

Epidemiological Study of *Rotavirus* Infection in the Diarrheic Neonatal Calves

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Abstract

Neonatal diarrhea is the main cause of morbidity and mortality in calves, and *Rotavirus* is the main viral etiology. The objective of the current study was to study the epidemiological role of *Rotavirus* infection in diarrheic neonatal cattle and buffaloes' calves in Assiut Governorate, Egypt. From December 2015 to November 2019, a total number of 315 neonatal calves belonged to different localities of Assiut Governorate, Upper Egypt, were clinically examined. Fecal samples of investigated calves were subsequently collected and serologically tested to reveal-up *Rotavirus* infection by using latex agglutination test (LAT) and immunochromatographic assay (ICA). The seropositive samples by LAT and ICA were 16.74% and 8.54%, respectively. The clinical findings of *Rotavirus* infection in enteric calves were pointed. The percentage of *Rotavirus* infection was 14.92% (44/295) of clinically diarrheic calves. However, 10% (2/20) of apparently healthy calves (n = 20) harbor *Rotavirus* in their feces suggesting carrier status. The high percentage of *Rotavirus* infection (20.13%) was obviously observed in 3 days-4 weeks old calves. There were no significance differences ($P < 0.05$) between the percentages of *Rotavirus* infection and sex, species (cattle and buffaloes) and breed (Native and Cross breed) of serologically tested calves. Moreover, there is no significant variations ($p < 0.05$) between calves under farmer's hand and calves bred in farm in susceptibility to *Rotavirus* infection. Based on climatologic conditions of Assiut, seropositive cases were more prevalent ($P < 0.001$) in cold months (22.67%) than the warm and hot months (7.27%) in Assiut.

Keywords: *Rotavirus*; Calves Diarrhea; Serological Testing; Epidemiology

Abbreviations

LAT: Latex Agglutination Test; ICA: Immunochromatographic Assay

Introduction

Newly born calves represent an essential source of animal production for either meat or breeding worldwide [1]. Neonatal enteritis appears to be a major problem of calves' rearing causing a considerable level of economic losses [2]. *Escherichia coli* is frequently incriminated as an outstanding pathogen causing enteritis in neonatal cattle and buffalo calves [3]. However, there are many pathogens were implicated as pathogens-causing enteritis in neonatal calves. In enteric calves, co-infection is observed frequently, but a single etiology can also be the cause in some cases [4]. The prevalence of each pathogen may vary from farm to farm and region to region, based on herd size, age, sex, breed, season and animal hus-

bandry practices [4]. Enteric viral infections particularly *Rotavirus* and *Coronavirus* infection cause acute viral enteritis [4,5]. *Rotavirus* is more prevalent ($P > 0.01$) than *Coronavirus* [6]. This virus, if not causing death in calves, warrants extra care and sometimes intensive care of calves [4]. *Rotavirus* mainly replicate in mature villous enterocytes [4]. Triple protein coat of this virus helps it to escape unaffected from acidic pH of stomach and digestive enzymes in the gut [4]. The mature enterocytes of duodenum villi are the first to become infected and to release significant number of virions to attack the enterocytes of mid and distal portion of small intestine [4]. *Rotavirus* infection leads to intestinal villous atrophy inducing mal-digestion followed by malabsorption with colossal economic losses of infected calves [7]. In calves, *Rotavirus* is predominately associated with neonatal enteritis mostly at 5-15 day old [4]. Practically, diagnosis of *Rotavirus* infection in enteric neonatal calves based on clinical background in association with serological testing using

LAT and ICA as field rapid tests [8]. Epidemiological data of *Escherichia coli* enteritis of neonatal calves was previously documented [3]. Inversely, reviewing of available literature indicated that epidemiological data of *Rotavirus* infection in diarrheic neonatal calves appears to be scarce. Consequently, the current work was carried-out to clear-up the epidemiological role of *Rotavirus* infection in diarrheic neonatal cattle and buffalo calves.

Materials and Methods

Animals and clinical examination

During the period of investigation, December 2015 to November 2019, a total number of 315 neonatal calves of different ages, sex, species and breed were admitted to Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University. Clinical examination of these investigated calves was carried out according to [9].

Sampling

Fecal samples of the enteric calves were collected and serologically tested. Moreover, fecal samples of random selection of apparently healthy neonatal calves were also collected and serologically tested [5].

Serological diagnosis

Serological screening of *Rotavirus* antigen in the collected samples was carried-out by two tests: LAT and ICA. Two-hindered thirty-three fecal samples were tested by LAT, a commercial kit (REF-M80 Rotascreen® kit Microgen Bioproducts limited, United Kingdom) and 82 samples tested by ICA (Rotascreen® Dipstick M580, Microgen Bioproducts limited, United Kingdom and Atlas Medical, United Kingdom). The serotesting was carried-out as per the manufacturer’s instructions [5,8].

Statistical analysis

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

Result

Clinical findings

Clinical findings revealed that the enteric calves showed classic signs of enteritis. 295 (93.65%) of investigated calves had diarrhea and 189 (60%) were suffered from dehydration. Weakness and reluctance to move were observed in 165 (52.38%) while recumbence, unable to stand and signs of comatose were showed in

24 (7.62%) of cases. 238 (75.56%) of cases had emaciation and 70 (22.22%) suffered from straining and arched back (Table 1). Fecal consistency of examined calves were differ from pasty watery, our result indicated that 20 (6.35%), 106 (33.65%) and 189 (60%) were suffering from normal consistency, pasty and watery. The color of feces varied from yellowish to greenish. 145 (46.03) had yellowish while 170 (53.97) had greenish color. Fecal samples contained mucus in 189 (60%) and mucus with undigested food in 17 (5.40%) of investigated calves (Table 2).

Clinical findings	Number of animal	%
Diarrhea	295	93.65
Dehydration	189	60
Weakness and reluctance to move	165	52.38
Recumbence, unable to stand and comatose	24	7.62
Emaciation	238	75.56
Straining and arched back	70	22.22
Apparently healthy	20	6.35

Table 1: Clinical findings in examined calves (n = 315).

Character	Number of animals	%	
Consistency	Normal	20	6.35
	Pasty	106	33.65
	Watery	189	60
Color	Yellowish	145	46.03
	Greenish	170	53.97
Abnormal content	Mucus	189	60
	Mucus with undigested food	17	5.40

Table 2: Character of fecal discharge in examined calves (n = 315).

Serological diagnosis

LAT indicated that 39 (16.74%) of 233 examined samples were serologically positive. ICA revealed that 7 (8.54%) of 82 tested fecal samples were serologically positive (Table 3).

Epidemiological findings

Percentage of *Rotavirus* infection

The present study indicated that percentage of *Rotavirus* infection was 14.60% (46/315) of examined calves (Table 4). The per-

Serological test	Number of animals	Positive	%
LAT	233	39	16.74
ICA	82	7	8.54
Total	315	46	14.60

Table 3: Serological detection of *Rotavirus* in the examined calves by LAT and ICA (n = 315).

centage of *Rotavirus* infection among enteric calves was 14.92% (44/295) and 10% (2/20) among clinically healthy calves (Table 4). Regarding to locality, infection of *Rotavirus* was 15.20% (26 of 171) of fecal samples collected from calves that came to Veterinary Teaching Hospital and was 13.89% (20 of 144) of tested fecal samples of calves of farms in Assiut Governorate (Table 5).

Calves	No.	Positive	%
Enteric calves	295	44	14.92
Apparently healthy calves	20	2	10
Total	315	46	14.60

Table 4: Percentage of *Rotavirus* infection in enteric and apparently healthy calves.

P value = 0.363. No significant variation at p < 0.05 (0.547).

Origin	No.	Positive	%
Private cases*	171	26	15.20
Farms	144	20	13.89
Total	315	46	14.60

Table 5: Distribution of *Rotavirus* infection among the examined calves.

* Admitted from various villages of Assiut to The Veterinary Teaching Hospital,

P value = 0.109. No significant variation at p < 0.05 (0.742).

Age susceptibility

The rate of *Rotavirus* infection was studied in calves at age groups of 3 days-4 week, 5-8 weeks and 9-12 weeks and yielding 20.13%, 9.45% and 10.27%, respectively of 315 of examined calves. The highest of *Rotavirus* infection was observed at age group 3 days-4 week (Table 6 and Figure 1).

Age groups	No.	Positive	%
3 days-4 weeks	149	30	20.13*
5-8 weeks	127	12	9.45
9-12 weeks	39	4	10.27
Total	315	46	14.60

Table 6: Age susceptibility to *Rotavirus* infection of investigated calves.

P value = 6.952 * Significant increase at p < 0.05 (0.031).

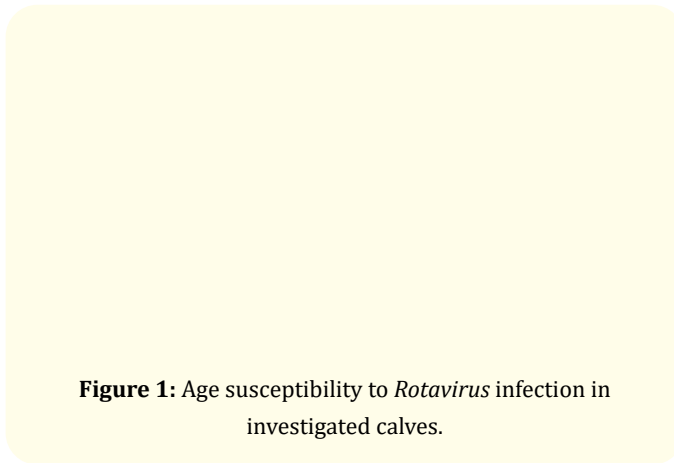


Figure 1: Age susceptibility to *Rotavirus* infection in investigated calves.

Effect of sex

The analytic results indicated that there was no significant difference in percentage of *Rotavirus* infection between male and female calves (Table 7).

Sex	No.	Positive	%
Male	181	31	17.13
Female	134	15	11.19
Total	315	46	14.60

Table 7: Effect of sex on *Rotavirus* infection of examined calves.

P value = 2.173. No significant variation at p < 0.05 (0.140).

Species susceptibility

Rotavirus infection was diagnosed in cattle and buffalo calves' fecal samples. The results revealed that 15.83% and 5.41% of cattle and buffaloes calves were positive, respectively (Table 8).

Species	No.	Positive	%
Cattle calves	278	44	15.83
Buffalo calves	37	2	5.41
Total	315	46	14.60

Table 8: Species susceptibility and *Rotavirus* infection of the investigated calves.

P value = 2.844. No significant variation at p < 0.05 (0.09).

Breed susceptibility

In the present study, there was no significant difference in percentage of *Rotavirus* infection between Native and Cross breeds (Table 9).

Seasonal variation

The obtained indicated that the percentage of *Rotavirus* infection in examined calves was higher in cold months (22.67%) than (7.27%) in warm and hot months (Table 10).

Breed	No.	Positive	%
Native (baldy)	113	14	12.39
Cross breed (Frisian/native)	202	32	15.84
Total	315	46	14.60

Table 9: Breed susceptibility and *Rotavirus* infection of the examined calves.

P value = 0.693. No significant variation at $p < 0.05$ (0.405).

Seasons	No.	Positive	%
Cold months (November-February)	150	34	22.67**
Warm and Hot months (March–October)	165	12	7.27
Total	315	46	14.60

Table 10: Seasonal variations and *Rotavirus* infection of the investigated calves.

P value = 14.931 ***highly significant increase at $p < 0.001$ (0.000).

Discussion

Neonatal calf enteritis is a multifactorial syndrome due to interaction between immune status of calves, environment, management, nutrition beside enteropathogens. The later appear to be the cornerstone of neonatal enteritis when the hygienic measures were sublevel [2]. The current work indicated the percentage of *Rotavirus* infection in enteric neonatal calves was 14.92% and the majority of serologically positive cases were occurred during the neonatal stage (3 days-4 weeks post parturition) referring to *Rotavirus* infection is neonates-linked-disease. However, the current serological examinations revealed that 10.27% of the serologically tested calves (9-12 weeks in age) was harbored *Rotavirus* in their feces. From clinical point of view, the current work revealed that *Rotavirus* infection was serologically positive in 46 cases. Watery to pasty yellowish diarrhea followed by dehydration was the prominent clinical findings. Similar findings were previously reported by [1,5,10,11]. These findings attributed to pathophysiological changes of the intestinal tracts associated with *Rotavirus* infection, in which *Rotavirus* can escape unaffected from acidic pH of stomach and digestive enzymes in gut due to presence of triple protein coat of this virus. *Rotavirus* can bind to host cell sialic acid receptor by VP7 and VP4 of outer layer of capsid. After contact with cellular receptor, VP4 spikes undergo conformational change through cleavage to VP5 and VP8 by protease enzyme (trypsin). So, *Rotavirus* invades surface epithelial cells of the small intestinal villi inducing stunts and exfoliates enterocytes' villi of small intestine inducing disorder in intestinal mucosal barriers in association with spectacular reduction in absorption capacity and in secretion of digestive enzymes resulting profuse viscous fluid containing undigested and

unabsorbed nutrients in intestinal lumen. Subsequently weakness and due to remarkable physiological alterations and electrolytes imbalances are established [11].

Serologically, LAT and ICA are rapid and easy diagnostic tests keeping laboratory viral detection is convenient and simplistic [2]. The current serological tests indicated that 16.74% of fecal samples of examined calves were positive by LAT and this result was higher than result obtained previously [12,13] that indicated that rate of serologically positive calves to *Rotavirus* infection was 6.76% and 10.83%, respectively. Highest rate of *Rotavirus* infection were reported previously [5,6] and concluded that the serologically positive cases reached-up to 21.43%. The present study showed that 7 (8.54%) of 82 diarrheic fecal samples of investigated calves were positive by ICA. Our result of ICA was lower than those of other studies [2,14,15] in which rate of *Rotavirus* infection in serologically positive enteric calves by ICA was 15.63%, 12.50% and 10%, respectively. Such variations in rate of *Rotavirus* infection in examined calves may be due to geographical variation, hygienic measures, environmental conditions, difference in timing of samples collection, clinical phase of the disease is a suitable time for sampling to obtain the optimum result from a test [11].

Epidemiologically, the present study found that percentage of *Rotavirus* infection of examined calves was 14.60% in Assiut Governorate. Previously, the high rate of *Rotavirus* infection in calves in Assiut was reported [5]. Such variation may be due to difference factors elucidated by [10]. However, the sublevel of hygienic measures plays an important role in prevalence of enteropathogens [5]. In the current work, *Rotavirus* infection was serologically detected in enteric (14.92%) and apparently healthy (10%) calves. The occurrence and distribution of *Rotavirus* were studied in enteric and clinically healthy calves [5]. The high rate of *Rotavirus* infection in enteric neonatal calves rather than healthy cases may be ascribed to *Rotavirus* destroys the enterocytes of small intestine leading to diarrhea, which is followed by a profuse fecal shedding of virus. Regarding to locality in the present study, there was no significant difference in percentage of *Rotavirus* infection in examined calves of Veterinary Teaching Hospital and farms of Assiut Governorate. This may be attributed to investigated calves were found under the same geographical, seasonal condition, hygienic measures and method of animals rearing. Statistical analysis of the obtained results showed that the rate of infection with *Rotavirus* was decreased by increasing the age of the examined calves and the peak of infection was at 3 days-4 weeks old. The rate of *Rotavirus* infection in calves was recorded at variant ages and results of other study concluded that rate of infection with *Rotavirus* was highest during the first 2 week and thereafter declining by increasing the age of calves [13]. This may be due to immune system of neonatal

calves is not fully mature to handle *Rotavirus* pathogen and susceptibility of calves to *Rotavirus* decreases with age probably due to loss of receptors on enterocytes [13,16].

In referring to the effect of sex on distribution of *Rotavirus* infection, there was no significant difference in rate of *Rotavirus* infection between male and female calves statistically. This may indicate that *Rotavirus* is non-sex-linked disease. This may be due to anatomical, functional and hormonal similarities of body systems of male and female calves in early ages that lead to non-particular resistance against *Rotavirus* infection but degree of contamination with virus, dose of virus, exposing to stress factors, consumption of colostrum or non, and another many environmental and management factors, all effect on rate of infection of *Rotavirus* and severity in both sex of calves in same or different periodic age [8].

Concerning to species susceptibility, the obtained results revealed that *Rotavirus* infection was diagnosed in both cattle and buffalo calves' fecal samples with no significant difference, although mathematically the higher percentage of *Rotavirus* infection in cattle's calves than buffalo calves. These findings were previously recorded [13] in which the susceptibility of cattle's calves to be infected with *Rotavirus* was higher than buffalo's calves and this may relate to difference of natural immunity of the two different species.

In the present work, there was no significant difference in percentage of *Rotavirus* infection between Native and Cross breeds but mathematically Cross breed had higher rate than Native breed. This finding was similar to [5] that concluded that there was no significant difference in prevalence of *Rotavirus* infection between different breeds of calves. The higher percentages of cross breed to *Rotavirus* infection in our result may be due to differences in digestive efficiency of absorption of antibodies in colostrum between different breeds beside differences in amounts of digestive enzymes, so suggests breed difference in susceptibility to *Rotavirus* infection [8]. Relationship between seasonal variations and rate of *Rotavirus* infection was studied and found that the percentage of infection was significantly higher in cold months (22.67%) than warm and hot months (7.27%). Similar results were reported by [1,4,13]. In Egypt, most calving occurs at the end of autumn and beginning of winter in which these neonatal calves are more susceptible to *Rotavirus* infection. Increased risk of *Rotavirus* infection during cold months may be attributed to the increased survivability of virus at low relative humidity and temperature. Additionally, the titer of immunoglobulins such as IgA, IgM and IgG in colostrum which act as protective factor against such infection in calves was decreased in autumn, winter and increased during spring, summer [1,4].

Conclusion

Rotavirus plays an outstanding role in neonatal calves' enteritis in different localities of Assiut governorate. LAT and ICA are reli-

able serological tests for detection of *Rotavirus* infection. *Rotavirus* infection is more prevalent in neonatal age group (3 days - 4 weeks). There was no significance difference in *Rotavirus* infection and sex, species and breed of examined calves. The higher percentage of *Rotavirus* infection was recorded in cold months than warm and hot months.

Acknowledgements

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

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