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ENTERIC ROTA AND CORONA VIRUSES INFECTION IN NEONATAL CALVES

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ABSTRACT

Calf diarrhea is a common syndrome causing colossal economic losses. *Rotavirus* and *Coronavirus* are major pathogens of calf diarrhea. During the period of investigation (13 consecutive months), a total number of 140 neonatal calves were carefully examined and thereafter subjected to serological and molecular diagnosis for the diagnosis of Rota and Corona viruses' infection. The serological and molecular diagnosis by using LAT and RT-PCR indicated that positive samples of *Rotavirus* infection were 21.43% (30/140) and 8% (4/50), respectively. ELISA and RT-PCR were used in diagnosing of BCoV infection and found that positive samples were 2.13% (2/94) and 4% (2/50), respectively. The Prevalence of *Rotavirus* and *Coronavirus* infection was 21.43 % (30/140) and 2.13 % (2/94) of the examined calves, respectively. It was found that there was a strong negative correlation between age of examined calves and *Rotavirus* infection. *Coronavirus* infection was found in calves at >4-7 weeks. There were no significance difference in Rota and Corona viruses' infection and sex, breed and species of examined calves. The most positive cases of *Rotavirus* and *Coronavirus* infection were reported in cold months. The lower infection rate of *Rotavirus* was recorded in hot months. It is concluded that Rota and Corona viruses play an outstanding role in causing enteritis in neonatal calves in different localities of Assiut governorate.

Key words: *Rotavirus, Coronavirus,* LAT, ELISA, RT-PCR, Epidemiology.

INTRODUCTION

Neonatal calves are the backbone of animal resources for dairy or beef industries (Andrews *et al.*, 2004). Pneumonia and enteritis appear to be the prominent problems deter the healthy condition of calves during the early days of their life. Enteritis appears to be more problematic and cause a considerable level of economic losses (Mushtaq *et al.*, 2013). Enteritis is a complex multi-factorial etiology, usually influenced by nutritional and environmental factors as well as management practices in association with infectious agents (Mohammed *et al.*, 2017). There are enormous pathogens encountered as enteropathogens. However, Enterotoxigenic K99+ *Escherichia coli*, followed by *Rotavirus*, *Coronavirus* and the protozoan *Cryptosporidium parvum* are commonly reported endemic micro-organisms associated with neonatal calf diarrhea (Rocha *et al.*, 2017). The infectious agents such as Rota and Corona viruses' appear to be more serious enteropathogens in neonatal calves (Cockcroft, 2015). Both *Rotavirus* and *Coronavirus* can induce intestinal villous atrophy which leads to malabsorption and maldigestion (Zachary and McGavin, 2013 and Ammar *et al.*, 2014). Bovine *Coronavirus* enteritis is more severe than bovine *Rotavirus* because it affects small and large intestine as well as it causes respiratory tract infection (Dash *et al.*, 2012 and Hansa *et al.*, 2012). Diagnosis of Rota and Corona viruses' enteritis depending on clinical signs is unfeasible. Consequently, the clinically suspected cases must be confirmed by laboratory tests (Mayameei *et al.*, 2010). Laboratory diagnosis of both viruses in calves' enteritis depends on identification of viral antigens then nucleic acids in fecal samples. Latex agglutination test (LAT) and Enzyme linked immunosorbent assay (ELISA) are rapid, sensitive, specific tests and need shorter time than viral isolation for Rota and Corona viral antigen detection. Virus confirmation can be done at genomic level by molecular method of reverse transcriptase polymerase chain reaction (RT-PCR) (Singh and Jhala, 2011; Dash *et al.*, 2012 and Sravani *et al.*, 2014). Detection of clinical findings, laboratory diagnosis and epidemiological studies of both BRV and BCoV in calves in Assiut appear to be scanty. Therefore the current work aimed to investigate the clinical findings of enteric calves with serological, molecular and epidemiological investigation of bovine Rota and Corona viruses in neonatal calves.

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MATERIALS AND METHODS

Animals

During the period of investigation, from December 2015 to December 2016, a total of 140 examined neonatal calves of different ages, sex, breed and species were belonged to different farms. Besides, individual cases were from different localities in Assiut Governorate that admitted to Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University.

Clinical examination

Clinical examination of investigated calves was carried out according to (Jackson and Cockcroft, 2002).

Sampling

All fecal samples were collected from enteric and clinically healthy neonatal calves in sterile plastic cups for serological and molecular diagnosis.

Serological diagnosis

a- Serological detection of *Rotavirus* antigen by LAT

All 140 fecal samples were subjected to LAT for detection of *Rotavirus* antigen by a commercial kit (REF-M80 Rotascreen® kit Microgen Bioproducts limited, United Kingdom).

b- Serological detection of *Coronavirus* antigen by ELISA

The 94 fecal samples were subjected to ELISA for detection of *Coronavirus* antigen by a commercial kit (Antigenic ELISA kit for detection of *Coronavirus*, Bio-x Diagnostics S.P.R.L., Belgium, BIO K 344/2) and following the protocol as per the manufacture's instruction. The results were interpreted by using OD value obtained at 450 nm using ELISA reader (Sunrise absorbance reader, Tecan, Austria).

Molecular diagnosis

Molecular detection of Rota and Corona viral nucleic acid by RT-PCR

a- RNA extraction

50 fecal samples were used for extraction of viral RNA by using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

b- RT-PCR

In case of *Rotavirus*, all extracted RNAs were denatured at 97°C for 3 min and immediately placed on ice (Fukuda *et al.*, 2013).Then, OneStep RT-PCR was performed by using Qiagen OneStep RT-PCR Kit (Qiagen, Hilden, Germany) according the manufacture's instruction in both Rota and Corona viruses. The primers pair used for amplification of VP7 gene of bovine *Rotavirus*. The sequences of these primers were as follows: upstream primer: 5′-GGC TTT AAA AGA GAG AAT TTC CGT CTG G-3′, downstream primers: 5′- GGT CAC ATC ATA CAA TTC TAA TCT AAG -3′ (Gouvea *et al.*, 1990 and Sravani *et al.*, 2014). But the primers pair used for amplification of N gene of bovine *Coronavirus*. The sequences of these primers were as follows: upstream primer: 5′-GCA ATC CAG TAG TAG AGC GT-3′, downstream primers: 5′-CTT AGT GGC ATC CTT GCC AA-3′ (Cho *et al.*, 2001). The amplified products were visualized on agarose gel stained with Ethidium bromide and photographed by a gel documentation system (BDA digital Biometra, Germany). The RT-PCR products of *Rotavirus* of 1062 bp were visualized on 1% agarose gel but the RT-PCR products of *Coronavirus* of 730 bp visualized on 1.5% agarose gel.

Statistical analysis

The data were analyzed by Chi-square of independence and correlation according to Statistical package for the social sciences (SPSS) version 16 software program (2007).

RESULTS

Clinical findings

Clinical findings revealed that the investigated enteric calves were showed classic signs of enteritis which suffering from variable degree of diarrhea, dehydration, weakness, reluctance to move, recumbence with signs of comatose. Some of examined calves were suffering from emaciation and straining with or without arched back (Fig. 1). In addition to enteritis, signs of respiratory embarrassment in form of nasal discharge, cough and signs of dyspnea were observed in some cases of enteric calves. Regarding to fecal characters of enteric calves, the fecal consistency differ from normal to profuse watery. The color of fecal discharge varied from pale yellowish, yellowish to greenish and some of fecal samples contained mucus with or without undigested food and clotted blood (Fig. 2).

Serological diagnosis

a- Serological diagnosis of *Rotavirus* antigen by using LAT

Of a total 140 examined fecal samples for *Rotavirus* antigen, 30 (21.43%) were serologically positive by LAT as indicated by clear agglutination of latex particles in tested samples (Fig. 3).

b- Serological diagnosis of *Coronavirus* antigen by using ELISA

Our result revealed that 2 (2.13%) of 94 examined fecal samples were serologically positive.

Molecular diagnosis

a- Molecular diagnosis of *Rotavirus* nucleic acid

Four (8%) of 50 fecal samples were molecularly positive. The specific band showed at 1062 bp after PCR amplification of VP7 gene of *Rotavirus* (Fig. 4).

b- Molecular diagnosis of *Coronavirus* nucleic acid

Our result revealed that 2 (4%) out of 50 fecal samples were molecularly positive. The specific band showed at 730 bp after PCR amplification of N gene of *Coronavirus* (Fig. 5).

Epidemiological findings

a- Percent of infection

The present study indicated that percentage of *Rotavirus* infection was 21.43% (30/140) of investigated neonatal calves, while percentage of *Coronavirus* infection was 2.13% (2/94) of examined neonatal calves (Table 1). The percentage of *Rotavirus* infection among the enteric calves was 23.33% (28/120) and 10% (2/20) among clinically healthy calves, while percentage of *Coronavirus* infection among enteric calves was 2.20% (2/91) and 0% (0/3) among clinically healthy calves (Table 2). Regarding to locality, the infection of *Rotavirus* was 23.08% (15 of 65) of fecal samples collected from calves that came to Veterinary Teaching Hospital, while prevalence was 20% (15 of 75) of tested fecal samples of calves of different farms in Assiut Governorate. The positive cases of *Coronavirus* infection was detected only in investigated enteric calves in farms (Table 3).

b- Age susceptibility

The rate of *Rotavirus* infection was studied in calves at age groups of 3 days-1week, >1-2, >2-3, >3-4, >4-5, >5-7 and >7-12weeks and yielding 57.14%, 45.46%, 33.33%, 16%, 14.29%, 19.23% and 10.26%, respectively of 140 of examined calves. *Coronavirus* infection was observed among enteric calves of >4-5 weeks old by 9.09% (1/11) and >5-7 weeks old by 6.25% (1/16) of investigated calves (Table 4 & Fig. 6).

c- Effect of sex

The analytic results indicated that there was no significant difference in percentage of both Rota and Corona viruses' infection between male and female calves (Table 5).

d- Breed susceptibility

In the present study, there was no significant difference in percentage of Rota and Corona viruses' infection between Native, Friesian ad Holstein breeds (Table 6).

e- Species susceptibility

Rotavirus infection was diagnosed in both cattle and buffalo calves' fecal samples. It's found 22.14% of cattle calves were positive to *Rotavirus*, where 11.11% of buffalo calves were positive. *Coronavirus* infection was diagnosed among cattle calves (2.25%) and no infection among buffalo calves was reported (Table 7).

f- Seasonal variation

Our result indicated that percentage of *Rotavirus* infection in examined calves was in cold months (26.97%) and was (11.77%) in hot months. Percentage of *Coronavirus* infection was detected in cold months only by 3.45% (Table 8 & Fig. 7).

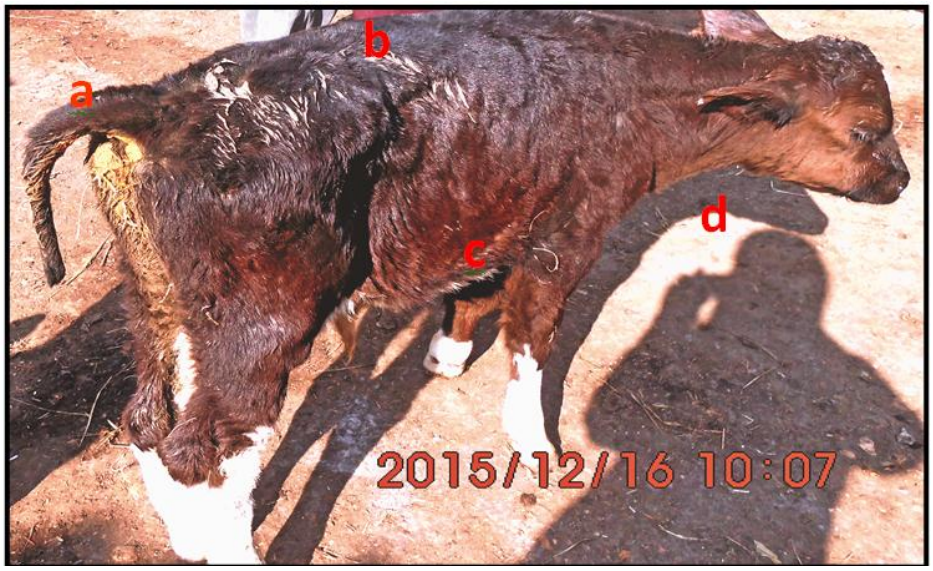

Figure 1: Frequent attempts of an enteric calf to evacuate the intestinal contents. Note: (a) Raising the tail with lateral deviation, (b) Arched back, (c) Abdominal tucked-up (d) Extension of head and neck.

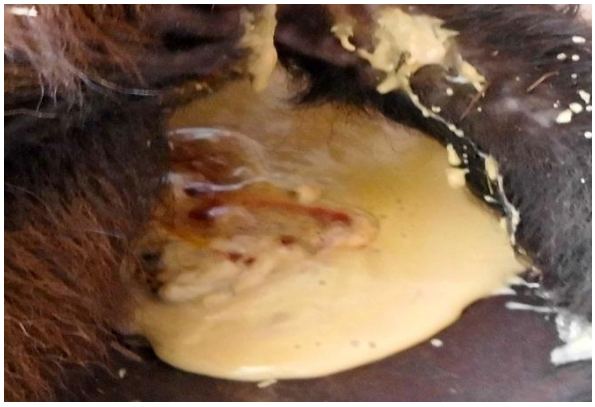

Figure 2: Close-up photograph on an enteric native breed calf discharging extra-mucoid yellowish fecal discharge similar viscous lentil soup containing various sizes of particles of indigestible food materials and flakes of blood clots.

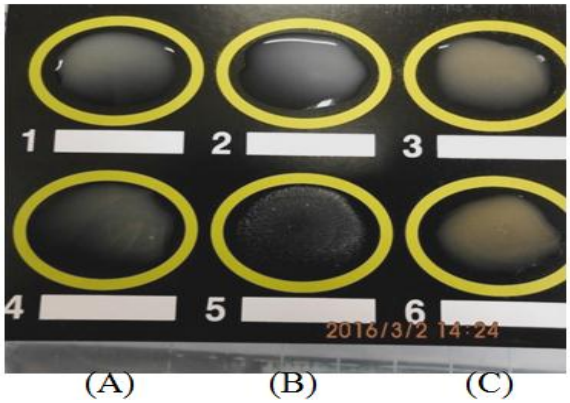

Figure 3: LAT for detection of *Rotavirus* antigen ($A_{1,4}$) and ($C_{3,6}$) Negative results (milky suspension). ($B_{2,5}$) Positive result (agglutination).

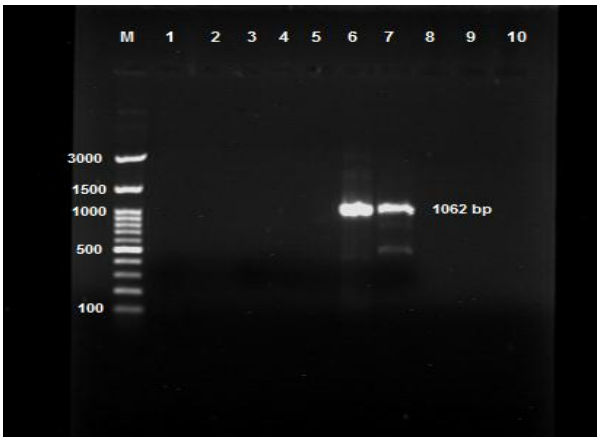

Figure 4: Agarose gel electrophoresis of RT-PCR amplification of VP7 gene of Rotavirus. Lane M: DNA Marker of 100 bp, lanes 6, 7: positive samples with amplified product at 1062 bp, lanes 1, 2, 3, 4, 5, 8, 9 & 10: negative samples.

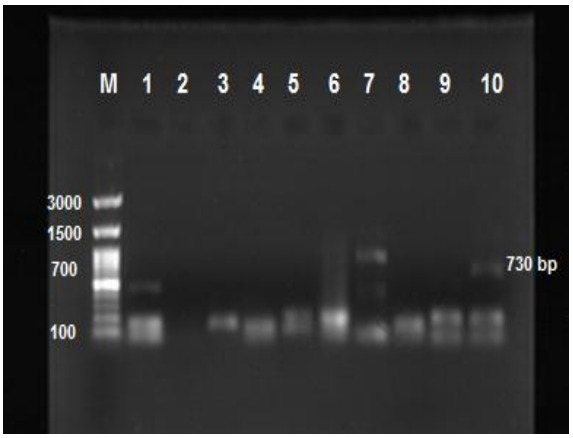

Figure 5: Agarose gel electrophoresis of RT-PCR amplification of N gene of Coronavirus. Lane M: DNA Marker of 100 bp, lanes 10: positive sample with amplified product at 730 bp, lane 1, 2, 3, 4, 5, 6, 7, 8 & 9: negative samples.

Table 1: Percentage of Rota and Corona viruses' infection in examined calves.

Viral enteropathogens	Number of examined calves	Positive	Percentage
Rotavirus	140	30	21.43 %
Coronavirus	94	2	2.13 %

Table 2: Occurrence of Rota and Corona viruses' infection in enteric and the clinically healthy calves**.**

	***Rotavirus* infection**			***Coronavirus* infection**		
	Nº.	**Positive**	**%**	**Nº.**	**Positive**	**%**
Enteric calves	120	28	23.33	91	2	2.20
Clinically healthy calves	20	2	10	3	0	0
Total	140	30	21.43	94	2	2.13

No significant variation at $p<0.05$

Table 3: Distribution of Rota and Corona viruses' infection in examined calves in different localities.

Origin	*Rotavirus* infection			*Coronavirus* infection		
	Nº.	Positive	%	Nº.	Positive	%
Individual cases*	65	15	23.08	38	0	0
Farms	75	15	20	56	2	3.57
Total	140	30	21.43	94	2	2.13

***** admitted from various area of Assiut to Veterinary Teaching Hospital
No significant variation at p<0.05

Table 4: Age susceptibility to Rota and Corona viruses' infection in investigated calves.

Age groups (weeks)	*Rotavirus* infection			*Coronavirus* infection		
	Nº.	Positive	%	Nº.	Positive	%
3 days-1week	7	4	57.14**	4	0	0
>1-2	11	5	45.46	8	0	0
>2-3	18	6	33.33	13	0	0
>3-4	25	4	16	15	0	0
>4-5	14	2	14.29	11	1	9.09
>5-7	26	5	19.23	16	1	6.25
>7-12	39	4	10.26	27	0	0
Total	140	30	21.43	94	2	2.13

** Highly significant negative correlation at P < 0.01

Table 5: Effect of sex on both Rota and Corona viruses' infection in examined calves.

Sex	*Rotavirus* infection			*Coronavirus* infection		
	Nº.	Positive	%	Nº.	Positive	%
Male	85	22	25.88	58	2	3.45
Female	55	8	14.55	36	0	0
Total	140	30	21.43	94	2	2.13

No significant at p<0.05

Table 6: Breed susceptibility to both Rota and Corona viruses' infection in investigated calves.

Breed	*Rotavirus* infection			*Coronavirus* infection		
	Nº.	Positive	%	Nº.	Positive	%
Native (balady)	54	10	18.52	35	2	5.71
Friesian	49	11	22.45	36	0	0
Holstein	37	9	24.32	23	0	0
Total	140	30	21.43	94	2	2.13

No significant variation at p<0.05

Table 7: Detection of Rota and Corona viruses' infection in investigated calves according to species.

Species	*Rotavirus* infection			*Coronavirus* infection		
	Nº.	Positive	%	Nº.	Positive	%
Cattle calves	131	29	22.14	89	2	2.25
Buffalo calves	9	1	11.11	5	0	0
Total	140	30	21.43	94	2	2.13

No Significant variation at $p<0.05$

Table 8: Detection of both Rota and Corona viruses' infection in examined calves according to seasonal variation.

Seasons	*Rotavirus* infection			*Coronavirus* infection		
	Nº.	**Positive**	**%**	**Nº.**	**Positive**	**%**
Cold months (from October to February)	89	24	26.97*	58	2	3.45
Hot months (from March to September)	51	6	11.77	36	0	0
Total	140	30	21.43	94	2	2.13

* Significant increase at $p<0.05$

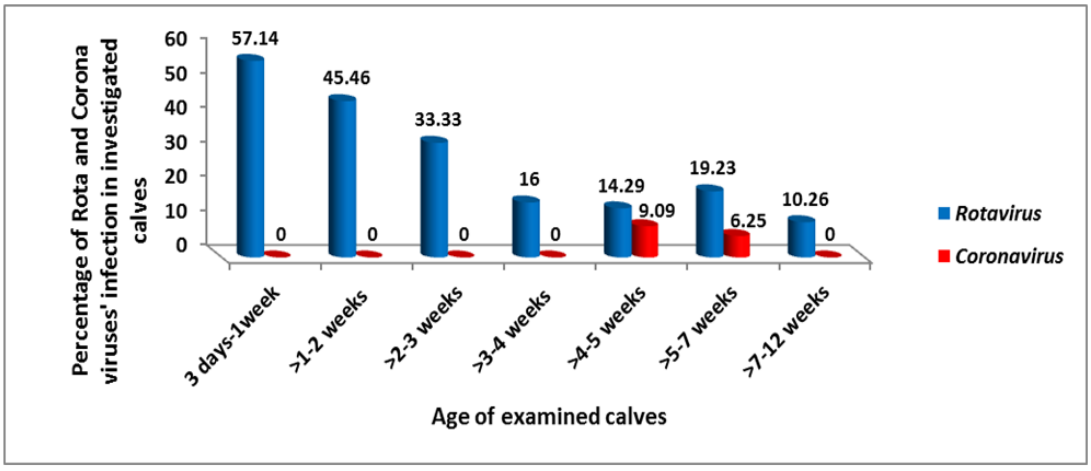

Figure 6: Age susceptibility to Rota and Corona viruses' infection in investigated calves.

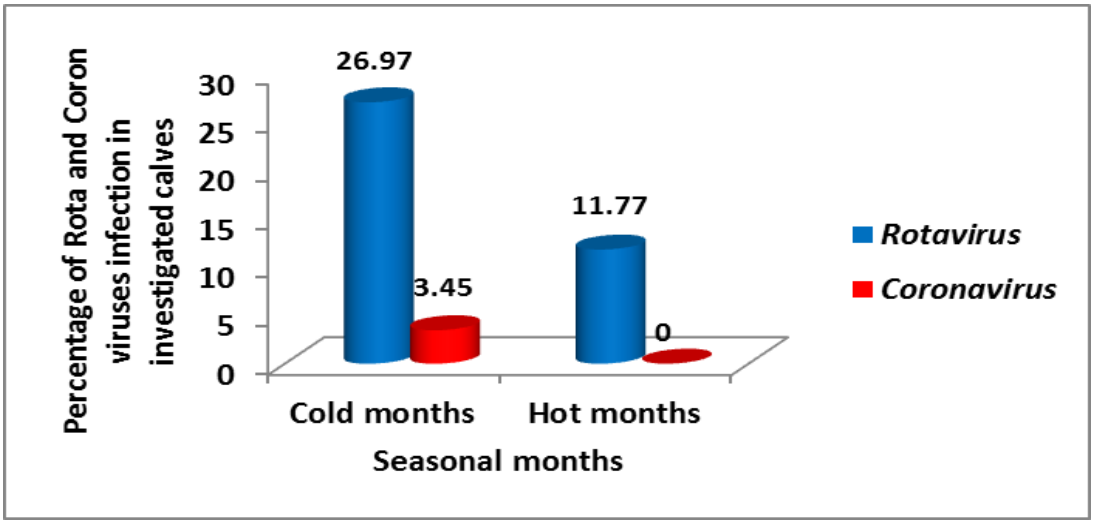

Figure 7: Detection of Rota and Corona viruses' infection in examined calves according to seasonal variation.

DISCUSSION

Neonatal calf diarrhea is multifactorial syndrome, resulting from an interaction between calf, managemental, environmental, and nutritional in association with infectious agents. The later plays an outstanding role in calf diarrhea particularly when the hygienic measures are sublevel or bad. Viral pathogens particularly *Rotavirus* and *Coronavirus* are the most implicated pathogen of calf diarrhea (Ammar *et al.*, 2014 and Mohammed *et al.*, 2017). From clinical point of view, the current work revealed that *Rotavirus* infection was serologically and/or molecularly detected in 30 cases of examined neonatal calves (n = 140) with characteristic clinical features of enteritis with variable degree of diarrhea, mostly yellowish mucoid discharge with or without flakes of clotted blood, followed by dehydration and ended by lateral recumbence with signs of comatose. Some calves had straining with or without arched back. The systemic reactions were varied from case to another. Similar findings were previously observed (Dash *et al.*, 2012 and Ammar *et al.*, 2014). Histopathlogically, *Rotavirus* invades stunts and exfoliates enterocytes' villi of small intestine inducing disorder in the intestinal mucosal barriers in association with spectacular reduction in absorption capacity and in secretion of the digestive enzymes resulting profuse viscous fluid containing undigested and unabsorbed nutrients in intestinal lumen. Subsequently weakness, dehydration and thereafter acidosis due to remarkable physiological alterations and electrolytes imbalances are established (Brown *et al.*, 2007). Bovine *Coronaviruses* infect calves all-over the world and are pneumotropic viruses in association with their pathological role in alimentary tract (Maclachlan and Dubovi, 2011). Moreover, Brown *et al.* (2007) added that BCoV produces villus atrophy by causing lysis or exfoliation of surface of mature enterocytes and causes microscopic and macroscopic alterations in colonic epithelium of infected calves. These may interpret the voluminous mucoid and slimy, green to light-brown in color of fecal discharge in association with signs of respiratory embarrassments, in form of nasal discharge, cough and signs of dyspnea, of calves infected by *Coronavirus* infection. Similar clinical findings and conclusions were previously reported (Zachary and McGavin, 2013).

Serodiagnosis of *Rotavirus* infection in fecal samples of examined calves depending on LAT. The present study revealed that 21.43% of fecal samples were positive and this result was higher than results obtained by Radhy (2015) and Dulgheroff *et al.* (2016). On contrary, highest rate of *Rotavirus* infection in enteric calves were offered by Faheem (1999); Singh and Jhala (2011) and Izzo *et al.* (2012). In regarding to *Coronavirus* infection, an antigen detecting ELISA technique was used for serological detection of *Coronavirus* infection in fecal samples of examined calves. The present study revealed that 2 (2.13%) of 94 cases were serologically positive. Similar results were reported by Safavi *et al.* (2012) and Bok *et al.* (2015). The obtained result was lower than that reported by Izzo *et al.* (2012) but the current result was higher than that reported by Yavru *et al.* (2016).Such variations in rate of infection of Rota and Corona viruses' infection in enteric calves may be attributes to geographical differences, difference in timing of samples collection, hygienic measures and environmental conditions.

Currently, fifty fecal samples of examined calves were molecularly tested by RT-PCR for detection of *Rotavirus* infection and revealed 8% of tested samples were positive. Comparatively, LAT and RT-PCR, The 50 fecal samples were examined by both techniques. Ten samples (20%) were positive by LAT while 4 only (8%) samples were positive by RT-PCR. Similar conclusions were reported by Kumar *et al.* (2011). The lower prevalence of RT-PCR than LAT in present work may ascribed to three plausible reasons; a) non-specific inhibition of PCR reaction by the components of fecal samples, b) mismatches in primer binding sites or c) the sequence variation in *Rotavirus* genome due to segmented of RNA genome, *Rotavirus* is continuously changing and leading to emergence of new genotypes (Gouvea *et al.*, 1990 and Kumar *et al.*, 2011).

Concerning *Coronavirus* infection, 50 fecal samples were examined by using RT-PCR for detection of *Coronavirus* infection in calves and found that 2 (4%) of 50 samples were positive. The current results indicated that both serological (ELISA) and molecular (RT-PCR) were efficient and sensitive {RT-PCR was 4% (2/50) – ELISA was 4% (2/50)}. Similar results were reported by Hansa *et al.* (2012) who reported that all ELISA tested fecal samples were found positive by RT-PCR.

From the epidemiological point of view, the present study indicated that percent of *Rotavirus* infection of examined calves was 21.43% in *Assiut* Governorate. Lower prevalence in Assiut was reported by Abd El-Rahim (1997) and Abou El-Ella *et al.* (2013) and higher prevalence was also reported by Faheem (1999) in *Assiut* Governorate. On the same manner, the rate of *Coronavirus* infection in the current work was 2.13% (2 of 94) of investigated neonatal calves in *Assiut* Governorate. This result was lower than that obtained by Abd El-Rahim (1997); Faheem (1999) and Abou El-Ella *et al.* (2013). Such diversities may attributed to differences in duration of samples collection, variation in samples size, farms management, hygienic status, environmental condition, using different techniques in diagnosis and difference in qualities and quantities of colostrum received by examined calves in the first few hours

after calving (Abd El-Rahim, 1997; Faheem, 1999 and Ammar *et al.*, 2014). The occurrence and distribution of *Rotavirus* in enteric and clinically healthy calves have been studied by (Safavi *et al.*, 2012). In the current study, the rate of *Rotavirus* infection of enteric and clinically healthy calves was 23.33% (28/120) and 10% (2/20), respectively. The obtained result indicated that *Rotavirus* infection was higher in enteric than clinically healthy calves. This may be attributed to *Rotavirus* destroys enterocytes of small intestine resulting in diarrhea which is accompanied by a profuse fecal shedding of virus. The rate of *Coronavirus* infection of examined enteric calves was 2.20% (2/91) and no rate of infection with clinically healthy calves. This may be attributed to *Coronavirus* destroys enterocytes of small and large intestines resulting in diarrhea, which is accompanied by a profuse fecal shedding of virus. Regarding to locality in the present study, there was no significant difference in percent of Rota and Corona viruses' infection in examined calves of Veterinary Teaching Hospital and farms of Assiut Governorate. This may be attributed to these calves were found under same geographical, seasonal condition, method of animals husbandry, hygienic measures, managemental system and the same animals breed.

Statistical analysis of the obtained results indicated that the rate of infection with *Rotavirus* was decreased by increasing the age of examined calves and peak of infection was at 3 days—1week old. Singh and Jhala (2011) and Ammar *et al.* (2014) studied rate of *Rotavirus* infection in calves at different ages and their results concluded that rate of infection was highest during the first 2 week and thereafter declining by increasing the age of calves**.** This may be due to immune system of neonatal calves is not fully mature to handle *Rotavirus* pathogen (Ammar *et al.*, 2014). Moreover, the susceptibility of calves to *Rotavirus* decreases with age probably due to loss of receptors on enterocytes (Udaykar *et al.*, 2013). Lactase enzyme present in the brush border of intestinal epithelial cells that may be act as a receptor and un-coating enzyme for *Rotavirus*, level of lactase is highest in early life and decreases as age of calf increases so rate of infection with *Rotavirus* was decreased by increasing age of examined calves (McNulty, 1978). *Coronavirus* infection was observed among enteric calves of >4-5 weeks old by 9.09% (1/11) and >5-7 weeks old by 6.25% (1/16) of investigated calves. Similar results was reported by Davoudi *et al.* (2014) who indicated that highest prevalence rate of *Coronavirus* infection was seen at 2-6 weeks and least during the first week and 6-8 weeks of age. This may due to decrease of colostral antibodies present in first weeks of age (Stipp *et al.*, 2009). In current study, absence of *Coronavirus* in age of 3 days-4 weeks may be due to presence of colostral antibodies and strong local immunity in intestinal lumen that protect newly born calves against viral infection (Mayameei *et al.*, 2010). The absence of *Coronavirus* in age of > 7-12 weeks may be attributed to activation of local host immunity so increase natural resist to viral infection (Mayameei *et al.*, 2010).

In referring to the effect of sex on distribution of infection, the analytic results indicated that there was no significant difference in rate of Rota and Corona viruses' infection between male and female calves. The same result was recorded by Radhy (2015) and Yavru *et al.* (2016). This may be due to the anatomical, functional and hormonal similarities of body systems of male and female calves in early ages that lead to non-particular resistance against Rota and Corona viruses' infection but degree of contamination with virus, dose of virus, exposing to stress factors, all effect on infection rate and severity in both sex of calves in same or different periodic age (Yavru *et al.*, 2016).

In the present work, there was no significant difference in percentage of Rota and Corona viruses' infection between Native, Friesian ad Holstein breeds. Similarly, Faheem (1999) revealed that no significant difference in prevalence of Rota and Corona viruses' infection between different breeds of calves. Concerning species susceptibility, the obtained results revealed that *Rotavirus* infection was diagnosed in both cattle and buffalo calves' fecal samples with no significant difference, although the higher percent of *Rotavirus* infection in cattle's calves than buffalo calves mathematically. These findings were previously reported by El-Bagoury *et al.* (2014). In the present study, *Coronavirus* infection was diagnosed among cattle calves (2.25%) and no infection among buffalo calves was reported. Abou El-Ella *et al.* (2013) found that BCoV was diagnosed in 8.45% of cattle calves and 5.13% in buffalo calves. This may attributed to lower susceptibility of buffalo calves than cattle calves and difference in immune status of calves.

Relationship between seasonal variations and rate of infection with Rota and Corona viruses was studied during the period of investigation and found that percent of *Rotavirus* infection in examined calves was high (26.97%) in cold months of Assiut Governorate and was low (11.77%) in hot months. Percent of *Coronavirus* infection was detected in cold months only by 3.45%. Similar results were reported by Mushtaq *et al.* (2013); Mayee and Alrodhan (2014) and Asadi *et al.* (2015). In Egypt, most calving occur at the end of autumn and beginning of winter in which these neonatal calves are more susceptible to Rota and Corona viruses' infections. Additionally, the intestinal and serum immunoglobulin's level were decreased in autumn, winter and increased during spring, summer (Mushtaq *et al.*, 2013 and Asadi *et al.*, 2015). Mayee and Alrodhan (2014) indicated that

BCoV was stable during cold months due to it's envelop nature, which was moderately sensitive to heat and *Coronavirus* was readily disseminated in winter months.

CONCLUSION

According to the results of this study and our field observation; Rota and Corona viruses play an outstanding role in causing enteritis in neonatal calves in different localities of Assiut governorate. Our attention should be directed toward these viruses in any control programs put to overcome enteritis in neonatal calves in Assiut.

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عدوى فيروسات الروتا والكورونا المعوية في العجول حديثة الولادة

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الإسهال فى العجول حديثة الولادة هومشكلة متعددة الأسباب حيث يسبب خسائر اقتصادية هائلة. وتعد فيروسات الروتا والكورونا المعوية من أهم المسببات الرئيسية للإسهال. تم استخدام ١٤٠ عجل حديث الولادة خلال فترة الدراسة (ثلاثة عشر شهرا متتالية) لتشخيص عدوى فيروسات الروتا والكورونا اكلينيكا بعناية ثم بعد ذلك تم تشخيصهم بواسطة الاختبارات السيرولوجية والجزيئية إلى جانب رصد الصورة الوبائية. كان معدل الإصابة بفيروسات الروتا و الكورونا ٢١.٤٣٪ (١٤٠/٣٠) و ٢.١٣٪ (٩٤/٢) فى العجول التى تم فحصها على التوالي. وأشار التشخيص السيرولوجى والجزيئي باستخدام اللاتكس و تفاعل البلمرة المتسلسل إلى أن العينات الإيجابية بعدوى فيروس الروتا كانت ٢١.٤٣٪ (١٤٠/٣٠) و ٨٪ (٥٠/٤) على التوالي. استخدمت الاليزا و تفاعل البلمرة المتسلسل في تشخيص عدوى فيروس الكورونا ووجد أن العينات الإيجابية كانت ٢.١٣٪ (٩٤/٢) و ٤٪ (٥٠/٢)على التوالي. وجد أن هناك ارتباطا سلبيا قويا بين عمر العجول التي تم فحصها وعدوى فيروس الروتا. ويتم العثور على عدوى فيروس الكورونا فى العجول فى الفئة العمريه <٤-٧ أسابيع. أشار هذا العمل الى أن الجنس فى العجول لا يلعب أي دور في نسبة العدوى بتلك الفيروسات من الناحية الاحصائية. لا يوجد أى فرق معنوي بين معدل الإصابة بتلك الفيروسات وسلالة العجول التى تم فحصها. كان معدل إصابة العجول البقرى والجاموسى بتلك الفيروسات متوازية إحصائيا. سجلت معظم حالاتنا الإيجابية بعدوى فيروسات الروتا والكورونا في الأشهر الباردة. سجل إنخفاض معدل الإصابة بفيروس الروتا في الأشهر الحارة. ولخص أيضا إلى أن فيروسات الروتا والكورونا تلعب دورا هاما في حدوث الالتهاب المعوى في العجول حديثة الولادة في مناطق مختلفة بمحافظة أسيوط.