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ISOLATION OF *BOVINE CORONAVIRUS* (BCOV) IN VERO CELLS AND ITS MOLECULAR CONFIRMATION BY RT-PCR FROM RUMINANTS IN ASSIUT GOVERNORATE

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ABSTRACT

Bovine Coronavirus (BCoV) is prevalent worldwide in young and adult dairy and beef cattle. It is a pneumoenteric virus that causes diarrhea and respiratory infections, resulting in significant financial losses for the cattle industry. Isolation of BCoV is challenging, and studies on virus isolation, molecular detection, and the epidemiology of BCoV, especially from respiratory tract infections in Assiut are limited. So, this study aimed to isolate BCoV, using the Vero cell line, followed by confirmation through RT-PCR. One hundred and sixteen samples (65 fecal and 51 nasal) were collected to isolate BCoV in Vero cells, then confirmed by RT-PCR, targeting the conserved N gene (730 bp). Out of the 116 samples, 27 (11 fecal and 16 nasal) samples exhibited specific CPE (syncytia formation). Sixteen samples (5 fecal and 11 nasal) were confirmed as BCoV positive via RT-PCR for the N gene. The prevalence of BCoV infection was 7.7% (5/65) in fecal samples and 21.6% (11/51) in nasal samples, with infections only observed in cattle. No infections were found in buffaloes, goats, or sheep. Additionally, no significant variation was observed in BCoV infections according to sex, locality, or season factors. In conclusion, the Vero monolayers effectively isolated BCoV, which is major in causing diarrhea and respiratory tract infections in Assiut. Further studies are needed to identify other vulnerable cell lines and confirm variations among the BCoV isolates at both antigenic and molecular levels.

Keywords: *BCoV, Epidemiology, N gene, RT-PCR, Vero cell line.*

INTRODUCTION

Bovine Coronavirus (BCoV) is prevalent among beef and dairy cattle globally. BCoV belongs to the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, and the genus *Betacoronavirus* (Group 2 Coronavirus, subgroup 2A). Like other enveloped viruses, BCoV is susceptible to lipid solvents such as ether and chloroform, as well as detergents. Heat and formaldehyde can readily deactivate it (Berezenko *et al.*, 2022). The BCoV genome is a positive ssRNA measuring between 26 and 32 kilobases. In addition to several non-

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structural proteins (NSPs), such as polymerase (Pol), it encodes several structural proteins, such as the nucleocapsid (N), membrane (M), hemagglutinin-esterase (HE), and spike (S) proteins. The BCoV's S, HE, and N proteins are essential for infection, replication, and pathogenesis (Lotfollahzadeh *et al.*, 2020).

It is among the largest RNA viruses, with a dual preference for intestine and pulmonary epithelial cells. Decreasing production in the meat and dairy industries, increasing mortality in young livestock, and driving up treatment costs, BCoV results in large economic losses. It is associated with winter dysentery (WD) in adult dairy cattle, neonatal calf diarrhea (CD) in young calves, and bovine respiratory disease complex (BRDC) (Wojciech *et al.*, 2022). CD primarily affects both dairy and beef cattle within the first three weeks of life, leading to severe and often bloody diarrhea. WD impacts adult dairy cattle, resulting in decreased milk production and significant economic losses. BRDC, related to various viral and bacterial pathogens, affects growing and feedlot calves, reducing their productivity and increasing the risk of secondary bacterial infections (Jakob *et al.*, 2020).

Even though there are some BCoV vaccinations available, reports of continuous viral infection outbreaks in cow populations around the world have raised awareness of the coronavirus's primary antigenic characteristics and the significance of laboratory isolation. However, it is difficult to isolate coronaviruses, which restricts our comprehension of their antigenic characteristics (Shah *et al.*, 2024).

Following its first isolation in the bovine kidney's primary culture (Mebus *et al.*, 1973), BCoV was subsequently propagated in several cell lineages: Vero (African green monkey kidney), MDBK (Madin-Darby bovine kidney), BEK-1 (bovine embryonic kidney), and HRT-18 (human rectal tumor) (Jerez *et al.*, 2005). The Vero cell is preferred for isolation because it produces a consistent cytopathic effect (CPE), which is characterized by gradual cell rounding progressed to cell fusion (syncytia) and monolayer detachment. RT-PCR, which targets the N gene, is frequently used to molecularly confirm the presence of BCoV in the infected tissue cultures. This gene, which is highly conserved across BCoV strains, is the most common gene in coronavirus-infected cells (Moussa *et al.*, 2021).

Studies in the epidemiology, isolation, and molecular detection of BCoV in Assiut, Egypt, are scarce. Although its association with respiratory diseases in Assiut has not been thoroughly investigated. However, its role in causing diarrhea, particularly in calves, is well-documented. Thus, the purpose of this study was to use the Vero cell line to isolate the BCoV that causes respiratory infections and diarrhea in Assiut and its molecular validation using RT-PCR. Furthermore, this study provided epidemiological data regarding the current situation of BCoV infections in Assiut.

MATERIALS AND METHODS

1. Ethical approval

Following the OIE guidelines for the use of animals in research, this work has been approved by the Faculty of Veterinary Medicine's Ethical Committee at Assiut University in Assiut, Egypt, under Approval No. (06/2024/0238).

2. Sampling

Samples were collected from 2022 to 2024. One hundred and sixteen feces (5 grams) and nasal swabs were collected from infected ruminants ranging in age from 7 days to 5 years from various Assiut Governorate regions that showed specific BCoV clinical symptoms, such as respiratory tract infections and diarrhea. The ruminants were 94 cattle, 6 buffaloes, 11 sheep, and 5 goats.

3. Specimens processing

Fecal samples (1 g) were diluted 1:10 in phosphate-buffered saline (PBS), and nasal swabs were suspended in 2 mL of PBS as part of the subsequent processing of the specimens. After 20 minutes of room temperature incubation, each sample was centrifuged for 10 minutes at 10000 rpm. After centrifugation, each sample's supernatant was stored for later examination at -80°C (Sevinc Temizkan and Alkan, 2021).

4. Virus isolation

A membrane filter measuring 0.22 µm was used to filter the samples. According to Hansa *et al.* (2013), these filtrates were then utilized to isolate BCoV using the Vero cell line (Vero-1008, ATCC-CCL-81), that was graciously obtained from the tissue culture unit (T.C), VACSERA, Egypt, and cultivated in 25 cm2 flasks. In short, 1 mL of the post-filtered sample was put onto the Vero monolayer and incubated for 1 hour at 37°C with 5% CO2. Cells were maintained on a maintenance medium that contained Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen, CA, USA), as well as 2% fetal bovine serum (Gibco, Invitrogen, CA, USA) after incubation. Cells were examined for any cytopathic effects (CPE) every day. The positive control virus was obtained from the Veterinary Serum and Vaccine Research Institute (SERVAC) in Egypt.

5. Molecular confirmation of BCoV by RT-PCR

RNA Extraction and Reverse Transcription

A QIAamp mini elute virus spin kit (Qiagen, Germany, GmbH, Cat. No. 52904) was used to extract viral RNA from the infected tissue cultures following the manufacturer's instructions. Ultimately, 100 µl of elution buffer was used to elute the nucleic acid. Following the kit's instructions, the RevertAid First Strand cDNA Synthesis Kit (Thermo ScientificTM, Cat. No. K1622, USA) was used to reverse-transcribe purified RNA.

For confirmation of BCoV, the established oligonucleotide primer sequence (F: 5′-GCA ATC CAG TAG AGC GT-3, genomic position 21-40 and R: 5′-CTT AGT GGC ATC CTT GCC AA-3, genomic position 731-750) were utilized to amplify the viral N gene, the conserved portion in the Mebus strain's genome, to a particular product size of 730 bp. The PCR condition was previously described as follows (Alkan *et al.*, 2011): Following a 5-minute initial denaturation at 94°C, cDNAs were subjected to 40 cycles, each of which included three steps: 45 seconds of denaturation at 94°C, 45 seconds of primer annealing at 58°C, and 1.5 minutes of extension at 72°C. This was followed by a final extension step at 72°C for 10 minutes. A 1.5% agarose gel was used to visualize the PCR results (AppliChem, Germany, GmbH). The fragment sizes were measured using a 100 bp DNA ladder (Fermentas, Thermo, Germany, Cat. No. SM0243). A gel documentation system (Biometra, Alpha Innotech, USA) was used to scan the gel, and computer software was used to analyze the results.

6. Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 16 software was used to perform the statistical analysis (2007). The Chi-square test was employed to evaluate the statistical distinctions between different variables.

RESULTS

1. Clinical finding

Clinical findings revealed that the ruminants under investigation displayed various digestive and/or respiratory symptoms, such as diarrhea, dehydration, weakness, and reluctance to move, and some were found lying down and appearing comatose. The diarrheal calves' feces varied in consistency from normal to extremely

watery. Some samples had mucus, undigested food, and clotted blood, and the fecal discharge ranged in color from pale yellowish to greenish. Along with enteritis, signs of respiratory distress, including nasal discharge, coughing, and difficulty breathing were also observed.

2. Isolation of BCoV in Vero cell line

The adaptation in Vero cells was evidenced by characteristic CPE (syncytium formation). The inoculated flasks were inspected daily. Positive control cells exhibited distinct specific CPE, while negative control cells showed no CPE. The observed BCoV CPE began with rounding 24 hours after inoculation, followed by granular, swollen, or enlarged cells within 48 hours. Following vacuolation and clumping of the expanded cell membranes at 72 hours, these membranes joined to form syncytia at 96 hours, indicating a viral infection, and eventually detached with the progression of CPE (Figure 1). This study revealed that 27 out of 116 samples (11 fecal and 16 nasal samples) showed syncytium formation (Table 1).

3. Molecular confirmation of BCoV by RT-PCR

The characteristic 730 bp band of the BCoV-specific N gene was detected by the RT-PCR testing **(**Figure 2) in 16 out of 27 specimens that exhibited CPE in Vero cells. The positivity rates for the fecal and nasal discharge samples were (5/11) and (11/16), respectively (Table 1).

4. Epidemiological findings

a- Percentage of BCoV infection among ruminant animals

Only cattle in this investigation had the BCoV infection but buffaloes, sheep, and goats did not have the virus (Table 2). In cattle, the percentage of BCoV infection was 28.9% (11/38) detected in nasal samples and 8.9% (5/56) in fecal samples.

b- Effect of sex

According to the findings, the rate of BCoV infection in females was 8.1% (3/37) and in males was 7.1% (2/23) in fecal samples, while in nasal samples, the rate of infection was 23.3% (7/30) in males and 19% (4/21) in females, with insignificant differences.

c- Age susceptibility

The prevalence of BCoV infection was examined across various age groups (<1 month, 1-3 months, 3-6 months, 6-12 months, and > 1 y). The infection was found in the fecal samples in only two groups: >1 month (8.9%) and 1-3 months (11.1%). In the nasal samples, the infection was detected in the following age groups: under 1 month (33.3%), 1-3 months (9%), 3-6 months (13.3%), and over 1 year (28.6%) with insignificant variations.

d- Seasonal variation

According to our findings, diarrhea and respiratory tract infections were more prevalent during the colder months than during the warmer ones. For fecal samples, the BCoV infection rate was 4% during hot months and 10% during cold months. Infection rates for nasal samples were 16.7% during the hot season and 22.2% during the cold season.

e- locality

The percentage of BCoV in fecal samples was 5% (1/20) from cattle at the Faculty of Agriculture farm, 8.3% (3/36) on the Abnoub El-Hamam farm, and 11.1% (1/9) from individual cases at the Veterinary Teaching Hospital. In nasal samples, the infection rate was 23.5% (4/17) at the Faculty of Agriculture farm, 21.7% (5/23) on the Abnoub El-Hamam farm, and 18.2% (2/11) from individual cases at the Veterinary Teaching Hospital.

Table 1: Positive results of BCoV by Vero cell line and RT-PCR.

	Positive No. (%)		
	Fecal samples No. (%)	**Nasal swabs No. (%)**	**Total No. (%)**
Vero Cells Inoculation	11 (16.9)	16 (31.4)	27 (23.3)
RT-PCR	5 (7.7)	11 (21.6)	16 (13.8)
Total	**65**	**51**	**116**

Table 2: Epidemiological findings of BCoV in this study according to RT-PCR result.

Epidemiological data	**Fecal samples**			**Nasal samples**		
	No of samples	**No of positive (%)**	**P value**	**No of samples**	**No of positive (%)**	**P value**
Species			**0**			
Cattle (n=94)	**٥6**	**5 (8.9)**		**38**	**11(28.9)**	
Buffalo (n=6)	**٤**	**0 (0)**	**-**	**2**	**0 (0)**	**-**
Sheep (n=11)	**3**	**0 (0)**		**8**	**0 (0)**	
Goat (n=5)	**٢**	**0 (0)**		**3**	**0 (0)**	
Sex						
Male	**٢٨**	**2 (7.1)**	**0.885**	**30**	**7 (23.3)**	**0.714**
Female	**٣٧**	**3 (8.1)**		**21**	**4 (19)**	
Age			**0.**			
<1 m	**٤٥**	**4 (8.9)**	**0.834**	**18**	**6 (33.3)**	
1-3 m	**٩**	**1 (11.1)**		**11**	**1 (9)**	**0.349**
3-6 m	**٦**	**0 (0)**		**15**	**2 (13.3)**	
6-12 m	**٣**	**0 (0)**		**0**	**0 (0)**	
>1 y	**٢**	**٠ (0)**		**7**	**2 (28.6)**	
Season						
Cold months**	**٤٠**	**4 (10)**	**0.377**	**45**	**10 (22.2)**	**0.756**
Hot months**	**٢٥**	**1 (4)**		**6**	**1 (16.7)**	
locality						
Faculty of Agriculture farm	**٢٠**	**1 (5)**	**0.830**	**17**	**4 (23.5)**	**0.945**
Abnoub El-Hamam farm	**٣٦**	**3 (8.3)**		**23**	**5 (21.7)**	
Individual cases	**٩**	**1 (11.1)**		**11**	**2 (18.2)**	
Total	**٦٥**	**5 (7.7)**		**51**	**11 (21.6)**	

****Cold months in Assiut are from October to February, and hot months are from March to September**

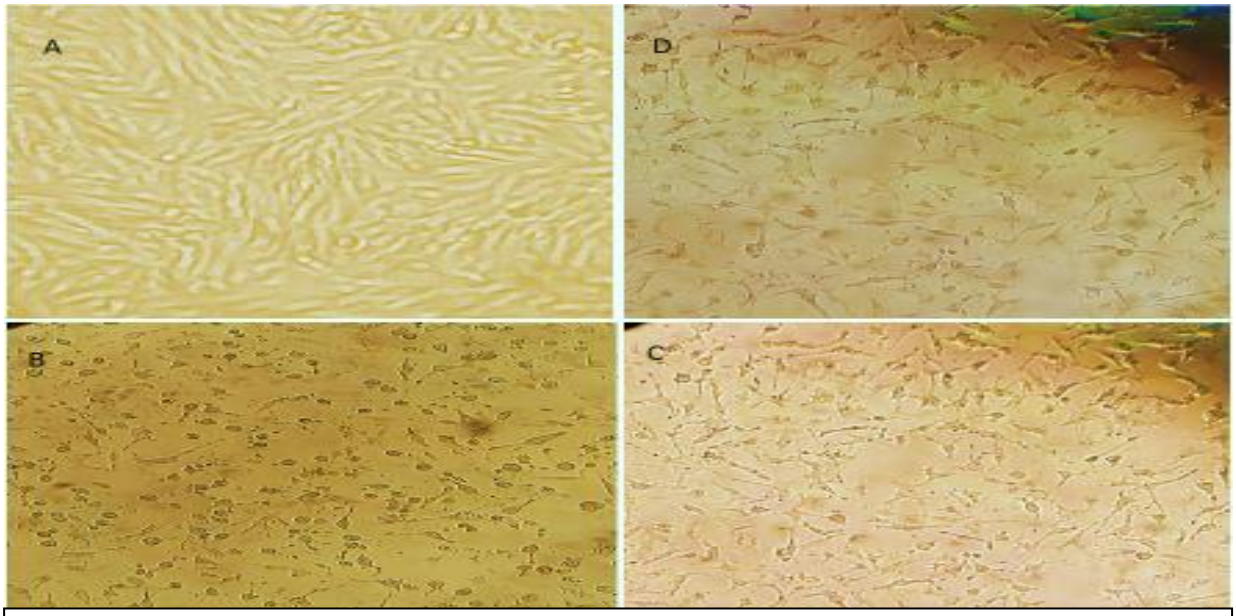

Fig. (1): At 100X magnification power, the following microscopic images show BCoV CPE in Vero cells: image A shows control, uninfected Vero cells; image B shows granulation and rounding of cells 24 Hours.; image C shows swelling and syncytium development 96 Hours.; and image D shows cell lysis and detachment 5 days after inoculation.

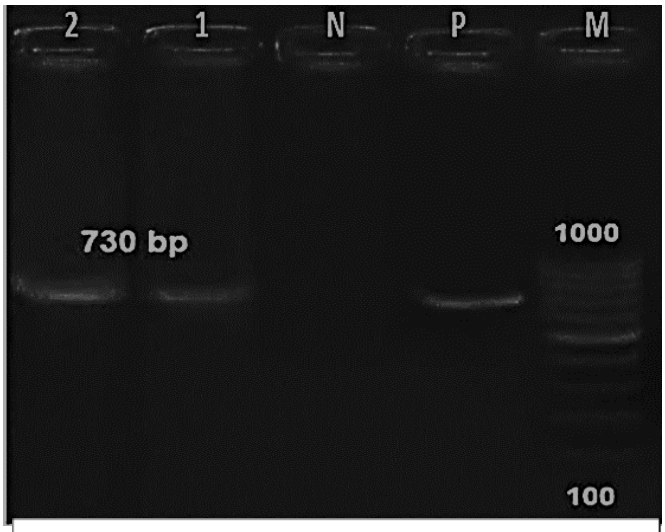

Fig. (2): Agarose (1.5%) gel electrophoresis showing amplification of a 730-bp fragment of the N gene. Lane M: DNA ladder marker (100 - 1000 bp), Lane P: positive control (Calfgaurd® vaccinal strain), Lane N: negative control, Lanes 1,2 are positive specimens.

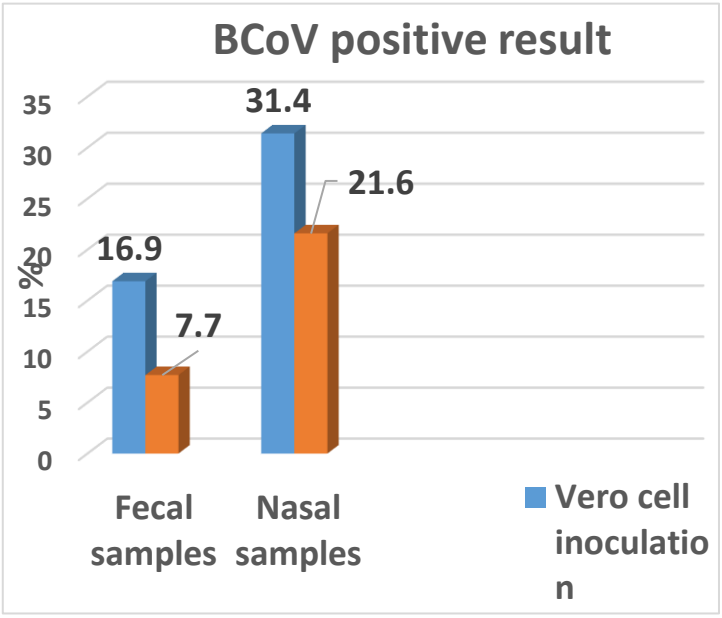

Fig. (3): Positive results of BCoV by Vero cell line and RT-PCR

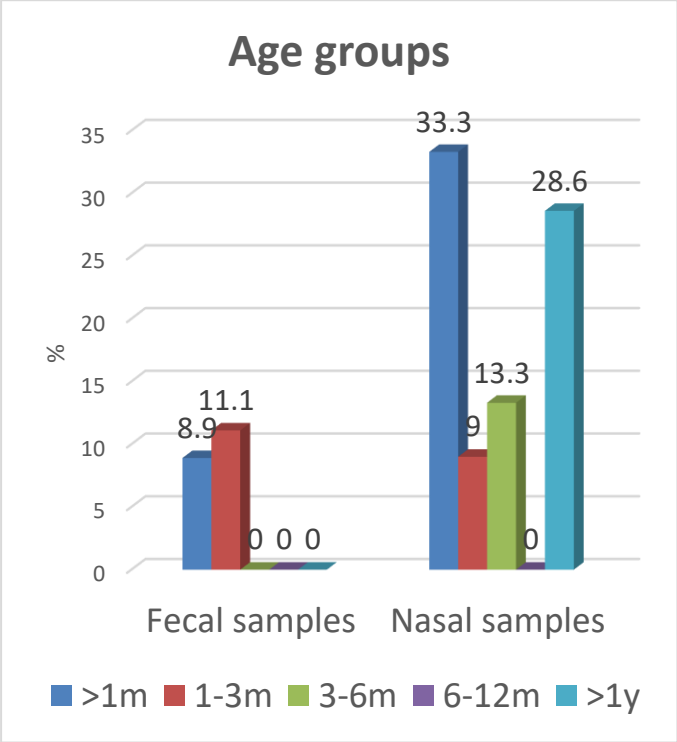

Fig. (4): Age-related BCoV susceptibility in fecal and nasal samples.

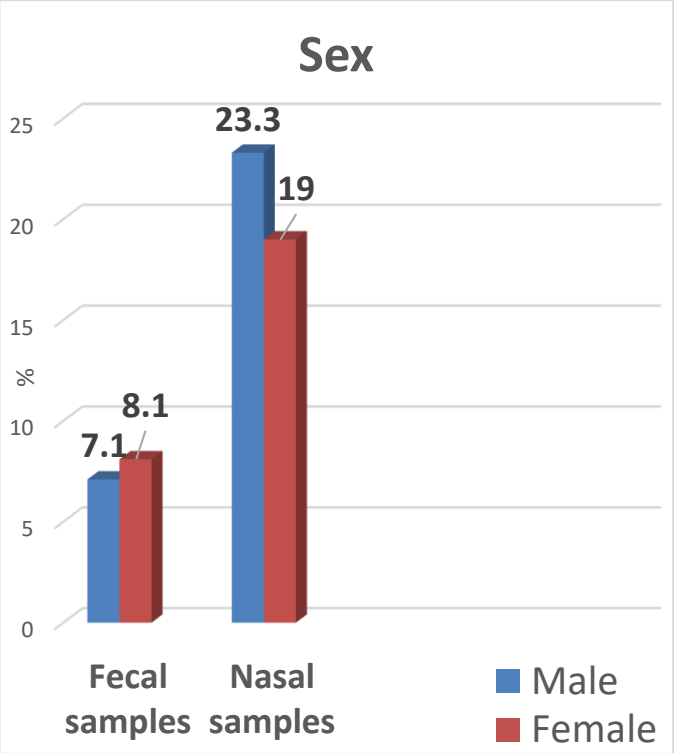

Fig. (5): The infection rate of BCoV in fecal and nasal samples based on sex differences.

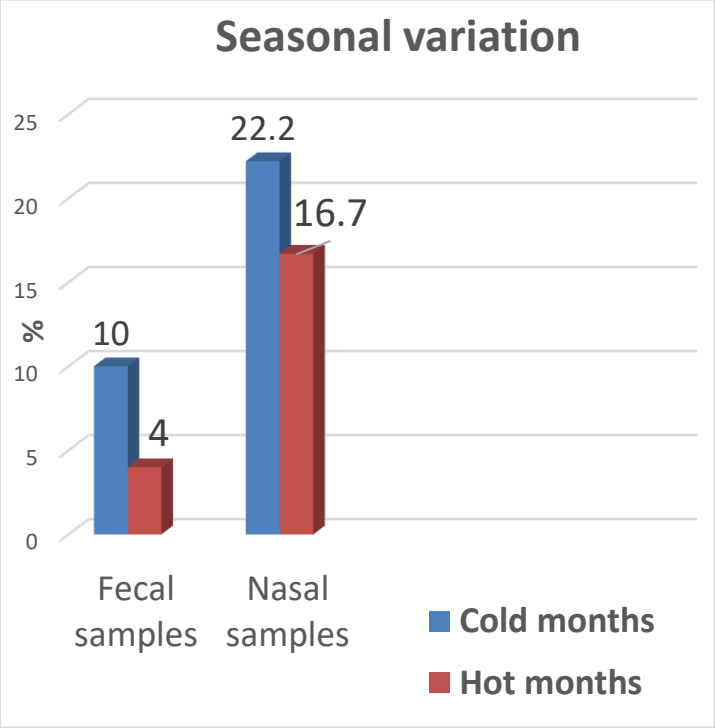

Fig. (6): The rate of BCoV infection in fecal and nasal samples according to seasonal variations.

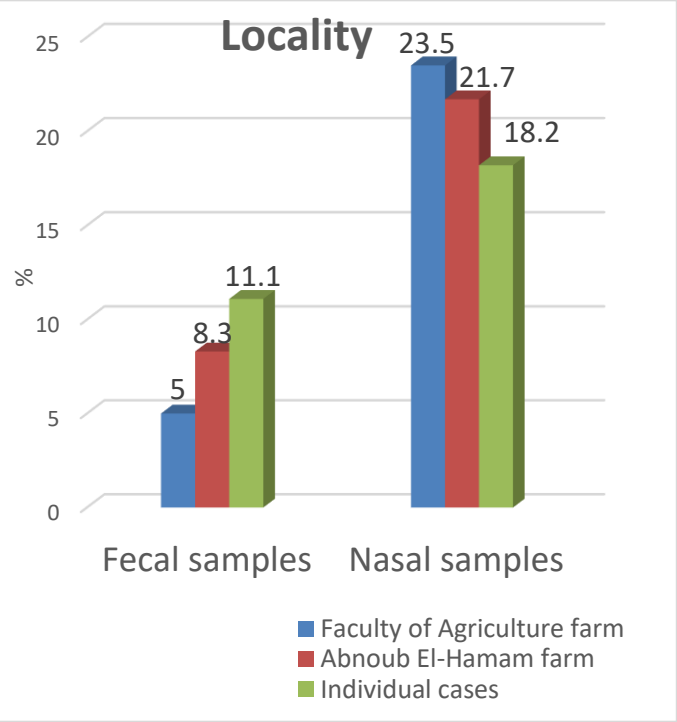

Fig. (7): The infection rate of BCoV in fecal and nasal samples based on locality.

DISCUSSION

Bovine coronavirus (BCoV) has dual tropism, causing both enteritis and respiratory tract infections. This results in significant economic losses due to treatment costs, reduced growth rates, and mortality, particularly in young animals. In livestock farms, enteric BCoV causes mortality in young calves, as it infects both the large and small intestines. (Çelik and Kozat, 2024). Respiratory-type BCoV has been found in growing calves and adult cows. According to Yilmaz *et al.* (2024), BCoV can cause concurrent infections with other pathogens, such as *Pasteurella multocida* and other respiratory pathogens, which can lead to severe pneumonia and a respiratory disease complex. BCoV has been known to induce fatal respiratory diseases in young cattle. The virus can infect both adult cows and calves, and infected animals frequently transfer the virus through their feces and nasal secretions (Vlasova and Saif, 2021).

There have been few studies on BCoV in Assiut, and they have mostly concentrated on calves' diarrhea while ignoring respiratory tract infections. So this study detected BCoV in both diarrhea cases and respiratory tract infections in young and adult cattle. Of the 116 samples that were included, 65 were from animals that had diarrhea and 51 were from animals who had respiratory tract infections.

Different diagnostic methods are employed to detect BCoV in various clinical samples. However, at the antigenic and genomic levels, tissue culture virus isolation is still the gold standard. Isolating BCoV in cell culture is regarded as being challenging, and typically requires a skilled analyst with specialized training. As a result of the difficulties associated with BCoV isolation in cell cultures, few reports exist on this virus's isolation. Nonetheless, these isolated BCoV strains can be beneficial for *in vivo* pathological research in biological models, including experimental infections (van den Hurk *et al.*, 2024).

In accordance with research by Clark (1993) and Tsunemitsu and Saif (1995), BCoV may be readily cultivated on a variety of cell lines, including the Vero cell line. Since the Vero cells consistently induce CPE in the form of syncytia cell rounding, and monolayer detachment, it had been specifically selected for isolation in this investigation. The results of El-Kenawy *et al.* (2019), Moussa *et al.* (2021), and previous studies by Tektoff *et al.* (1983) were all in agreement with the CPE that was developed.

In this study, 27 test samples (11 fecal and 16 nasal samples) showed the characteristic CPE (in the form of syncytia). Then to detect the BCoV RNA in the culture supernatant, RT-PCR that targets the BCoV N gene was used, which is highly conserved among BCoV strains. The N gene is also known to be the most common gene in coronavirus-infected cells because it includes the smallest RNA template and the most sgRNA (subgenomic RNA) during transcription. This indicates that compared to other BCoV genes, the N gene has more RNA accessible. The detection of the N gene RNA may be beneficial due to its abundance in cells. This may raise the sensitivity of the diagnostic method (Hofmann *et al.*, 1990, Takiuchi *et al.*, 2006).

Out of the eleven fecal samples and sixteen nasal samples that demonstrated CPE in the Vero monolayer cell culture, five fecal samples, and eleven nasal samples were identified by RT-PCR as BCoV. This could be explained by the presence of other viruses capable of inducing similar CPE. For instance, it is noteworthy that Rotavirus infection in Vero cells manifests as cellular rounding, granulation, and syncytial CPE (Sharawi and DOKHAN, 2010). Similarly, the Peste des Petits Ruminants (PPR) virus also causes CPE, which usually starts with cell rounding, aggregation, and then syncytial formation (Hegde *et al.*, 2009). Also, the Bovine Respiratory Syncytial Virus (BRSV) CPE begins with cell

rounding and continues to syncytia formation 3–4 days after infection (dpi) (Spilki *et al.*, 2006).

The study's overall BCoV prevalence of 13.8% indicates that *Beta coronavirus 1* BCoV has a significant role in the epidemiology of respiratory tract infections and diarrhea cases in Assiut, Egypt. This prevalence is higher than that of a previous study conducted in the same area, which found that 2.2% (2/91) of the tested enteric calves had BCoV (Zaitoun *et al.*, 2018). This disparity may be attributed to diminishing passive immunity resulting from diverse factors and the absence of natural resistance to infection owing to the lack of established vaccination protocols (Lanz Uhde *et al.*, 2008, Moussa *et al.*, 2021).

The prevalence of BCoV in the fecal samples was 7.7%. Several research have shown similar outcomes. For example, research by Lotfollahzadeh *et al.* (2020) in Iran found that 7.2% of neonatal calves had coronavirus in their fecal samples. Additionally, Çelik and Kozat (2024) detected BCoV 7 (7.29%) among the 96 diarrheic calves. On the other hand, Bok *et al.* (2015) used an ELISA test and found a higher incidence of the virus in diarrhea cases of dairy calves in Argentina, at 12.13% (29 out of 239). Using sandwich antigen ELISA, Singh *et al.* (2022) reported that 7 (0.85%) of 816 infected dairy calves in Central and North India that had diarrhea under the age of 3 months tested positive for BCoV.

The positive rate for BCoV in nasal samples was 21.6%. This result is similar to a previous study in Northeast China by Zhu *et al.* (2022), which found BCoV in 21.53% (79/367) of nasal swab samples. In contrast, other studies have reported a higher occurrence of coronavirus in nasal samples. For example, a study by Sevinc Temizkan and Alkan (2021) in Turkey detected BCoV in 33.3% of respiratory cases. On the other hand, a lower prevalence than our study has been suggested by other studies, including the study by Wojciech *et al.* (2022) in Poland, which reported a prevalence of 10.5%.

The varying rates of BCoV infection in cattle may be attributed to differences in sample collection duration, sample size, farm management practices, hygiene conditions, environmental factors, diagnostic techniques, and the amount and quality of colostrum that calves consumed in the initial hours following calving (Ammar *et al.*, 2014).

From the epidemiological view, BCoV infections were observed only among cattle (8.9% in fecal samples and 28.9% in nasal samples). No infection appeared among buffaloes and small ruminants. This could be due to the few samples taken from these animals and their lower susceptibility, as well as differences in their immune status that need to be ascertained in large-scale studies. These findings were consistent with the earlier research conducted in Assiut and published by Zaitoun *et al.* (2018), who found that buffalo calves had no coronavirus infection, while cattle calves had an infection rate of 2.25 %. However, Abou El-Ella *et al.* (2013) found that BCoV was present in 5.13% of buffalo calves and 8.45% of cow calves. Furthermore, a study conducted in Ghana by Burimuah *et al.* (2020) found BCoV in 1 out of 1,328 cattle, 3 out of 66 goats, and no sheep.

Analysis of the impact of the animals' sex on the rate of BCoV infection revealed no discernible difference in the rates of coronavirus infection in male and female cattle. This finding is consistent with research conducted by Zaitoun *et al.* (2018) and Yavru *et al.* (2016). This could be explained by the early biological, functional, and hormonal resembles between the male and female bodily systems, which lead to a similar resistance to coronavirus infection. However, the rate and severity of infection in both sexes of cattle, whether at the same or different ages, are influenced by the level of virus

contamination, the virus dose, and exposure to stressors (Yavru *et al.*, 2016).

The animal age had an impact on the infection susceptibility of cattle. Only two age groups had the microorganism in their fecal samples. These groups were >1 month (8.9%) and 1-3 months (11.1%). These findings were in line with earlier findings by Davoudi *et al.* (2014) and Lotfy *et al.* (2020), who found that enteric BCoV was more common in young calves. The following age groups had the infection found in the nasal samples: those under 1 month (33.3%), those 1-3 months (9%), those 3-6 months (13.3%), and those over 1 year (28.6%). This supports the claim that the respiratory-type bovine coronavirus has been described in growing calves and adults (Liu LiHong *et al.*, 2006).

Regarding the impact of seasonal fluctuations on the incidence of BCoV infection, prior research has demonstrated that the virus frequently manifests during the winter months as a result of cold stress, including chilling from low temperatures. Cold stress is a significant risk factor for winter dysentery, since it can be caused by drinking cold water or experiencing extreme temperature swings (Saif, 1990). According to this study, cattle had a high percentage of BCoV (10%) in the cold months and low (4%) during the hot months. These findings align with the research of Zaitoun *et al.* (2018), who found that 3.45% of cattle calves had enteric coronavirus infections only during the cold months. They explained this finding by pointing out that the majority of calvings in Egypt take place at the end of autumn and the beginning of winter, when these newborn calves are more vulnerable to enteric coronavirus infections. Moreover, intestinal and serum immunoglobulin levels increased in the spring and summer and decreased in the fall and winter. Additionally, because of its envelop nature, which made it somewhat sensitive to heat, the BCoV remained stable during the cold months, while the coronavirus spread easily during the winter.

Cattle that are infected can release BCoV into the environment, and the virus can survive for 120 hours in low temperatures and high relative humidity, and for at least 96 hours in feces (Chan *et al.*, 2009). Conversely, other research has demonstrated that there is no correlation between low temperatures and diarrhea caused by BCoV (Chouljenko *et al.*, 2001, Li *et al.*, 2024). In South Korea, for instance, Park *et al.* (2006) found that BCoV is endemic in adult cattle having diarrhea throughout the year. They also suggested that the virus is endemic in adult cattle that have diarrhea during the warmer months. This might be because the BCoV's biological characteristics have changed, perhaps increasing the virus's ability to survive in warmer climates.

Regarding location, the percentage of coronavirus infection in the cattle analyzed at the Veterinary Teaching Hospital and the Assiut Governorate farms did not differ significantly in the current study. This lack of variation may be explained by the calves' similar upbringing in the same geographic and seasonal circumstances, with the same management systems, hygienic practices, and animal husbandry techniques. This outcome is consistent with the Zaitoun *et al.* (2018) investigation.

In conclusion, this study found that the Vero cell line can be used to isolate BCoV. The BCoV produced distinct CPE in Vero cells up to five days after infection. Furthermore, BCoV was detectable in Vero-infected cells using RT-PCR. However, additional research is required to isolate different strains, find additional vulnerable cell lines, and validate differences between isolates at the genomic and antigenic levels.

REFERENCES

A Abou El-Ella, G.; M Mohamed, A. and A Aamer, A.J.A.V.M.J. (2013): Prevalence of enteropathogens associated with neonatal calf scour in cattle and buffalo calves using (FASTEST® Strips) rapid field test. 59**,** 19-26.

Alkan, F.; Ozkul, A.; Bilge-Dagalp, S.; Karaoglu, T.; Oguzoglu, T.; Caliskan, E.; Burgu, I.J.T. and Diseases, E. (2011): The detection and genetic characterization based on the S1 gene region of BCoVs from respiratory and enteric infections in Turkey. 58**,** 179-185.

Ammar, S.S.M.; Mokhtaria, K.; Tahar, B.B.; Amar, A.A.; Redha, B.A.; Yuva, B.; Mohamed, H.S.; Abdellatif, N. and Laid, B.J.A.P.J.O.T.B. (2014): Prevalence of rotavirus (GARV) and coronavirus (BCoV) associated with neonatal diarrhea in calves in western Algeria. 4**,** S318-S322.

Berezenko, A.; Vabishchevych, F.; Godovskyi, O. and Nedosekov, V.J.A.B. (2022): Investigation of bovine coronavirus strain CV-315 cultural properties. 24, 7.

Bok, M.; Miño, S.; Rodriguez, D.; Badaracco, A.; Nuñes, I.; Souza, S.P.; Bilbao, G.; Uriarte, E.L.; Galarza, R. and Vega, C.J.V.M. (2015): Molecular and antigenic characterization of bovine Corona-virus circulating in Argentinean cattle during 1994–2010. 181**,** 221-229.

Burimuah, V.; Sylverken, A.; Owusu, M.; El-Duah, P.; Yeboah, R.; Lamptey, J.; Frimpong, Y.O.; Agbenyega, O.; Folitse, R. and Emikpe, B.J.B.V.R. (2020): Molecular-based cross-species evaluation of bovine coronavirus infection in cattle, sheep and goats in Ghana. 16**,** 1-7.

Çelik, V. and Kozat, S.J.J.O.I.V.S. (2024): Prevalence of rotavirus, coronavirus, Cryptosporidium spp, Escherichia coli K99 and Giardia lamblia in neonatal calves with diarrhea in Burdur and its districts. 8**,** 92-91.

Chan, C.M.; Lau, S.K.; Woo, P.C.; Tse, H.; Zheng, B.-J.; Chen, L.; Huang, J.-D. and Yuen, K.-Y.J.J.O.V. (2009): Identification of major histocompatibility complex class IC molecule as an attachment factor that facilitates coronavirus HKU1 spike-mediated infection. 83**,** 1026-1035.

Chouljenko, V.N.; Lin, X.; Storz, J.; Kousoulas, K.G. and Gorbalenya, A.E.J.J.O.G.V. (2001): Comparison of genomic and predicted amino acid sequences of respiratory and enteric bovine coronaviruses isolated from the same animal with fatal shipping pneumonia. 82**,** 2927-2933.

Clark, M.J.B.V.J. (1993): Bovine coronavirus. 149**,** 51-70.

Davoudi, Y.; Nourmohammadzadeh, F.; Abdollahpour, G.; Nouri, A. and Nowrouzian, I.J.I.J.O.V.M. (2014): The prevalence of coronavirus in fecal samples of neonatal calf diarrhea using electron microscopic examination. 8**,** 85-89.

El-Kenawy, A.; Shahein, A. and Yassen, A.J.M.V.M.J. (2019): Isolation and identification of Bovine coronavirus (BCoV) from diarrheatic calves in Dakahlia Governorate, Egypt. 20**,** 27-34.

Hansa, A.; Rai, R.; Dhama, K.; Wani, M.; Saminathan, M. and Ranganath, G.J.P.J.O.B.S.P. (2013): Isolation of bovine coronavirus (bcoV) in vero cell line and its confirmation by direct FAT and RT-PCR. 16**,** 1342-1347.

Hegde, R.; Gomes, A.R.; Gowda, S.B.; Santhosh, A. and Renukaprasad, C.J.V.W. (2009): Cytopathic effect of PPR vaccine virus strains in Vero cells. 2**,** 93.

Hofmann, M.A.; Sethna, P.B. and Brian, D.A.J.J.O.V. (1990): Bovine coronavirus mRNA replication continues throughout persistent infection in cell culture. 64**,** 4108-4114.

Jakob, H.J.; Jožica, J.; Jože, S.J.T.A.H. and Production (2020): Coronaviruses in cattle. 52, 2809-2816.

Jerez, J.A.; Gregori, F.; Brandão, P.E.; Rodriguez, C.A.R.; Ito, F.H.; Da Glória Buzinaro, M. and Sakai, T. (2005): Isolation of bovine coronavirus (BCoV) in monolayers of HmLu-1 cells Isolamento de coronavírus bovino (BCoV) em monocamada de cultura de células da linhagem HmLu-1.

Lanz Uhde, F.; Kaufmann, T.; Sager, H.; Albini, S.; Zanoni, R.; Schelling, E. and Meylan, M.J.V.R. (2008): Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. 163**,** 362-366.

Li, S.; Huang, J.; Cai, X.; Mao, L.; Xie, L.; Wang, F.; Zhou, H.; Yuan, X.; Sun, X. and Fu, X.J.V.S. (2024): Prevalence and Evolutionary Characteristics of Bovine Coronavirus in China. 11**,** 230.

Liu Lihong, L.L.; Hägglund, S.; Hakhverdyan, M.; Alenius, S.; Larsen, L. and Belák, S. (2006): Molecular epidemiology of bovine coronavirus on the basis of comparative analyses of the S gene.

Lotfollahzadeh, S.; Madadgar, O.; Reza Mohebbi, M.; Reza Mokhber Dezfouli, M.; George Watson, D.J.V.M. and Science (2020): Bovine coronavirus in neonatal calf diarrhoea in Iran. 6, 686-694.

Lotfy, A.; Selim, A.; Ibrahim, A.M.M. and Salem, S.J.B.V.M.J. (2020): Seroprevalence and Molecular characterization of Bovine viral diarrhea, Rota and corona viruses in neonatal cattle and buffalo calves in some governorates in Egypt. 38**,** 5-9.

Mebus, C.; Stair, E.; Rhodes, M. and Twiehaus, M. (1973): Neonatal calf diarrhea: propagation, attenuation, and characteristics of a coronavirus-like agent.

Moussa, S.; Afify, A.; Salah, S.; Hamed, A.J.H. and Viruses (2021): Isolation and molecular characterization of bovine coronavirus from calves with acute gastroenteritis in Egypt. 8**,** 29-38.

Park, S.-J.; Jeong, C.; Yoon, S.-S.; Choy, H.E.; Saif, L.J.; Park, S.-H.; Kim, Y.-J.; Jeong, J.-H.; Park, S.-I. and Kim, H.-H.J.J.O.C.M. (2006): Detection and characterization of bovine coronaviruses in fecal specimens of adult cattle with diarrhea during the warmer seasons. 44**,** 3178-3188.

Saif, L. (1990): A review of evidence implicating bovine coronavirus in the aetiology of winter dysentery in cows: an enigma resolved?

Sevinc Temizkan, S. and Alkan, F.J.A.O.V. (2021): Bovine coronavirus infections in Turkey: molecular analysis of the full-length spike gene sequences of viruses from digestive and respiratory infections. 166**,** 2461-2468.

Shah, A.U.; Gauger, P.C. and Hemida, M.H.J.B. (2024): Isolation and molecular characterization of an enteric isolate of the Genotype-I Bovine coronavirus with notable mutations in the receptor binding domain of the spike glycoprotein and deletion downstream the RNA binding domain of the nucleocapsid protein.2024.08. 03.606496.

Sharawi, S. and DOKHAN, K.Z.J.A.V.M.J. (2010): Detection And Isolation Of Rota (Group A) And Corona (Group 2) Viruses From Nomadic Camels With Special Reference To Pathological Alterations. 56**,** 1-19.

Singh, S.; Singh, R.; Singh, K.; Singh, V.; Malik, Y.; Kamdi, B.; Singh, R. and Kashyap, G.J.B.R.R. (2022): Prevalence of bovine coronavirus infection in organized dairy farms of Central and North regions, India. 53**,** 351-357.

Spilki, F.R.; De Almeida, R.S.; Campalans, J. and Arns, C.W.J.J.O.V.M. (2006): Susceptibility of different cell lines to infection with bovine respiratory syncytial virus. 131**,** 130-133.

Takiuchi, E.; Stipp, D.T.; Alfieri, A.F. and Alfieri, A.A.J.J.O.V.M. (2006): Improved detection of bovine coronavirus N gene in faeces of calves infected naturally by a semi-nested PCR assay and an internal control. 131**,** 148-154.

Tektoff, J.; Dauvergne, M.; Durafour, M. and Soulebot, J.J.D.I.B.S. (1983): Propagation of bovine coronavirus on Vero cell line: electron microscopic studies. 53**,** 299-310.

Tsunemitsu, H. and Saif, L.J.A.O.V. (1995): Antigenic and biological comparisons of bovine coronaviruses derived from neonatal calf diarrhea and winter dysentery of adult cattle. 140**,** 1303-1311.

Van Den Hurk, S.; Regmi, G.; Naikare, H.K. and Velayudhan, B.T.J.P. (2024): Advances in Laboratory Diagnosis of Coronavirus Infections in Cattle. 13**,** 524.

Vlasova, A.N. and Saif, L.J.J.F.I.V.S. (2021): Bovine coronavirus and the associated diseases. 8**,** 643220.

Wojciech, S.; Magdalena, L.; Jerzy, R. and Dariusz, B. (2022): Occurrence of bovine coronavirus and other major respiratory viruses in cattle in Poland.

Yavru, S.; Yapici, O.; Kale, M.; Sahinduran, S.; Pehlivanoglu, F.; Albay, M.K. and Avci, O.J.A.S.V. (2016): Bovine coronavirus (BoCV) infection in calves with diarrhoea and their dams. 44**,** 1-7.

Yilmaz, S.G.; Aydin, O.; Tali, H.E.; Karadag, G.; Sonmez, K.; Bayraktar, E.; Yilmaz, A.; Turan, N.; Mutlu, Z. and Iqbal, M.J.J.O.V.S. (2024): Brain invasion of bovine coronavirus: molecular analysis of bovine coronavirus infection in calves with severe pneumonia and neurological signs. 25.

Zaitoun, A.; Abdel-Hakim, O. and Youssef, Z.J.A.V.M.J. (2018): Enteric Rota and Corona viruses infection in neonatal calves. 64**,** 8-17.

Zhu, Q.; Su, M.; Li, Z.; Wang, X.; Qi, S.; Zhao, F.; Li, L.; Guo, D.; Feng, L. and Li, B.J.V.R. (2022): Epidemiological survey and genetic diversity of bovine coronavirus in Northeast China. 308**,** 198632.

عزل فيروس كورونا البقري في خلايا كلية القرد الأفريقي الأخضر وتأكيده الجزيئي بواسطة تفاعل البلمرة المتسلسل العكسي من المجترات في محافظة أسيوط

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ينتشر فيروس كورونا البقري على نطاق واسع في أبقار الألبان واللحوم الصغيرة منها والبالغة في جميع أنحاء العالم. وهو فيروس رئوي معوي يسبب الإسهال والتهابات الجهاز التنفسي، مما يؤدي إلى خسائر اقتصادية كبيرة في صناعة الماشية. يمثل عزل فيروس كورونا البقري تحديًا كبيرًا، كما أن الدراسات حول علم الأوبئة وعزل الفيروس والكشف الجزيئي لفيروس كورونا البقري، خاصة من التهابات الجهاز التنفسي في أسيوط، محدودة. لذلك هدفت هذه الدراسة إلى عزل كورونا البقري باستخدام خلايا كلية القرد الأفريقي الأخضر(Vero cells)، متبوعًا بتأكيده الجزيئي بواسطة تفاعل البلمرة المتسلسل العكسي (RT-PCR). تم استخدام مجموعة ١١٦ عينة من المجترات (٦٥ عينة براز و٥١ عينة من الانف) لعزل فيروس كورونا البقري في خلايا Vero cells، ثم تم تأكيدها بواسطة RT-PCR باستهداف الجين N (730bp). من بين ١١٦ عينة، أظهرت ٢٧ عينة (١١ عينة براز و١٦ عينة من الانف اعتلال خلوي محددًا في صورة خلايا مجمعة (سينستيا). ومن بين هذه العينات، تم التأكد من أن ١٦ عينة (٥عينة براز و١١ عينه من الانف) إيجابية لفيروس كورونا البقري عبر اختبار RT-PCR الذي يستهدف الجينN . وجد أن معدل انتشار العدوى بفيروس كورونا البقري بلغ ٧,٧٪ (٦٥/٥) في عينات البراز و٢١,٦٪ (٥١/١١) في عينات الأنف، مع ملاحظة العدوى في الماشية فقط. ولم يتم الكشف عن أي إصابات في الجاموس أو الماعز أو الأغنام .ولم يكن هناك فرق إحصائي ملحوظ في معدلات الإصابة بفيروس كورونا البقري على أساس الجنس أو المنطقة أو الموسم. وفي الختام، كانت خلايا كلية القرد الافريقي الأخضر (Vero cells) فعالة في عزل فيروس كورونا البقري، والذي يلعب دورًا كبيرًا في التسبب في الإسهال والتهابات الجهاز التنفسي في ماشية محافظة أسيوط. هناك حاجة إلى مزيد من الدراسات لتحديد الخلايا الحساسة الأخرى لعزل فيروس الكورونا البقري وتأكيد الاختلافات بين عزلات الفيروس على المستويين الانتجينى والجيني.