefully cleaned of any fixed nitrogen contaminants. In such conditions, the nitrogen content of the dry weight of *Rhodotorula* can drop as low as 1%, compared to around 14% for most bacteria growing in normal conditions. *R.rubra*on Sabouraud's dextrose agar cultures are coral pink, usually smooth, sometimes reticulate, rugose or corrugated, moist to mucoid yeast-like in appearance. Microscopic morphology shows spherical to elongate budding yeast-like cells or blastoconidia:Physiological tests: Germ Tube test is Negative, Hydrolysis of Urea is Positive, Growth on Cycloheximide medium is Negative and Growth at 37 ℃ is Variable(Postgate and John,1994).

2.5.3.1.Clinical significanceof Rhodotorula:-

Rhodotorularubra is a common airborne contaminant of skin, lungs, urine and faeces. *R. rubra* is a known cause of fungal peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD). This is usually due to saprophytic colonization of catheters or dialysis machinery and removal of the source of contamination usually leads to clearing of the symptoms. It has also been reported as causing fungemia, endocarditis and meningitis in

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patients undergoing chemotherapy for cancer and those with indwelling intravenous catheters(Postgate and John,1994).

CHAPTER THREE

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MATERIALS AND METHOD

3.1. Sample Collection: -

A total of 100 nasal swabs were collected from the Camels Research Centre University of Khartoum at Shambat, Camel Research Centre at (Tamboul) and from camels brought for slaughter to Tamboul market.Tamboul (about) 150 Kilometers South East of Khartoum. Specimens were taken by sterile cotton swabs(15cm) into the walls of the nasal cavities and samples were transferred to the laboratory in a container with ice. On arrival to laboratory they were cultured on Sabourauds dextrose agar plates, incubated at 25℃±1℃ and examined daily for 7days. Plates showing yeast- like growth were examined for the presence of yeast by making smears, which were stained with Gram's stain and examined microscopically. Subcultures were made from the plates showing yeast-like growth on Sabourauds dextrose agar to obtain pure cultures. Stock cultures were maintained on Sabourauds dextrose agar slopes in universal bottles and kept at 4℃ with monthly subcultureing.

3.2. Media preparation : -

Media used in this study were prepared according to the manufacturers recommendations.

3.2.1. Corn Meal Agar(Oxoid):- Code No.CM 30

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Used for morphological studies of yeast and Consisted of:

Corn meal extract (oxoid) ……………….2 g

Agar ……………………………………..15 g

Distilled water ……………..……… 1000 ml

Seventeen grams of the powder were suspended in 1000ml of distilled water, dissolved by heating and sterilized by autoclaving at 121℃ at 15 Ibs/sq-inch for 15 minutes, thepH adjusted to 6.

3.2.2.Czapek Dox Agar+ Tween80(Oxoid):-Code No.CM 97

Consisted of:

Sucrose ………………………… 40 g

Sodium Nitrate ………………….. 2 g

Di potassium sulphate ………….. 1 g

Magnesium sulphate ………….. 0.5 g

Potassium chloride …………….. 0.3 g

Ferrous ……………..………… 0.01 g

Agar ……………………….…… 15 g

Distilled water ……………... 1000 ml

Forty nine grams of the powder were suspended in 1000ml of distilled water and dissolved by heating. Then10ml of tween 80 were added. Sterilization was done by autoclaving at 121℃ at 15 Ib/sq-inch for 15minutes.

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3.2.3. Sabourauds Dextrose Agar (SDA) (Supplemented with Choloramphenicol)(Oxoid):Code No.CM 41

Consisted of:

Mycological peptone (oxoid) …….……..10 g

Dextrose ………………………..……..40 g

Agar (oxoid) ………………………….... 15 g

Chloramphenicol …….……………… .0.05 g

Distilled water ……………………… 1000 ml

Ingredients were suspended in1000 ml of distilled water, dissolved completely by heating and 0.05ml of chloramphenicol was added. The medium was sterilized by autoclaving at 121℃ at 15Ibs/sq-inch for 15minutes,the pH adjusted to 5.6.

3.2.4. Sugar Fermentation Medium (Oxoid): Code No. M 028

Formula:

Peptone water ……………… 10 g

Carbohydrates ……………….10 g

Andrade's indicator...………..10 g

Sugar ……………………..….20 g

Solid ingredients were dissolved in 1000 ml distilled water. The pH was adjusted to 7.1- 7.3 before the addition of andrade's indicator. The complete medium was mixed, distributed in to 2 ml volumes into test tubes containing

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Derham's tube and sterilized by autoclaving at 115℃ for 10 minutes. The sugars used were : Glucose , Galactose , Sucrose , Maltose , Lactose , Trehalose ,Starch , Inulin and Inistol.

3.2.5. Urea Agar(Oxoid): Code No. CM53

Urea hydrolysis test was performed using urea agar medium:

Code No. CM53

Consisted of :

Peptone(oxoid L37) …………………… 1 g

Dextrose ……………………………... 1 g

Sodium chloride ……………………... 5 g

Disodium phosphate ……………….... 1.2 g

Potassium dihydrogen phosphate ….. 0.8 g

Phenol red ……………………….. 0.012 g

Agar No.3(Oxoid L13) …………….… 15 g

Distilled water ……………...……. 950 ml

Twenty four grams of the powder were suspended in 95 ml of distilled water, dissolved completely , autoclaved at 115℃ for 20 minutes and left to cool to 50℃ in a water bath. Five mililiter of sterilized 40% urea solution were added aseptically , mixed well and distributed into sterile bottles and allowed to set in a slope position, the pH adjusted to 6.8.

3.2.6. Yeast Carbon Base: -(Difco)Code No. 0391-15-0

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Used for nitrogen assimilation test and consisted of :

Bacto yeast carbon base ………………………… 117 g

Agar ………………………………………………. 15 g

Distilled water ……………………………….. 1000 ml

Hundred and seventeen grams of the powder were suspended in1000mlof distilled water , distribution into universal bottles and autoclaved at 121℃ at 15 Ibs/sq-inch for 15minutes,the pH adjusted 4.5.

3.2.7. Yeast Nitrogen Base : -(CDH) Code No.0392

It was used for carbon assimilation and consisted of:

Bacto yeast nitrogen base ……………………. 67 g

Agar ……………………………………….…. 15 g

Distilled water ………………………….…. 1000 ml

Sixty seven grams yeast nitrogen base powder were suspended in 1000 ml of distilled water, dissolved completely, distributed in sterile universal bottles and sterilized by autoclaving at 121℃ at 15 Ibs/sq- inch for 15 minutes, the pH adjusted to 4.5.

3.3. Identification of the Yeast Isolates:-

Single colonies from each yeast isolated wassubcultured. Odor, consistency, colour and edge of each colony were studied. Identifications were performed on pure cultures according to the criteria described by lodder(1974).

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3.3.1. Microscopic Colonial Morphology:-

This was determined by inoculating plates of corn meal agar and CzapekDox Agar + Tween 80 with young cultures of yeast isolates (48hours old). The media were inoculated with a loopful of each isolate by making parallel streaks onto the agar across the diameter of the plate.Sterile cover slips were placed over portions of the streaks before the plates were incubated at 25℃ for 7days. Each plate was examined microscopically using a low power lens along the streaks for the presence of pseudomycelia and blastospores or chlamydospores or yeast cells only. The test was carried out as described by (Gerald collec*et al.*,1996).

3.3.2.Germ Tube Test: -

The test was carried out as described by Gerald collec*et al.*,(1996). This test was performed by inoculating 0.5 ml of a sterile rabbit serum with a loopful of a 48hours old yeast culture and incubated at 37℃ for two hours. One drop was placed on a slide and covered with a cover slip and examined microscopically for the presence of germ tubes ,if germ tubes were not noticed cultures were re-incubated for another 2hours and re-examined.

3.3.3.Carbon Assimilation Test: -

The test was carried out as described by Gerald collec and *et al.*,(1996).The ability of a yeast species to utilize a specific carbohydrate compound as the sole carbon source was tested by the auxanographic method using yeast nitrogen base as the basal medium. This test was prepared by making aqueous suspensions of the yeast from 3-4days old cultures on SDAgrown at

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25℃,part of the culture was suspended in 3ml of a sterile distilled water ,then1ml of the suspension was added to 20 ml of yeast nitrogen base medium (cooled to 45℃ in water bath) and shaken to distribute the suspension .The medium was poured into sterile petri dishes and left to solidify. Filter paper prepared as disk was impregnate by 10% sugar solution. Four types of sugar impregnated disks were placed on the one plate of agar and the plates were incubated at 25℃ for two weeks with daily examination. The capability of the yeast to assimilate a specific sugar as a carbon source was indicated by a heavy growth around the disk. Primary sugars used were : - glucose, galactose, sucrose, lactose, maltose, inositol, inulin, trehalose and starch.

3.3.4.Nitrogen Assimilation Test : -

The test was carried out as described by Gerald collec*et al.*,(1996). The ability of a yeast species to utilize nitrate as the sole nitrogen source was tested by the auxanographic method using yeast carbon base as the basal medium. Yeast carbon base medium was used, potassium nitrate(KNO_3) in the form of impregnated disks was used, with peptone as a positive growth control. To 10ml volumes of melted yeast carbon base agar cooled to 45℃,1ml of the yeast suspension was added using a sterile Pasteur pipette. The yeast suspension and agar were mixed, poured into sterile petri dish and allowed to solidify. The nitrate and peptone- impregnated disks were placed on the inoculated agar surface and the plates were incubated at 25℃, with daily examination, for a week. A heavy growth around the nitrate disk was regarded as positive test. In doubtful cases the test was repeated.

3.3.5.Sugar Fermentation Test: -

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The test was carried out as described by Gerald collec*et al.*,(1996). Sugar fermentation test was performed using sugar fermentation medium. Each tube of medium was inoculated with a 48hours old culture from SDA and inoculateusing a sterile Pasteur pipette. The tubes were incubated at 25℃ for two weeks , shaken and examined daily .Reactions were recorded as production of acid which was indicated by appearance of reddish color ,acid and gas production.

3.3.6.Urea Hydrolysis Test: -

The test was carried out as described by Barrow and Feltham (1993). The hydrolysis of urea was determined by streaking a loopful of a 48hours old of S.D.A. cultures of yeasts on urea agar slopes and incubated at 25℃ for a week. Production of urease was indicated by colour change of the medium to pink.

3.3.7.Growth at 37℃:-

Young cultures of all yeast isolates were inoculated onto S.D.A. plates , incubated at 37℃ for 48hours and examined for the presence of the growth.

3.4.Vitek2Compact System: Department of Microbiology -soba

vitek2 is automated microbiology system used for the identification of microorganisms.David,(1978).

3.4.1.Principles:

- Using fresh and pure cultures contained single colonies .

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-Using sterile swab or applicator stick to transferred sufficient number of colonies and suspended into 3.0 ml of sterile saline (pH adjusted6) in sterile plastic test tube.

-Measuring the turbidity by using turbidity meter ,the range of turbidity of the yeasts (1.8-2.2).

-Put the test tubes in special rack ,after that put the identification cards (the cards contained transferred tube and 64 wells each wells contained tested substrate).

-Put the rackmanually in vacuum chamber station. After the vacuum was applied and air was re-introduced into the station.

-The yeasts suspension was forced through the transfer tube into micro-channels that filled all the test wells.

-The cards were passed by mechanism, which cut off the transfer tube and sealed ,the cards incubated at 35.5 ± 1 ℃ this steps took 15 minutes. After that transported to the optical system for reaction reading (each test reaction took15 minutes) and then returned to the incubator until the next read time.

- Data collected at 15 minutes.

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Figure (1):Vitek2 Compact System:

CHAPTER FOUR

RESULTS

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Field investigation:

Of the nasal swabs taken from 100 apparently healthy camels ,40 from the Camel Research Centre, Faculty of Veterinary Medicine, University of Khartoum at Shambat,25 from the Camels in the Research Station at Tamboul and 35 from camels brought for slaughter to Tamboul market. Fourty four swabs yielded positive cultures for yeasts whereas 56 swabs were negative. The yeasts isolated belonged to the genus; *Cryptococcus*, and were identified as *Cr.albidus*26,*Cr.laurentii 6* and Cr.*terreus*3isolatesand the remaining9isolates wereunidentified black yeasts. This results were confirmed by Vitek 2 compact system and as the same results in the conventional method.

colonial morphology of the Cryptococcus:-

Colonies of Cryptococcus are soft, glistening, smooth, mucoid and creamy color. It was taking 48 to 72 hours to grow well at 25 ℃.

Table(1): Yeasts isolated from the nasal cavity of apparently normal camels:

No of	Spcies	location	No of	Spcies	location

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isolate			**isolate**		
1	Cr. Albidus	Shambat	19	Cr. albidus	Tamboul
2	Cr. Albidus	"	20	Cr. albidus	"
3	Cr. Albidus	"	21	Cr. albidus	"
4	Cr. Albidus	"	22	Cr. albidus	"
5	Cr. Albidus	"	23	Cr. albidus	"
6	Cr. Albidus	"	24	Cr. albidus	"
7	Cr. Albidus	"	25	Cr. terreus	"
8	Cr. Albidus	"	26	Cr. albidus	"
9	Cr. laurentii	"	27	Cr. albidus	"
10	Cr. Albidus	"	28	Cr. albidus	"
11	Cr. Albidus	"	29	Cr. albidus	"
12	Cr. Albidus	"	30	Cr. albidus	"
13	Cr. laurentii	"	31	Cr. albidus	"
14	Cr. laurentii	"	32	Cr. albidus	"
15	Cr. laurentii	Tamboul	33	Cr. albidus	"
16	Cr. laurentii	"	34	Cr.terreus	"
17	Cr. Albidus	"	35	Cr.terreus	"
18	Cr. laurentii	"			

Table (2): Percentage of the yeasts isolated from the nasal cavity of apparently normal camels:

Species	**No. of isolates (%)**
Cr. albidus	26 (59.1%)

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Cr.laurentii	6 (13.6%)
Cr.terreus	3 (6.8%)
Black yeasts	9 (20.5%)
Total Number	44 (100.0%)

Table(3):Carbohydrates and nitrate assimilation patterns of 35 yeast isolates from the nasal cavity ofapparently normal camels.

Species	Gluc.	Malt.	Suc.	Lact.	Galac.	Inos.	Treh.	Star.	Inul.	Nitrate
Cr. albidus	+	+	+	_	W	+	+	+	_	_
Cr. laurentii	+	+	+	+	+	+	+	+	_	_
Cr. terreus	+	W	_	_	W	+	W	+	_	_

Key to symbols:

+ =assimilated.

- =not assimilated.

W =weekly assimilated.

Table (4): Sugar fermentation, urease production, growth at 37°C and germ tube production of yeast isolates:

Species	Gluc.	Malt.	Suc.	Lact.	Glact.	Inos.	Treh.	urease	Growth at 37°C	Germ tube

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Cr. albidus	-	-	-	-	-	-	-	+	+	-
Cr. laurentii	-	-	-	-	-	-	-	+	W	-
Cr. terreus	-	-	-	-	-	-	-	+	W	-

Key to symbols:

- = not fermented

W= weak growth

Table (5):Results of the yeasts identification by the vitek 2 compact system.

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No of isolate	Spcies	Identification	No of isolate	Spcies	Identification
1	Cr. albidus	Excellent	19	Cr. albidus	Acceptable
2	Cr. albidus	Excellent	20	Cr. albidus	Excellent
3	Cr. albidus	Very good	21	Cr. albidus	Very good
4	Cr. albidus	Excellent	22	Cr. albidus	Very good
5	Cr. albidus	Excellent	23	Cr. albidus	Very good
6	Cr. albidus	Very good	24	Cr. albidus	Excellent
7	Cr. albidus	Excellent	25	Cr. terreus	Good
8	Cr. albidus	Very good	26	Cr. albidus	Acceptable
9	Cr. laurentii	Excellent	27	Cr. albidus	Acceptable
10	Cr. albidus	Excellent	28	Cr. albidus	Acceptable
11	Cr. albidus	Acceptable	29	Cr. albidus	Acceptable
12	Cr. albidus	Very good	30	Cr. albidus	Acceptable
13	Cr. laurentii	Excellent	31	Cr. albidus	Acceptable
14	Cr. laurentii	Excellent	32	Cr. albidus	Acceptable
15	Cr. laurentii	Excellent	33	Cr. albidus	Acceptable
16	Cr. laurentii	Excellent	34	Cr. terreus	Acceptable
17	Cr. albidus	Very good	35	Cr. terreus	Acceptable
18	Cr. laurentii	Acceptable			

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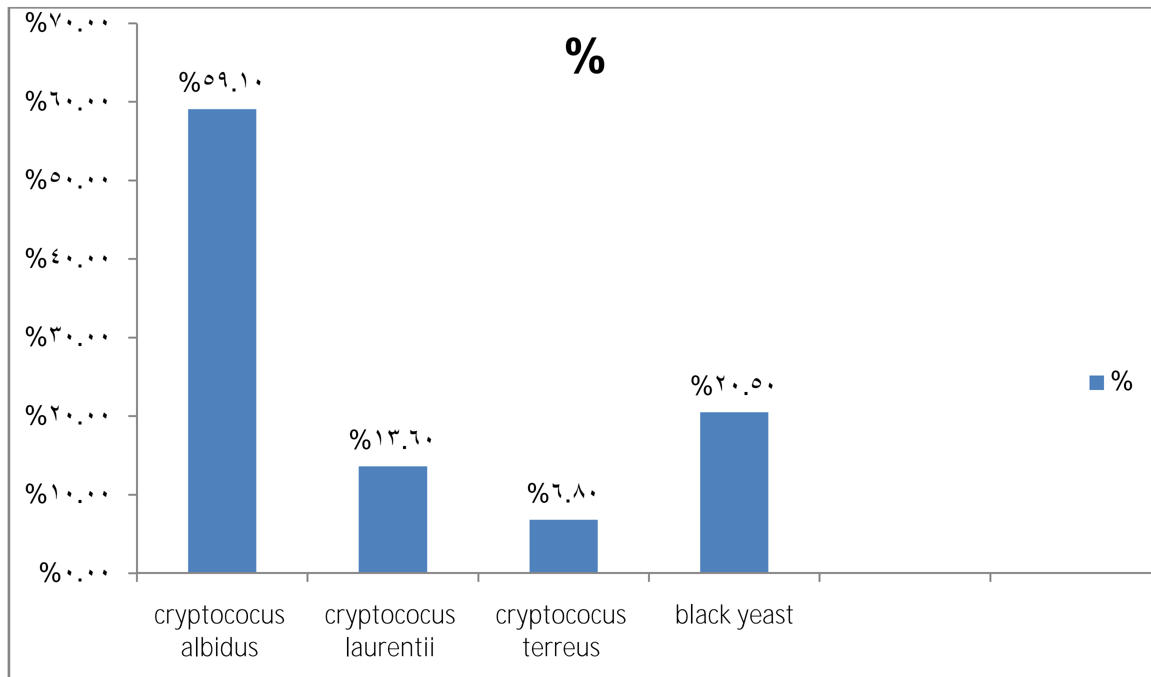

Fig (2) :Frequency of isolation of yeasts from the nasal cavity of apparently normal camels:

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CHAPTER FIVE

DISCUSSION

This study documented the colonization of apparently healthy camels with yeast flora. There have been few studies, and little information about yeast flora in the nasal cavity of camels. Several studies have confirmed the existence of yeast florain thenasal cavity of healthy camelsand other animals (Al-bashan and Al-banki, 1999, Buzina,2003).

Hundred samples of nasal swabs were examined in this study and thirty five obtained in this study were found to belong to the genus *Cryptococcus* and included the species *Cryptococcus albidus*26(59.1%),*Cryptococcus laurentii*6(13.6%)and*Cryptococcus terreus*3(6.8%). Nine (20.5%) of the isolates were unidentified black yeasts.

The present study showed high presentation of *Cryptococcus spp.* isolated from nasal cavity. This result disagrees with the finding of Al-bashanand Al-banki, (1999) who studied the yeasts flora of the nasal cavity of camels, they wereisolate*Candida albicans*.Garelick,(2004) who described *Cryptococcus laurentii* as the most important cause ofhuman and animalpulmonary infection, andNimir, (1980) isolated *Cr.laurentii* and *Cr. albidus* from the nasal turbinates of dogs and cats.

In this study *Cryptococcusterreuswas isolated by*Alangaden*et al,*(1994) isolated the same organismfrom the bone marrow of abnormal pathogenic.

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Hala*et al*.,(2011) isolated *Cryptococcus neoformans* from thenasal cavity of Cattle, buffalo and goats while Van Uden*et al.,*(1958) isolated itfrom the intestine contents of apparently healthyhorses, but in this study we cannot isolate*Cryptococcus neoformans* probably due to different environmental conditions and the endogenous origin of the yeast isolates.

It is known that, yeast flora reach the respiratory tracts of different animal species mainly through inhalation of the contaminated dusts. Also the environmental events act as being specificrisk factors for invasive fungal diseases as a result ofbad ventilationPound,*et al*.,(2002).

In this study the frequency of isolation of isolates brought from Tamboul which were: *Cr. albidus, Cr. Laurentii and Cr. Terreus*were found similar to that isolated from Shambat except *Cr. Terreus* thatcould not be isolated,this may be due to environmentalchanges and extremes of climatic conditions.

CONCLUSION

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This study of swabs from the nasal cavity of apparently healthy camels, contained yeasts of the genus *Cryptococcus*which included three species:*Cr.albidus,Cr.laurentii and Cr.terreus*. Nine of the isolates were unidentified black yeasts.*Cr.albidus*and *Cr.laurentii*are potential pathogens that cause systemic diseases of man and animals whereas,*Cr.terreus*is not known to be a pathogen. The results of conventional method used in this study as same as result confirmed by Vitec2 Compact System.

RECOMMENDATION

More intensive studies using large samples are required to investigate the normal yeast flora of the upper respiratory tract of apparently healthy camels kept in different location and under different environmental conditions.

REFERENCES

Please purchase PDFcamp Printer on http://www.verypdf.com/ to remove this watermark.

Abdelsamed, G. H. (1995).*Yeast Flora in the Digestive Tract of the one Humped Camel*.M.SC Thesis, University of Khartoum.

Ahearn, D. G. (1974). Identification and Ecology of Yeasts of Medical Importance, p.129-146.*In*:Opportunistic Pathogens. E.d. Jame, E., Prier and Herman Frierdman. University Park Press, , London.

Akpan, A. and Morgan, R. (2002). Oral Candidiasis. *J. of Postgrad. med.,* **78** (922): 455–9.

Alangaden, G.; Chandrasekar, P.H.; Bailey, E.; and Khaliq, Y., (1994). Bone Marrow Transplantation: *Bone Marrow Transplant,* **14**: 919-924.

Al-ani, F. K.,(2004). The Respiratory System. *In*:*Camel Management and Diseases,* Ed.1st, 269-270. published by Al-ani.

Al-bashan, M. M. and A l-banki A. I., (1999). A Microbiological Study on the Nasal Cavity Swabs of Apparently Healthy Camels.*J.of Agric. Sc.,* **15**.

Albert, (1991).*Manual of Clinical Microbiology,* 5th ed. American Society for Microbiology, 628**.**

Barrow and Feltham (1993). Manual for the identification of medical bacteria**.** 3edit, published by the press syndicate of the university of Cambridge**.**

Bekele, T., (1999). Studies on the Respiratory Disease ‘Sonbobe’ in Camels in the Eastern Lowlands of Ethiopia. *J.Trop. Anim. Health Prod.* **9** (31): 333-345.

Please purchase PDFcamp Printer on http://www.verypdf.com/ to remove this watermark.

Buzina, W.; Braun, H.; Freudenschuss, K.; Lackner, A.; Habermann, W. and Stammberger, H.,(2003). Fungal Biodiversity as Found in Nasal Mucus. *J. Med. Mycol.,* **41**: 149-61.

Carmo-Sousa and Dol., (1969). Distribution of Yeasts in Nature. *in*: *The Yeasts,* **1**: 101-105. Ed. A.H. Rose and J.S. Harrion. Academic press, London.

Clapper, W. E. and Meade, G. H., (1962). Normal Flora of the Nose, Throat and Lower Intestine of the Dogs, *J. of Bacteriology.,* **85**: 643-648. publication 18 October 1962.

David, H. and Pincus, M.S.,(1978).*Microbial Identification Using The BiomerieuxVitek2 System* , *J. of Clinic. Microbio. and Med. Myco.*

Dwigh, C. Hirsh; James, N. Maclachlan and Richard, L. Walkar, (2004). Respiratory System. Veterinary Microbiology. 2 edit, 488-publishing profissinal2121State Avenue,Ames,Lowa50014,USA.

Ebbens, F.A. and Fokkens, W.J., (2008). The Mold Conundrum in Chronic Rhinosinusitis: Where do we stand today? Curr Allergy Asthma. **8**(2): 93-101.

Fidel, P.L. (2002). Immunity to Candida. *Oral Diseases,* **8**: 69–75.

Frothingham, L.,(1902). A tumer- like lesion in the lung of a horse caused by a *Blastomycosis*(Torula). *J. of med. Res*., **8**: 31- 43.

Garelick, J. M.,(2004). Scleral Ulceration Caused by *Cryptococcus albidus*in a Patient with Acquired Immune Deficiency SyndromeCornea, **23**: 730-1.

Please purchase PDFcamp Printer on http://www.verypdf.com/ to remove this watermark.

Gerald collec, J.; Barrie, P. Marmion; Anthony simmons and Andrew, G.Fraser,(1996). *Practical Medical Microbiology,*ed.14.

Guilliermond, A. (1912). Les Levures. in: EncyclopedieScientifique. Ed.: A.A. Toulousse. OetaveDoin, Paris.

Hala, A. Saleh; Amgad, A. Moawad; Mohmoud el- Hariri and Mohamed K. Refai, (2011). Prevalence of Yeasts in Human, Animals and Soil Sample at ElfayoumGovernaarate in Egypt. *International J. of Microbio. Res.,* **2**(3): 233-239.IDOSI Publications, 2011.

Harrison, F. C., (1928). A systemic Study of Some Torulae. Proc. and Trans. Roy. Soc., Canada, **22**: 187- 225.

Hernande, C.R.; Martinez, C.H.L.; Daz, A.A.; Romero, O.A.; Godinez, R.C.; Zavala, G.A. and Verdugo, R.A., (2004). Aerobic Bacterial Flora of the Nasal Cavity in Gulf of California Sea Lion. *J. of Vet.*, **170**: 359-63.

Ingraham, J. L. and Ingraham, C. A.(2000). Introduction to Microbiology. 2nd. ed. Ed. Broks /Cole.

Jorgensen, A.,(1886). In :Microorganisms and Fermentation. Translated and edited by Hansen, A., Grffin and Co. Ltd. London.

Kreger-van Rij NJW.,(1984).*The yeasts a taxonomic study,* 3rd. ed. Elsevier Science Publishers B.V. Amsterdam.

Lodder ,J., (1970). *The Yeasts*. A taxonomic Study .Editor :Lodder,J.2nd Ed., North-Holland Publishing Co., Amsterdam ,Oxford.

Lodder, J.,(1974).*The Yeasts*, Ataxonomic study 3nd.ed. North Holland Publishing Company, Netherlands.

Please purchase PDFcamp Printer on http://www.verypdf.com/ to remove this watermark.

Meyer, K. F., (1913). A pathogenic Blatomyces from the horse. Pronc. Path. Soc. Philad., **16**: 28.

Nimir , A. H., (1980).*Studies on Certain Yeast Associated with Animals*. Ph.D. Thesis, University of Glasgow ,United Kingdom.

Olivia Labrecque; Doris Sylvestre and Serge Messier, (2005). Systemic *Cryptococcus albidus*Infection in a Doberman Pinscher. *J. of Vet. Diagn. Invest.*,**17**: 598-600.Published by:http://www.sagepublications.com.

Parle, J. N. (1957). Yeast Isolated from the Mammalian Alimentary Tract. *J. gen. microbiol.*, **17**: 363- 367.

Pasteur, L.(1876). Etudes sur la Biere. Gauthier-villars, Paris.

Phaff, H. J. and Fell, J. W., (1974). *Cryptococcus*Kutizing Emend. Phaff and Spencer. 1088-1145, *in*: *The Yeasts*: Ataxonomic study; 2nd ed., 3rd printing; ed.: J. Lodder; North- Holland publishing Co., Amsterdam.

pirone, A.; Castagnetti, C.; Mariella, J.; Bonoli, C.; Stancampiano,L; Tempieri, M.P. and Galuppi, R.,(2011). Yeast Flora in Oropharyngeal and Rectal Mucous Membranes of Healthy and Critically ill Neonatal Foals. *J.ofequi. vet. sc.*

Postgate and John,(1994). The Outer Reaches of Life, 132-134. Cambridge University Press.

Pound, M.W.; Drew, R.H. and Perfect, J.R., (2002). Recent Advances in the Epidemiology, Prevention, Diagnosis and Treatment of Fungal Pneumonia. *J. of Curr. Opin. Infect. Dis.,* **15**: 183-194.

Please purchase PDFcamp Printer on http://www.verypdf.com/ to remove this watermark.

Rodríguez, J.M; Mendoza, M.H.; Ramírez, E.A. and Roca,G.S.,(2006). Cryptococcosis by *Cryptococcus gattii*in immunocompetent goats in Spain and review of the literature, *J. Acta Scientiae Veterinariae*, **34**(3): 245-253.Pub. 683.

Rose, A.H. and Harrison, J. S., (1987-1993).*The Yeasts*: Academic Press, London, **1-5**.

Saez, H. and Rinjard, J., (1973): *Trichosporuncapitatum*A member of the Fungal Flora of the Digestive Tract of Certain Suidae. *Annls. J. of Med. Vet.*, **117**: 177-182.

Saito, K. (1922). Untersuchungenuber die atmospharischenpilzkeime. Jap. *Bot*., **1**: 1- 54.

Schiefer, H.G., (1997). "Mycoses of the urogenital tract". *Mycoses,***40** (Suppl 2): 33–6.

Shokri, H.; Khosravi, A. ;Sharifzadeh, A.and Tootian, Z. (2010). Isolation and Identification of Yeast Flora from Genital Tract in Healthy Female Camels, *J. of Vet. Microbio.*, **144**: 183-186.

Sneller, M. R. and Swatek, F. W., (1974). Distribution of the Genus *Cryptococcus* in Southern California Soils, **12**: 46- 53.

Sorum, H. and Sunde, M., (2001). Resistance to Antibiotics in the Normal Flora of Animals. *J. of Vet. Res*., **32**: 227–241.

Spencer, J. F. T. and Spencr, D. M.,(1997). Yeasts in Natural and Artificial Habitats. Springer-Verlag Berlin Heidelberg, 381.

Sridhar Rao, P. N.,(2009). www.microrao.com.

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