Investigation of Rota And Corona Viruses as Causative Agents for Diarrhea in Egyptian Calves

El-Sadek F. Esraa[1], Shahein A. Momtaz[2], Shahira A. Abdelwahab [3] and El- Shahidy S. Mohamed[4]

[1]vetsoso@yahoo.com, [2]momtaz.shahein@yahoo.com,
[3]Shahira_mohamed@vet.suez.edu.eg, [4]el_shahidy@yahoo.com
[1,2]Animal health research institute, Dokki, Giza, Egypt.
[3,4]Virology department, Faculty of Veterinary Medicine, Suez Canal University, Egypt

Abstract

Rota and Corona viruses are the major causes of calf scour involved in 27% to 36% of calf diarrheic in Egypt. This study was aimed to investigate frequency of Rota and Corona viruses in diarrheic cattle and buffalo calves in Egypt. A total of 82 fecal samples were collected from 0-1-month-old in three provinces in Egypt (Ismailia, Alexandria and Al-Sharqia). All fecal samples were tested by probe based real time polymerase chain reaction technique (qRT-PCR) to determine the presence of BRV and BCoV nucleic acids by using specific generic primers. Fourteen (17.1%) and 22 (26.8%) out of 82 fecal samples were positive for BRV and BCoV, respectively. Positivity percentage of different investigation parameters about both BRV and BCoV were calculated depending on our historical data. Corona and Rota viruses' infection in cattle were more predominant than buffalo calves. Infection rate of Coronavirus is more than Rotavirus infection in cattle calves of Ismailia than other studied provinces. Single infection of cattle or buffalo calves with both viruses is mostly predominant but double infection in the same animal species at the same time is possible. Infection rate of BRV and BCoV in cattle and buffalo calves is higher in winter than autumn, summer and spring.

Key Words: Rotavirus, Coronavirus, calves, diarrhea.

Introduction

Acute calf diarrhea is multifactorial syndrome caused by bacteria, viruses and protozoa; these pathogens are involved in 75%-95% of worldwide calf diarrheic cases. Rota and Corona viruses are the major causes of calf scour and have been detected in 27-36% of diseased cases (*Gumusova et al., 2007 & Dhama et al., 2009*). Farm management as colostrum intake, calf housing and vaccination regime are important risk factors that enhance the effect of ubiquitous infectious causes of diarrhea (*Lorenz et al., 2011 & Cho and Yoon, 2014*).

Bovine coronavirus (BCoV) causes digestive disease in young and adult cattle that result in worldwide significant economic losses. The virus was first reported in the United States in the early 1970s from calves with diarrhea *(Stair et al., 1972 & Mebus et al., 1973)*. It still remains a major cause of neonatal calf diarrhea (NCD), together with Escherichia coli, Rotavirus, Cryptosporidium parvum and Salmonella species *(Reynolds et al., 1986)*. BCoV is also frequently involved in outbreaks of winter dysentery in adult cattle *(Saif et al., 1988)*.

BCoV have emerged as worldwide viral pathogens that annually cause enormous economic losses in dairy and beef herds. BCoV infection manifests as diarrhea with varying degrees of severity that consequently may result in mortalities in severe cases *(Saif, 2010 & Larsen, 2000)*. BCoV is a major welfare and economic burden affecting both beef and dairy cattle, and costing around a billion dollar per annum in the United States *(Cliver, 1973)*.

BCoV has single stranded ribonucleic acid (ssRNA) genome which causes low fidelity of RNA replication machinery; this results in a high mutation rate during virus replication *(Moya et al., 2004)*. Additionally, coronavirus are characterized by genetic recombination *(Woo et al., 2009 & Zhang et al., 2005)* that resulted in high genomic diversity among coronaviruses *(Smith and Denison, 2012)* which consequently causes emerging of new variant that may replace the old one and become the dominant virus *(Woo et al., 2009)*.

Rotaviruses infects young age of wide range of species including human, mammals (piglets, calves, goats, lambs and foals) and birds *(Dhama et al., 2009 & Estes and Greenberg, 2013)*. Bovine rotaviruses group A (Bovine RVAs) are the most worldwide prevalent viral agent in diarrheic calves aged less than 6 weeks. It causes economic losses due to retarded growth, increased susceptibility to other infections, treatment cost, and calf mortalities *(Mawatari et al., 2004)*.

Due to genetic variability of bovine Rota VP6, at least 8 different groups (from A to H) are differentiated *(Matthijnssens et al., 2012 & Mihalov-Kovács et al., 2015)*; Furthermore, within each group, Rotaviruses are classified into serotypes and genotypes based on antigenic and genetic variations of the VP4 and VP7. The VP7 protein is glycosylated and its analysis classify RVA into G groups, while VP4 is a protease sensitive polypeptide and assigns the P groups *(Estes and Greenberg, 2013)*.

The goal of this study was to investigate the epidemiology and distribution of Rota and Corona viruses in diarrheic cattle and buffalo calves in different provinces of Egypt. Seasonal and

environmental risk factors affecting the infection rate of corona and rota viruses are involved. To achieve this aim, fecal matters were collected from diarrheic cattle and buffalo calves in different provinces and the viruses in the samples were screened by probe based real time RT-PCR.

Materials and methods
1.   Clinical samples:
From September 2014 to May 2019, a total of 82 diarrheic cattle and buffalo calves below one month of age were clinically examined and fecal samples were collected from them. Briefly; 69 fecal samples were collected from different dairy and beef farms in Ismailia, Al-Sharqia and Alexandria governorates. Additionally, 13 samples were gathered from individual cases of native cattle and buffalo calves in Ismailia governorate.
Fecal samples were collected from calves of 12 dairy cattle and buffalo farms, Friesian cattle (25), native breed cattle calves (12) and native buffalo calves (45). All historical data were taken concerning to bread, species, sex, age, season and vaccinal status. The samples details are described in (**Table 1**). The samples were collected in sterilized plastic tubes and kept at -80°C till further processing. Sample preparation begins with samples were came out from the refrigerator (-80°C) and completely thawed. Homogenization was made by suspending the fecal materials in equal amounts of saline and 300 µl of suspension was used for RNA extraction.

2.   Clinical history, collection and preparation of samples:
Clinical examination of Rota and/or Corona infected calves in 12 examined farms revealed loss of appetite, depression, reluctance to stand, severe watery diarrhea, dehydration and loss of weight. All examined cattle and buffalo calves were below one month of age. Calves and/or their dam in farm 1, 4, 5, 6, 8, 9, 10, 11, 12 and individual cases were not vaccinated against bovine Rota and Corona viruses. Pregnant dams in farm 2, 3 and 7 were vaccinated with inactivated Rota and Corona viruses vaccine by I/M injection during late stage of pregnancy, however, neonates were administered live attenuated bovine Rota and Corona viruses vaccine at the first day of age by oral administration.

3.   RNA extraction:
Total RNA was extracted from 300 µL of fecal samples using ABT Total RNA Mini Extraction Kit (Applied biotechnology Co. LTd., Egypt) according to manufacturer instruction.

4.   One step real time qRT-PCR:
Five microliters of extracted RNA was used for both BRV and BCoV detection, using SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) in

Applied Biosystems™ 7500 real-time PCR system. The amounts of total and individual reactants were mixed according to the instructions of manufacturer.

For BRV detection, sequences of primers and probes used in qRT-PCR for amplification of NSP-5 are listed in **(Table 2)** and the reaction component and cycling condition were done according to **Soltan et al., 2016**.

For BCoV detection, qRT-PCR was used for amplification of conserved M gene of BCoV. Primers and probe sequences **(Table 2)** were designed and the procedure was done according to **Decaro et al., 2008.**

Table (1): *Number, breed and provinces of fecal samples collected in the study.*

Farms	Animal species	Governorates	No. of samples
Farm 1	Friesian Cattle	Ismailia	13
Farm 2	Buffalo (native breed)	Ismailia	6
Farm 3	Buffalo (native breed)	Ismailia	10
Farm 4	Friesian Cattle	Ismailia	7
Farm 5	Buffalo (native breed)	Ismailia	10
Farm 6	Buffalo (native breed)	Ismailia	5
Farm 7	Buffalo (native breed)	Ismailia	5
Farm 8	Friesian Cattle	Alexandria	5
Farm 9	Buffalo (native breed)	Ismailia	2
Farm 10	Cattle (native breed)	Ismailia	2
Farm 11	Cattle (native breed)	Ismailia	3
Farm 12	Cattle (native breed)	Al-Sharqia	1
Individual cases	Cattle (native breed)	Ismailia	6
	Buffalo (native breed)	Ismailia	7
Total			**82**

Table (2): Primers and probe sequences used in qRT-PCR for amplification of NSP-5 of BRV and M gene of BCoV.

Primer/probe	Sequence	Position*[a]
Rota-F	5′-TTCTGCTTCAAACGAYCCACTC-3′	221-234
Rota-R	5′-GAGAAATCYACTTGRTCGCA-3′	353-334
Rota-probe	5′-FAM- TCCATAGAYACRCCAGYRTCTGCRTTTGTC- BHQ-3′	296-267
BCoV-F	5′-CTGGAAGTTGGTGGAGTT-3′	29026-29043
BCoV-R	5′-ATTATCGGCCTAACATACATC-3′	29090–29110
BCoV-Prob	FAM[b]-CCTTCATATCTATACACATCAAGTTGTT-BHQ1[c]	29058–29085

*According to GenBank accession number GU937876. [a]Oligonucleotide position is referred to the sequence of BCoV strain Mebus (GenBank accession no.: U00735).[b] FAM: 6-carboxyfluorescein.   [c]BHQ1: black hole quencher 1.

Results

Fecal matter of diarrheic cattle and buffalo calves were collected from 12 farms and other individual cases and tested for Corona and Rota viruses' infection by qRT-PCR. 14 (17.1%) and 22 (26.8%) samples out of 82 were positive for BRV and BCoV, respectively. Positivity percentages of BRV and BCoV in different farms by qRT-PCR were illustrated in **Figure 1**.

Positivity percentages of Corona and Rota viruses in Ismailia, Al-Sharqia and Alexandria were illustrated in **table 3**. Highest infection rate of Coronavirus were observed in Friesian cattle of Alexandria and native breed cattle of Ismailia province than the other examined provinces with a positivity percentage of 60% and 54.5%, respectively. Rotaviruses infection rate in Ismailia Friesian cattle is more than Coronavirus infection with a rate of 30% and 25% respectively. A total of 22 samples out of 82 stool samples were positive to Coronavirus infection with a rate of 26.8% However, the infection rate of Rotavirus is low (17.1%).

Generally, in this study Corona and Rota viruses' infection in cattle was more common than buffaloes with total percentages of 75.7 and 17.8 respectively **(Table 4).** Infection rate of Coronavirus equal Rotavirus infection in cattle of Ismailia with percentages of 14.5, however, infection rate of Coronavirus in buffaloes of Ismailia more than Rotavirus **(Table 4).**

Concerning to single infection or double co-infection of cattle or buffalo calves with Rota and Corona viruses, it was found that only 2 fecal samples from Friesian cattle of Ismailia and Alexandria were positive for both viruses with a total percentage of 2.4, while buffaloes do not show double Rota and Corona viruses' infection **(Table 5).** Single infection with either Rota or Corona viruses is mostly predominant in cattle and buffaloes with a total percentage of 41.5 **(Table 5).**

Coronavirus was detected most frequently in one week old cattle calves with the highest infection rate of 53.8%, however, the lowest rate was shown in 2 weeks old calves with a percentage of 40. Highest infection rate of Rotavirus in cattle calves was observed in 2 and 4-week-old with a rate of 40% for both ages **(Table 6).**

Distribution of Rota and Corona viruses in different age groups of buffalo calves, revealed that Coronavirus was predominant in 4-week-old buffalo calves with a rate of 46.7%, while the highest

distribution rate for Rotavirus was observed in the first week of life of diarrheic buffalo calves with percentage of 12.5 **(Table 6).**

Distribution of Rota and Corona viruses in relation to sex is of little value. Percentages of positive corona and Rota viruses in male and female animals are nearly parallels to each other. Coronavirus in male cattle calves is more comman than female with high percentages of 40 and 38.9 respectively. Male buffalo calves showed high Rotavirus infection than female with a rate of 5.6% and 0% **(Table 7).**

Control measures and prevention of diarrhea in calves caused by Corona and Rota viruses depend on hygiene and vaccination of dam to get maternal immunoglobulins to their calves. In this study, both Rota and Corona viruses were detected in feces of calves which their dam vaccinated against both viruses. Unfortunately, Corona and Rota viruses are more predominant in non-vaccinated calves than vaccinated one. Coronavirus infection is showing high infection rate in non-vaccinated cattle calves than Rota virus with percentages of 58.9 and 47 respectively, meanwhile vaccinated buffalo calves also showed high infection rate of 16.7% than non-vaccinated **(Table 8).**

Positivity percentages of BRV and BCoV in cattle and buffalo calves in different season were investigated. Coronavirus is the most predominant in diarrheic cattle calves in autumn seasons with the highest infection percentages of 55.6 followed by Rotavirus in cattle calves in winter with a percentage of 38.5. Infection rate of Rota and Corona viruses in buffalo calves in autumn season are equal percentage of 14.3. No Rota or Corona viruses were recovered from cattle or buffalo calves in summer or in spring **(Table 9).**
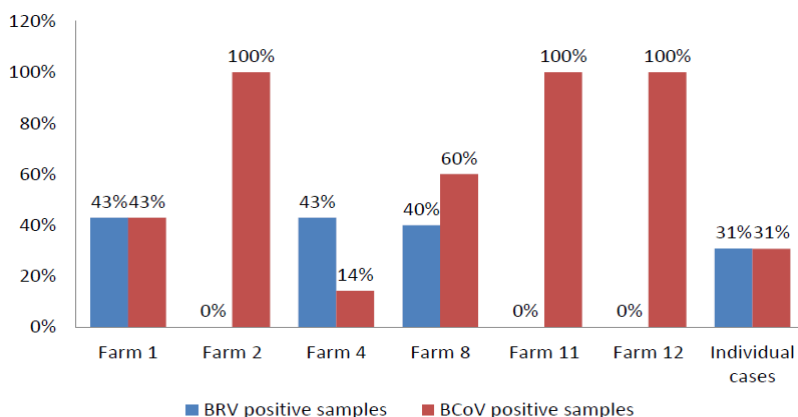
Figure (1): Positivity % of BRV and BCoV in different farms by qRT-PCR.

Table (3): *Number of bovine Rota and Corona viruses' positive samples in Ismailia, Al-Sharqia and Alexandria governorates using qRT-PCR.*

Provinces	Breed	Total	+ve Rotavirus		+ve Coronavirus	
			No.	%	No.	%
Ismailia	Friesian cattle	20	6	30	5	25
	Native cattle	11	5	45.5	6	54.5
	Native buffalo	45	1	2.2	7	15.6
Alexandria	Friesian cattle	5	2	40	3	60
Al-Sharqia	Native cattle	1	0	0	1	100
Total		**82**	**14**	**17.1**	**22**	**26.8**

Table (4): *Positivity percentages of Rota and Corona viruses in cattle and buffaloes in Ismailia, Alexandria and Al-Sharqia province by quantitative real time PCR.*

Province/Total	Virus	Total +ve		Cattle		Buffalo	
		No.	%	+ve	%	+ve	%
Ismailia	Rota	12	15.8	11	14.5	1	1.3
(76)	Corona	18	23.7	11	14.5	7	9.2
Alexandria	Rota	2	40	2	40	0	0
(5)	Corona	3	60	3	60	0	0
Al-Sharqia	Rota	0	0	0	0	0	0
(1)	Corona	1	100	1	100	0	0
Total (82)		**36/82**	**43.9**	**28/37**	**75.7**	**8/45**	**17.8**

Table (5): *Simultaneous infection of cattle with corona and Rota viruses*

Provinces	Breed	Total	Single infection		Double co-infection	
			No.	%	No.	%
Ismailia	Friesian cattle	20	10	50	1	5
	Native cattle	11	11	100	0	0
	Native buffalo	45	8	17.8	0	0
Alexandria	Friesian cattle	5	4	80	1	20
Al-Sharqia	Native cattle	1	1	00	0	0
Total		**82**	**34**	**41.5**	**2**	**2.4**

Table (6): *Distribution rate of Corona and Rota virus in different age groups of cattle and buffalo calves.*

Age	Species							
	Cattle				Buffalo			
	Rotavirus		Coronavirus		Rotavirus		Coronavirus	
	+ve	%	+ve	%	+ve	%	+ve	%
1 wk	5/13	38.5	7/13	53.8	1/8	12.5	0/8	0
2 wk	4/10	40	4/10	40	0/10	0	0/10	0
3 wk	0/4	0	2/4	50	0/12	0	0/12	0
4 wk	4/10	40	2/10	20	0/15	0	7/15	46.7
Total	**13/37**	**35.1**	**15/37**	**40.5**	**1/45**	**2.2**	**7/45**	**15.6**

Table (7) *Distribution of Rota and Corona viruses in male and female diarrheic calves*

Sex/Species	Rotavirus		Coronavirus	
	+ve	%	+ve	%
Male cattle calves	5/20	25	8/20	40
Female cattle calves	8/18	44.4	7/18	38.9
Male Buffalo calves	1/18	5.6	3/18	16.7
Female buffalo calves	0/26	0	4/26	15.4
Total	**14/82**	**17.1**	**22/82**	**26.8**

Table (8*): Distribution of Rota and Corona virus in relation to vaccine status*

Vaccine status/Species	Rotavirus		Coronavirus	
	+ve	%	+ve	%
Vaccinated cattle calves	0/20	0	5/20	25
Non vaccinated cattle calves	8/17	47	10/17	58.9
Vaccinated Buffalo calves	0/36	0	6/36	16.7
Non vaccinated buffalo calves	6/9	66.7	1/9	11.1
Total	**14/82**	**17.1**	**22/82**	**26.8**

Table (9): *Distribution of BRV and BCoV in cattle and buffaloe calves in relation to season:*

Season	Cattle		Buffalo	
	No. & %+ve Rota	No. & % +ve Corona	No. & %+ve of Rota	No. & % +ve Corona
Spring	0/0 (0%)	0/0 (0%)	0/7 (0%)	0/7 (0%)
Summer	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
Autumn	3/9 (33.3%)	5/9 (55.6%)	1/7 (14.3%)	1/7 (14.3%)
Winter	10/26 (38.5%)	10/26 (38.5%)	0/31(0%)	6/31 (19.4%)
Total	**13/37 (35.1%)**	**15/37 (40.5%)**	**1/45 (2.2%)**	**7/45 (15.6%)**

Discussion

BRVs constitute the major cause of neonatal calf diarrhea worldwide *(Kapikian, 1996)*. Rotavirus, are medium-sized, non-enveloped viruses. Their dsRNA genome consists of 11 RNA segments that encode six structural (VP1-4, VP6 and VP7) and six non-structural proteins (NSP1-6) *(Greenberg and Estes, 2009)*. Based on their common group antigen (VP6), Rotaviruses are classified into eight antigenically distinct groups (from A to H) *(Matthijnssens et al., 2011)*.

Coronaviruses are large, enveloped, positive-strand RNA viruses that have become increasingly important causes of human and animal diarrhea *(Lai and Holmes, 2001)*. BCoVs contain five major structural proteins: nucleocapsid (N), transmembrane (M), spike (S), small membrane (E), and hemagglutinin/esterase (HE) *(Kumar et al., 2004)*. Both the S and HE glycoproteins hemagglutinate red blood cells by binding to N-acetyl-9-O-acetylneuraminic acid as a receptor determinant *(Vlasak et al., 1988)* and produce virus neutralizing antibodies *(Dea et al., 1990)*. In addition, they are involved in determining the virus tissue and host tropism *(Spaan et al., 1988)*.

Real-time RT-PCR assay proved to be sensitive and rapid test for detection of BRV and BCoV RNA. This assay can be used as powerful tool for the simultaneous analysis of several samples (up to 96 samples in the same plate) in a short time. Considering the difficulties of BCoV and BRV isolation and titration on cell cultures, the established assay can be helpful in BCoV-pathogenesis studies and vaccine trials *(Decaro et al., 2008)*.

In Egypt bovine Rota and Corona virus were discovered for the first time in 1981 and 1990, respectively *(Shalaby et al., 1981 & Abd El-Karim et al., 1990)*, later on the studies on both viruses were increased in the period from 1996 till now *(Byomi et al., 1996; Abd El-Rahim, 1997; Hussein et al., 2001, Gabr et al., 2014&*

Kassem et al., 2017). The prevalence of Rota and Corona viruses in 2016 in Egypt were 48% and 0% of diarrheic calves *(Mohamed et al., 2017).*

Regarding to infection rate of BRV and BCoV in Egyptian cattle and buffaloes in our study as shown in **(Table 3),** it was concluded that, Coronaviruses is more predominant than Rotaviruses in both cattle and buffaloes with a total percentage of 26.8 and 17.1 respectively. This result was in disagreement with a previous paper carried by *Rai et al., 2011*, who recorded higher infection rates of Rotaviruses than coronaviruses with percentages of 15.68 and 11.76, respectively.

Infection rate of bovine Rota and Corona viruses in calves of three Egyptian provinces presented in **table 4** showed that, coronavirus is more common in Ismailia than Rotaviruses. Cattle calves are more susceptible to Rotavirus than buffalo calves, on contrary buffalo calves more susceptible to coronavirus infection than cattle. These results are coincided with *Basera et al. (2010)* who stated that buffalo calves are more resistant to BRV than cattle calves. Unfortunately, infection rate of both viruses in Alexandria and Al-Sharqia provinces is very low; these may be due to small number of samples collected from these provinces.

Concerning to distribution of Rota and Corona viruses in different age groups of buffalo calves documented in **(Table 6)**, it was found that, Coronavirus was predominant in 4 week old buffalo calves with a rate of 46.7%, while the highest distribution rate for Rotavirus was observed in the first week of life of diarrheic buffalo calves with percentage of 12.5**.** The same results were obtained by many authors in many countries due to the immune system of newly born calves is not fully matured and lacking of sufficient maternal antibodies *(Saif, 2010 and Paton et al., 1998).* On contrary our results were disagree with *Dash et al. (2011)*, who stated that a low infection rate of BCoV in cattle farms was observed.

Data recorded in **Table 6** also showed that**,** Coronavirus was detected most frequently in one week old cattle calves with the highest infection rate of 53.8%, however, the lowest rate was shown in 2 weeks old calves with a percentage of 40. Highest infection rate of Rotavirus in cattle calves was observed in 2 and 4 week old with a rate of 40% for both ages**.** The same results were obtained by *Sravani et al. (2015)***,** in India who stated that calves below 20 days of age and buffalo calves were found to be more susceptible to Rotavirus infection. Although, it can affect calves up to several months of age *(Gay et al., 2012).*

Distribution of Rota and Corona viruses in relation to sex, **Table 7** showed that, gender have a little value for both Rota and Corona viruses in both cattle and buffalo calves. Percentages of positive corona and Rota viruses in male and female animals are nearly parallels to each other. Rotavirus in female cattle calves is more common than male with high percentages of 44.4 and 25 respectively**.** These results disagree with *Sravani et al. (2015(,* who reported that male diarrheic calves were found to be more susceptible to Rotavirus infection than female diarrheic calves.

Control measures and prevention of diarrhea in calves caused by Corona and Rota viruses depend on hygiene and vaccination of dam to get maternal immunoglobulins in colostrum to their calves *(Saif and Fernandez, 1996).* In this study, Coronavirus was detected in feces of calves which their dam were vaccinated against Rota and Corona viruses. High positivity percentage of Coronavirus was observed in vaccinated cattle calves than Rotavirus with percentages of 25. Unfortunately, Vaccinated buffalo calves showed high infection rate of 16.7% with coronavirus **(Table 8)**. These results coincide with the fact that vaccination with inactivated Coronavirus vaccine represents a strong selective positive pressure

sites observed in the receptor-binding subunit of the S protein gene of BCoV strains indicate a natural mode of evolution that is mainly due to exposure to the host immune system *(Fulton et al., 2013).*

Seasonal pattern of Corona and Rota viruses in Cattle and buffaloes presented **in table 9** revealed that, Coronavirus is the most predominant in diarrheic cattle calves in winter and autumn season with the highest infection percentages of 55.6 and 38.5. These results are compatible with that obtained by *Saif et al. (1991).* NCD has a peak incidence in winter, presumably because BCoV is moderately sensitive to heat and exposure to cold stress and drinking cold water which is an important risk factor for calf diarrhea *(Saif and Jackwood, 1990).* Other investigators in Korea detect and characterize BCoV in fecal specimens of adult cattle during warmer seasons *(Park et al., 2006)*

Conclusion

Corona and Rota viruses' infection in cattle were more predominant than buffalo calves. Infection rate of Coronavirus is more than Rotaviruses infection in cattle calves of Ismailia than other studied provinces. Single infection of cattle or buffalo calves with both viruses is mostly predominant but double infection in the same animal species at the same time is possible. Coronavirus

was detected most frequently in one-week old cattle calves but the highest infection rate of Rotavirus in 4-week-old. Infection rate of BRV and BCoV in cattle and buffalo calves is higher in winter than autumn, summer and spring.

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الملخص العربى

فيروسات الروتا والكورونا هي الأسباب الرئيسية لاسهال العجول الرضيعة حيث تمثل ٢٧ ٪ إلى ٣٦ ٪ من اسباب الاسهال فى العجول في مصر. هدفت هذه الدراسة إلى التحقق من تواتر فيروسات الروتا والكورونا في إسهال عجول الابقار و الجاموس في مصر. حيث جمعت ٨٢ عينة برازية من عمر صفر إلى ثلاثة أشهر في ثلاث محافظات في مصر (الإسماعيلية والإسكندرية والشرقية). تم اختبار جميع عينات البراز بواسطة تقنية تفاعل سلسلة البلمرة في الوقت الحقيقي القائمة على مسبار (qRT-PCR) لتحديد وجود الأحماض النووية لفيروسى الروتا و الكرونا باستخدام البوادئ الخاصه لكل فيروس. كانت أربعة عشر (١٧٫١ ٪) و ٢٢ (٢٦٫٨ ٪) من ٨٢ عينات البراز إيجابية لفيروس الروتا و فيروس الكرونا على التوالي. تم حساب نسبة الإيجابية من المعلمات التحقيق المختلفة حول كل من فيروس الروتا و فيروس الكرونا اعتمادا على البيانات التاريخية لدينا. كانت الإصابة بفيروس الكورونا والروتا في عجول الابقار أكثر انتشارًا من عجول الجاموس. معدل الإصابة بفيروس الكورونا أكثر من عدوى فيروس الروتا في عجول الابقار بالإسماعيلية مقارنة بالمناطق الأخرى التي تمت دراستها. غالبًا ما تكون العدوى الفردية لعجول الابقار أو الجاموس مع كلا الفيروسين سائدة ، لكن العدوى المزدوجة في نفس النوع الحيواني ممكنة في نفس الوقت. معدل الإصابة بفيروسات الروتا و الكرونا في عجول الأبقار والجاموس أعلى في فصل الشتاء من الخريف والصيف والربيع.