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Molecular Detection of *Candida tropicalis* Isolated from Buffalo's Genitalia in Wasit Province

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CANDIDA tropicalis produces biofilms and other virulence factors like attachment to endothelial and buccal epithelial cell and release of lytic enzymes such as hemolysins, phospholipases, proteinases, and transformation from buds to hyphae (also known as morphogenesis). The aim of this study was to identify *Candida tropicalis* using API 20 Candida as well as using conventional polymers chain reaction technique for the detection of the genotype and virulence in isolates causing mycotic vaginitis in buffaloes in Wasit Province during the period (May 2021 to October 2021). In this study, one hundred vaginal swab samples were collected from buffalo. The results showed that Candida species were found in 40 out of 100 (40%) of the mycotic vaginal swabs and 30/40 (75%) of the isolated *Candida species* showed positive results using API 20 Candida test for *Candida tropicalis*. PCR amplification of the 18S rRNA gene for identification of *Candida tropicalis* revealed that this gene was detected in 16/30 samples (53.3%). Phospholipase (*PLB*) gene and hyphal formation (*CPH1*) gene were both found in 10/16 (62.5%) and 8/16 (50%) of samples respectively. In conclusion, the PCR was a useful tool and excellent alternative method for the early detection and diagnosis of the most frequent Candida species causing infection of genital tract in buffalo where culture was not required.

Keywords: *Candida tropicalis*, Bovine vaginitis, Conventional PCR, API 20C test.

Introduction

Candida usually resides on the pores and skin and inside the physique (in mouth, throat, stomach, and vagina) and causes candidiasis [1]. Animal's reproductive failure could be attributed to fungal infections or synthesis of poisonous compounds [2]. Pneumovagina, continual endometritis and recurrent intrauterine antibiotic therapy are predisposing factors that assist fungus to invade the uterus. The special situation that permit the fungus to colonize the uterus is unknown [3].

Fungal endometritis has no longer been linked to mating with diseased stallions, despite the fact that fungal isolates have been cultivated from the urethra and semen of these animals, even extra pathological genital tract complications including metritis, vulvo-vaginitis, and cervicitis have been linked to fungi [2-4]. In terms of epidemiology and pathogenicity, *Candida tropicalis* appears to be one of the important Candida species. Real hyphae are successfully produced, which is unique to *Candida albicans* and its related species *Candida dubliniensis*. Moreover, *C. tropicalis* is a species that produces a lot of biofilm and is unexpectedly adherent to endothelial and epithelial surfaces [5]. Furthermore, a wide variety of current research have documented the recovery of *C. tropicalis* resistance to presently

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available antifungal drugs, such as amphotericin B, azoles derivatives, echinocandins as first-line therapy, with extended-spectrum triazoles as applicable options [6,7]. As an osmotolerant microbe, *C. tropicalis* may also additionally be essential for fungal persistence in saline settings, inclusive of the development of virulence factors in vitro and resistance to antifungal medicines [8]. Species of Candida identification using traditional morphology and assimilation examinations can take three to five days or perhaps longer for more challenging or rare species [9]. Detection of candidemia was done by PCR method using species-specific probes targeted to the ITS2 region of the rRNA-encoding gene, and multicopy gene targets (rDNA) [10, 11]. Phospholipase C (PLC) enzymes are important in regulating a variety of essential cellular elements in eukaryotes such as yeasts [12]. These enzymes are divided into phospholipase A, B, C, and D [13,14]. The phospholipases B, C, and D genes have been located. With both hydrolase (releasing fatty acids) and lysophospholipase-transacylase activity, phospholipase B is the essential activity of phospholipase in Candida species [15].

The objective of the study was to isolate and identify *Candida tropicalis* which lead to vaginitis in buffalo by using of standard molecular strategies and detection of some virulence genes.

Material and Methods

Samples Collection

A total of 100 vaginal swap from buffalo suffering from vaginitis during the period (May 2021 to October 2021) in Wasit province. The samples were cultured on Sabouraud's dextrose agar supplemented with 0.05 mg/ml chloramphenicol and incubated at 37˚C for 24 hours to one week, *Candida tropicalis* was conducted in order to determine the genus and species level by molecular identification.

Molecular Identification

DNA extraction

Yeast genomic DNA was isolated from *Candida tropicalis* using the G-spin DNA extraction kit in accordance with the manufacturer's instructions [22]. PCR primers (forward and reversal) were constructed using the NCBI-Genbank database, 18S rRNA, two significant virulent genes were investigated in this study, which include *C. tropicalis* phospholipase *PLB* gene and hyphal formation *CPH1* gene Table (1).

PCR Amplification

Utilizing PCR master mix reaction training (Maxime PCR premix kit i-Taq protocol).The master mix was once made in accordance with the manufacturer›s guidelines (Table 2). PCR results were processed for 1 hour at 100 volts on an agarose gel electrophoresis, and the DNA bands were detected using a gel documentation system.

Results

About 100 vaginal samples analyzed from the buffalo with vaginitis, *Candida tropicalis* based on cultural (Sabouraud dextrose agar) morphological aspects, API 20 Candida identified and conventional PCR.

The presence of 18S rRNA gene in 16/30 (53.3%) Yeast isolates of *C. tropicalis* with a PCR product size of 542 bp. DNA concentration ranged between 5.9 and 70.2 ng/ml using a Nano-Drop spectrophotometer, with a purity of (1.8 – 2.08) Fig.illustrates (1).

TABLE 1: Primers for the amplification of the 18S rRNA, PLB, and CPH1 genes in Candida tropicalis.

Gene (systematic name) primer		Sequence (3´-5´)	Product Size (bp)
18S rRNA gene	F	TCTGACGTGCTGGGGATAGA	542
	R	TGGAATACCAAAGGGCGCAA	
Phospholipase *PLB* gene	F	GGTGGGTCATGGTTAGTGGG	247
	R	CGGCCATCATTTTAGCAGCC	
Hyphal formation *CPH1* gene	F	ACTGCTCCAGCTAATTGGCA	528
	R	ACCAAGACCAACAGCAGCAT	

TABLE 2. Setting PCR program for *Candida tropicalis*.

Step	Temperature			Time	Cycles
	18S rRNA	*Phospholipase PLB gene*	*Hyphal formation CPH1 gene*		
Initial denaturation	95°C	95°C	95°C	2 min	
Denaturation	95°C	95°C	95°C	30 sec	Repeat steps 2-4 for 29 more times
Annealing	58.3°C	60.3°C	57.2°C	30 sec	
Extension	72°C	72°C	72°C	50-60 sec	
Final Extension	72°C			5 min	
Hold	4° C			10 min	

Table 3. Comparative results of *Candida tropicals* isolated from buffalo gentile with vaginitis between sabouraued dextrose agar, API 20 Candida and conventional PCR.

No.	Isolates species	No isolates	Sabouraued dextrose agar		API 20 C		Conventional PCR	
			No	%	No	%	No	%
1	*Candida tropicalis*	100	40	40%	30/40	75%	16/30	53.3%

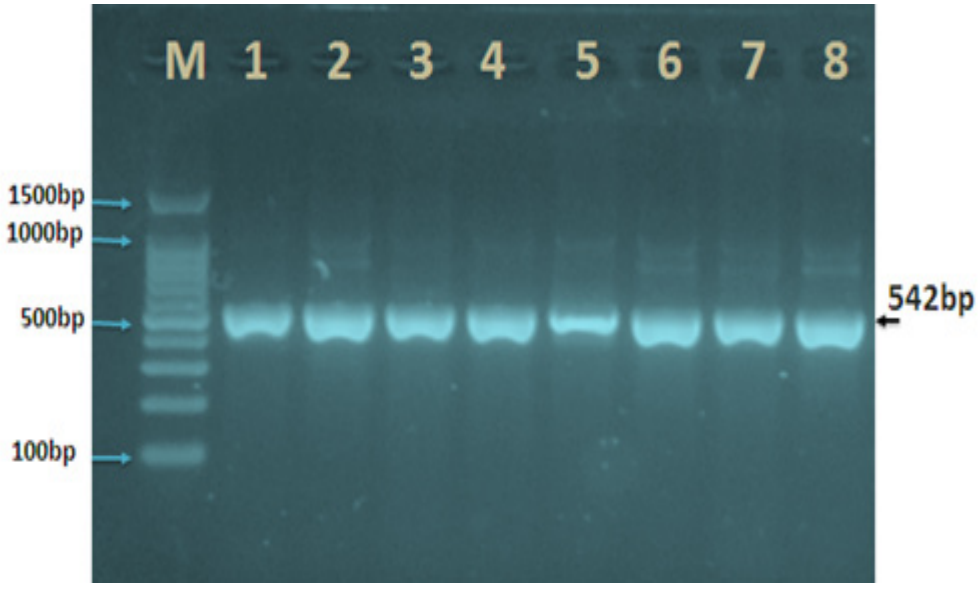

Fig. 1. Agarose gel electrophoresis image shows PCR product analysis of pathogenic *Candida tropicalis*. Lane M Marker ladder (1500 bp), lanes (1-8): *18SrRNA* gene of *Candida tropicalis* isolate with 542 bp.

PCR Assay of Virulence Genes (Phospholipase PLB and Hyphal Formation (CPH1) for Candida tropicalis:

PCR amplification, Phospholipase (*PLB*) 10/16 (62.5%) was detected in 10 isolates of *C. tropicalis* yeast. 8/16 (50%) were positive for hyphal development gene (*CPH1*), Fig.(2).

Discussion

This study described the occurrences of (mycotic vaginitis) along with participatory investigations of constraints and potentials of buffalo manufacturing in Wasit province/ Iraq. Seven samples were excluded due to bacterial growth and only 40/100 (40%) of growth detected *Candida tropicalis*. The giant fungal infection recognized as vulvovaginal candidiasis (VVC) is caused with the aid of means of the Candida species, most commonly *Candida albicans*. It is diagnosed by means of the presence of inflammatory symptoms in the vulva and vaginal mucosa that are associated with the overgrowth of Candida species, which had been usually present as vaginal commensals [16,17].

Even though *Candida albicans* is the pathogen that reasons VVC the most frequently, the identification of non-Candida albicans (NCAC) species, generally *Candida glabrata* as the motive of this sickness seems to be rising gradually. Because the diagnosis and therapy are oftentimes especially based totally on the symptoms and no longer be confirmed by microscopic examination culture methods, finding out its prevalence is difficult [18]. In the current study, the percentage of *C. tropicalis* 30 (75%) out of 40 samples that was higher than those obtained in a study in Basrah province, Iraq / 2011 by (10), that verified the percentage of *C.tropicalis* used to be 9 (22%) out of 41samples and greater than study carried out through some investigators [19] in Basrah province, Iraq / 2000 that established the percentage was 2 (16.66) out of 12 whole vaginal isolation [20]. The occurrence of opportunistic yeast in different types of samples reflects the ability of the microorganism to colonize these biological niches then invade the different localities in appropriate conditions due to the potential ability of these microorganisms to cause opportunistic infection which reflects finally on economic and public health aspects

This study confirmed that the predominant isolation from buffaloes' vagina used to be *C.tropicals* which did not agree with a study carried out in Anbar province, Iraq , 2008 by way of Al-Maadidhi on some Candida kind infection of reproductive tract in ewes, which determined three species of Candida, *C. albicans*, *C. krusei*, and *C. tropicals* at the proportion of 33.33%, 20.83 and 29.16% respectively, and detected that *C. albicans* was the predominant isolate from vaginal swabs [21].

Conventional PCR for screening

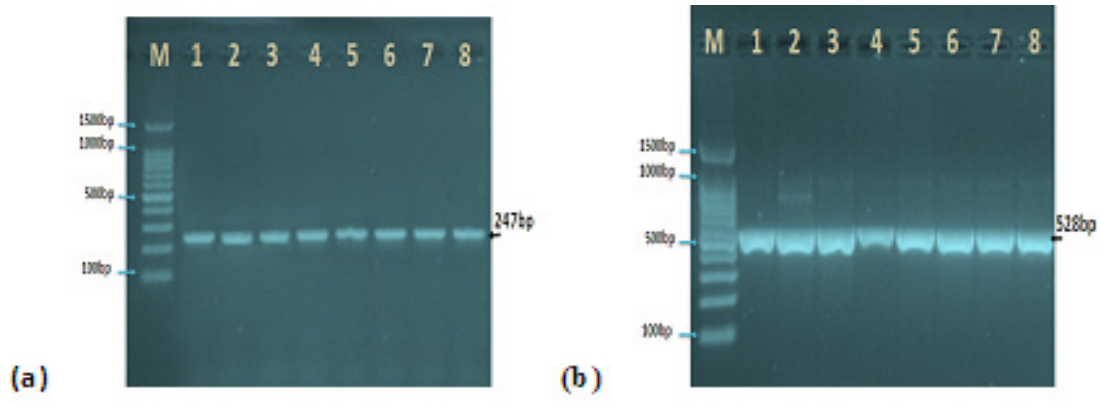

Fig.2. (A)- Agarose gel electrophoresis image shows PCR product analysis of pathogenic *Candida tropicalis* Lane M Marker ladder (1500-100bp), lanes (1-8): phospholipase *PLB* gene of *Candida tropicalis* isolate with 247 bp. (B):Agarose gel electrophoresis image shows PCR product analysis of pathogenic *Candida tropicalis* Lane M Marker ladder (1500-100bp), lanes (1-8): hyphal development gene *CPH1* of *Candida tropicalis* isolate with 528 bp.

virulence factors by using *C. tropicals phospholipase PLB* gene was once detected in 10/16 (65.2%) and *hyphal formation CPH1 gene* was once detected in 8/16 (50%). Candida species are the second most common cause of vulvovaginitis worldwide [22]. Vulvovaginal candidiasis is becoming more frequent as end result of the significant use of broad-spectrum antibiotics and extended cases of immunocompromised people [23]. Unfortunately, techniques like single and direct PCR or multiplex PCR have not been widely adopted despite being more sensitive and specific and having a quicker turnaround time [30]. Earlier studies showed that the expression of *HWP1* and genes from the *ALS*, SAP, *LIP*, and *PLB* gene families is connected to the development of biofilm on mucosal surfaces [24]. Fungal species that belong to Cryptococcus, Candida, Aspergillus, Histoplasma, and Pneumocystisare are responsible for more than 90% of all reported fungal-related deaths [25]. These variations in the proportions of every study can also come from the distinction in the primers used for PCR techniques, the discrepancy in the quantity of isolates enrolled in every study and the abilities of laboratory investigators. PCR has been more and more used to diagnose Candida, as it is fast, simple, specific, sensitive and reliable.

Conclusion

The PCR was a useful tool and excellent alternative method for the detection of the most common Candida species causing infection of genital tract in buffalo. Phenotypically, results showed that the strains, *Candida tropicalis* were the most common cause of mycotic vaginitis in buffalo.

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Conflict of interest

The authors state that they have no conflicts of interest.

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Ethical approval

Project approval number for data collection was UM.VET.2021.070.

References

1. Bruna, G., Carina, F., Carlos, T. A., Mariana, H., Joana, A. and Sónia, S. Vulvovaginal candidiasis: epidemiology, microbiology and risk factors external icon. *Criti. Rev. Microbiol*., **42**,905-927 2016**(**). DOI: 10.3109/1040841X.2015.1091805

2. Garoussi, M.T., Khosrave, A.R. and Havareshti, P. Mycoflora of cervicovaginal fluids in dairy cows with or without reproductive disorders. *Mycopathologia*., **164**, 97–100(2007). DOI: 10.1007/s11046-007-9031-x

3. Stout, T.A.E. Fungal endometritis in the mare. *Pferdeheilkunde*, **24**(1), 83–87 (2008). DOI: 10.21836/PEM20080117.

4. Pinki, S., Madhumeet, S. and Pravesh, K. Fungal endometritis in bovines. *Open Vet. J.,* **9**(1), 94–98(2019). Doi: 10.4314/ovj.v9i1.16.

5. Marcos-Zambrano, L. J., Escribano, P., Bouza, E. and Guinea, J. Production of biofilm by Candida and non-Candida spp. isolates causing fungemia: comparison of biomass production and metabolic activity and development of cut-off points. *Int. J. Med. Microbiol*., **304**, 1192–1198(2014). doi: 10.1016/j.ijmm.2014.08.012.

6. Choi, M. J., Won, E. J., Shin, J. H., Kim, S. H., Lee, W. G. and Kim, M. N.,

1. Lee, K., Shin, M.G., Suh, S.P., Ryang, D.W and Im Y.J. Resistance mechanisms and clinical features of fluconazole-nonsusceptible Candida tropicalis isolates compared with fluconazole-less-susceptible isolates. *Antimicrob. Agents Chemother*., **60**, 3653–3661(2016). doi: 10.1128/AAC.02652-15.

7. Seneviratne, C. J., Rajan, S., Wong, S. S., Tsang, D. N., Lai, C. K. and Samaranayake, L. P and Jin, L. Antifungal susceptibility in serum and virulence determinants of Candida bloodstream isolates from Hong Kong. *Front. Microbiol*., **7**, 216 (206). doi: 10.3389/fmicb.2016.00216.

8. Zuza-Alves, D. L., de Medeiros, S. S., de Souza, L. B., Silva-Rocha, W. P., Francisco, E. C. , de Araújo, M. C., Reginaldo, G. , Lima-Neto, R.G., Neves, R.P., Melo, A.S. De. A., Guilherme, M. and Chaves, G.M. Evaluation of virulence factors in vitro, resistance to osmotic stress and antifungal susceptibility of Candida tropicalis isolated from the coastal environment of Northeast Brazil. *Front. Microbiol.*, **7**,1783(2016). doi: 10.3389/fmicb.2016.01783

9. Warren, N. G. and Hazen, K .C. Candida, Cryptococcus, and other yeasts of medical importance. In: Murray, P. R., Baron, E .J., Pfaller, M. A., Tenover, F. C. and Yolken, R. H. editors. Manual of Clinical Microbiology. 6th ed. Washington, D.C: *American Society for Microbiology*, 723–737(1995). https://scholar.google.com/scholar?q=related:OQnrPk3vDHUJ:scholar.google.com/&scioq=&hl=ar&as_sdt=0,51995).

10. Désirée, E. B., Christine, E. Mc. and David, C. C. Genetic characterization of a phospholipase C gene from Candida albicans: presence of homologous sequences in Candida species other than Candida albicans. *Microbiology*, **144** (1), 55-72(1998). doi: 10.1099/00221287-144-1-55.

11. Fujita, S-I., Lasker, B. A., Lott, T. J., Reiss, E. and Morrison, C.J. Microtitration plate enzyme immunoassay to detect PCR-amplified DNA from Candida species in blood. *J. Clin. Microbiol.,* **33**, 962–967(1995). DOI: 10.1128/jcm.33.4.962-967.

12. Shin, J. H., Nolte, F. S. and Morrison, C .J. Rapid identification of Candida species in blood culture by a clinically useful polymerase chain reaction method. *J. Clin. Microbiol*., **35**,1454–1459(1997). DOI: 10.1128/jcm.35.6.1454-1459.1997.

13. Mukherjee, P.K. and Ghannoum, M.A. Secretory proteins in fungal virulence. In : Calderone RA, Cihlar RL, eds. Fungal pathogenesis: principle and clinical applications. New work. *Marcel. Dekker*., 51-79(2002).

14. Yang, Y.L. Virulence factor of Candida species. *J. Microbial. Immunol. Infect*., **3**(36), 223-228(2003).

15. Hawraa, F.H. Al-abedi., Bassam, Y. K. and Azhar, A.F.A. Conventional and molecular detection of candida albicans and candida parapasilosis isolated from bovine mastitis in basrah-Iraq, *Biochem. Cell. Arch*., **19**(2), 3285-3289 (2019). DOI: 10.35124/bca.2019.19.2.3285

16. Jack, D. Sobel. Vulvovaginal candidosis. Lancet., 9 (369), 1961-1971(2007). Doi: 10.1016/S0140-6736(07)60917-9

17. Latéy, B., Marcus, C. C., Bing, M., Vincent, B. and Jacques, R. Vaginal Candida spp. genomes from women with vulvovaginal candidiasis. *Pathog. Dis*., **75**(6), 1-3(2017). DOI: 10.1093/femspd/ftx061

18. Rad, M. M., Zafarghandi, S., Abbasabadi, B. and Tavallaee, M. The epidemiology of Candida species associated with vulvovaginal candidiasis in an Iranian patient population, *Eur. J. Obstet*., **155**, 199–203(2011). DOI: 10.1016/j.ejogrb.2010.11.022.

19. Basil, A A., Khudor, M.H. and Touhali, I.S. Isolation, identification and immunological study of yeast from cattle and buffaloes in Basrah province, Iraq. *Iraqi Journal of Science*, **52**(1),125-131،2011).

20. Isa, T.S. Survey of some opportunistic yeasts from cattle and buffaloes with histopathological and immunological study of candida albicans in Basrah governorate, A Thesis Submitted to the Council of the College of Veterinary Medicine-University of Basrah in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Medicine in Microbiology, B.V.M. (2000).

21. Al-Maadidhi, A.H.A. Study of some Candida type infection reproductive system in ewes.Anbar, *Vet. J.*, **1**(1), 29-30(2008). ISSN:1999-6527.https://iasj.net.

22. Yücesoy, M. and Marol, S. Performance of CHROMAGAR candida and BIGGY agar for identification of yeast species. *Annals of Clinical Microbiology and Antimicrobials,* **2**(8),1-7 (2003).

23. Fujita, S.I., Senda, Y., Nakaguchi, S. and Hashimoto, T. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *Journal of Clinical Microbiology*, **39**(10), 3617–3622(2001).

24. Mohammed, N. A., Ajah, H. A. and Abdulbaqi, N. J. Detection The Prevalence of Adhesins and Extracellular hydrolytic enzymes genes in Candida albicans Biofilm Formation. *Iraqi Journal of Science*, **58**(2C), 988–1000(2022). Retrieved from https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/5931.

25. Mohsin, Z. A. and Ali, W. S. Antagonistic Activity of Bacteriocin-producing Lactobacillus Against Candida spp. *Iraqi Journal of Science*, **7**, 2153–2162 (2021). https://doi.org/10.24996/ijs.2021.62.7.4.

الكشف الجزيئي عن المبيضات الاستوائية المعزولة من الأعضاء التناسلية للجاموس في محافظة واسط

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المبيضات الاستوائية ينتج هذا النوع أغشية حيوية وعوامل ضراوة أخرى مثل الارتباط بإفرازات الخلايا البطانية و الخلايا الظهارية الشدقية من الإنزيمات المحللة مثل الهيموليسين والفوسفوليبيز والبروتينيز والتحول من البراعم إلى الخيوط (المعروف أيضًا باسم التشكل), في هذه الدراسة ، تم استخدام Candida ٢٠ API للتعرف على المبيضات الاستوائية ، واستخدمت تقنية تفاعل البلمرة التقليدي للكشف عن التركيب الوراثي والضراوة في العزلات التي تسبب التهاب المهبل الفطري في الجاموس. في محافظة واسط خلال الفترة (مايو ٢٠٢١ - أكتوبر ٢٠٢١), تم أخذ عينات مسحة مهبلية من الجاموس المصاب بأنواع المبيضات والتي ظهرت في ٤٠ من ١٠٠ عينة (٤٠٪) من المسحات المهبلية الفطرية ، أظهرت نسبة ٤٠/٣٠ (٧٥٪) من أنواع المبيضات المعزولة نتائج إيجابية وفقًا لاختبار Candida ٢٠ API الخاص بالمبيضات الاستوائية. التضخيم لجين الرنا الرايبوزي ١٨S لتحديد المبيضات الاستوائية ، كان هذا الجين موجودًا في ٣٠/١٦ عينة (٥٣,٣٪). تم العثور على كل من جين(Phospholipase (PLB وجين تكوين hyphal (CPH١) في ١٦/١٠ (٦٢,٥٪) و ١٦/٨ (٥٠٪) من العينات على التوالي. يعد تفاعل البلمرة التقليدي طريقة مفيدة وطرق بديلة ممتازة للكشف عن أنواع المبيضات الأكثر شيوعًا التي تسبب إصابة الجهاز التناسلي في الجاموس ، فإنه يتجنب متطلبات ثقافة الزرع حيث ممكن ان نبدأ من العينة السريرية ويفضل التشخيص المبكر لداء المبيضات.