Detection of bovine corona viral antigen in neonatal claves with diarrhea in Kalubya Governorate

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Abstract
Neonatal diarrhea is an important cause of economic losses for cattle farms among calves. One of the main viral etiology of enteric diseases is the Bovine Corona Virus (BCoV). In the present study, 192 fecal samples were collected fromdiarrheic and nondiarrheic apparently healthy calves from dairy farms in Kalubeya Governorate. Samples were analyzed using immune captured ELISA and direct fluorescent antibody technique (DFAT) for detection of BCoV.
Corona viral antigens were found in percentage of 68.2% (131/192) by ELISA test and were 24% (6/25) by fluorescent antibody technique .
The results shows that corona virus is wide spread, mainly among neonatal calves up to 1 week of age and among cattle > ١-٢years that may shed virus without clinical symptoms. These results emphasis the significant role of corona virus in neonatal enteric infection in Kaluobya Governorate in Egypt.

Key words: *Corona virus, ELISA, Direct fluorescent anti-body technique (FAT).*

Introduction

Neonatal diarrhea is one of the main causes of calf mortality worldwide. The disease causes major economic losses in many dairy and beef cattle herds that result from treatment costs and calf deaths. Calf diarrhea is considered to be a multifactorial disease. Several environmental, management-related, nutritional, and physiological factors may occur either alone or in synergy with different infectious agents such as protozoan, bacteria, and/or viruses. (**Alfieri *et al.,* (2006); Oliveira Filho *et al.,* 2007).**
Bovine corona virus (BCOV) was first identified as the agent of severe diarrhea in neonatal calves (neonatal calf diarrhea).**(Snodgrass *et al.,* 1986)** as well as the primary etiology of Winter Dysentery (WD) of adult cows. Enteritis, diarrhea, pneumonia, dehydration and some death particular neonatal cases **(Wunschmann *et al;* 2002)**
Heavy losses as a result of corona virus infections in animal husbandry industries are well documented **Cebra *et al .,* 2003)**.
Corona viruses (CoVs) belonging to family **Coronaviridae** within the order Nidovirales, and are classified into 4 groups on the basis of antigenic and genetic properties **(Enjuanes *et al.,* 2000)**.
The virus is a large enveloped, positive-sense single-stranded, RNA viruses with a club-shaped surface **(Derbyshire and Wood, 1978 and Kalica *et al.,* 1978).**
The diagnosis of BCOV infection is usually carried out by identifying the virus infeces because isolation of BCoV in cell culture is difficult **(Clark1993)**.
Distinguishing between different BCoV isolates with monoclonal antibodies is difficult. Most Bovine Corona Viruses isolates and wild ruminant strains can be distinguished on the basis of haemagglutination inhibitiontest using mouse erythrocytes.
Enteric BCoV infections generally are diagnosed by examination of fecal samples or intestinal contents by electron microscopy (EM) or ELISA**.** In respiratory disease, the viral anti-

gen can easily be demonstrated in washed nasal epithelial cells by direct fluorescent antibody test. Demonstrating the antigen in the lower respiratory tract is difficult **(Decaro *et al*., 2008)**

Neutralization test and enzyme-linked immunosorbent assays (ELISA) remain important, although new techniques with great potential, like nucleic acid hybridization, sequencing, and reverse transcription polymerase chain reaction (RT-PCR), are being developed. Detection of corona virus in fecal samples were confirmed with fluorescent antibody technique, ELISA and RT-PCR **(Park *et al.*, 2006)** .

The aim of this study is detection of corona viral antigen in newly born calves of dairy cattle in some dairy farms of Kalubeya governorate by captured ELISA and FAT.

Materials and methods.

Fecal samples: 192 fecal samples were collected from diarrheic calves for detection of corona viral antigen from some dairy farms in Kalubeya Governorate. Fecal samples were diluted and prepared according to manufacture

Table (1). Total Number of collected fecal samples of different ages from different localities in Kalubeya Governorate.

Localeties	Age of calves	No. of collected samples
Toukh	Up to 1 week	80
Kaloub	1-2 weeks	50
Benha	2-4 weeks	47
Shibeen El Kanater	1-2 years	15
	Total	192

Direct fluorescent anti-body technique (FAT).

The luminal surface of intestinal segment was cellular scraped, and 1ml PBS was added. The sample centrifuged at 5000rpm\m for 5 minute. The supernatant was decant and sediment cells were resuspended in 1ml PBS then pipette (50ul) over microscopic slide, dried at 37C, then fixed at aquas acetone ethanol (1:1) over night at 4C,then washed three times with PBS.50ul of Hyperimmune serumlabeledflourescine (flourescine isothianate) for corona virus are added for each slide and incubated at 37C for 1h,follwed by washing of slides with PBS containing 0.1%bovine albumin three times, then mounted in glycerol saline and examined under fluorescent microscope **(Burlesone *et al.,* 1997)**

Immune captured enzyme linked immune sorbent assay (ELISA).

All fecal samples were analyzed using commercial ELISA kits (Bio-x Diagnostic, Belgium), for detection of bovine corona viral antigen in fecal samples. Positive results were expressed by calculation of NET OD of sample =OD of the sample – OD of –ve control Divide the OD of the sample on the OD of positive control x100. Positive sample => 5.97**(Park *et al.,* 2006).**

Results

Table(2) shows Bovine corona viral antigen detected in fecal samples by immune captured ELISA, were 68.2%(131/192)of total examined fecal samples. The examination of total 25 intestinal sediment cells using direct Fluorescent antibody technique revealed that 24% (6/25) samples were positive to corona virus as showed in table (3).

Table (2). Detection of Bovine Corona Viral antigen by using immune captured ELISA.

Total no. of 192 fecal samples	Age	No. of +ve Samples	% of +ve samples	No. of –ve samples	%of –ve samples
80	Up to 1 week	60	41.6%	20	10.4
50	1-2 weeks	35	24%	15	7.8
47	2-4 weeks	29	24.5%	18	9.4
15	1-2 years	7	7.8%	8	4.2
192		131	68.2	61	31.8

Table(3). Results of detection of Bovine Corona Virus by using direct immunoflourescet antibody technique.

Total no. Of samples	No. of +ve samples	%of +ve samples
25	6	24%

Discussion

Bovine coronavirus is now recognized as an important causeof Winter Dysentery in adult cattle because it is always identified in sickanimals **(Lai *et al.,* 2001).** BCoV might play a key role in the occurrence of epidemic diarrhea in herds of adult cattle in the warmer seasons, its precise role in the epizootic diarrhea of adult cattle **(Jeong *et al.,* 2005)**.

A simple and reliable technique is needed for detection of Bovine Corona Virus in clinical specimens. Currently, examination of intestinal contents by Direct Fluorescent antibody Technique (DFAT) and staining of intestinal tissue are the most widely used techniques for the diagnosis of BCOV infection **(Naeemand Goyal, 1988**). Although (DFAT) staining of intestinal tissue isa rapid and simple technique as compared with virus isolation, **(Reynolds *et al.,* 1985)**, direct fluorescent antibody technique (DFAT) and staining of fecal or rectal smears is not as reliable because of the presence of fewer cells in fecal smears and because of non specific fluorescence. This method is laborious and time consuming when done in large scale **(WHO, 1980)**.

In this work, Direct fluorescent antibody technique has been used to stain intestinal cells from infected calves, our studying results revealed that,6 positive cases from 25 which agreed with those obtained by **(Sharawi and Dokhan, 2010)** Out of out of 30 samples7 positive with ELISA test which agreed with **(Park *et al.,* 2006)**.

A simpler method is needed for detection of corona viral antigen in fecal samples of calves. ELISA is reproducible, rapid and quantitative method for detection of corona viral antigen and suited for large scale testing of fecal samples. **(Barros and Borges., 2007)**

The prevalence corona viral antigen was higher by ELISA than Electron microscope (EM) **(Dea and Tijssen., 1988)**.

Table (2) reveled that corona viral antigen in fecal were 68.2% (131/192) that agreed with those by **(Raafat *et al.,* 1994)** (75%) and **(Park *et al.,* 2006)** (58.2%). This result may be related to virus shedding in outbreaks in non vaccinated populations of calves **(Barreiros *et al.,* 2004)**. The presence of BCoV might decrease the intestinal immunity and make corona virus infection easier, even with virus strains that are not usually associated with high pathogenicity **(Brandão *et al.,* 2007and**

Oliveira Filho *et al.*, 2007).
Herd vaccination aims to decrease clinical signs of infection by these enteric pathogens, it is impossible to ascertain if the severity of some bovine diarrhea outbreaks is high due to a more pathogenic virus strain or mixed infection and it is difficult to determine which viral pathogen is more prevalent in vaccinated herds. It is also unknown if the vaccine-induced immune response is maintained in mixed infections. However, prophylactic vaccination against neonatal calfdiarrhea in dairy and beef cattle herds is still recommended to be used and application of sanitary measurementat large scale.
From this study we can advice the owners to fulfill: good hygienic measures, vaccination of the pregnant dams and continuous evaluation of immune state of the flock against corona virus.

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