



Frequency of rotavirus detection by a sandwich ELISA in feces of diarrheic bovine calves from Qalubia province, Egypt

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ABSTRACT

This work aimed to study the prevalence of rotavirus in diarrheic feces from bovine calves and the sensitive's parameters such as age group and seasonal pattern. A total of 250 fecal samples were collected from diarrheic bovine calves in different localities at Qalubia province, Egypt all over the four seasons of the years 2013 and 2014. These samples were tested for rotavirus antigen by sandwich Enzyme linked immunosorbent assay (ELISA). Rotavirus antigen was detected in 30% (75/250) of examined samples with a prevalence of 32.9% (47/143) in cow calves and 26.2% (28/107) in buffalo calves. The highest rate of infection was recorded in the diarrheic calves within one week (46.6%) and two weeks (41.4%) of age. Examined samples all over the four seasons of the years, 2013 and 2014 indicated the prevalence of Rotavirus antigen by 43.1% (56/130) during winter, 20% (5/25) during summer, 21.4% (12/56) during spring and 5.1% (2/39) during autumn. In conclusion, rotavirus was involved in the neonatal calves' diarrhea, where its frequency was clearly higher in calves up to 2 weeks of life during winter season.

Keywords: Rotavirus, Sandwich ELISA, Bovine calves, Seasonal pattern

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(BVMJ-27(2):341-347, 2014)

1. INTRODUCTION

Rotavirus diarrhea in calves is a high morbidity syndrome causing important economic losses for the farmers in terms of treatment cost and reduction of weight gain in affected animals (Bartels et al., 2010). Rotaviruses form a genus rotavirus within the Reoviridae family (Estes and Kapikian 2007). They are characterized by non-enveloped triple-layered viral particles with a genome composed by 11 double-stranded RNA segments (dsRNA). Based on the genetic and antigenic variation of the two outer capsid proteins VP7 (Glycoprotein) and VP4 (Protease sensitive protein), rotaviruses are classified into G and P types, respectively (Estes and Kapikian, 2007). To date, 27 G

types and 37 P types have been recognized (Matthijssens et al., 2011; Trojnar et al., 2013). Calf rotavirus infection has a worldwide distribution and associated with 40-48% of the cases of neonatal calf diarrhea (Morin et al., 1976). In Ethiopia, 16.7% of 108 diarrheic bovine calves examined were rotavirus positive (Abraham et al., 1992), and in Sudan, rotavirus associated calf diarrhea was reported in 66.4% out of 116 diarrheic calves from Friesian dairy farm by El Nur.,(1994). In Egypt, bovine rotavirus was firstly isolated and identified in 66.6% of fecal samples collected from calves with diarrhea (Shalaby et al. , 1981) and recorded in 48.2% of fecal samples obtained from diarrheic buffaloes calves in Ismailia

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governorate by monoclonal antibodies (Mabs) based ELISA (Hussein et al., 2001). Rotaviruses are transmitted by the fecal-oral route. Clinical signs range from mild to severe diarrhea resulting in depression, dehydration and occasionally death (Radostitis et al., 2007). Two vaccination strategies have been developed to control rotavirus infection in young calves. First, the induction of active immunity in young calves by using modified live attenuated or inactivated rotavirus vaccines. However, this strategy was failed under field condition (Saif and Jackwood, 1990). Second, the induction of passive immunization to prevent bovine rotavirus (BRV) infection in young calves through immunization of pregnant cows with live attenuated or inactivated adjuvanted rotavirus vaccines to increase the colostrums antibodies (Rousic et al., 2000). Under field conditions, the efficiency of the commercial maternal vaccines in cattle varied and many researchers are not satisfied with the efficiency of these vaccines in enhancing the rotavirus antibody titers and protecting calves against rotavirus infection (Myers and Snodgrass, 1982 and Saif et al., 1983). Diagnosis is done through collecting feces of animals suffering from diarrhea by a rectal swab or collecting intestinal contents (Castro and Heuschele 1992) and using laboratory diagnostic tests such as direct electron microscopy, Enzyme-linked immunosorbent assay (ELISA), latex agglutination, polyacrylamide gel electrophoresis, reverse transcription polymerase chain reaction, and also immuno-electron microscopy (Parwani et al. 1992; Radostitis et al., 2007). ELISA is used in most diagnostic laboratories (Christensen and Howard, 1999) which was more sensitive in detecting rotavirus group A antigen (Benfield, et al., 1984) and has a shorter test time than virus isolation (VI). The most common type of ELISA for

rotavirus detection is antigen capture ELISA (Rouch., et al 1984, Kapikian and Chanock. 1990, Al-Yousif et al., 2001). The high frequency and persistence of calves' neonatal diarrhea in farming has gained the interest of many researchers. Thus; the purpose of the present study is to report and estimate the prevalence of rotavirus infection using ELISA assay in diarrhetic feces from calves and the sensitive's parameters like age-group and seasonal distribution in Qaluobia province, Egypt during 2013 and 2014.

2. MATERIALS AND METHODS

2.1. Specimen collection:

A total of 250 fecal samples were collected from diarrhetic bovine calves in different localities at Qaluobia province, Egypt all over the four seasons of the years 2013 and 2014 (table 1). No record of rotavirus vaccination or any serologic evidence of rotavirus infection has ever been reported in this province. These samples were collected in sterilized plastic container, transported under ice and stored at -20°C till further processing.

2.2. Preparation of fecal samples

A 10% (w/v) fecal suspension was prepared in phosphate buffered saline containing 0.4% (v/v) of 20 000 U/ml penicillin, 0.4% (v/v) of 20 mg/ml streptomycin and 0.8% (v/v) of 12.5 mg/ml mycostatin, and buffered to a pH of 7.2 using 7.5% (w/v) sodium bicarbonate. It was centrifuged at 10.000 rpm for 30 min. The supernatant fluid was filtered through a 0.45 µm pore diameter membrane filter and then stored at -80°C until use in indirect sandwich ELISA.

2.3. Sandwich ELISA

It was performed to detect rotavirus antigen in the fecal supernatants as described by the

Table (1). Number of fecal samples of cattle and buffalo calves in relation to the age and season.

Species	Age									season				Total/ season
	≤1m**					2 m	3 m	6 m	Total /age	Winter	Summer	Spring	Autumn	
	1 w*	2 w	3 w	4 w	Total ≤1m									
Cow calves	36	20	11	6	73	28	19	23	143	79	8	35	21	143
Buffalo calves	22	9	6	2	39	13	28	27	107	51	17	21	18	107
Total	58	29	17	8	112	41	47	50	250	130	25	56	39	250

* weeks of age, ** month of age.

kit manufacturer (Rotavirus ELISA kit, Bio-X Diagnostics). The 96 well plate provided by the kit contains two different capture antibodies. Rows A, C, E, and G were coated with rotavirus specific capture antibodies and rows B, D, F, H coated with non specific antibodies. The detection antibody present in the kit is a peroxidase labeled anti rotavirus specific monoclonal antibody. The net optical density of each sample was calculated by subtracting the reading for each sample well from corresponding negative control. Net optical density (O.D.) = O.D. of specific binding - O.D. of non-specific binding

Any sample that yielded a difference of 0.15 or greater in optical density was considered positive.

3. RESULTS

It is Rotavirus antigen detection in 250 fecal samples of diarrheic bovine calves (143 cattle and 107 buffaloes) by indirect sandwich ELISA revealed that 30% (75/250) of samples were positive. From examined samples for each species 32.9% (47/143) cow calves and 26.2 % (28/107) buffalo calves were positive for Rotavirus antigen (table 2).

In correlation to age, rotavirus antigen was highly distributed among bovine calves within one month old reached 38.4%

(43/112) while bovine calves with 2, 3 and 6 months olds showed Rotavirus antigen in their feces by 31.7% (13/41), 17% (8/47) and 22% (11/50) respectively. At these ages, higher percentages of antigen were detected in cow calves than buffalo's calves except for 3 month old bovine calves where cow calves represents 6.4% (3/47) and buffaloes calves represents 10.7% (5/47) (table3).

Table (2): Prevalence of rotavirus infection in bovine calves as determined by sandwich ELISA

Animal Species	No of fecal samples	No. of Positive	Percent Positive
cow calves	143	47	32.9
Buffalo calves	107	28	26.2
total	250	75	30

Table (3): Age distribution of rotavirus infection in diarrheic bovine calves (≥ one month) assessed by sandwich ELISA.

Age/month	Total examined faeces	No. of Positive		Total Positive
		cow calves	Buffalo calves	
1	112	27 (24.1%)*	16 (14.3%)	43 (38.4%)
2	41	9 (22.0%)	4 (9.8%)	13 (31.7%)
3	47	3 (6.4%)	5 (10.7%)	8 (17%)
6	50	8 (16%)	3 (6%)	11 (22%)

*Numbers in bracket indicate percent positive from total examined sample

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A critical observation of (Table-4) represents distribution of rotavirus infection in diarrheic bovine calves up to one month of age. Bovine calves within one week old showed higher antigen detection 46.6% (27/58) while bovine calves with 2, 3 and 4 weeks old showed Rotavirus antigen in their feces by 41.4% (12/29), 17.6% (3/17) and 12.5% (1/8). Also, cow calves revealed higher percentages of antigen detection than buffalo's calves at these periods of age.

Table (4): Age distribution of rotavirus infection in diarrhoeic bovine calves (\leq one month) assessed by sandwich ELISA.

Age/week	Total examined faeces	No. of Positive		Total Positive
		cow calves	Buffalo calves	
1	58	17 (29.3%)*	10 (17.2%)	27 (46.6%)
2	29	7 (24.1%)	5 (17.2%)	12 (41.4%)
3	17	2 (11.8%)	1 (5.9%)	3 (17.6%)
4	8	1 (12.5%)	0 (0.0%)	1 (12.5%)

*Numbers in bracket indicate percent positive from total examined sample

Table (5): Seasonal pattern of rotavirus infection in diarrhoeic bovine calves assessed by sandwich ELISA.

season	Total examined faeces	No. of Positive		Total Positive
		cow calves	Buffalo calves	
Winter	130	35 (26.9%)*	21 (16.2%)	56 (43.1%)
Summer	25	4 (16%)	1 (4.0%)	5 (20%)
Spring	56	8 (14.3%)	4 (7.1%)	12 (21.4%)
Autumn	39	0 (0.0%)	2 (5.1%)	2 (5.1%)

*Numbers in bracket indicate percent positive from total examined sample

As shown in Table (5) from examined fecal samples all over the four seasons of the year 2013 and 2014 indicates prevalence of rotavirus infection by 43.1% (56/130) during winter, 20% (5/25) during summer, 21.4% (12/56) during spring and 5.1% (2/39)

during autumn with increase prevalence among cow calves than buffalo's calves.

4. DISCUSSION

Rotavirus destroys small intestinal enterocytes resulting in diarrhea which is accompanied by a profuse fecal shedding of virus (Radostits et al., 2007, Badiei et al., 2010). In fact, such vast numbers of rotavirus particles occur in diarrheal faeces. Thus, it is possible to diagnose this infection by negative staining, electron microscopic examination of fecal specimens (Radostits et al., 2007, Ali et al., 2008). Till today a variety of methods are used to detect bovine rotaviral infection in faeces like electron microscopy (Ali et al., 2008), Latex agglutination test (Al-yousif et al., 2001, Khafagi et al., 2010), virus isolation in cell culture (Al-yousif et al., 2001), ELISA and PCR (Dhama et al., 2009, Khafagi et al., 2010).

ELISA is one of the essential methods in the determination of viral antigens. It is used widely in calves with diarrhea for determination of rotavirus in faeces (Duman and Aycan 2010, Badiei et al., 2010, Ali et al., 2008, Khafagi et al., 2010). ELISA had the advantage of being inexpensive for examination of many samples and has the probability of being much more sensitive (Duman and Aycan 2010, Badiei et al., 2010).

In our study, Screening of bovine faecal samples was done to know the prevalence of rotavirus infection in Qaluobia province. Sandwich ELISA revealed that 30% (75/250) of samples were positive for Rotavirus antigen with a prevalence of 32.9% (47/143) in cow calves and 26.2% (28/107) in buffalo calves. This clarified that cow calves more affected by rotavirus infection as reported by Singh et al., (1993).

Our findings showed rotavirus antigen was highly distributed among bovine calves within one month old reached 38.4% while bovine calves with 2, 3 and 6 months olds showed Rotavirus antigen in their feces by 31.7%, 17% and 22% respectively. This emphasizes that the susceptibility to rotavirus infection in new born calves' decreases as the animal becomes older and calves under 1 month of age group are more susceptible to infection with BRV (Bridger, 1980, Saif et al., 1994).

The highest rate of infection was recorded in the diarrheic calves within one week (46.6%) and 2 weeks (41.4%) of age. Rotaviruses play a sizable role in the aetiology of neonatal calf diarrhea and found in the faeces of diarrheic calves up to 3rd week of life (Radostits et al., 2007). Calves are known to excrete the virus through faeces by the secondary infection which continues for 7-8 days and susceptible calves of 2-3 weeks age may get infected (Radostits et al., 2007, Steele et al., 2004)

Incidences of rotavirus diarrhea have been reported year round (2013 and 2014), the highest percentage of rotavirus positive samples occurred during winter months (43.1%) followed by the summer season (20%). These results supported the previous results of Mittal et al., (1986), and Samad and Ahmed (1990) who found that rotavirus display a seasonal pattern of infection in temperate regions.

The present study confirms the circulation of rotavirus in Qalubia province and further studies are required to characterize the genotypes prevalent and their zoonotic implications.

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تزامن الكشف عن فيروس الروتا بالسندويتش اليزا في براز العجول المسهلة لفصيلة الابقار بمحافظة القليوبية بمصر.

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الملخص العربي

لدراسة الانتشار لفيروس الروتا فى الاسهالات البرازية لعجول فصيلة الابقار والمؤشرات الحساسة مثل المجموعة العمرية ونموذج الفصول السنوية. تم جمع إجمالي 250 عينة برازية من اسهالات لعجول الفصيلة البقرية من مختلف الاماكن لمحافظة القليوبية بمصر خلال الاربع فصول لاعوام 2013 و2014. تم اختبار تلك العينات بواسطة السندويتش اليزا. تم الكشف عن الانتيجين لفيروس الروتا فى 30% (250/75) من العينات المفحوصة وبمعدل انتشار 32.9% (143/47) فى عجول الابقار و 26.2% (107/28) فى عجول الجاموس. سجل اعلى معدل للعدوى بالفيروس فى الاسهالات للعجول بين اسبوع (46.6%) و اسبوعين (41.4%) فى العمر. وقد اشارت العينات البرازية التى فحصت خلال الاربع فصول لاعوام 2013 و2014 انتشار الانتيجين لفيروس الروتا ب 43.1% (130/56) اثناء الشتاء و 20% (25/5) اثناء الصيف و 21.4% (56/12) اثناء الربيع و 5.1% اثناء الخريف. و استنتجا لذلك فان فيروس الروتا كان مسببا لاسهالات العجول الصغيرة حيث كان متزامنا بوضوح كبير فى العجول حتى اسبوعين من العمر اثناء فصل الشتاء.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(2):241-347, ديسمبر 2014)