ISOLATION, IDENTIFICATION AND IMMUNOLOGICAL STUDY OF YEAST FROM CATTLE AND BUFFALOES IN BASRAH PROVINCE, IRAQ

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Abstract

Two hundred samples taken from cattle and buffaloes (50 samples from each urine, blood, tracheal and vaginal swabs) were collected. The study showed that *C.albicans* was the most common isolates from the total samples. In cattle, it was isolated in a percentage of 50% out of 52 samples, followed by *C.krusei* 19%, *C.parapsilosis* 11.5%, C.tropicalis 10%, and *C.rugosa* 1.9%, on the other hand other isolated yeasts were *Cryptococcus neoformans* 3.8%,*Geotricum candidum* 1.9%, and *Rhodotorula mucilaginosa* 1.9%. In buffaloes *C.albicans* was also found as a predominant isolates. The total isolates of *C.albicans* was 43.9% out of 41 samples, followed by, *C.tropicalis* 22%, *C.krusei* 19.5%, *C.parapsilosis* 4.9% and *C.rugosa* 2.4%. Other yeasts were also identified such as *Geotrichum candidum* 4.9% and *Trichosporom cutaneum* 2.4%. *C.rugosa* was firstly isolated from urine samples taken from cattle and buffaloes and considered as newly recorded species from Iraq. Statistical analysis showed significant difference ($P<0.01$) between *C.albicans* isolation comparable with other yeasts in cattle and buffaloes. Using Indirect Immunofluorescent antibody technique, the causative agent can be detected after 12 and 20 days after experimental infection of mice with *C.albicans*. The result of using disc diffusion method for seven antifungal drugs showed significant differences $P<0.01$ and $P<0.05$ on susceptibility of the tested isolates toward antifungal drugs.

عزل وتشخيص ودراسة مناعية للخمائر المعزولة من الأبقار والجاموس في محافظة البصرة –العراق

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الخلاصة

تم جمع ٢٠٠ عينة من الابقار والجاموس تمثل ٥٠ عينة من كل من البول والدم ومسحات القصبه الهوائية والمسحات المهبلية. أشارت النتائج الى ان خمائر C. albicans كانت الأكثر شيوعا وقد بلغت نسبة ظهورها في عينات الأبقار ٥٠% من ٥٢ عينه تتبعها الخمائر C. krusei ١٩% و C.parapsilosis ١١.٥% و C.tropicalis ١٠% و C.rugosa ١.٩%. أما الخمائر الاخرى من غير جنس الـ Candida فقد كانت نسب ظهورها كما يلي: Cryptococcus neoformans ٣.٨% و Geotricum candidum ١.٩% و Rhodotorula mucilaginosa ١.٩%. أما في العينات المأخوذه من الجاموس فقد كانت C.albicans هي السائدة أيضا وكانت نسبة ظهورها ٤٣.٩% من أصل ٤١ عينة تتبعها خمائر

C.tropicali ٢٢% و C.krusei ١٩.٥% و C.parapsilosis ٤.٩% و C.rugosa ٢.٤%. كما تم عزل خمائر أخرى أيضا مثل Geotrichum candidum ٤.٩% و Trichosporom cutaneum ٢.٤%. إن عزل خميرة C.rugosa من عينات البول في الابقار والجاموس يسجل لأول مرة في العراق بحسب الدراسات المتوفرة. لقد أشارت النتائج الأحصائية الى وجود اختلافات معنويه لعزل خميرة C.albicans بالمقارنة مع الخمائر الأخرى في الأبقار والجاموس. وباستعمال تقنية التألق المناعي فأنه تم تحديد العامل المسبب للاصابة صاحبه معيار عالي من الأجسام المضادة وخصوصا بعد اليوم الثاني عشر والعشرون من الصابة التجريبية للفئران المختبرية بخمائر C.albicans. وأشارت نتائج استخدام الأقراص لسبعة من المضادات الفطرية الى وجود اختلافات معنوية لحساسية الخمائر تجاه المضادات الحيوية

Introduction

Candidiasis is a primary or secondary mycotic infection caused by members of the genus *Candida* .Chiefly *Candida albicans* is responsible for about 70 to 80% of all *Candida* infection .The *Candida* as opportunistic yeast pathogen which increases predominantly in patients with predisposing conditions. Animals candidiasis is common and it causes economic loss and it is considered as one of the causes of dairy cattle abortion that leads to low quality and production of milk due to mastitis as a result of *Candida* infection [1, 2].

The *Candida* as an opportunistic yeast pathogen which increase predominantly in patients with predisposing condition, including immunodeficiencies such as hepatic infection virus HIV, AIDS, Prolong use of broad-spectrum antibiotics, corticosteroids –ect. [3, 4]. Antigenic variation on the surface of *Candida* cells may help the organism to invade host defenses, furthermore, mannan is one of the cell wall components of *Candida albicans* which had been shown to suppress cellular immunity [5].

Candidiasis is commonly treated with antimycotics, the antifungal drugs used to treat candidiasis are topical clotrimazole, topical nystatin, fluconazole and ketoconazole, and other type of yeast infection may require different treatment like Amphotericin B, caspofungin, variconazole, may be used [6]. The use of nystatin in mucocutaneous candidiasis and local infection has been shown to decrease the incidence of systemic disease [7]. The ketoconazole, fluconazole, itraconazole and voriconazole within azole antifungal agent used for systemic mycoses. Ketoconazole is active against *Candida* spp .and *Cryptococcus neoformans* but due to its limited penetration to cerebrospinal fluid, it is clinically ineffective in meningeal cryptococcosis [6]. Fluconazole a triazole that inhibits fungal ergosterol synthesis has excellent absorption and cerebrospinal fluid (CSF) penetration and is safe .The effective dose is 400 mg /day. Fluconazole is generally considered to be a fungistatic agent [8]. Itraconazole and fluconazole are used for most systemic mycoses including aspergillosis and candidiasis [4].

Materials and Methods

Samples Collection

A total of 400 animals samples, 200 from cattle and 200 from buffaloes were collected. The samples were distributed as 25 male and 25 female for urine, blood and tracheal samples. The vaginal samples were collected from 50 cattle and 50 buffaloes. All samples were collected from the government slaughter house in Basrah province during the period from October 2008 up to May 2009.

Urine samples were directly collected after slaughtering of animal from urinary bladder by sterile syringe. About 10 ml were dispensed into sterile test tube and transmitted to the laboratory for diagnosis [9, 10].

Five milliliter of blood samples were directly collected after the slaughter of animal from jugular veins by sterile tube with anticoagulant, and transmitted to the laboratory for diagnosis. Blood were concentrated by centrifuge and inoculated into tubes containing brain heart infusion medium. The tubes incubated at 37^{o}C for 1-2 days. 0.5 ml of the cultures were evenly spread onto the surface of Sabouraud dextrose agar containing chlormphenicol [9, 10].

The tracheal swabs were collected by sterile cotton swab after slaughter of animal and empted of viscera, incision made was in the lower part of tracheal and the sterile cotton swab was entered in the lumen of trachea. These swabs were transferred to the laboratory for diagnosis after adding few drops of sterile distilled water.

Vaginal samples were collected by sterile cotton swab from vagina, and then transferred to the laboratory for diagnosis after adding few drops of sterile distilled water in the laboratory.

Identification

The identification of yeasts depended on morphological characteristics, germ tube production, palman plate technique, surface growth, urease test, fermentation, assimilation test and Api 20C AUX diagnostic strips [10, 11].

Yeast Inocula

The local isolates of *C.albicans* from buffaloes blood sample were grown on the Sabouraud dextrose agar for 48 hours at 30°C followed by aseptically sub culturing of a heavy inoculum to tubes containing 10 ml Sabouraud dextrose broth, incubated for 24 hours at 30°C.Then it concentrated by centrifugation at 3000 rpm for 15 minutes. The yeast cells were washed 3 times with sterilized normal saline for 5 minutes neglecting the floating fluid each time, and diluted with sterilized normal saline to form 1:100 which were shaken smoothly. The cells were counted by using a hemocytometer and re-adjusted to 1×10^6 cell /ml, simultaneously. Plating the inoculums on SDA was conducted to assure inocula viability [12, 13].

Experimental infection

Expermental animals (Balb/c mice) were divided into four groups include eight equal individual group. Eight males and eight females were inoculated Intra-peritoneum (I/P) with 0.2 ml from 1×10^6cell /animal using a thin small gauche syringe, other eight males and eight females inoculated Intra-peritoneum (I/P) with normal saline as a control groups. Two animals from each group were killed every 4 days for 3 period. The fourth period was extende to 20 days [12].

Indirect immunoflurescence antibody test (IFAT)

Sera were collected from 4 infected mice directly after killing, and from 4 mice of control group. The blood were collected from their heart puncture, and centrifuged at 3000 rpm. for 15 minutes, sera were stored at -20 °C until use. For preparation of *C.albicans* whole antigen, *C.albicans* were harvested from SDA and added to 10 ml SDB in a test tube, incubated at 30°C for 24 hr. then it concentrated by centrifugation at 3000 rpm for 15 minutes, the yeast cells were washed 3 times with PBS for 5 minutes with neglecting of the floating fluid each time and diluted 1:100 by PBS solution [12]. Serum samples were treated with rabbit anti-mouse IgG Kit as the following steps listed by manufacturer's.

1. Dilutions of serum samples (1\2, 1\4, 1\8, 1\16, 1\32, 1\64, 1\128) were made by blocking solution.
2. 25 µl of antigen were added to each well of slide, (each slide contain 8 wells).
3. Slides were incubated in a humid chamber for 30 minutes at 37°C.
4. Slide were washed with PBS and dipped briefly in distilled water.
5. Slides were allowed to air dry.
6. Then 25 µl of serum dilutions were added to each well (serum from treatment and control group up to last well .
7. Step 4, 5, 6. were repeated.
8. 25µl of rabbit anti-mouse IgG was added to each well include control well also.
9. Step 4, 5, 6. were repeated.
10. Small drops of glycerol were added to each well and carefully cover with a cover slip.
11. Slides were examined as soon as possible in a fluorescence microscope at absorption wavelength 495 nm, emission wavelength 528 nm.

Antifungal Agents:

The following antifungal drugs were used Amphotericin B, nystatin, clotrimazole, itraconazole, ketoconazole, fluconazole, and miconazole. Each antifungal agent was prepared at initial concentration (Working stock drug solution) of 10,000 µg/ ml by dissolving 50 mg of each drug in 5 ml of dimethyl sulphoxide (DMSO) in clean sterile screw – capped glass vials. [14].

Batches of 100 small discs of absorbent paper (about 6.5 mm diameter) were dispensed in screw capped vials and sterilized by autoclaving and later were left in the electric oven for 50 minutes at 150 °C to be dried. A concentration of the 1000 µg / ml was prepared from the original stock solution (10,000 µg / ml). One ml of the 1000 µg / ml was added to each vial which containing 100 discs to obtain a concentration of 10 µg / disc. These discs were stored in wet condition in screw capped vials tightly screwed and kept at -20 °C until used [14, 15, 16].

Yeast inocula suspensions (0.2 ml), were evenly spread with sterilized L-shaped spreader on the surface of the Emmon's modified Sabouraud's dextrose agar (ESDA) plates. The inverted inoculated plates were left on the bench for 1 hour. The antifungal discs (10 µg / disc) were placed on the surface of the medium. The plates were left in the refrigerator for 1-3 hours prediffusion. At the same time growth controls without antifungal discs of all strains on ESDA were prepared. The inoculated plates incubated at 30 ^{o}C and the assay was recorded after 24-48 hours of incubation. The zones of inhibition of the growth were expressed as clear zones around the antifungal discs in millimeters. Duplicate plates were used [13, 14, 15].

Results

Candida albicans was the most common been isolated from animal samples including urine, blood, tracheal and vaginal swabs. In cattle *C. albicans* represent 26(50%) out of 52 samples, followed by *C.krusei* 10(19 %), *C.parapsilosis* 6(11.5%), *C.tropicalis* 5(10%), and *C.rugosa* 1(1.9%). Other yeast had been isolated was *Geotricum candidum* 1(1.9%), *Rhodotorula mucilaginosa* 1(1.9%) and *Cryptococcus neoformans* 2(3.8%) (Table 1).

Table 1: Number and percentage of *Candida* species and other yeasts isolates from cattle

species	Urine samples	Blood samples	Tracheal swabs	Vaginal swabs	Total positive	% Total positive
C . albicans	3	6	8	9	26 a	50%
C. krusei	2	2	4	2	10 b	19%
C. parapsilosis	1	1	1	3	6 bc	11.5%
C. tropicals	2	1	1	1	5 bc	10%
C.rugosa	1	0	0	0	1 c	1.9%
G.candidum	0	0	1	0	1 c	1.9%
R.mucilaginosa	0	0	1	0	1 c	1.9%
C.neoformans	0	0	1	1	2 c	3.8%
Total isolation	9	10	17	16	52	100%
% Total positive	17.3	19.2	32.7	30.8		

RLSD=0.1369 P<0.01

In buffaloes *C.albicans* also the most common isolate. *C.albicans* were represent 18(43.9%) out of 41 samples, followed by, *C.tropicalis* 9(22%), *C.krusei* 8(19.5), *C.parapsilosis* 2(4.9%) and *C.rugosa* 1(2.4%). *Trichosporom cutaneum* had also been isolated which represent 1(2.4%) and *Geotricum candidum* 2(4.9%), (Table 2).

Table 2: Number and percentage *of Candida* species and other yeasts isolates from buffaloes.

Species	Urine samples	Blood samples	Tracheal swabs	Vaginal swabs	Total positive	%Total positive
C. albicans	4	3	3	8	18 a	43.9%
C.tropicals	6	0	1	2	9 b	22 %
C.krusei	5	1	1	1	8 bc	19.5 %
C. parapsilosis	1	0	1	0	2 bc	4.9 %
C.rugosa	1	0	0	0	1c	2.4 %
T. cutaneum	0	0	0	1	1c	2.4 %
G . candidum	0	0	2	0	2bc	4.9 %
Total isolation	17	4	8	12	41	100 %
%Total positive	41.5	9.8	19.5	29.2		

RLSD=0.1710 P<0.01

Candida albicans was predominant isolate in cattle and buffaloes from vaginal swabs, tracheal swabs, blood and urine respectively. The statistical analysis showed significant differences (P<0.01) in the frequency of *C.albicans* comparable with other yeasts in both cattle and buffaloes.

Candida rugosa was isolated from urine samples of cattle and buffaloes which regarded the new record of this isolate from animals in Iraq. Whereas *T.cutaneum* only from vaginal samples of buffaloes, while *R.mucilaginosa*, and *C.neoformans* were isolated from cattle only.

Sera were collected from 16 mice after 4 days of intraperitoneal injection with 1×10^{6} cells\ mice of *C.albicans* were used to test their antibody by IFAT. The yeast (*C.albicans*) was detected by immunofluorecent microscope (Table 3, Figure 1).

Table 3: Results of immunofluorescent examination of 16 sera with *C.albicans*

Days	No of animals	IgG Antibody Titer	Evaluation
4 day	4	0	Negative
8 day	4	2	Weak
12 day	4	32	Moderate
20 day	4	64	Strong
Total animals used= 32	Total infected Mice=16		
	Control =16	0	Negative

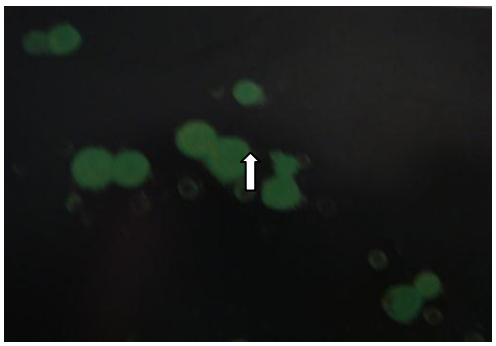

Figure 1: positive result of Immunoflourescenc antibody against *Candida albicans*

Using disc diffusion method 60 isolates from different species of yeasts were tested for their antifungal susceptibility toward antifungal drugs (Table 4 and 5).

Table (4): *In vitro* antifungal susceptibility test for *C.albicans*

animals	No *C.albicans*	samples	No isolates	Inhibition zones in millimeters						
				A	N	C	I	K	F	M
Cattle	1	urine	1	10	20	13.5	18	32	33	21
	2		6	12	18.5	15	17	30	30	20
	3	blood	2	9	20	15	16	30	32	20
	4		5	10	21	15	17.5	31	33	20
	5		10	10	20	16	17	33	30	19
	6	tracheal	2	11	21	15	18	28	31	19
	7		7	10.5	18	15.5	17	30	30.5	18
	8		15	8	20	15.5	16	31	32	20
	9		18	10	21	15	17	27	31	22
	10	vaginal	2	10	17	15	18	33	30	20
	11		5	10	22	16	17	30	32	21
	12		9	11	20	14.5	17.5	30	30	20.5
	13		12	9	20	13	18	28	30.5	20.5
	14		16	10	23	15.5	17	32	30	20
Buffaloes	15	urine	14	10	21	15.5	17	28	33	19
	16		17	11	19.5	17	18	30	15	18
	17	blood	2	9	22	15	15	27	32	19.5
	18		4	9	18.5	15	17	32	31	17
	19	tracheal	8	10.5	20	14.5	17	30	30.5	22
	20		1	11	19.5	15.5	20	31	33	19
	21	vaginal	4	9.5	20	15	17	30	33	17
	22		4	10	20	15.5	16	30	32	20
	23		6	11	22	16	15.5	26	31	16
	24		8	10.5	19	13.5	16	32	15	20
Total mean				10	20.1	15	17	30	30	19.5
X^2= 16.51				P<0.01						

Table 5: *In vitro* antifungal susceptibility test for yeasts isolates (other *Candida* spp. and other yeasts).

animals	No *C.krusei*	samples	No isolates	Inhibition zones in millimeters						
				A	N	C	I	K	F	M
Cattle	25	urine	7	11	12	9	4	15	16	10.5
	26	blood	2	10.5	11.5	10	4	15	15	11
	27	tracheal	4	11.5	12	10	5	14	15	10
	28		9	12	11	10	3.5	15	14	9.5
	29		1	11	12.5	9	4	16	15	9
	30	vaginal	1	12	10.5	11	4	15	15.5	11
buffaloes	31	urine	2	10	13.5	10.5	7	14	15	11
	32		5	11	12	11	4.5	15	14.5	10
	33		13	11	12	10	4.5	15	15	9
	34	blood	1	10	12	9	4	16	15	10
	35	tracheal	5	10	12	9.5	4	14.5	16	10
	36	vaginal	9	12	13	11	6	16.5	14	9
Total mean				11	12	10	4.5	15	15	10
X^2= 6.97				P<0.05						
C.tropicals				**A**	**N**	**C**	**I**	**K**	**F**	**M**
Cattle	37	urine	5	14	13.5	15	0	14	0	9
	38	blood	6	15	15	15	0	14	0	10
	39	tracheal	10	15	14	14.5	0	15	0	10
	40	vaginal	12	16	15	15	0	14	0	9
buffaloes	41	urine	3	15	15	15	0	13	0	10
	42		6	14	15.5	15	0	14	0	10
	43	tracheal	1	15	16	15	0	15	0	11
	44	vaginal	12	16	16	15.5	0	13	0	11
Total mean				15	15	15	0	14	0	10
X^2= 29.51				P<0.01						
C.parapsilosis				**A**	**N**	**C**	**I**	**K**	**F**	**M**
Cattle	45	urine	4	12	14	15	20	34	24	18
	46	blood	1	13	15	15	19	35.5	25	18
	47	tracheal	5	12.5	14	15	20	35	24.5	19
	48	vaginal	6	12	14	16	21	33.5	25	17
	49		8	12	14	14	21	36	25	19
buffaloes	50	urine	10	13	15	15	19	36	26	17
	51	tracheal	3	13	12	15	20	35	25.5	18
Total mean				12.5	14	15	20	35	25	18
X^2= 18.63				P<0.01						
NO *C.rugosa*		**samples**	**No isolates**	**Inhibition zones in millimeters**						
				A	**N**	**C**	**I**	**K**	**F**	**M**
cattle	52	urine	1	10	14.5	17	16	32	27	15
buffaloes	53	urine	2	10	15.5	17	17	32	28	15
Total mean				10	15	17	16.5	32	17.5	16.5
X^2= 15.43				P<0.01						
T.cutaneum				**A**	**N**	**C**	**I**	**K**	**F**	**M**
buffaloes	54	vaginal	10	10	15	10	15	25	0	15
Total mean				10	15	10	15	25	0	15
X^2= 26.67				P<0.01						
R.mucilogonsa				**A**	**N**	**C**	**I**	**K**	**F**	**M**
cattle	55	tracheal	14	10	17	8	0	32	0	10
Total mean				10	17	8	0	32	0	10
X^2= 66.36				P<0.01						
G.candidium				**A**	**N**	**C**	**I**	**K**	**F**	**M**
Cattle	56	tracheal	13	10	14	19	19.5	29	27	19
Buffaloes	57	tracheal	2	9.5	13	19	18	29	27	18
	58		4	10.5	15	19	18	30.5	27	19
Total mean				10	14	19	18.5	29.5	27	19
X^2= 14.22				P<0.01						
C.neoformans				A	N	C	I	K	F	M
cattle	59	tracheal	8	12	18	13	19	32.5	33	23
	60	vaginal	14	12	18	12	17	32.5	33	21
Total mean				12	18	12.5	18	32.5	33	22
X^2= 21.21				P<0.01						

A=Amphotericin-B, N=Nystatin, C=Clotrimazole, I=Itraconazole, K=Ketocnazole, F=Fluconazole, M=Miconazole

The results obtained in this study (total mean values) revealed that ketoconazole, nystatine, miconazole, and amphotericine B inhibit all yeasts isolates, while clotrimazole gave lower inhibition zones against *R.mucilagiosa*. Itraconazole and fluconazole resisted by *C.tropicalis* and *R.mucilaginosa*, while *T.cutaneum* isolates were able to resist fluconazole only. The results of statistical analysis showed significant differences ($P < 0.01$ and $P < 0.05$) on susceptibility of the tested isolates to antifungal drugs.

Discussions

Candida albicans was the predominant from total samples (urine, blood, tracheal swabs, and vaginal swabs). In cattle it represented 26(50%) out of 52 samples, followed by *C.krusei* 10(19 %), *C.parapsilosis* 6(11.5%), *C.tropicalis* 5(10%), and *C.rugosa* 1(1.9%). Other yeasts were also found these were *Geotricum candidum* 1(1.9%), *Rhodotorula mucilaginosa* 1(1.9%) and *Cryptococcus neoformans* 2(3.8%) were also been isolated.

In buffaloes *C.albicans* was also found as the most common isolate from samples. The total isolates of *C.albicans* 18(43.9%) out of 41 samples, followed by, *C.tropicalis* 9(22%), *C.krusei* 8(19.5), *C.parapsilosis* 2(4.9%) and *C.rugosa* 1(2.4%). Other yeasts were also identified these were *Trichosporon cutaneum* 1(2.4%) and *Geotricum candidum* 2 (4.9%). The results of statistical analysis showed significant differences ($P<0.01$) in isolation of *Candida albicans* comparion with other yeasts in cattle and buffaloes.

The current study is inconsistent with Samaka [17], who isolated some fungi from cattle and sheep in Baghdad city. He found *C.albicans* in cattle at a percentage of 48.1%, while *C.krusei*, 37%, *C.parapsilosis*, 14.8%, but he couldn't isolate *C.tropicals* and *C.rugosa* from any samples. The results approximately similar in relation with the isolation of *G.candidum*, *C. neoformanse* and *Rhodotorulla*, which who isolated in percentage 2.9% for each one from tracheal swabs.

Al-Maadidhi [18] through studies of some *Candida* type infection of reproductive system in ewes vaginal swabs, found three species of *Candida, C.albicans, C.krusei,* and *C.tropicals* at the percentage of 33.33%, 20.83 % and 29.16% respectively, and showed *C.albicans* more common isolate from vaginal swabs. This result in line with our result about *C.albicans* which was predominant isolation from vaginal in cattle and buffaloes.

Candida albicans is the species most frequently isolated as colonize superficial and invasive infection agent at different anatomical sites all over the world. It has a well known pathogenic potential and its main pathogenicity and virulence factors, are capacity to adhere to different mucosa and epithelia, dimorphism, with production of pseudohyphae helping tissue invasion, thermotolerance, and exoenzymes like proteinase and phospholipase and germ tube formation with consequent development of the filamentous form [11]. The mannan (glycoprotein present on the cell surface of *C.albicans*), adhesion responsible for the attachment of *C.albicans* to host cells more strong than *C.tropicalis* and *C.prapsilosis* (19).

In Brazil, Cleffi [20], were isolated of *Candida* species from vaginal healthy canine females during estrous cycle. He found the percentage of isolation of *Candida* species (37%) out of total samples, he found six species of *Candida: C.albicans, C.krusei C.parapsilosis, C.guilleromondii, C.kefyr, and C.glabrata*. The physiologic changes like estrous cycle and pregnancy were also considered predisposing factor for *Candida* spp. proliferation [21]. The high estrogen level in proestrus can account for *Candida* isolation in this phase fungi development is enhanced by this hormone which increases glycoproteic epithelial complex exposition acting as receptors for fungal agent [20].

Other study which consistent with our result indicate that *Candida* species where isolated from urinary tract infection of dog and cat when examination thirty –five animals (23 dogs, 12 cats) found seven species of yeast identified in the affected animals, *C.albicans* was the most common isolate [22].

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