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Detection of *Histoplasma capsulatum*in bats dropping by using polymerase chain reaction(PCR) at the first time in Iraq

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Abstract

In the present we isolated the fungus *Histoplasmacapsulatum* as a first time from bats dropping in AL-Qadisiyah province .Results show that the macro and micro characteristicsreferred that these characteristics was related to the fungus *Histoplasmacapsulatum*,also this result wasconfirmed by PCR technique.

Key words: *Histoplasmacapsulatum*, bats dropping,PCR

Introduction:*Histoplasmacapsulatum* is a fungal pathogen that can result in a wide range of clinical presentations, from asymptomatic through fatal infection. It usually causes lung disease called Histoplamosis or Darling's disease. It is called Darling's disease because it was found by Samuel Darling in histopathological specimens about a century ago.(Robert,2001) *Histoplasmacapsulatum* is a biologically interesting inhabitant of soil and mammalian hosts, a clinically significant cause of respiratory and systemic infection, and an excellent fungal model of dimorphic cell development and facultative intracellular pathogenesis. (Chang Ryan, 2007)*H. capsulatum* is unique in its dimorphism. Dimorphism allows *H. capsulatum* to infect mammals by going through three significant development stages depends on the temperature shift from 25 °C to 37 °C.(Bossche,1993) In the moist soil that is rich in bird or bat guano at temperature about 25 °C, *H. capsulatum* exists in a filamentous mycelia form. However, when humans inhale *H. capsulatum* into their respiratory tracks, in order to replicate its DNA in the host at 37C, the pathogen has to be able to convert its tissue from one form to another. In this case, *H. Capsulatum* changed from fungi to yeast when it's growing in the human bodies(Kauffman, 2007).*Histoplamosiscapsulatum* is found throughout the world. It is endemicin certain areas of the United States, particularly in states bordering the Ohio River valley and the lower Mississippi River. The humidity and acidity patterns of soil are associated with endemicity. Bird and bat droppings in soil promote growth of *Histoplasma*. Contact with such soil aerosolizes the micro conidia, which can infect humans. It is also common in caves in southern and East Africa. (RyanandRay, 2004).Regarding thepresence ofthe fungusinIraqis not registeredaccordingavailable references we have and thatthe current studyis thefirstin the countryto recordthe presence ofthisfungus.

Material &Methods:

Culture media: The following media were used for isolated and identification of fungus from samples (Beneke and Rogers,1980):

*Sabouraud'sDextrose Agar &chloramphenicol

*Brain Heart Infusion Agar with cycloheximide&chloramphenicol*Salvin's YP medium

Collection of sample : Samples of bats dropping were collected from bats nests were it's found .These sampleswere placed in sterile taines and transported to laboratory for culturing and testing .

Isolation &Identification of fungus: By using of method that reported by Lopez-Martines&Castaron-Oliver(1995) was isolated the fungus from samples that collected .make suspension from the samples and cultured on the media that reported above .After the incubator period (at 25C for two weeks and at 37C for seven days)the fungus was identification as a *Histoplasmacapsulatum* according to macro and micro features that reported by (Ellis ,1994; Larone,1995;St-Germain & Summerbell,1996 ;Frey *et.al*.,2002)

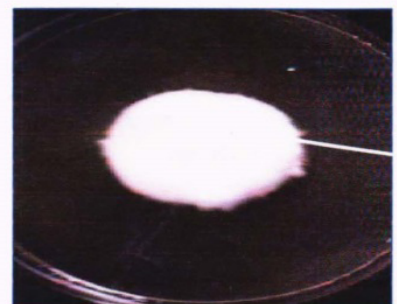
PCR Technique:

Extract DNA: the DNA extracted from the isolates that selective on SDA medium, and Thenfollowed the stepsinstalledthatsupplied withthe kitfittedfrom the company(Bioneer)to extractDNA, then blending part of it with dye Ethidium bromide on gel Agarose record in a concentration of 1.5% (100) ml buffer solution TEB Buffer concentration (1X) Molar according of the company (Bio Basic) for the purposeofpuritywayelectricdeportation.

Prepare the reaction solution to a polymerasechain reactionsPCR: attended the reaction mixture (size 50 Micro liters) for a polymerase**chain** reactions following the steps installed and supplied with the kit AccuPower ® PCR PerMix processed from the company (Bioneer) and by using of the*H. capsulatum*-specificprimers HC-1 and HC-2 (HC-1: 5-GAGCCTCTGACCGGGAC-3;HC-2: 5-GCACGTCCCAC CCGGTCAG-3).(White*et.al.,*1990)

Results&Discussion:

Dimorphic fungi, such as *Histoplasma*, undergo a temperature induced transition between growth phases, which means that it has two distinct phenotypes. This dimorphism makes these organisms difficult to correctly identify using traditional microbiological methods. At ambient temperatures below 30°C in contaminated soils, *Histoplasma* grows in itsmycelial form with the appearance of typical molds. Once the spores or micro conidiahave been inhaled, the organism then undergoes a metamorphosis to its yeast or parasiticform. It is at this stage that *Histoplasma* becomes virulent. At normal body temperatures,around 37°C, the organism looks and behaves as yeast with the typical budding off ofcells. This phase change, induced by the elevated temperatures of the body, is requiredfor virulence**.** (Benekli,2004 ; Robert Brooks **,**2011). Fig.(1) show colony with slow growing and cottony appearance , white in color .On other hand, Fig.(2) show septate hyphae withmacro conidia, or tuberculate conidia, are 9 m in diameter .Identification of the tuberculate macro conidia allows to diagnosis of *Histoplasma* (Bhatti, *et.al*., 2004)Also, this result accordant with (Kauffman, 2003) that reported , the tuberculate conidia, are 8 to 15 m in diameter and have distinctive projections on their surface. Fig (3) show moist, folded creamy colony at 37C ,that represented parasitic phase and fig(4) show , oval , unicellular budding yeast cells and buds arise at narrow neck . This results reported by (Beneke ,2004) whose reported that conversion to the yeast phase at 37C on brain heart infusion agar are necessary criteria for identification of *Histoplasmacapsulatum* . Many species offungi otherthan*H. capsulatum*produce similar colony and sporulation characteristics. Some examples are *BlastomycesdermatitidisChrysosporiumsp., andSepedoniumsp*. Therefore, additional testingis needed to definitively identify the organism. One method is to convert the fungus colony from thefilamentous, mycelial phase to theyeast phase by subculture to highly enriched cysteine-containing media and incubation at 35° to 37°C..(Wheat,1989).



Fig(1)White cottony colony on SDA(at 28C)

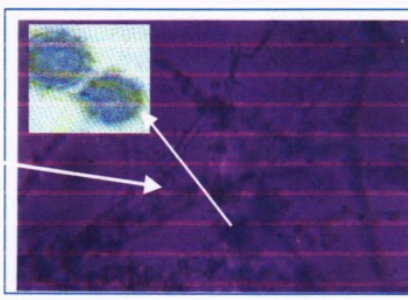
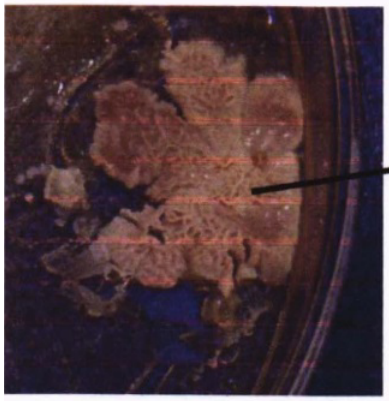


Fig.(2)Tuberculated macroconida &small microconidia(640X)



Fig(3) Folded creamy colonyon BHI (at 37C)

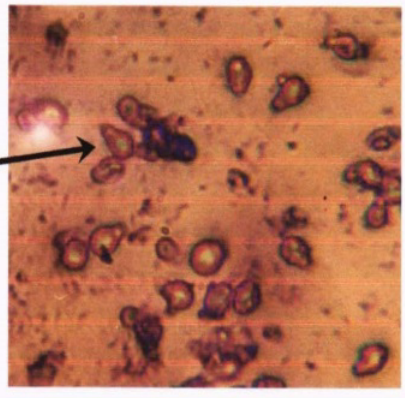
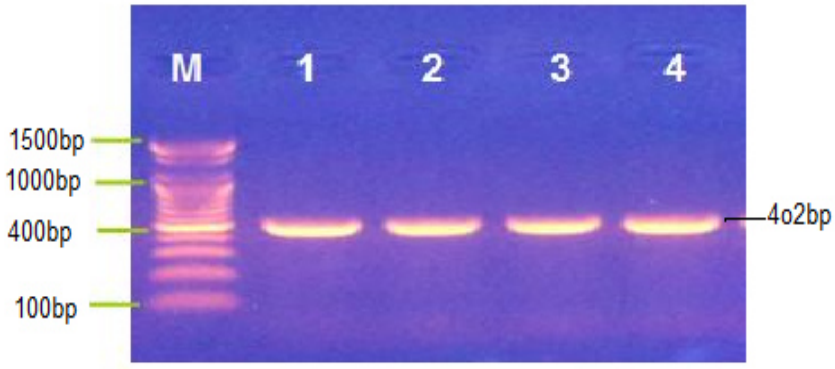


Fig.(4)yeast cells with capsule (640X)

DNA based PCR technology, which uses probes specific to these organisms, is a quicker, ore accurate and cost-effective way of determining their presence or absence.Sequences that are found only in the genome of *Histoplasma*are targeted by the probes, allowing aprecise, definitive result and do not require a viable organism for detection.(Hopfer,*et.al*., 1993 ; Reid and Schafer ,1999).Fig.(5) show , Polymerase chain reaction method (PCR) have been used to detection and identification of fungus , gaverise to a single 402bp PCR product .This result accordant with Reid and Schafer(1999) that reported A polymerase chain reaction (PCR) method was developed thatallows the direct detection of*H. capsulatum* in soil and gave to a single 400 bp PCR product diagnostic of*H. cap.sulatum* .



Fig(5):gel electrophoresis that the results of polymerasechain reaction to DNAgenediagnosis of*Histoplasma sp*. Whereis theM: Marker ladder 100-1500 bpandsamplesofNo.1-4representpositive samplesfor testingan outputlength of402bp.

Conclusion:

The data presented in this study for our knowledgeis considered the first screening data regarding theincidence of *Histoplasma* in Iraq and the high incidence wasobserved in bats dropping samples . PCR technique have important role of diagnosis of this fungus and this assay that correctly identified *H. capsulatum*from among avariety of fungi grown in the laboratory appears promising.

References:

Beneke,E and Rogers ,1980. Medical Mycology Manual with humanmycosis monograph .4thed.Burges pub.comp.

Benekli,M. 2004. Pulmonary histoplasmosis in patients with a chronic obstructive pulmonary disease.Diagnosis J. Thrac .Cadiovasc. 127:585-586.

Bhatti, S., L. Vilenski, R. Tight, and R. A. Smego, Jr.2004.Histoplasmaendocarditis: clinical and mycologic features and outcomes. J. Infect.51:2–9

Bossche, Hugo, Frank Odds, and David Kerridge.1993.Dimorphic Fungi in Biology and Medicine. 1st ed. New York and London: Plenum Press.

Chang Ryan. 2007.Histoplasmosis. medicine.10 (1): 114–122.

Ellis ,D.H. 1994.Clinical Mycology :The Humans opportunistic Mycosis .Gillingham Printers Ldt.Asturalia.
Frey ,D.; Oldfield,R. &Bridger,R. 2002. A color atlas of pathogenic fungi. Wolfe Medical Publication P.285.
Hopfer, R. L., Walden, P., Setterquist, S. &Highsmith, W. E. 1993. Detection and differentiation of fungi inclinical specimens using polymerase chain reaction(PCR) amplification and restriction enzyme analysis. Journal of Medical Veterinary Mycology 31, 65–75
Kauffman, C. A.2003. Histoplasmosis, p. 285–298.InW. E. Dismukes, P. G.Pappas, and J. D. Sobel (ed.), Clinical mycology. Oxford University Press,New York, NY
Kauffman, CA. 2007."Histoplasmosis: a clinical and laboratory update. Clinical Microbiology Reviews.20 (1): 115–132.
Larone,D.H.1995.Midecally important fungi –A guide to identification .3rd ed.ASM Press , Washinton,D.C.
Lopez-Martinez , M. &Castaron-Oliveres,L. 1995.Isolation of Cryptococcus neoformans from birds dropping , fruits and vegetable. Mycopathologia,129:25-28.
ReidT. M and M. P. Schafer .1999.Direct detection of Histoplasmacapsulatumin soilsuspensions by two-stage PCR. Molecular and Cellular Probes . 13, 269–273
Rippon, J. W.1988. Histoplasmosis. p. 381-423. InMedical mycology, the pathogenicfungi and the pathogenic actinomycetes.3rd ed. W. B. Saunders Company.
Robert Brooks. 2011.pcr detection of Histoplasmacapsulatum in environmental samples. Microbac.7:14-18.
Robert,G.2001.Enviromental Health Services:Systemic fungal infections .Coloradostate university
Ryan KJ, Ray CG. 2004. Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 674–6
St-Germain,G.&Summerbell,R. 1996. Identification filamentous fungi :Aclinical laboratory hand book. 1st ed. Star publishing company,Belmont,Californinia.
Wheat, L. J.1989.Diagnosis and Management ofHistoplasmosis. Eur. J. Clin. Microbiol. Infect. Dis. 8:480-490.
White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990).Amplification and direct sequencing of fungal ri-bosomal RNA genes for phylogenetic. InPCR Protocols—a Guide to Methods and Applications (Innis,M. A., Gefland, D. H., Sninsky, J. J. & White, T. J.,Journaleds) pp. 315–22. San Diego: Academic Pres
Woods, J. P., E. L. Heinecke, J. W. Luecke, E. Malsonado, J. Z. Ng, D. M.Retallack, and M. M. Timmerman. 2001. Pathogenesis of Histoplasmacapsulatum .Semin.Respir. Infect.16:91–10

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