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### Original Paper

# Seroprevalence and molecular characterization of bovine viral diarrhoea, Rota and corona viruses in neonatal cattle and buffalo calves in some governorates in Egypt

Lotfy, Asmaa<sup>1</sup>, Selim Abdelfattah<sup>1</sup>, Moustafa Abdel Moneim<sup>1</sup>, Salem, Sayed<sup>2</sup>

<sup>1</sup>Department of animal Medicine, Faculty of Veterinary Medicine, Benha University

<sup>2</sup>Animal Health Research Institute (AHRI)

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### ABSTRACT

The present study aims to detect the prevalence and molecular characterization of Bovine Viral Diarrhoea, Rota and corona viral diseases as major causes of diarrhoea that mainly leads to neonatal deaths among calves in some governorates in Egypt (Kafr El Sheikh, El Menofya, Sharkia, Bahaira and El-Gharbia). The infection rate of the three examined viral diseases showed non-significant difference between sex and age of cow calves, and the infection more common in female young calves. Selective positive samples undergo sequencing and compared with other strains in GenBank. The results showed that the Egyptian BVD strain is closely related to BVD strain from Argentina (DQ088995) and Colombia (MH198305), while Bovine Rota (BRV) showed high similarity with Bovine Rota strain of South Korea and Turkey, but Bovine Corona virus (BCoV) was closed to Bovine Corona viruses (BCoV) strain isolated from France.

## 1. INTRODUCTION

Diarrhoea causes serious economic damages by increasing mortality rate and reducing weight gain. So, it is considered one of the most serious diseases in neonatal calves with great risk especially in first months of life. Neonatal calf diarrhoea is a complex syndrome with various etiologies (Asadi et al., 2014). Multi-factorial nature of calf diarrhoea leads to problem in prevention and control so it should be based on a good understanding of the disease complications such as management and feeding during the calving period before disease outbreaks occurs (Yong-il Cho et al., 2014).

Bovine Viral Diarrhoea viruses (BVDV) infection can lead to enteric, respiratory, and reproductive disorders ease in bovine accompanied by immune suppression and increased susceptibility to subsequent viral or bacterial infections. There are variable clinical signs in infected animal, range from diarrhoea, nasal discharge, cough, pyrexia, and depression to hemorrhagic syndrome and death (Houe, 2003; Ridpath and Fulton, 2009; Taxis et al., 2019)

The main viral etiologies of enteric diseases are group A Rota viruses (GARV) and bovine corona virus (BCoV). Although, both viruses infect calves of the same age (Barry et al., 2009). Bovine Rota virus (BRV) is considered as a major agent of neonatal calf diarrhoea that causes severe diarrhoea, anorexia and deaths due to dehydration in calves that lead to major economic losses. (Jin et al., 2014). Bovine corona virus is a pneumoenteric virus that causes diarrhoea and respiratory disease in calves (Takiuchi et al., 2009). Infection is primarily

via fecal-oral and to a lesser extent, the respiratory (aerosol) route. Most often, transmission of enteric BCoV is horizontal, and transmission to young animals occurs from a carrier dam to their offspring postpartum or from clinically or chronically infected calves housed in proximity to immunologically naïve animals (Rocha et al., 2018). The prophylactic measures such as good management, hygiene and vaccination of dam cows. for prevention of neonatal diarrhoea caused by coronavirus and rotavirus. This occur through enhancement of antibody titres in the colostrum and milk of vaccinated cows to avoid high frequency, persistence infection, difficulties in the clinical diagnosis and failure of the treatment in young calves (Selles et al., 2014)

Therefore, the present study aims to investigate the seroprevalence of the three pathogens (BVD, BCoV and Rota virus) as a causative agent for calf diarrhoea and molecular characterization of the identified Egyptian isolates.

## 2. MATERIAL AND METHODS

### 2.1. Samples

A total of 118 serum samples were collected from clinically suspected un-vaccinated cow and buffalos' calves of different age (ranged between one month to more than six months) during summer and winter seasons of 2018 and 2019 year (Table 1)

In addition, a total of 119 fecal samples were collected from diarrheic bovine calves at age day-up to three months during winter and summer seasons of the 2018 and 2019 year (Table 2).

\* Corresponding author: Lotfy, Asmaa, Department of animal Medicine, Faculty of Veterinary Medicine, Benha University

Table 1 Locality, species and ages of farm animals used in the study of BVD (serum samples).

Locality	Species		Age /month		Total*
	Cattle calves	Buffalo calves	1-6m	>6m	
Menofyia	18	15	20	13	33
Kafr El Sheikh	15	10	18	7	25
Behaira	12	8	10	10	20
Sharkia	9	7	10	6	16
Gharbia	14	10	10	14	24
Total	68	50	68	50	118

\* Total number of animal in each province

Table 2 Locality, species and ages of farm animals used in the study of Rota and Corona viruses (fecal samples)

Locality	Species		Age /month		Total*
	Cattle calves	Buffalo calves	≤1m	≤3m	
Menofyia	12	13	8	17	25
Kafr El Sheikh	15	6	7	14	21
Behaira	13	7	10	10	20
Sharkia	10	18	10	18	28
Gharbia	12	13	15	10	25
Total	62	57	50	69	119

\* Total number of animals in each province

## 2.2. Serological analysis

The collected serum samples were examined using commercial ELISA kit for detection of BVDV antibodies (INGENASA Immunological Genetica Aplicada, Madrid, Spain) and for detection of BVDV antigen using IDEXX (USA) BVDV-Ag/serum plus antigen –capture ELISA.

The antibodies against Rota and Corona viruses were investigated in serum samples using Rotavirus ELISA kit, Bio-X Diagnostics (Sandwich ELISA) sandwich ELISA and Coronavirus ELISA kit, Bio-X Diagnostics (Sandwich ELISA).

## 2.3. Molecular diagnosis and characterization of three pathogens

RNA of BVDV was extracted from serum samples using QIAamp Viral RNA Mini Kit (QIAGEN, USA). The Extracted RNA were examined according to Zulauf (2007) with specific pair of primer for BVV

P1 5' - GGGNAGTCGTCARTGGTTCG - 3' (forward primer)

P2 5'- GTGCCATGTACAGCAGAGWTTT - 3' (reverse primer).

The primer sequence of BVDV 5'UTR gene supposes to amplify 177 bp fragments was selected for amplification of DNA. RNA of Rota and Coronavirus was extracted from collected fecal samples using QIAamp DNA stool Mini Kit (Qiagen, Germany). The RNA of Rota virus was examined using specific pair of primer

P15' - GAT ATT GGA CCA TCT GAT TCT GCT TCA AA - 3' (forward primer)

P2 5'- GAA ATC CAC TTG ATC GCA CCC AA - 3' (reverse primer)

The primer sequence of BRV5'UTR gene supposes to amplify 155 bp fragments was selected for amplification of DNA. The primer for Corona virus was

P1 5' - TGGATCAAGATTAGAGTTGGC - 3' (forward primer)

P2 5'- CCTGTCCATTTCTGACC - 3' (reverse primer)

The primer sequence of BCV5'UTR gene supposes to amplify 236 bp fragments was selected for amplification of

DNA. The PCR product was analyzed on 1.5% agarose gel electrophoresis.

The PCR product of one positive sample from each virus was partially sequenced. The purified PCR products were sequenced directly using the ABI PRISM® Big Dye™ Terminators Ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences were edited, and alignment was done with Benedict software. Also, the phylogenetic analysis for of obtained sequence was performed by Mega 7 software using the neighbor-joining tree method with 1000 bootstrap replicates.

## 3. RESULTS

The infection rate of three viral agents (BVD, Rota virus and Corona virus) as a causative agent of calve diarrhea in relation to sex of cow calves showed non-significant difference as shown in Table (1). The result revealed that the prevalence of BVD was higher in female cow calves (15%) than in males (9%). In contrast, the infection rate was higher in female (37.1%) and (11.4%) than male 26% and 11% for Rota and Corona viruses in cow calves, respectively.

Regarding the age, the infection rate was higher in young calve (1-6 month). The infection rate was 14%, 40% and 6.6% for BVDV, Rota and Corona viruses, respectively (Table 3). On another hand, the infection rate of BVD, Rota and Corona virus was higher in female buffalo calve where it was 34%, 13.3% and 16.6% (Table 4). Moreover, the infection rate was higher in young buffalo calve of 1-6-month-old; it was 26%, 25% and 20% for BVDV, Rota and Corona viruses, respectively.

The purified PCR product of each virus was sequenced and alignment with other reference strains in database of GenBank. The results revealed that the Egyptian BVD strain in the present study was closely related to BVD strain from Argentina (DQ088995) and Colombia (MH198305) (Figure 1). The phylogenetic analysis of obtained BRV strain revealed that Egyptian BRV was closely related to MF940692 BRV from South Korea (KX212878), Turkey (KF697087), India (KF636255.1) and USA (Figure 2). In addition, Figure (3) revealed that our isolated BCV was closed to BCov strain isolated from France (KX982264).

## 4. DISCUSSION

Diarrhea is considered the most important cause of disease in calves that lead to major economic losses in cattle production. (Stanton et al., 2013). The present results revealed that the prevalence of BVD was higher in female calves either in cattle (15%) or buffalo (34%) than in male (9%) in cattle and (18%) in buffalo. The obtained results came in accordance with results previously reported by Mockeliünien et al. (2004), who reported that sex of animal had no influence on the prevalence of BVDV.

Regarding to age of examined calves, the infection rate of BVD was non-significantly differed between two age groups (1-6 months and ≥ 6 months). The obtained result revealed that the percentage of antibodies against BVD virus was higher in younger calves (14% in cattle calve and 26% in buffalo calve) than older age group. These results was in harmony with results recorded by Guthmann (2019), who reported that serological testing of

calves with maternal antibodies leads to false-positive test results.

Serological investigation of antibodies against Rota virus in fecal samples which collected from diarrheic calves showed non-significant difference in infection rate between male and female. The infection rate was higher in

female calves (37.1% in cattle calve and 13.3% in buffalo calve) in comparison with male calve either cattle or buffalo. The results are dissimilar to that reported by (Sharma 2004; Dash et al., 2011; Umer et al., 2019), who reported that the males were more susceptible to Rota virus infection as compared to female calves.

Table 3 Infection rate of BVDV Rota virus and Corona virus among cow calve in relation to sex and age

Factor	BVD			Rota virus			Corona virus		
	No of examined	No of positive (%)	P value	No of examined	No of positive (%)	P value	No of examined	No of positive (%)	P value
Male	22	2 (9%)	0.13	27	7 (26%)	0.25	27	3 (11%)	0.64
Female	46	7 (15%)		35	13 (37.1%)		35	4 (11.4%)	
Age									
1-6 m	35	5 (14%)	0.539	30	12 (40%)	0.002	30	2 (6.6%)	0.66
≥6m	33	4 (12%)		32	2 (6.2%)		32	2 (6.2%)	

Table 4 Infection rate of BVDV, Rota virus and Corona virus among buffalo calve in relation to sex and age

Factor	BVD			Rota virus			Corona virus		
	No of examined	No of positive (%)	P value	No of examined	No of positive (%)	P value	No of examined	No of positive (%)	P value
Male	27	5 (18%)	0.246	27	2 (7.4%)	0.387	27	2 (7.4%)	0.258
Female	23	8 (34%)		30	4 (13.3%)		30	5 (16.6%)	
Age									
1-6 m	30	8 (26%)	0.582	20	5 (25%)	0.41	20	4 (20%)	0.493
≥6m	20	5 (25%)		37	7 (18.9%)		37	6 (16.2%)	

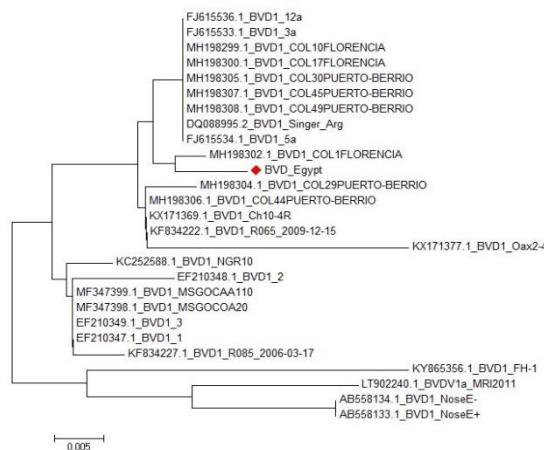


Figure 1 Alignments of nucleotide sequences of 5'UTR of BVDV local isolate and reference published BVDV strain in GenBank

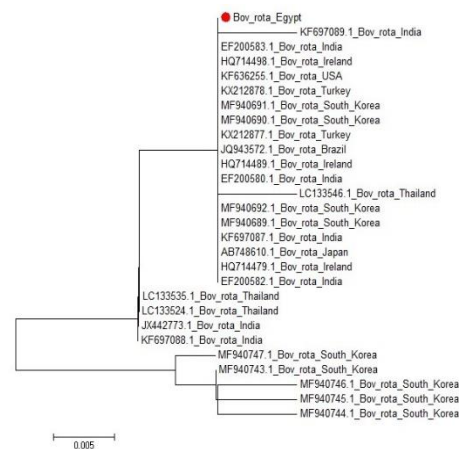


Figure 2 Alignments of nucleotide sequences of Rota virus local isolate and reference published Rota virus in GenBank

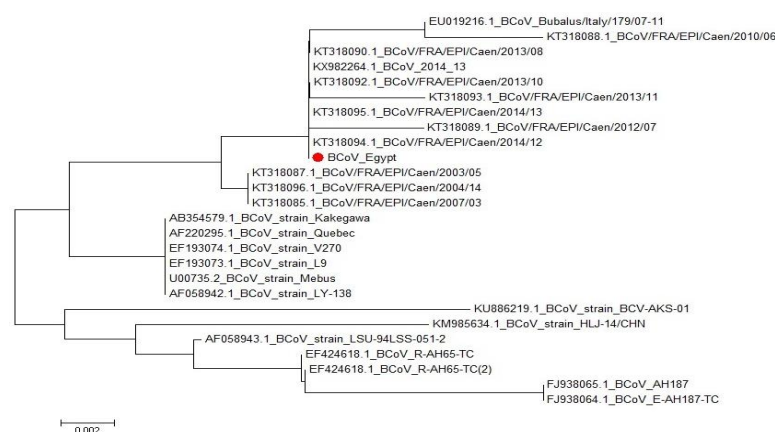


Figure 3 Alignments of nucleotide sequences of corona virus local isolate and reference published corona virus in GenBank

Moreover, the infection rate of Rota virus was significant higher in young age cattle calve 40% than older one 6.2%. In contrast, it was non-significant higher in younger buffalo calve 25% than older one (18.9%). The results agreed with that reported previously (Chase et al., 2008; Coura et al., 2015; Rocha et al., 2017), who found that all calves infected with Rota virus presented an episode of diarrhea in the first month of life. This finding was attributable to the immune status of calves, which is higher in the first week of life due to passive immunity provided by colostrum, and gradually decreases through a natural process of catabolism of immunoglobulins, making calves more susceptible to infection in the second and third weeks of life. Since most cases of RVA diarrhea occur within these age groups, at 4 weeks of age, calves are normally immune to infection through natural resistance

In addition, the infection rate of corona virus among calve showed non-significant difference between male and female or between young and older age calves. The obtained result is harmony with Abd El Rahim (1997), who mentioned that there was no significant difference in the susceptibility between cattle and buffalo calves or sex to bovine enteric coronavirus. The highest occurrence of infection decreased with advanced age.

The phylogenetic analysis of the obtained BVD strains revealed that Egyptian BVD strains present in one Clade with MH198302 from Colombia. The previous evidence come in agree with Habit et al. (2005), Emran et al. (2014), and Lysholm et al. (2019), who reported that BVDV-1 genotype, which according to previous studies is more common in Africa than BVDV-2. The phylogenetic analysis of Rota virus isolate that obtained the present study has higher homology percentage more than 98.4% with BRV isolated from Thailand and BRV isolated from South Korea. The obtained results disagree with Rondelli et al. (2018), who reported that Bovine RVA has shown genomic variability and complexity, so it difficult to implement efficient control and prophylaxis programs for this enteric infection. In the present study, the genotypes found through molecular characterization of RVA strains suggest a high genotypes diversity involvement in calf diarrhea.

The phylogenetic analysis revealed that our isolated BCV was grouped with BCV-1 reference strains available in the GenBank database and closest to (BCoV) France 2013,

(BCoV) France 2014. This may be attributed to the role of trade in transmission of the diseases with pregnant females from France and Italy.

## 5. CONCLUSION

From the previous results, we conclude that, BVD, BRV, and BCV, is endemic disease in Egypt and causes severe economic losses in cattle industry. This study showed that BRV was strongly distributed specially among calves aged 1-30 days. However, further investigations to identify the need for in-depth epidemiological studies. Therefore, rearing system, and colostrum intake is recommended for preventing and controlling calf diarrhea.

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