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MOLECULAR DIAGNOSIS OF CASEOUS LYMPHADENITIS IN SHEEP AT DAKAHLIA GOVERNORATE, EGYPT

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ABSTRACT

The aim of the present study was to diagnose caseous lymphadenitis in sheep by PCR assay. A total of 1257 sheep from different flocks in Dakahlia governorate were clinically examined during the period of January 2014 to February 2015. sheep were classified according to the status of infection in the flocks to infected , incontact and free ones. 37 needle biopsy samples were randomly collected from 26 diseased, 7 incontact and 4 free examined sheep for PCR assay. rpoB gene was detected in 37 samples (100%) while PLD gene was in 18 samples (48.64%), from which 16 from diseased sheep and two from incontact ones, while not detected in free sheep. The PCR assay was rapid, specific and significant technique as bacterial culture in detecting corynbacterium pseudotuberculosis in needle biopsy samples from sheep.

Keywords: *Caseous lymphadenitis. Corynebacterium pseudotuberculosis . Sheep Polymerase chain reaction . Lymph nodes biopsy .*

INTRODUCTION

Caseous Lymphadenitis (CLA) is a chronic, contagious, transmissible disease of sheep and goat caused by *Corynebacterium pseudotuberculosis* **(Droppa-Almeida et al., 2016; Oreiby, 2015).** CLA causes severe economic losses of animal industry all over the world **(Williamson, 2001).**

The disease is characterized by chronicity and in many cases appears in subclinical form in small ruminants across the world **(Dorella et al., 2006).** CLA is manifested clinically by large swellings of superficially located lymph nodes that contains infectious agent, which may be discharged through ruptured abscesses **(O'Reilly et al., 2006).** Mature lesions are characterize by concentric lamellations which located in the center of the lesion . These lesions usually are seen in the external lymph nodes, lungs and, less commonly in other organs **(Severini et al., 2003).**

Polymerase chain reaction (PCR) is a promising method, which used for detection of *corynebacterium pseudotuberculosis* in bacterial colonies or in pus samples **(Çetinkaya et al., 2002; Pacheco et al., 2007)**. The obstacles facing PCR to detect caseous lymphadenitis cases are inability to take samples from visceral lesions and the questionable results of PCR when performed on blood samples.PCR significantly improves rapid detection of *C.Pseudotuberculosis* and could supersede bacteriological culture for microbiological and epidemilogical diagnosis

of CLA **(Pacheco et al., 2007)**. As microbiological and biochemical methods are not always straightforward, the development of a rapid and specific diagnostic tool is imperative for the diagnosis of CLA **(Çetinkaya et al., 2002)** , therefore we used PCR .

This study aimed to detect *C. Pseudotuberculosis* in needle biopsy samples by PCR .

MATERIAL &METHODS

1-Animals:

A total of 1257 sheep from 34 different flocks in Dakahlia Governorate were clinically examined during the period of January 2014 to February 2015. Sheep were classified according to the status of infection of flock into infected , incontact and free **(Radostitis et al., 2007)** .

2-Needle biopsy samples :

A total of 37 needle biopsy samples obtained under complete aseptic condition from superficial lymph nodes were obtained for DNA extraction and PCR assay (infected,n = 26; normal,n=11) . Fine needle biopsy (FNB) is a procedure in which a small –caliber single use needle is placed into mass (lymph node tissue) and cellular material is removed **(Zajdela et al., 1987)** .Tissue taken by needle was placed in sterile epindorf containing PBS and sent to lab in ice box and kept at – 20 ºC until processed for PCR diagnosis .

3-Polymerase chain reaction (PCR):

PCR was performed in the Biotechnology Laboratory of Animal Health Research Institute, El-Dokki, Egypt. It was applied to detect *C . pseudotuberculosis* in needle biopsy samples based on two specific genes (rpoB and PLD), according to **(Sammra et al., 2014)** and **(Ilhan, 2013),** respectively.

3.1. DNA extraction for PCR :

QIAamp DNA Mini Kit (Catalogue no.51304) . Using.

3.2. Oligonucleotide primers used in cPCR

They have specific sequence and amplify a specific product as shown in Table (1).

RESULTS

Clinical findings I-

Applied on 1257 sheep . 37 sheep were randomly selected, from which 26 clinically diseased , 11 incontact and 4 free sheep.The clinical signs appeared on diseased sheep were abscessiation of superficial lymph nodes.Closed abscesses were ranged in size from bean's size up to orange size **(Figure 2)**, where they can be palpated by hand.In opened abscesses the pus color in some cases was creamy **(figure 3)** and other cases may be greenish.Consistency was varied from fluid to hard caseous materials and lamellations may be noticed and wool over abscesses lost**(Figure 1)** .Some animals showed signs of internal abcessiation like chronic ill-thrift and poor condition .

Molecular findings: II-

Out of 37 needle biopsy examined samples for PCR assay, 37 (100%) were positive rpoB indicated at 406 bp **(Figures 4 and 5)**, while 18 (48.64%) were positive PLD results indicated at 203 bp(**Figures 6 and 7**) , from which 16 infected sheep and 2 incontact sheep but not detected in free sheep.

Table (1) Oligonucleotide primers sequences

Source: **Metabion (Germany)**

Gene	Sequence	Amplified product	Reference
Pld	ATA AGC GTA AGC AGG GAG CA	203 bp	**(Ilhan, 2013)**
	ATC AGC GGT GAT TGT CTT CCA GG		
rpoB	CGWATGAACATYGGBCAGGT	406 bp	**(Sammra et al., 2014)**
	TCCATYTCRCCRAARCGCTG		

3.3 PCR Master Mix used for cPCR

Table (2): according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit as shown in table (2):

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	12.5 µl
PCR grade water	4.5 µl
Forward primer (20 pmol)	1 µl
Reverse primer (20 pmol)	1 µl
Template DNA	6 µl
Total	25 µl

Table (3): Cycling conditions of the different primers during cPCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
PLD	94˚C 5 min.	94˚C 30 sec.	56˚C 30 sec	72˚C 30 sec	35	72˚C 5 min.
rpoB	94˚C 5 min.	94˚C 30 sec.	52˚C 45 sec	72˚C 45 sec	35	72˚C 5 min.

3.4. Agarose gel electrophoreses

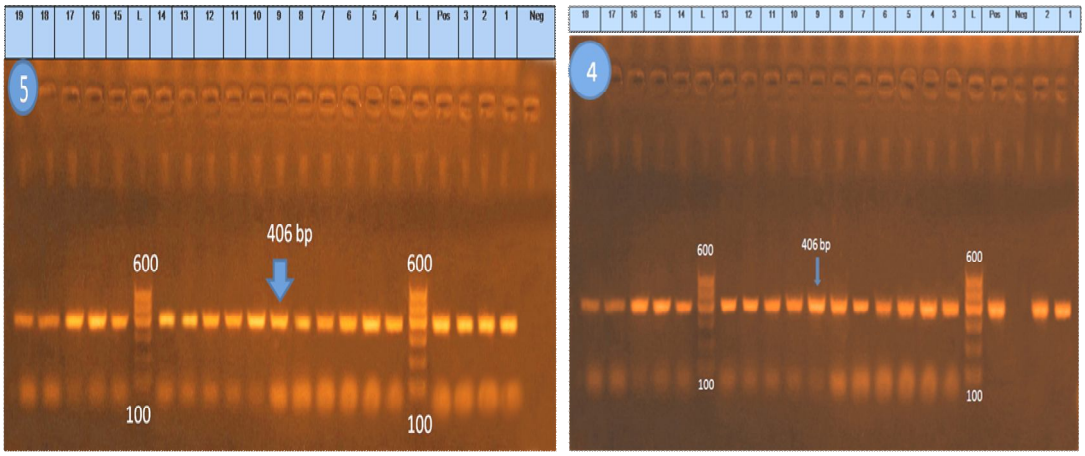
Modified from **(Sambrook et al., 1989).**

Figure(1): abscess in parotid lymph node, **Figure(2):** abscess in prescapular lymph node**. Figure(3)** open abscess creamy pus.

Table (4): Direct detection of *C. pseudotuberculosis* in needle biopsy samples of CLA-affected and apparently normal animals using the mPCR assay.

Herd Status	No of samples	rpoB		PLD	
		+ve	%	+ve	%
Infected	26	26	100%	16	**61.53.%**
Incontact	7	7	100%	2	**28.5%**
Free	4	4	100%	-ve	**0%**
Total	37	37	100%	18	**48.64%**

mPCR assay result for both genes rpoB (Figures 4 and 5)and PLD (Figures 6 and 7)



Figures 4 and 5 : Positive samples at band size of 406 bp fragment

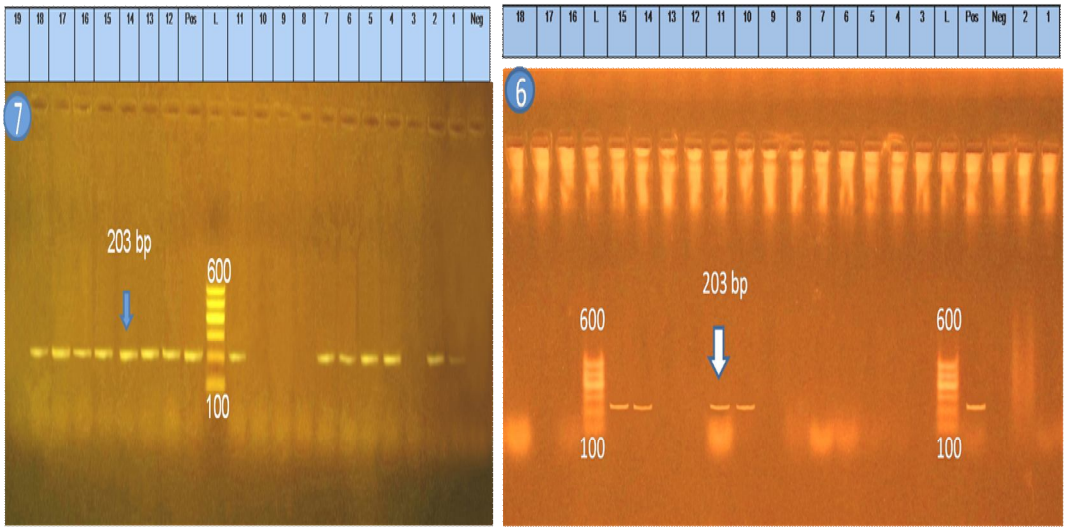


Figure 6 and 7: positive samples at band size of 203 bp fragment

DISCUSSION

Caseous lymphadenits is a chronic contagious disease of sheep and goats all over the world ,characterized by formation of abscesses in superficially located lymph nodes and may affect internal organs and visceral lymph nodes **(Baird and Fontaine, 2007; Paule et al., 2004)** .The disease is endemic in Egypt **(Al-Gaabary et al., 2010; Oreiby et al., 2014; Oreiby, 2015).**

Regarding to clinical examination, 1257 sheep were clinically examined , from which 37 sheep were randomly selected (26 clinically diseased , 7 incontact and 4 free sheep).The clinical signs appeared on diseased sheep were abscessiation of superficial lymph nodes and in some cases wool was lost over the lesion. These results were in conformity with**(Binns et al., 2007)** and **(Radostitis et al., 2007) ,** who mentioned that the signs of CLA are prominent enlargement of superficial lymph nodes such as submandibular, prescapular, prefemoral, supramammary,popliteal, or in visceral organs such as lungs**.**

The PCR assay was rapid, specific and as significant as bacterial culture in detecting bacteria directly in the clinical samples. The PCR assay developed in the study can be applied for the diagnosis and control of CLA. Furthermore, the assay can also be applied to detect C. pseudotuberculosis in various clinical samples. **(Kumar et al., 2013)**.

Regarding to PCR examination,37 needle biopsy samples were examined for PCR assay, 37 (100%) were positive rpoB indicated at 406 bp and this in harmony with **(Sammra et al., 2014),** who use the rpoB gene Primer to diagnose *Arcanobacterium phocisimile* and said that ***rpoB*** gene is non specific gene encodes the β subunit of bacterial RNA polymerase , while 18 (48.64%) were positive PLD results is indicated at 203bp from which 16 infected sheep and 2 incontact sheep but not detected in free sheep and this agree with **(Ilhan, 2013)** who used PCR assay protocol for the direct detection of *C. pseudotuberculosis* in 147 samples of lymph nodes (prescapular and mediastinal) from carcasses of naturally infected sheep and to

compare its performance with the traditional bacteriological culture technique. *C. pseudotuberculosis* was isolated in 81 samples mainly from prescapular nodes and a specific 203 bp PCR amplified **PLD** gene ,DNA fragment was detected in 85 samples.

Our result revelead that 2 samples were positive come from apparently normal lymph node from apparently normal animal and the explanation is due to contact with affected animal during close confinement or, indirectly, via contaminated shearing equipment **(Baird and Fontaine, 2007**).

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الملخص العربي

استخدام تفاعل البلمرة المتسلسل في تشخيص مرض السل الكاذب في الاغنام المصابة و المخالطة في محافظة الدقهلية بمصر

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** قسم بحوث البكتريولوجي –معهد بحوث صحة الحيوان بالدقي

الهدف من هذا البحث هو تشخيص مرض السل الكاذب في الاغنام في الحيوانات المصابة والحيوانات المخالطة لهاوالتي لم يظهر عليها اعراض باستخدام تفاعل البلمرة المتسلسل .

تمت هذه الدراسة علي ١٢٥٧ حيوان من قطعان مختلفة في محافظة الدقهلية تم فحصهم في الفترة من يناير ٢٠١٤ وحتي فبراير ٢٠١٥ وتم تقسيم القطعان الي قطعان مصابة وقطعان مخالطة وقطعان لم تظهر بها الاعراض سابقا وتم اخذ ٣٧ عينة باستخدام الوخز بابرة قاطعة في غدد الحيوانات منهم ٢٦ عينة من حيوانات مصابة و ٧عينات من حيوانات مخالطة و ٤ عينات من حيوانات من قطعان لم تظهر بها الاعراض باستخدام تفاعل البلمرة المتسلسل وكانت النتائج كالتالي ٣٧ عينة (١٠٠%) ايجابي لجين rpoB و ١٨ عينة (٤٨,٦٤%) ايجابي لمكيروب الكورينيبكتريم سيدوتيوبركولوزيس منهم ١٦ عينة من حيوانات مصابة وفقط حالتان من حيوانات مخالطة .