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DETECTION OF ROTAVIRUS AND ESCHERICHIA COLI AS A CAUSE OF NEONATAL CALF DIARRHEA IN EL-WADY EL-GEDID GOVERNORATE

By

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ABSTRACT

Rotavirus and Escherichia coli are the most common causes of scours, rotavirus alone accounts for about 27- 36% of calf scours incidence worldwide. A total of 65 fecal samples were randomly collected from diarrheic calves up 5 weeks of age in different localities in El- Wady El-Gedid governorate. All fecal samples were examined to determine the presence of bovine rotavirus (BRV) and *E. coli,* as causes of calf diarrhea. Twelve (18.46%) and 18 (27.69%) out of 65 fecal samples were positive to rotavirus using Lateral flow and ELISA testes respectively. The infection rate of *E. coli* was (46.15%).The Age with higher incidence of Rotavirus infection was recorded in 8-15 days of age (with percentage of 12.31%), but higher incidence of *E. coli* infection was recorded in 1-7 days of age (with percentage of 16.92%), and incidence of co-infection was (10.77%). Results also revealed that the infectious rate of BRV, *E. coli* and co-infection were higher in winter with percentages of 18.46 %, 24.62 % , and 7.69 % than autumn (7.69%, 12.31% and 3.08%).In spring the infections were 1,53%, 6.15 % and zero % while in summer the rates were zero %, 3.08 % and zero % respectively. Serotyping of *E. coli* revealed the presence of O_{142}, O_{55}, O_{111}, O_{27}, O_{157}, O_{119}, O_{26} and O_{127} by a percentage of 26.67%, 20%, 13.3%, 10%, 6.6%, 3.3%, 6.6% and 6.6%, respectively. Multiplex PCR was applied for detection of the virulence genes *stx1 (5/10), stx2 (3/10) and eae (6/10) in E.coli isolates.*

Key words:

Calf diarrhea, Rotavirus, ELISA, *E.coli* virulence and resistance genes

INTRODUCTION

Neonatal Calf diarrhea is one of the most common problems of cattle industry all over the world and a major cause of productivity and economic losses, directly through mortality and indirectly through poor growth after clinical disease, increased susceptibility to other infections and treatment costs **(Barua *et al*., 2019 and El-Seedy *et al.*, 2016).** About 57% of weaning calf mortalities are due to diarrhea and most cases occurred in calves less than 1 month old **(USDA. 2007)** Various pathogenic agents (*e.g*., viruses, bacteria, and protozoa) are involved in the development of this disease, these pathogens are involved in 75%-95% of worldwide calf diarrheic cases. The most common enteric pathogens known to cause calf diarrhea are bovine Rota virus (BRV) and bovine Corona virus (BCV), enterotoxigenic Escherichia (E.) coli,) and Cryptosporidium parvum **(Radostits *et al.*, 2007**; **Bartels *et al*., 2010 and Izzo *et al*., 2011).** Combination-infection is frequently observed in diarrheic calves, although a single primary pathogen can be the cause in some cases. Rotavirus alone accounts for about 27- 36%. **(Barua, *et al*., 2019 and Uhde *et al*., 2008)**.Other factors including both the environment, nutritional status and management practices influence the disease severity **(Izzo *et al*., 2011)**

Bovine rotavirus (BRV) is a primary etiological agent of calf diarrhea. The virus belongs to the genus *Rotavirus* within the family Reoviridae. Rotavirus is a non-enveloped virion possessing 11 double-stranded RNA segments and is very stable over a wide range of pH with heat labiality **(Fenner *et al*., 2011).**

There are eight serogroups (A through H) of rotaviruses based on antigenic and genetic variability of the intermediate capsid protein (VP6), and most BRVs (95%) belong to group A **(Matthijnssens *et al*., 2012 and Mihalov-Kovács *et al*., 2015).** Moreover, within each group, Rotaviruse*s* 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 classifieds RVA into G groups, while VP4 is a protease-sensitive polypeptide and assigns the P groups **(Estes and Greenberg, 2013).**Group A (BRV) is the major cause of acute viral gastroenteritis in neonatal calves and usually causes diarrhea in calves at 1 to 2 weeks of age.The milk uptake by calves can provide a good environment for rotavirus survival under a wide range of gastrointestinal pH levels and infection of the intestinal epithelial cells **(Dhama *et al*., 2009 and Cho and Yoon, 2014).**

The virus infection has a very short incubation period (12-24 h) and induces peracute diarrhea in the affected calves **(Steele *et al*., 2004)** and the infected calves shed a large amount of the virus via feces for 5-7 days. BRV replicates in the cytoplasm of epithelial cells of small intestinal villi, destroys mature enterocytes in the villi causes' villous atrophy and usually affects the caudal part of the small intestine **(Cho and Yoon, 2014).**

BRV infects the young age of a wide range of species including humans, mammals (piglets, calves, goats, lambs, and foals) and birds **(Dhama *et al*., 2009 and Estes and Greenberg, 2013**). Some human rotaviruses contain genomic segments of bovine rotaviruses as a result of direct transmission to human or reassortment **(El - Sherif *et al*., 2011).**

The symptoms of BRV infection appear as watery feces which may be discolored yellowish-green. Infected calves are often quite depressed and lose their appetite. Many cases of Rotavirus are fatal, and the fatality rate increases with decreasing age. BRV causes economic losses due to calf mortalities, increased susceptibility to other infections, retarded growth, and treatment costs,(**Mawatari *et al*., 2004 and Das, *et al*., 2018**).Specific and sensitive detection methods are required and several tests are used routinely in diagnostic laboratories for the detection of rotavirus in fecal samples. Including Electron microscopy, enzyme linked immunosorbent assay,(**Czeruy and Eichhorn, 1989**), virus isolation, immunoelectrophoresis, latex agglutination tests (**Kaminjolo and Adesiyun, 1994**), reverse transcriptase polymerase chain reaction (RT-PCR) **(Maes *et al*., 2003)** and next-generation DNA sequencing (NGS) for detection of Bovine group A Rotavirus, (**Minami-Fukuda *et al*., 2013**).

In Egypt bovine Rota virus was isolated and identified from diarrheic calves for the first time in 1981 **(Shalaby *et al*., 1981).** Later, the studies 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 and Kassem *et al*., 2017).** The prevalence of Rotavirus in 2016 in Egypt represented 48% of diarrheic calves **(Mohamed *et al*., 2017)** and 17.1% in 2019 **(El-Sadek *et al*., 2019).** On the other hand *E. coli* is one of the most common causes of scours, **(Kolenda *et al*., 2015)** particularly in calves between 1 and 10 days old. *E. coli* can be classified into six pathogenic groups based on virulence scheme, some of them affect directly through damage the intestinal lining, and others cause diarrhea by releasing toxin. **(Kaper *et al*., 2004).**

Neonatal calves are most susceptible to enterotoxigenic Escherichia coli (ETEC) infection during the first 4 days after birth and develop watery diarrhea **(Foster and Smith 2009 and**

Nataro and Kaper 1998).Low pH (less than 6.5) of the distal portion of the small intestine is a suitable environment for ETEC colonization. *E. coli* expresses K99 antigen for attachment**,** a heat-stable toxin that leads to the up-regulation of chloride secretion into the gut, this osmotically pulls water into the intestinal lumen and leads to the development of secretory diarrhea (**Francis *et al*., 1989; Ata *et al*. 2013;ChoandYoon 2014**).The most important serogroups of *E.coli* causing disease in animals and human are O_{157}, O_{26}, O_{103}, O_{111}, O_{145}, O_{45}, O_{91}, O_{113},O_{119}, O_{121} and O_{128} which are mostly belonging to shiga toxin producing *E. coli* (STEC) pathotype (**Jenkins *et al*., 2003 and Lin *et al*., 2011**).

Multiplex PCR includes simultaneous amplification of more than one target gene including more than one set of primers in the same reaction mixture (**Chandra *et al*., 2013**). It has been widely used in various studies for differentiation of *E.coli* pathotypes based on presence of genes encoding virulence factors (**Müller *et al*., 2007**) and serogrouping of *E. coli* is based on presence of genes encoding serogroups **(Fakih *et al*., 2016).**

The aim of the present work is to investigate the incidence of Rota virus and Escherichia Coli as causes of neonatal calf diarrhea in El-Wady El-Gedid governorate that helps in control the disease and enhance cattle production in the study region.

MATERIAL AND METHODS

Samples:

A total of 65 calf fecal samples were randomly collected from different localities in El-Wady El-Gedid governorate in 2019 and early 2020. The calves had diarrhea, fever and variable degrees of dehydration and weakness. Diseased calves did not all respond to antibiotic therapy, no mortalities were found during the time of sample collection. Calves ages were time of birth to 5 weeks. All data were taken concerning breed, age, and season. Samples were collected in a sterile plastic bag and transported to laboratory in ice box. All fecal samples were initially screened by Rotavirus rapid test cassette then stored at -70 C for processing by other tests to investigate the cause of diarrhea.

Rotavirus Rapid Test Cassette (Feces):

The Rotavirus Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay (Right Sign Biotest-Rotavirus Rapid Test Cassette REF IROT-C61, and LOT .NO**.** ROT19110001) for the qualitative detection of rotavirus in fecal specimen, providing results in 10 minutes. The test utilizes antibody specific for rotavirus to selectively detect rotavirus from feces

specimens according to the manufacturer's instruction. In this test, a membrane is pre-coated with anti-rotavirus antibody on the test line region of the strip. During testing, the specimen reacts with the particle coated with anti-rotavirus antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-rotavirus antibody on the membrane and generate a red line in the test line region. The presence of red line in the test region indicates a positive result, while its absence indicates a negative result .To serve as a procedural control, a red line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

ELISA kit for detection of rotavirus antigen:

Using a double antibodies sandwich ELISA kits Bio-X Diagnostics .Belgium) **(De Beer *et al.*, 1997)** following the manufacturers protocol.

Interpreting the results:

The net optical density of each sample was calculated by subtracting from the reading for each sample well the optical density of the corresponding negative control. The test is validated only if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than the value given on the quality control (QC) data sheet. The signal read for each sample well was divided by the corresponding positive control signal and multiply this result by 100 to express it as a percentage.

$$\text{Value} = \frac{\text{Delta OD of sample}}{\text{Delta OD of Positive}} \times 100$$

Isolation and identification of *E. coli* (Quinn *et al.*, 2011):

The collected samples were inoculated in buffered peptone water and incubated aerobically at 37ºC for 18-24h, then 1 ml of inoculated buffered peptone water tube was inoculated into MacConkey broth and incubated aerobically at 37ºC for 18-24h. A loopful of inoculated MacConkey broth was plated onto sheep blood agar and MacConkey agar. The inoculated plates were incubated aerobically for 24-48 hours at 37ºC. The suspected colonies were picked up, purified and identified by cultural, morphological characters and biochemically by VITEK2 compact according to **(Chatzigeorgiou *et al.*, 2011).**

Antimicrobial sensitivity test:

Thirty isolates were subjected to antibiotic sensitivity test against ampicillin, amoxicillin piperacillin, cefalexin, cefpodoxime, ceftiofur, ceftiofur imipenem, amikacin, gentamicin tobramycin, enrofloxacin, marbofloxacin, tetracycline, nitrofurantoin, chloramphenicol trimethoprim and sulfamethoxazole **(Chatzigeorgiou *et al.*, 2011)**.

Serological identification:

Serogrouping of pathogenic *E.coli* isolates was carried out by slide agglutination method using specific polyvalent and monovalent anti-sera for *E.coli* (DENKA SEIKEN CO., LTD., Chuo-ku, and Tokyo, Japan) **Edwards and Ewing (1972).**

Molecular examination:

Multiplex PCR was used to amplify two virulence genes *(stx1and stx2)* and uniplex PCR was performed for amplification *the eae* of 10 *E. coli* isolates by using sepecific primers (Table 1). DNA was extracted by QIAamp® DNA Mini Kit instructions (Catalogue no. M501DP100) following instructions of the manufacturer. For Multiplex PCR, DNA samples were subjected to 35 PCR cycles, each consisting of 1 min. denaturation at 95°C; 2 min. annealing at 65°C for the first 10 cycles, then to 60°C in the next 15 cycles; and 1.5 min elongation at 72°C, then 2.5 min from cycles 25 to 35. DNA amplification was carried out in a Perkin-Elmer thermocycler 2400. Amplicons were then visualized by ethidium bromide through electrophoreses in 2% agarose.

Table (1): Primers used for the detection of virulent genes of *E.coli* Forward (3'-5'), R: Reverse (5'-3').

Target Gene	Primers sequences	Amplified Segment (bp)	Reference
stx1	F:ACACTGGATGATCTCAGTG G	614	Dipineto *et al.*(2006)
	R:CTGAATCCCCCTCCATTATG		
stx2	F:CCATGACAACGGACAGCAGTT	779	
	R:ACACTGGATGATCTCAGTGG		
Eae	ATG CTT AGT GCT GGT TTA GG R: GCC TTC ATC ATT TCG CTT TC	248	Bisi-Johnson *et al*. (2011)

RESULTS

Virological Examination:

Detection of Rotavirus Antigen (RVA) by Rapid Test Cassette:

Fecal samples were identified as positive for RVA using the Rotavirus Rapid Test Cassette as a qualitative, lateral flow immunoassay for the detection of rotavirus in feces, twelve fecal samples (18.46%) were detected (Table2). where two red lines were observed on each positive sample, one in the control line region (C) and the other colored line appear in test line region (T) and in negative sample only one red line appeared in the control line region (C). as shown in figure ($1_{A\&B}$).

Fig. (1): (A) Negative (B)Positive

Detection of bovine rotavirus antigen by ELISA test:

Bovine rotavirus antigen were detected in 27.69% of the diarrheic fecal samples by using a double antibodies sandwich ELISA kit as shown in (Tables 2, 3).The highest incidence of calf diarrhea (12.31 %) was recorded at the age of 8-15 days and decreased with the increased ages with lowest incidence (1.54%) at age of 29-35 days as shown in (Table 4). Infection rate was the highest in winter (18.46 %) followed by autumn (7.69%) while the lowest infection rate was recorded in spring (1.54 %) (Table 5).

Table (2): Matching of Rotavirus Rapid Test Cassette and ELISA Test for detection of rotavirus antigen.

No. of fecal Samples	Rapid test Cassette		ELISA test	
	+ ve %	-ve %	+ ve %	-ve %
65	**12**	**53**	**18**	**47**
%	**18.46 %**	**81.54 %**	**27.69 %**	**72.31 %**

Table (3): Positivity percentagesof Rotavirus and *E.coli* of single infection andco- infection.

Total No. of fecal Samples	No. of +ve of Rota	No. of+ve of *E.coli*	No of +ve Co-infection
65	**18**	**30**	**7**
ve %+	**27.69**	**46.15**	**10.76**

Table (4): Distribution of infection rates of Rota virus and *E.coli* in different ages of calves.

Age (days)	No. of tested samples	+ve No. of Rota & +ve % related to total No=65		+ve No. & +ve % of *E.coli* related to total No=65	
1-7 d.	**18**	**4**	**(6.15%)**	**11**	**(16.92%)**
8-15 d.	**14**	**8**	**(12.31 %)**	**7**	**(10.77%)**
16-21 d.	**11**	**3**	**(4.62%)**	**5**	**(7.69 %)**
22-28 d.	**12**	**2**	**(3.08%)**	**5**	**(7.69%)**
29-35 d.	**10**	**1**	**(1.54%)**	**2**	**(3.08%)**
Total	**65**	**18**	**(27.69%)**	**30**	**(46.15%)**

Table (5): Distribution of single infection rate of both BRV and *E.coli* and co-infection in calves in relation to season.

Season	No. of tested samples	+ve No. of Rota& +ve % in related *to total No=*65		+ve No. of *E.coli* +ve % % in related to total No=65		+ve No. & +ve % of co- infection related to total No = 65	
Winter	**28**	**12**	**18.46 %**	**16**	**24.62**	**5**	**7.69%**
Spring	**11**	**1**	**1.54 %**	**4**	**6.15**	**0**	**(0%)**
Summer	**8**	**0**	**0 %**	**2**	**3.08**	**0**	**(0%)**
Autumn	**18**	**5**	**7.69**	**8**	**12.31**	**2**	**3.08%**
Total	**65**	**18**	**27.69**	**30**	**46.15%**	**7**	**10.77%**

Bacteriological examination:

A total of 65 calf fecal samples *E. coli* was identified in 46.15% (30/65) of the diarrheic fecal samples (Table 3). Included in this study. The highest incidence of calf diarrhea was recorded at the ages of 1-7 days (Table 4). The frequency of positive diarrheic calves because of *E.coli* infection was the highest in winter (24.62 %) followed by autumn (12.31%) while the lowest frequency was recorded in summer (3.08%) as shown in (Table 5).

Antimicrobial sensitivity test:

Of 30 *E. coli* isolates tested for antimicrobial sensitivity; 7.7% were resistant to marbofloxacin, 15.4% were resistant to cefpodoxime, ceftiofur, imipenem and tobramycin and nitrofurantoin meanwhile 23% of isolates were resistant to amikacin and enrofloxacin. High level of resistance was recordedagainst ampicillin,amoxicillin,chloramphenicol and trimethoprim/ sulfamethoxazole; 46.2%, 53.8, 38.5% and 53.8% respectively. About 33.3% of isolates were multidrug resistant, i.e. resistant to three or more antimicrobials (Table 6).

Table (6): Resistance patterns of *E. coli* isolates.

Resistance patterns	*E. coli* of isolates (30)	percentage %
To only one drug	**5**	**16.67 %**
To two drugs	**-**	**-**
To three drugs	**6**	**20 %**
To more than three drugs	**4**	**13.3%**
To all drugs	**-**	**-**

Serotyping of *E. coli* isolated from diarrheic calves:

Serogrouping of *E. coli* isolates (30) revealed presence of 8 serogroups ; O_{142}, O_{55}, O_{111}, O_{27}, O_{157}, O_{119}, O_{26} and O_{127} with percentage of 20.69%, 17.24%, 13.8%, 6.9%, 6.9%, 6.9%, 3.45% and 3.45% , respectively (Table 7).

Molecular characterization of *E. coli* isolates:

The distribution of both virulence genes were showed in (Table 7)**,** four isolates were positive for *stx1* and 3 isolates were positive for *stx2* (one isolate was found to contain both *stx1* and *stx2*) and two isolates were negative for both *stx1&stx2* Fig.(2,3) of the10 investigated *E. coli* isolate*s* O142, O26, O119, O55 and O157 were positive to the intimin gene (*eae)* while O27, O111 and O127 were negative Fig. (4).

Table (7): Serotyping of *E. coli* isolated from diarrheic calves.

E. coli serotypes	Number (out of 30)	% of serotypes	Virulence genes		
			Stx1	*Stx2*	*Eae*
O142*	**8**	**26.67**	**2**	**-**	**2**
O55 *	**6**	**20**	**-**	**1**	**1**
O111	**4**	**13.3**	**1**	**-**	**-**
O27	**3**	**10**	**-**	**-**	**-**
O127	**2**	**6.6**	**1**	**-**	**-**
O119	**1**	**3.3**	**-**	**1**	**1**
O157	**2**	**6.6**	**1**	**1**	**1**
O26	**2**	**6.6**	**-**	**-**	**1**
untypable	**2**	**6.6**	**-**	**-**	**-**
Total	**30**	**100 %**	**5**	**3**	**6**

No.: Number of isolates. %: Percentage in relation to No of tested isolated strain (*E.coli* (30)

***Indicate that two isolates were investigated from serogroup for virulotyping.**

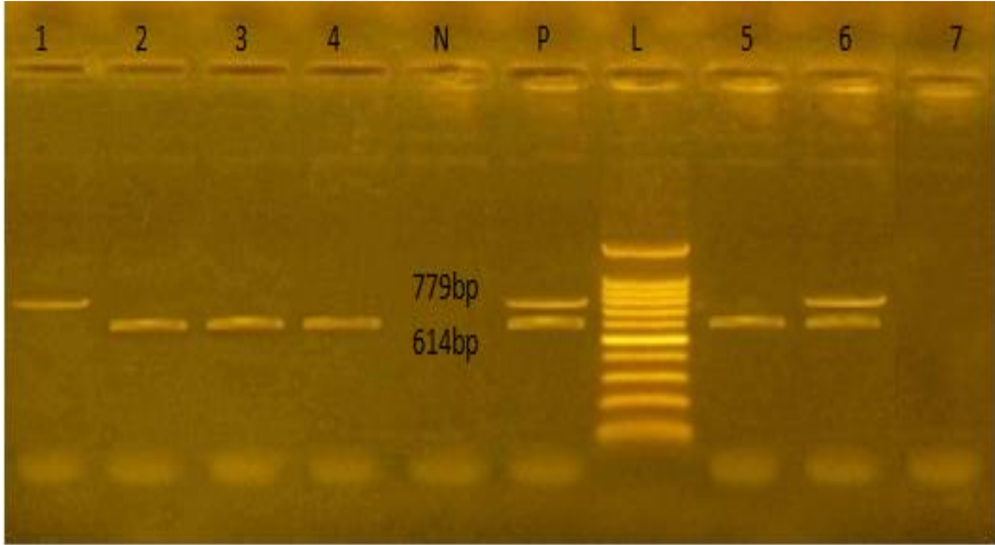

Fig. (2): Agar gel electrophoresis of multiplex PCR for detection of *stx1 (*614 bp) and *stx2* (779 bp) genes from *E.coli* isolates L: represent the molecular size marker (100bp ladder).

Lane 1 positive to *stx2* (O119)   Lane 2,3,4,5 positive to *stx1* (O127- O111-O142-O142)

Lane 6 positive to *stx1*&stx2 (O157) -Lane 7 negative tostx1&*stx2*

N: control negative   P: control positive

Fig. (3): Agar gel electrophoresis of multiplex PCR for detection of stx1 and stx2 genes from *E.coli* isolates.

L: represent the molecular size marker (100bp ladder) lane 3: negative for stx1 & stx2 (O55) lane 4: positive of stx2 779 bp (O55). Lane 5- negative for stx1 & stx2 (O26).

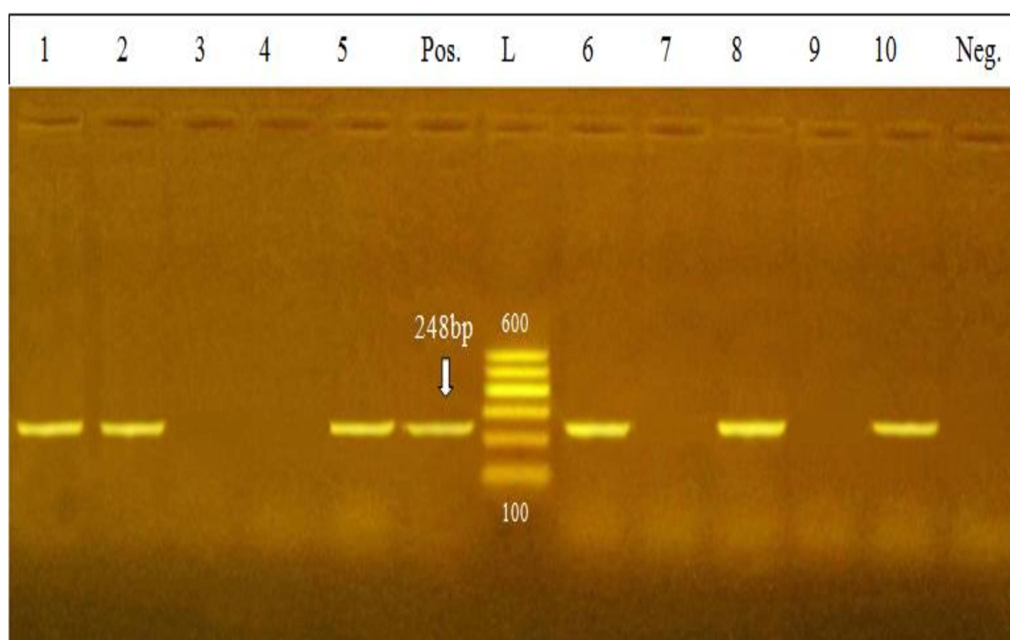

Fig. (4): Agarose gel electrophoresis of uniplex PCR for detection of *eae* gene.

Neg: Negative control. Pos: Positive control of *eae* gene (248 bp)

L: represents the molecular size marker (100bp ladder).

(Lane1, 2): Positive for O142 Lane 6 Positive for O119

(Lane 3.7) Negative for: O27&O127 Lane 8: Positive for O55

(Lane 4) negative for, O111 Lane 9: Negative for O55

(Lane 5) Positive for, O26 Lane10, Positive for O157

DISCUSSION

Diarrhea is a major cause of mortality in young cattle under one month. It is one of the most common problems of cattle industry all over the world and a major cause of productivity and economic losses. Bovine neonatal gastroenteritis is a multifactorial disease including infectious and noninfectious agents. The diagnosis of the etiological agent of diarrhea can only be performed in the laboratory because clinical signs do not allow differentiating between different microorganisms.

Many factors including, various laboratory techniques, number of specimens per day and equipment's influence the choice of protocols used for diagnostic testing. Rapid, simple and accurate diagnosis of BRV is required for the detection BRV in feces.

The ELISA technique proved to be sensitive, specific and rapid test for detection of BRV.

ELISA is commonly performed because small amount of BRV can be detected in feces 4-9 days after onset of diarrhea the test cane be completed in less than 4 hours **De Beer *et al*.(1997**).Commercial ELISA kits are available for the routine diagnostic screening of large numbers of samples.

In the present study BRV antigen were detected with Rotavirus Rapid Test Cassette as a lateral flow immunoassay which has some advantages compared with other diagnostic methods including cost, specificity, rapidity, providing results in 10 minutes, easy to use and reading. No equipment's are needed and no requirement for training, so it can be used for diagnosis in the field. Unlike the latex agglutination test, the formation of permanent red lines allowing the results to be read at times convenient for the technician. **Iman *et al.,* (2009**). The current study revealed that 12 (18.46%) and 18 (27.69%) fecal samples were positive for BRV antigen detection by rapid teste cassette and ELISA as a confirmatory test respectively as shown in (Table 2) these results agree with **Gumusova *et al*., (2007)** who mentioned that BRV infection is the major cause of acute gastroenteritis and the most worldwide prevalent viral agent in diarrheic calves aged less than 6 weeks. Also **Patel *et al.,* (2019**) **and Soltan *et al.,* (2016)** recorded high positivity of Rotavirus from diarrheic calves by using ELISA.

Regarding to infection rate of BRV and *E. coli* as shown in **(**Table 3),it was (27.69%) and (46.15%) respectively, *E. coli* was more predominant than Rotaviruses which may be due to low PH (lower than 6.5) in the distal portion of the small intestine which is a suitable environment for ETEC colonization. or due to environmental condition, nutritional status, poor sanitation and management practices, these non- infectious factors increase susceptibility to infection **.Ata *et al*.,(2013) ,Cho and Yoon (2014**).

The distribution of both infections in different ages as shown in (Table 4). It was noticed that the highest infection rate of BRV was observed in 8-15days with a rate of (12.31%). These results are coincided with **Cho and Yoon (2014), Khamees, (2015) and Patel *et al.,* (2019)** who recorded higher infection rates of Rotaviruses usually in calves at 1 to 2 weeks of age and this may be due to immature immune system of very young calves to fight infection and the milk uptake by calves can provide a good environment for rotavirus survival under a wide range of gastrointestinal pH levels and infection of the intestinal epithelial cells. **Dhama *et al.,* (2009) and Cho and Yoon (2014).** *E. coli* was more predominant than Rotaviruses in

the first week (1-7d) of age with the highest infection rate (16.92%). This is in agreement with **Foster and Smith (2009)** who recorded that neonatal calves are most susceptible to ETEC infection during first 4 days after birth and **Ata *et al*. (2013) and Cho and Yoon (2014)** who mentioned that typical symptoms appear in calves less than 7 days of age and as early as 12 h of life. However, the lowest rate was shown in the age of 22 -28 days in rotavirus and *E.coli* was shown in 29-35 days old calves with a percentage of (3.08 %). These coincided with **Dash *et al*., (2011) and Kumari *et al*., (2019)** who reported that, the susceptibility of bovine calves to rotavirus infection decreases with age which may be due to loss of receptors on enterocytes. **Ammar *et al*., (2014) and Suresh *et al*., (2013**) recorded that calves acquire natural resistance increased against infection with progression of age.

Concerning single infection or double co-infection of calves with Rotavirus and *E. coli*, it was found that 7 fecal samples were positive for both Rotavirus and *E. coli*, with a total percentage of 10.77%, which agrees with **Cho (2012)** who stated that Co-infection with two pathogens were the most common finding (31%). Diarrhea caused by *E. coli* can occur as early as 24 h after birth, but seldom occurs after three days of age unless it occurs as part of a mixed infection with rotavirus.

Single infection with either Rotavirus with a total percentage of 27.69 or *E. coli* with a total percentage of 46.15 were recorded (Table 4).These results are compatible with that obtained by **Barua, *et al*.,(2019)** who stated that Rotavirus infection alone accounts for about 28- 36%. and the results agrees with that described by **Islam *et al*. (2015)** who isolated *E.coli* with an incidence of 45.2%. **Uhde *et al*.,(2008)** stated that combination-infection is frequently observed in diarrheic calves, although a single primary pathogen can be the cause in some cases.

Distribution of single infection rate of both BRV and *E.coli* and double co-infection in calves in relation to season as shown (Table 5) revealed that, the highest infection rate was in winter for both single BRV and *E. Coli* infection or double co-infection with percentages of 18.46%, 24.62% and 7.69%, respectively, followed by Autumn with percentage of 7.69%, 12.31% and 3.08% , In spring, the percentages were 1,53%, 6.15% and zero % while in summer zero %,3.08 and zero percentage were recorded respectively .These results coincided with **Barua, *et al*., (2019) and Mukhtar *et al*.,(2017**) who detected higher prevalence of BRV in winter season compared to summer season. *E. coli*. was the most predominant in diarrheic

calves in winter and autumn season with the highest infection rate of 24.62% and 12.31% where infection rate of BRV was 7.69 % and double co-infection was 3.08% in autumn season, these may be due to cold weather which leads to stress on younger calves, In addition to poor sanitation, overcrowding in the calf pens and other non-infectious factors, such as insufficient uptake of colostrum and presence of more than one age tougher, increasing the exposure to infection and lowering the defense mechanism within the calf in early life due to its poor immune capability.

In the present study *E.coli* isolates showed two of them resistant to at least one antimicrobial agent (Table 6). Multidrug resistance appeared in 10 strains similar to that obtained by **Messaï *et al.* (2013).**

Out of eight identified serogroups, O_{142} was the most prevalent serogroup (26.67 %) followed by O_{55} and O_{111} at rates of 20% and 13.3% respectively, then O_{27} with a rate of 10% then $O_{26,}$ O_{157} and O_{127} at a rate of 6.6% and the last serogroup O_{119} was found at a rate of 3.3 %.

The above- mentioned results agree with results of **Lin *et al.*, (2011)** who detected O_{157}, O_{26}, O_{142} and O_{111} and **Aisha (2001)** who isolated O_{26}, O_{127} and O_{27} from diarrheic calves.

Molecular characterization of *E. coli* isolates recovered from diarrheic calves was carried out through applying different conditions of uniplex Fig. (4) and multiplex Fig. (2&3) PCR assays for detection of genes encoding virulence factors (*stx1*, *stx2* and *eae*). (Table 8).

The tested E.coli isolates carried different virulence genes, as the negative isolates of *E. coli* for tested virulence genes may be nonpathogenic and the animals had diarrhea caused by other infectious agents or these isolates may carry other virulence genes not included in this study **Pourtaghi *et al.*, (2013).**

In this study, the rate of *stx* gene existence in isolated *E.coli* from cattle calves was 30%.

Multiplex PCR assays approved the presence of intimin *(eae)* 6/10 and Shiga toxins ($STx1_{5/10}$ *and* $STx2_{3/10}$) in *E.coli* strains $_{(10)}$ **Gharieb *et al.* (2015).** In the current study *E. coli* O_{157} was positive to *stx1, stx2* and eae genes, which agree with **Karmali, (2004).**

CONCLUSION

It can be concluded that BRV and *E.coli* play major role in cases of diarrhea in the examined calves in El-Wady El-Geded Governorate were rotavirus and *E. coli* infection .Virulence genes of *E. coli* were *stx1, stx2* and *eae* which played an important role in its pathogenicity. On the other hand, neonatal calf diarrhea (NCD) has a peak incidence in winter and in the first two weeks of age. Accurate and rapid early confirmation of the etiology in the disease as well as improving the various management factors are advised, for effective control and prevention of enteric disease, in addition to vaccination of newborn calves .

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الكشف عن فيروس الروتا وبكتريا الايشيرشيا كولاى كمسببات الاسهال فى العجول حديثة الولادة بمحافظة الوادى الجديد

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الملخص العربى

يعتبر فيروس الروتا البقرى وبكتريا الايشيرشيا كولاى من اهم اسباب الاسهال فى العجول حديثة الولادة حيث يمثل فيروس الروتا وحده حوالى27% الى 36 % من اسباب الاسهال فى العجول فى العالم . فى هذه الدراسة تم جمع 65عينة براز من عجول بقرى من الولادة وحتى عمر5 اسابيع من اماكن مختلفة فى محافظة الوادى الجديد , تم فحص جميع العينات فيرولوجياوبكتريولوجيا لتحديد مدى الاصابة بفيروس الروتا وبكتريا الايشيرشيا كولاى كاحد اهم مسببات الاسهال فى العجول حديثة الولادة. وقد اوضحت النتائج ان 12عينة بنسبة (18.46%) و 18عينة بنسبة (27.69) من 65 عينة ايجابية لوجود فيروس الروتا باستخدام الاختبار السريع للكشف عن الروتا واختبار الاليزا على التوالى. وكان معدل الاصابة بالايشيرشيا كولاى عالى وبنسبة (46.15٪) والاصابة بالايشيرشيا كولاى هى السائدة عن الروتا، وسجلت اعلى معدل اصابة بالفيروس فى الاعمار من 8الى 15 يوم من العمر (بنسبة12.31٪) ولكن اعلى معدل اصابة بالايشيرشيا كولاى كان فى عمر يوم الى 7 ايام بنسبة (16.62٪) ومعدل الاصابة المزدوجة بكل من الروتا والايشيرشيا كولاى (10.77٪). واوضحت النتائج ايضا ان معدل الاصابة الفردية بالروتا والايشيرشيا كولاى والاصابة المزدوجة عالية فى فصل الشتاء بنسبة 18.46٪ و24.62٪ و7.69٪ عن الخريف وبنسبة (7،69٪ و 12،31٪ و3،08٪) والربيع بنسبة (1،53٪ و6،15٪ وصفر٪) اما في الصيف كانت النسبة (صفر٪ و 3،08٪ و صفر٪) على التوالى بينما اوضحت الاختبارات البكتريولوجية الاخرى ان معزولات الايشيرشيا كولاى كانت متعددة المقاومة للمضادات البكترية. وقد كان الفحص السيرولوجى لعترات الايشيرشيا كولاى كالتالى , O55 , O111 , O27 ,O157, O119, O26, OO127 O142 بنسب 26.67٪، 20٪، 13.3٪، 10٪ ، 6.6٪ ، 3.3٪ ، 6.6٪، 6.6٪ على التوالى.

باستخدام تفاعل البلمرة المتسلسل للكشف عن جينات الضراوة فى 10 عينات عشوائية لميكروب الايشيرشيا كولاى وجد 5 عينات ايجابية *Stx1* و3 عينات إيجابية *stx2* و6 عينات جين *eae*.