Original Research Article

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Clinical and diagnostic studies on Rota and Corona viral infection in calves in New Valley governorate

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ABSTRACT: *Bovine Rotavirus* and *Bovine Coronavirus* are considered the most important causes of diarrhea in neonatal calves. May lead to many losses for the breeder and the animal economy. This work was aimed for isolation and identification of Rota and Corona viruses at New Valley governorate. During the period from August 2022 to December 2023, 102 fecal samples were collected from diarrheic (n=92) and healthy calves (n=10) and isolate on Madin Darby bovine Kidney (MDBK), cell line. All positive samples on tissue culture were examined for presence of BRV and BCoV by using an antigen Enzyme Linked Immune Sorbent Assay (ELISA). The results was 22 (21.5%) and 24 (23.5%) for BRV and BCoV, respectively. The prevalence rate of BRV was highest in the age groups from one day to one month and there is no difference between male and female but BCoV infection of age up to 1m in female more than male

KEYWORDS: *Rotavirus, Coronavirus*, prevalence, risk factors, New Valley governorate. preservative.

1. Introduction

One of the most dangerous conditions affecting newborn calves is diarrhea. Diarrhea is most common in the first month of life for calves. It represents a threat to animal health and dairy industry [1, 2]. Neonatal calf diarrhea (NCD) is referred to as a multi-factorial disease, resulting from the interplay between environmental including management, nutritional, physiological factors and calves it selves either alone or in synergy with different etiologic agents such as protozoans, bacteria and viruses [3, 4, 5]. The most common viral causes of NCDs, which are economically significant for the cow sector due to mortality, medicine costs, labour costs associated with treating ill calves, and delayed calves' growth, are bovine rotavirus (BRV) and bovine coronavirus (BCoV) infections. [6]. A non-enveloped virus with a diameter of 65–70 nm, rotaviruses are members of the Reoviridae family, Sedoreovirinae subfamily, and are distinguished by their segmented genomes, which are made up of 11 segments of double-stranded RNA and encased in a triple-layered icosahedral capsid layer. Their genomes encode six structural proteins (VP1, VP2, VP3, VP4, VP6, and VP7) and six NSP (NSP1 to NSP6). [7]. Coronavirus is a single-stranded RNA virus with a lipid envelope belongs to Nidovirales order, Coronaviridae family, Orthocoronavirinae subfamily, Betacoronavirus genus, and Embecovirus subgenus. It is linked to severe diarrhea in newborn calves and respiratory illnesses in cattle.[6, 8]. The five main structural proteins found in BCoV are hemagglutinin/esterase (HE), spike (S), small membrane (E), transmembrane (M), and nucleocapsid (N).[9]. The detection of viral antigens and subsequently nucleic acids in faecal samples is necessary for the laboratory diagnosis of both viruses in calves' enteritis. Cell culture or other methods are frequently used to diagnose infections.[10], antigen detection using ELTSA kit and genome detection by using polymerase chain reaction assay[11]. The primary goal of this study is to isolate and identify the viruses that cause NCDs using tissue culture and direct ELISA methods.

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2. Material and Methods

2.1. Ethical approval:

This research was conducted according to the guideline of the Institutional Review Board of the Faculty of Medicine, Assuit University, Egypt (04-2023-200261).

2.2. Study area:

El-Kharga and El-Dakhla, two distinct locations in the New Valley governorate in the southwest region of Egypt, were the sites of this study.

2.3. Clinical examination:

A total of 210 calves were analysed in this study based on clinical manifestations, diarrhoeal calves, and calves that seemed healthy.

2.4. Animal and Sample collection:

A total of 102 fecal swabs collected from diarrheic (n92) and non-diarrheic (n10) calves during the period from August 2022 to December 2023.calves divided according to their age to three groups, the 1st group from 1 Day to 1 Months of age, 2nd group from 1 to 2 Months and 3rd group from 2 to 4 Months. Fecal samples were prepared in 0.01 M phosphate buffered saline (PBS) at pH 7 then centrifuged at 1500 x g and the supernatants were stored in sterile eppendorf at -20 ° C at Veterinary Serum Vaccine Research Institute (VSVRI) till the time of viral isolation.

2.5. Virus isolation and identification

2.5.1. Virus isolation on tissue culture:

Madin Darby bovine Kidney (MDBK), cell line was used to isolate the viruses from all samples to identify the characteristic cytopathic effect (CPE) of both viruses [12].

2.5.2. Virus identification by using rapid ELISA kits:

All positive samples on tissue culture were examined for presence of BRV and BCoV by using an antigen ELISA (antigen captured ELISA, IDEXX Rota-Corona-k99, USA) test.

Figure 1: Close up pictures of examining calves suffering from sever yellow, watery diarrhea

Table 1: Distribution of BCoV and BRV in calves of various ages based on clinical manifestation:

Age group	Total examined calves (102)		*BRV*		*BCoV*		Mixed infection
	Healthy	Diseased	+ve	%	+ve	%	%
1D-1M (74)	8	66	18	24.3%	17	22.9%	0
1M-2M (16)	2	14	3	18.7%	4	25%	0
2M-4M (12)	0	12	1	8.3%	3	25%	0
Total	10	92	22	21.4%	24	23.4%	0

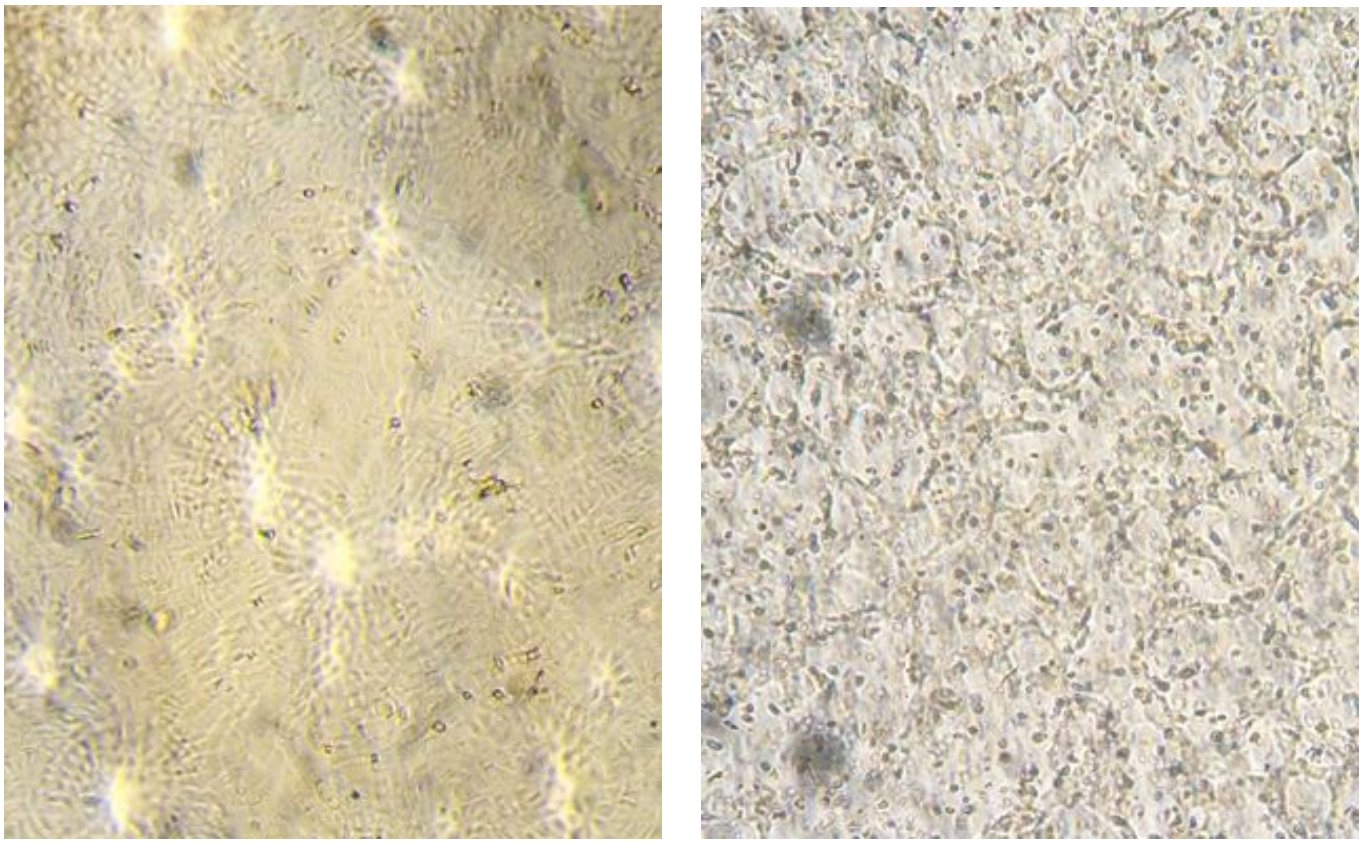
3. Results

3.1. Clinical examination

Clinical examination were carried out for signs of diarrhea and revealed that Calves showing watery, yellow or greenish diarrhea may containing mucus or blood, loss of appetite, weakness, loss of body weight, pale mucus membrane, unable to stand, depression, different degree of dehydration. Signs of pneumonia, coughing, and nasal discharge are also indicators of respiratory discomfort in addition to enteritis.

3.2. Results of Isolation of BRV and BCoV in MDBK Cell Line

Out of 102 fecal samples cultured on MDBK cell line and characteristic CPE of BRV and BCoV was observed after 48 hours in only 46 samples (22 for BCoV and 24 for BRV). Infected cells did not exhibit any CPE in the first



(a) Normal cell culture

(b) 24 hr. post infection.

Figure 2: Isolation of BRV and BCoV in MDBK Cell Line

Table 2: Distribution of BCoV and BRV in calves, both male and female

Sex	No.	*BRV*		*BCoV*	
		+ve	%	+ve	%
Male	55	12	21.8	9	16.3
Female	47	10	21.2	15	31.9
Total	102	22	21.4	24	23.5

Table 3: Species susceptibility to BRV and BCoV infection of investigated calves

Species	No.	*BRV*		*BCoV*	
		+ve	%	+ve	%
Cattle calves	100	22	22	24	24
Buffalo calves	2	0	0	0	0
Total	102	22	21.5	24	23.5

passage. However, the infected cells began exhibiting distinctive CPE after the second passage. Within 72 to 96 hours, the infected cells rounded, the cell wall shrank, and the granularity increased, eventually forming a cluster of grapes.

3.3. Results of rapid ELISA kits test

Out of 102 samples were confirmed by using rapid ELISA kits. 22 (21.5%) fecal samples were positive for BRV and 24 (23.5%) positive for BCoV. The percentage of BRV and BCoV infection were 90.2% (92/102) of clinically diarrheic calves. However, only 20% (2/10) of apparently healthy female calves infected with BCoV at age from 1d to 1m (1st group).

4. Discussion

One of the most serious illnesses affecting newborn calves is infectious calf diarrhea. The most common cause of

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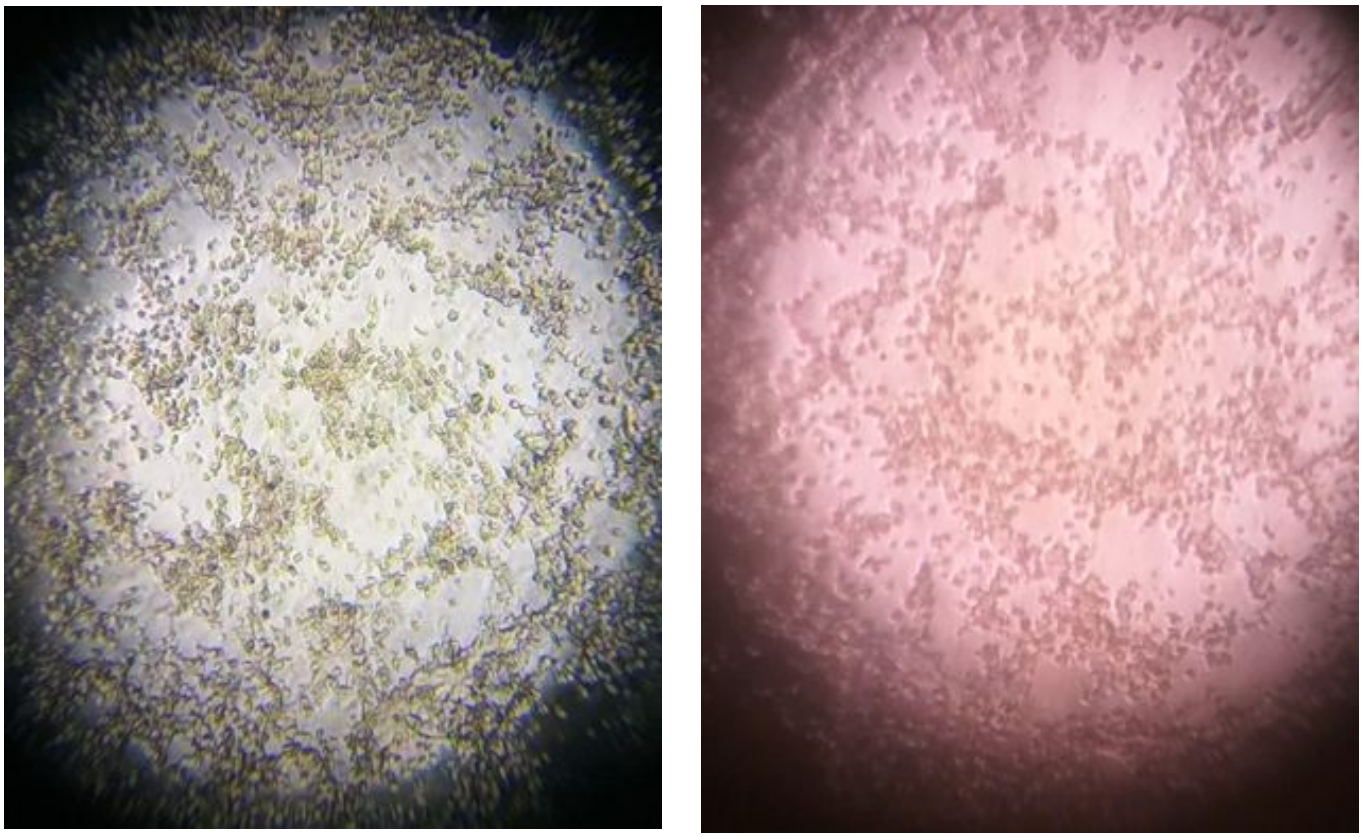


(a) 48 hrs. post infection

(b) 72 hrs. post infection.

Figure 3: Isolation of BRV and BCoV in MDBK Cell Line

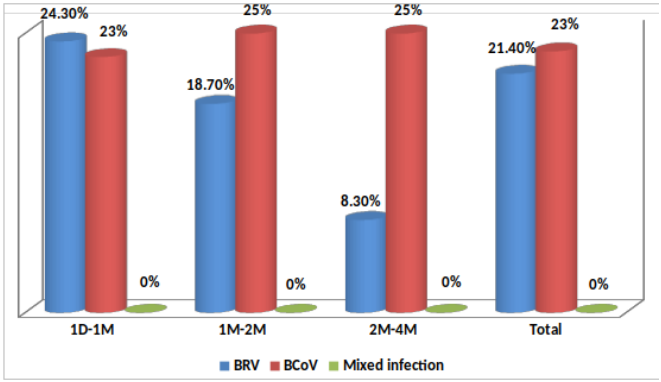


Figure 4: : Distribution of BCoV and BRV in calves of various ages based on clinical presentation.

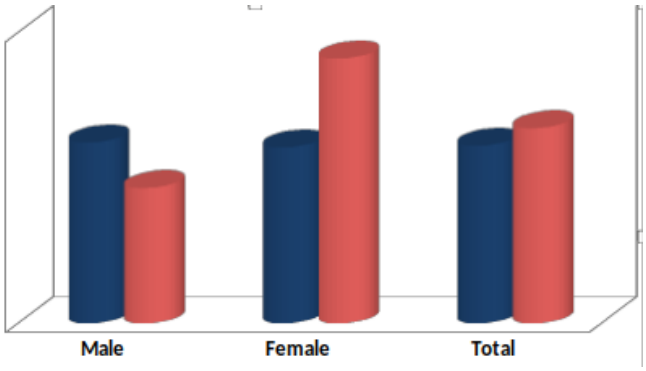
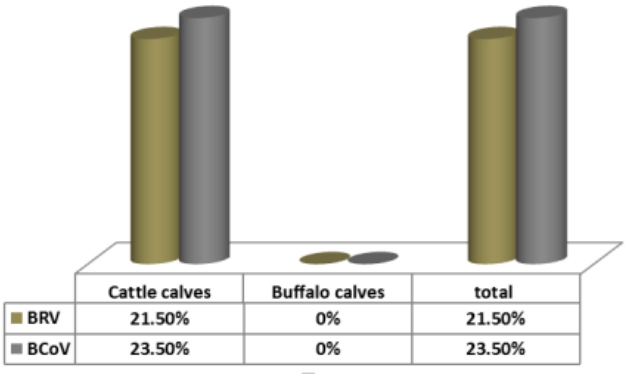


Figure 5: Distribution of BRV and BCoV in male and female of calves

calf diarrhea is viral infections, specifically BRV and BCoV. [13]. This study clarified the most important symptoms that appear in a calves infected with BRV and BCoV infection such as sever watery, yellow or greenish diarrhea may containing mucus or blood, sometimes fever, loss of appetite, weakness, loss of body weight, pale mucus membrane, unable to stand, depression, different degree of dehydration. Signs of respiratory discomfort, such as coughing, nasal discharge, and pneumonia, in addition to enteritis. These symptoms are consistent with the previous study [14, 15]. In this study, inoculation cells were examined for CPE in order to measure viral growth in cell culture. Only 46 injected MDBK cells produced distinctive CPE at the second passage level out of 102 samples.



	Cattle calves	Buffalo calves	total
BRV	21.50%	0%	21.50%
BCoV	23.50%	0%	23.50%

Figure 6: species susceptibility to BRV and BCoV infection of investigated calves:

Cells that were infected exhibited rounding, cell wall shrinkage, and increased granularity before developing into a cluster of grapes. The study's CPE results were consistent with earlier findings. [16, 17] The current work study revealed that BRV and BCoV infection were 22 (21.5%) and 24 (23.5%), respectively. Regarding to BRV infection, This result was agreed with previous study [18] who recorded that the prevalence of Assuit governorate had a BRV of 21.5%, while Qaluobia, Sharkia, and Giza governorates had varying locales with BRVs of 24.5%, according to [19]. However, These result disagree with the result of [20] who reported that the prevalence rate of BRV tested by ELISA kits was 10.82%. But regarding to BCoV infection, [21] agree with our results of BCoV, the prevalence rate was (25.5%) but on the other side, there are other studies that differ from our results in terms of the BCoV rate which is considered much lower, 3.17%, 1.04%, 7.2% [22, 11, 23], respectively. On the other side, the results obtained completely opposite to our results of both BRV and BCoV, the results of infection were 7.2 and 1.2%, respectively[17]. In the current work the BCoV was present in both diarrheic and healthy calves, this results go in the same way with results obtained by [24] who found that the BCoV may affect diarrheic calves and calves that look in a good healthy. The samples under examination show no signs of mixed infection. The incidence of the BRV-BCoV mixed infection, however, is higher than anticipated based on the ratio of the occurrence of both individual infections in calves with diarrhoea, as demonstrated by [5]. Based on the calves' ages, the first group had the greatest BRV infection rate (24.3%) between the ages of one day and one month. This is similar with the results of [25, 26, 15], who proved that BRV infection was obviously observed in first days to weeks old calves of age. In BCoV, the infection rate in three groups nearly to each other (1st group 22.9%, 2nd group 25% and 3rd group 25%) this means the infection rate of BCoV occurs in wide range of ages. This findings agree with [24] results they concluded that the prevalence rate of BCoV is between wide range of ages from 2 to 6 months of age. This could be because, in addition to causing gastrointestinal sickness, BCoV can also replicate in the pulmonary system. The rate of infection regarding to sex of calves in BRV was (21.8% in male and 21.2% in female) this indicate that there is no difference in the ratio between male and female in BRV infection. This is consistent with the research of [27] but other studies [28, 29] revealed that male calves had higher possibility of BRV. However, a study conducted in Egypt revealed higher prevalence of BRV among female calves than males[25]. Infection rate in BCoV was (31.9% in female which higher than in male 16.3%). This results agreed with [30] but varies with [31, 25] reported that BCoV higher in male than female. In this study, it was found that the prevalence or BRV (21.5%) and BCoV (23.5%) viral infection is higher only in the cattle species than in the buffalo species, and this may be due to the lack of buffalo breeding in the New Valley governorate.

Conclusion

According to the findings of our investigation and our field analysis, BRV and BCoV contribute significantly to diarrhea in newborn calves in two distinct regions within the New Valley governorate, with corresponding percentages of 21.5 and 23.5%. Certain risk factors, including age, sex, and species, can affect the prevalence rates of BRV and BCoV in calves that have diarrhea.

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