

## Detection of bovine corona virus in some governorate of Iraq

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### Abstract

In the present study we used Reverse transcription polymer chain reaction (RT-PCR) assay for detecting of bovine corona virus ,We evaluated presence of bovine corona virus ( BCoV) in diarrheic fecal samples in age (1 – 30)day . (152) faecal samples from diarrheic calves were collected from four Iraqi governorates (Al-Qadisiya, Babylon ,Wassit and Najaf) .10 (6.57%) out of 152 were positive to bovine corona virus . This study is the first detection of bovine corona antigen in Iraq .The results suggest that RT-PCR is more sensitive than other method to detect BCoV, especially in subclinical cases and Because these animals shed a low amount of virus in faeces and more animal show clinically normal we need to apply sensitive techniques, such as RT-PCR .

**Key word: bovine corona virus, BCoV., RT.PCR**

### Introduction

Bovine Coronavirus (BCoV), a member of the family Coronaviridae, order Nidovirales (1)The Animal coronaviruses are divided into 3 antigenic groups: Group 1 has no hemagglutinin-esterase (HE), and important members of this group are feline infectious peritonitis and transmissible gastroenteritis virus in swine; Group 2 has HE and contains BCoV; and Group 3 contains avian virus-like infectious bronchitis (2) Coronaviridae comprises at least two genera. One, the genus Coronavirus, contains a substantial number of pathogens of mammals and birds that individually cause a remarkable variety of diseases , Bovine coronavirus infections are associated with three distinct clinical syndromes in cattle: calf diarrhea, winter dysentery (hemorrhagic diarrhea) in adult cattle, and respiratory infections in cattle of various ages, including the bovine respiratory disease complex (shipping fever) in feedlot cattle (3) . Coronaviruses were first reported as a cause of diarrhea in calves in the United States in 1973. (4) in other report showed that the Coronaviruses are responsible for a number of economically important diseases. Avian infectious bronchitis virus (IBV) was the first coronavirus to be isolated, from the domestic

fowl, and propagated in the 1930s ( 5). Transmission of infection occurs by the fecal–oral route is the presumed method of transmission but aerosol transmission may also occur. Bovine coronavirus causes diarrhea in both dairy and beef calves world wide ranging in age from 1 day to 3 months of age but mostly between 1 and 2 weeks of age. In young calves Disease is more common during the winter months, which may reflect enhanced survival of the virus in a cool, moist environment. The coronavirus may be present in both diarrheic and healthy calves; the incidence rates range from 8-69% and 0-24% for diarrheic calves and healthy calves, respectively( 6) There are different methods to detect BCoV, but a high degree of sensitivity is required, especially in subclinically infected calves and chronic shedders of BCoV in faeces( 7). The RT-PCR assay is useful to detect small quantities of nucleic acid and is widely used for the diagnosis of infectious disease. The aim of this study to determine the prevalence of bovine coronavirus in instance of calves' diarrhea in some Iraqi governorate.Materials and methods.Collection of sample .A total of (152) fecal samples were collected from diarrheic calves , aged from 1 day to four

weeks this sample collected from four governorate during winter month . for collection of feces , rectal stimulation was made for the calves then collected directly in to disposable closed plastic container , transported under cold condition were the required tests were done or stored at -20 C<sup>0</sup>.RNA extraction .RNA from faeces was extracted using AccurZol virus RNA mini kit (Bioneer)as instructed by the manufacturer. RNA was extracted with TRIzol LS reagent (Invitrogen) according to the manufacturer's instructions. 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 12 000 g 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. RT-PCR master mix Detection of BCoV RNA was carried out using Accurpower ,rocket script RT-PCR virus RNA mini kit (Bioneer)as instructed and primers specific for the N gene protein that are able to detect BCoV. (8). Table(1)

Table(1) : RT-PCR master mix

RT-PCR master mix		Volume
Viral RNA template		2 µL
Primers	F.Primer	1.5 µL
	R.Primer	1.5 µL
PCR water		15 µL
Total		20

Preparation of oligonucleotide primers. The oligonucleotide primers used in the RT-PCR were designed according (9) sequence of N gene of Mebus strain (Gen Bank accession No.M16620). The sequence of primers were as follows: upstream primer: 5'-GCA ATC CAG TAG TAG AGC GT-3' (21-40), downstream primers: 5'-CTT AGT GGC ATC CTT GCC AA-3' (731-750). The predicted RT-PCR product size was 730

bp.RT-PCR thermocycles conditions. Detection of BCoV 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) reverse transcription. The PCR products were detected by electrophoresis through a 1.5% agarose gel and visualisation under UV light after bromide ethidium staining.

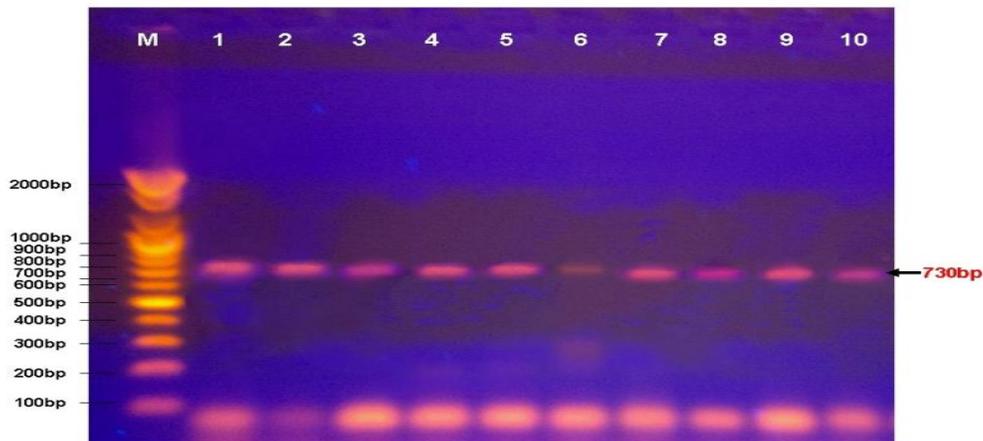
Table(2) RT-PCR thermocycles conditions

Primer step	Universal primer	Number of cycles
cDNA synthesis	45°C 30 min	1
Predenaturation	95°C 5 min	1
Denaturation	95°C 5 min	35
Annealing	57°C 30 sec	35
Extension	72°C 40 sec	35
Final extension	72°C 5 min	1

## Result and discussion

clinical signs of bovine corona virus . In the present study most calves showed different clinical signs , including diarrhea with or without flask of blood ; The feces were pale yellow, mucoid, A yellow to blood-stained mucus, profuse watery diarrhea, and other clinical signs severe dysentery, dehydration, loss of weight ,depression and these signs were similar to study (10). This study showed the presence of coronavirus, in fecal sample from diarrheic and were the causative agents in neonatal calf diarrhea.this results agreed with (11) whom by other techniques detected of bovine corona and rota virus and is agreement with (12) using techniques(one step PCR) to detect bovine corona virus. Detection of corona virus antigen by using one step RT-PC .In the present study we used one step RT-PCR assay for detecting small quantities of BCoV, using a universal primer to detect

and target the 730 bp fragment of the nucleocapsid (N) gene of BCoV and amplify the BCoV strains. We detected the presence of BCoV in diarrheic samples. Figure (1). In the present study a universal published primer for the detection of the N gene( nucleocapsid gene) that was conserved among BCoV strains was used , When we used the one step RT-PCR method we observed that the of the specific gene increased. RT-PCR bovine corona virus was excreted in 6.57% of tested bovine fecal samples from clinically infected calves which is in contrast to other studied from other countries which were as low as (3.9%) to( 8-69%), (13 and 14 ).Figure (1): Agarose gel electrophoresis image showing the positive results of Bovine Corona Virus in RT-PCR, where M: DNA marker (100-2000bp), and 1-10 positive results of BCV primer at specific RT-PCR product size (730bp).



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## تشخيص فايروس bovine corona virus في بعض محافظات العراق

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### الخلاصة

تم في هذه الدراسة استخدام تفاعل سلسلة البلمرة المنعكس للتحري عن وجود فايروسات الكورونا المسببة للاسهال في العجول المولودة حديثا ، حيث تم تقييم وجود فايروسات الكورونا في براز العجول المصابة بالاسهال وفي اعمار تتراوح بين (1 - 30) يوم ، تم جمع 152 عينة براز من اربع محافظات عراقية ( القادسية ، بابل ، واسط ، النجف )، كان 10 (6.57) من بين (152). ان هذه الدراسة تمثل الاولى في العراق لتحديد مستضدات فايروسات الكورونا في العجول المولودة حديثا باستخدام تقنية تفاعل سلسلة البلمرة المنعكس. اظهرت النتائج ان تفاعل سلسلة البلمرة اكثر حساسية من الفحوصات الاخرى لتشخيص فايروس الكورونا ، وخصوصا في الحالات التي لا تظهر عليها علامات سريرية وتطرح الفايروس بكميات قليلة لذلك نحتاج الى تقنية عالية الحساسية مثل تفاعل سلسلة البلمرة المنعكس.

**الكلمات المفتاحية:** فايروسات الكورونا، تفاعل سلسلة البلمرة المنعكس للتحري