Detection of mixed infection of bovine rotavirus group A with bovine corona virus in diarrheic calves by using (RT-PCR)

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In this study we used more sensitive techniques (reverse transcription polymerase chain reaction) and specific primers VP7 to Detection the mixed infection bovine rota virus group A in diarrheic fecal samples which taken from calves in age (1 -30)day .diarrheic fecal samples were collected from different area Iraqi province (Al-Qadisyia, Babylon ,Wassit and Najaf) during the winter season. The result of convential PCR for detection bovine rotavirus group A (specific primer Vp7) showed , only ١١ out of ١٥٢ samples positive to bovine corona virus(They were examined before), and when those 11 samples were tested by One step RT-PCR ,only four samples were shown to be positive for rotavirus group A .

Key word: bovine rotavirus, corona virus, mixed infection, calves diarrhea , RT.PCR.

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تشخيص الاصابة المختلطة بفايروس الروتا نوع A مع فايروس الكورونا في العجول المصابة بالاسهال باستخدام تقنية تفاعل البلمرة المنعكس

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الخلاصة

يعتبر فايروس الروتا نوع A من اهم المسببات الفيروسية الشائعة التي تسبب الاصابة بالإسهال بالعجول المولودة حديثا ونوع A هو من اهم الانواع وله نسب حدوث عالية واهمية سريرية ..يصنف فايروس الروتا نوع A الى سبعة انماط مصلية من G ال P حسب الخصائص الوراثية أو المستضدات للبروتينات VP7و VP4 الموجودة في الغلاف الخارجي للفايروس .

تم في هذه الدراسة استخدام تقنية حساسة(تفاعل سلسلة البلمرة المنعكس) وباستخدام بادئات خاصة للبروتين الخارجي VP7 للتحري عن وجود الاصابة المختلطة بفايروس الروتا نوع A في عينات اسهال تحتوي على فايروس الكورونا في اعمار تتراوح بين (١-٣٠) يوم. جمعت عينات الاسهال من مناطق مختلفة من اربع محافظات عراقية (القادسية ،بابل ،واسط، النجف)خلال

ISSN: 2072-3875

فصل الشتاء .اظهرت تفاعل سلسلة البلمرة المتسلسل المنعكس التقليدي وباستخدام البادي البروتيني VP7 ان من بين 11 عينة من بين عينة١٥٢ انها كانت موجبة لفيروس الكورونا (كانت مفحوصة مسبقا) وعند فحص هذة (١١) كانت فقط اربع عينات انها تحتوي على فايروس الروتا .

الكلمات المفتاحية: فايروس الروتا ، فايروس الكورونا ، الاصابة المختلطة، تفاعل البلمرة المنعكس .

Introduction

Diarrhea is consider one of the major causes of calf loss in beef calves and are the most cause of loss in dairy heifers born alive. Mortality from diarrhea, in dairy calves diarrhea and other digestive diseases account for about 5% of total mortality (USDA.,1997).

Since diarrhea in calf and other animals is a great problem which involves economic losses, several diagnostic methods are used to detect enteropathogenic agents. Diagnosis is done through collecting feces of animals suffering from diarrhea by a rectal swab or collecting intestinal contents (Castro *et al*.,1992).

Rotavirus, bovine coronavirus (BCoV), *Escherichia coli* F5 (*E. coli*), and *Cryptosporidium* species are internationally recognized as the most important Enteropathogens in acute diarrhea in young calves(Gulliksenl *et al.*,2009).

In calves, bovine group A rotavirus (GARV) and BCoV are the most commonly associated viruses with neonatal diarrhea and it is not unusual that both viruses can concomitantly infect calves(Barry *et al*., 2009).

Mixed infections caused by rotavirus and coronavirus can lead to severe form of diarrhea. The most commonly recognized viral causes of neonatal calf diarrhea are rotavirus, coronavirus (Bouda *et al*.,1997).

(Acheson, 2007). Rotaviruses are the most commonly diagnosed cause of neonatal diarrhea, they affect calves 4 to 14 days old. Bovine coronavirus (BCV) causes diarrhea in both dairy and beef calves in 4 to 30 day-old calves (1,7)

There are different methods to detect Rotaviruses, but a high degree of sensitivity is required, especially in subclinically infected calves and chronic shedders of Rotaviruses in faeces (Parwani *et al*.,1992).The RT-PCR assay is useful to detect small quantities of nucleic acid and is widely used for the diagnosis of infectious disease (Jeong *et al*., 2005) .

The objective of this study were designed to useful the RT-PCR assay to detect small quantities of nucleic acid bovine rotavirus which mixed infection with coronavirus in diarrheic calves .

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الخلاصة

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Material and methods

Samples . 11 0ut 152 fecal samples were contain bovine corona virus were collected from diarrheic neonatal calves, their age was 1-30 day from both sex ,the investigated sample were collected from four province(Babylon , AL-Qadisyia , Wassit , Najaf) during winter month. Diarrheic feces were collected directly into disposable plastic containers , sample were transported under cold condition to the laboratory where the required test were done or storage at -20 C^{o}.

RNA Extraction :This 11 samples carried to RNA extraction submitted to RNA extraction with TRIzol. The genomic RNA of Bovine Rota virus were extracted by using Trizol RNA extraction Kit (Bioneer) and done according to kit instructed by the manufacturer .briefly 250 µl of sample material was mixed with 750 µl of TRIzol and incubated for 5 min at room temperature. Thereafter, 200 µl of chloroform was added and the combination was mixed. Following centrifugation at 12 000g for 15 min, the aqueous phase was transferred to a new tube with 500 µl of isopropanol and incubated overnight for RNA precipitation at -20°C. After centrifugation for 20 min at 4°C and washing with 1 ml of cold 75% ethanol, the pellets were air dried, resuspended in 30 µl of dimyristoylphosphatidylcholine (DMPC) water, and stored at - 20°C.

PCR amplification :RT–PCR master mix Detection of bovine rota virus RNA was carried out using Accurpower ,rocket script RT-PCR virus RNA mini kit (Bioneer)as instructed and primers specific for the VP7 gene protein that are able to detect bovine rota virus. Table(1)

Table(1) : RT-PCR master mix

RT–PCR master mix		Volume
Viral RNA template		5 µL
Primers	F.Primer	2 µL
	R.Primer	2 µL
PCR water		11 µL
Total		20 µL

All these components of RT PCR master mix reaction were added into *AccuPower* RT PCR PreMix tube that contain PreMix pellet of all other components of one step RT PCR such as (Revese transcription enzyme for cDNA synthesis, Taq DNA polymerase, dNTPs, MgCl2, KCl, and

Green loading dye). Then mixed by vortex for resuspension of PreMix pellet. The oligonucleotide primers for bovine rotavirus were designed in this study using the published sequence of VP7 gene found NCBI-Gene Bank and Primer 3 design online. All primers provided by (Bioneer) company. The sequence of primers were as follows:

upstream primer:5′ GTATGGTATTGAATATACCAC-3′

downstream primers: 5′GATCCTGTIGGCCATCC-5′.

Detection of bovine rota RNA was carried out using Accurpower rocket script RT-PCR virus RNA mini kit (Bioneer)as instructed . The following thermal protocol was used table (2) .

Table(2) R T–PCR thermocycles conditions.

Primer step	Universal primer	Number of cycles
cDNA synthesis	50	1
Predenaturation	95	1
Denaturation	55	35
Annealing	72	35
Extension	72	35
Final extension	4	1

All the RT PCR products were subjected to gel electrophoresis the samples that showed as positive bands for VP7gene of Bovine Rota Virus visible at 344bp in the PCR product on UV light.

Result and discussion

The result showed only four samples were positive for rotavirus group A from 11 samples that contain bovine corona virus.

ISSN: 2079-2670

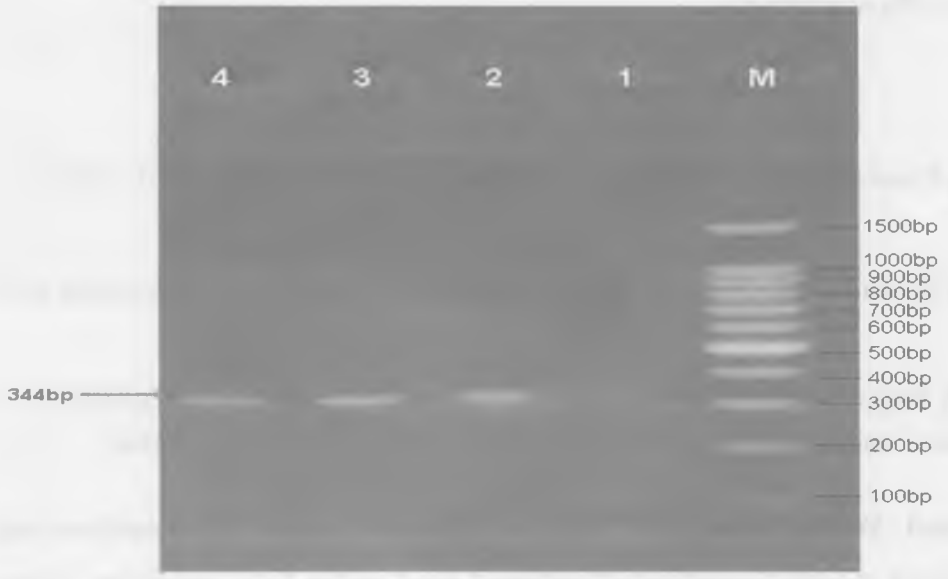

Figure (1) Ethidium bromide- stained Agarose gel of PCR amplified product from extracted RNA bovine rota virus DNA amplified with primer VP7 .
Lane marker (M), DNA molecular size marker (100 – 1500 bp ladder).
Lane (1 –4) showing the positive results of Bovine rota Virus primer at specific RT-PCR product size (344 bp).
The electrophoresis was perfored at 80 m Ampere for 1 hour product.

T ic calves with coronavirus are known as causative agents in neonatal calf diarrhea , bovine coronavirus and type A rotavirus were the principal viral particles present in samples of diarrheic

while rotavirus is also thought to play main an etiological as a single causative agent .This result agreed with many other studies (2,6) who reported the prevalence of BRV and BCV in scouring calves.

The presence of BRV in four and BCV in two samples out of nine fecal samples from diarrheic calves with 10–60 days of age was reported in Sao Paulo, Brazil (Brandao *et al.*,2007). The other study in Turkey showed the presence of BRV antigen and BCV antigen in diarrheic calves, 41.17% and 1.96%, respectively. In healthy calves, BRV was detected in 4.08% and BCV was not detected (Gumusova *et al.*,2007).

In the present study by using One step TR-PCR , the mixed infection of coronavirus in diarrheic samples was 6.57% and bovine rotavirus was 40% out of 10 samples positive for corona, in diarrheic fecal samples . our results were closed to result of (10,13) who found calves younger than

ISSN 2072-3875

3 months the last researchers mentioned that calves mainly of 1– 21 days old were affected with a percentage of 58.7% and 7.8% respectively .

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ISSN 2072-3875

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ISSN 2072-3875

Republic of Iraq

Ministry of Higher Education and Scientific Research

Al – Qasim Green University – College Agriculture

ISSN 2072 - 3875

EUPHRATES JOURNAL OF AGRICULTURE SCIENCE

REFEREED SCIENTIFIC JOURNAL

PUBLISHED BY AGRICULTURE COLLEGE

AL-QASIM GREEN UNIVERSITY

Second veterinary Conference_2017

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Republic of Iraq
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ISSN 2072-3875

EUPHRATES JOURNAL OF AGRICULTURE SCIENCE

Vol. ( ) No. ( )

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