



Clinical Study with Rapid Serological Detection of *Rotavirus* Infection in Diarrheic Neonatal Calves

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Abstract

Rotavirus is an enteritis causing pathogen in neonatal calves. A total number of 175 neonatal enteric calves were clinically examined. Their feces were sampled to rapidly serotested for *Rotavirus* detection. The clinical findings on *Rotavirus* diarrheic calves were anorexia, weakness, arched back with straining during defecation, variable degree of dehydration. Feces were pasty yellowish with and/or without flakes of clotted blood. Body temperature, respiratory and heart rates were varying. The collected fecal samples were serotested by Latex agglutination test (LAT) and Immunochromatographic assay (ICA). Results of LAT and ICA indicated that the positive samples of *Rotavirus* infection were 9.68% and 8.54%, respectively. It is concluded that *Rotavirus* infection plays an outstanding role in enteritis of neonatal calves of Assiut Governorate and control measures should be attained. LAT and ICA are effortlessly field serotests in screening of *Rotavirus* infection in diarrheic neonatal calves.

Keywords: *Rotavirus*; Calf Diarrhea; LAT; ICA

Abbreviations

LAT: Latex Agglutination Test; ICA: Immunochromatographic Assay

Introduction

Enteritis in newborn calves is an outstanding problem with an economic dimension to cattle herds [1]. Enormous pathogens were incriminated as an etiologic agent of neonatal calves' enteritis. *Rotavirus* occupied the second grad of calves' enteropathogens following *Escherichia coli* causing intestinal villous atrophy ensuing malabsorption and maldigestion of the infected cases [2-4]. Diagnosis of enteritis due to *Rotavirus* infection based on clinical signs is currently unachievable. The neonatal enteric cases must subject to laboratory tests. Laboratory detection of *Rotavirus* infection depends on identification of viral antigen in fecal samples [5]. Latex agglutination test (LAT) and Immunochromatographic

assay (ICA) are commercially available rapid methods for Rota viral detection. Currently, neonatal calves' enteritis associated with *Escherichia coli* were documented. Contrariwise, data on *Rotavirus* infection in diarrheic calves in Assiut Governorate appears to be scanty [2]. Consequently, the current work performed to carry-out a rapid sero-screening of neonatal enteric calves associated with *Rotavirus* infection coming The Veterinary Teaching Hospital (VTH) of Assiut University, Assiut, Egypt.

Material and Method

Animal and clinical examination

During the period of investigation, Jan. 2018 to Nov. 2019, a total of 175 enteric neonatal calves aged from 3 to 60 days were clinically examined at VTH and bloodsera of the examined cases were tested serologically. Clinical examination of the enteric calves was carried out based on [6].

Sampling

The fecal samples of the enteric cases were subsequently collected in sterile plastic cups and stored for serological testing.

Serological testing

Serodetection of *Rotavirus* antigen by LAT

Ninety-three fecal samples were subjected to LAT for detection of *Rotavirus* antigen by a commercial kit (REF-M80 Rotascreen® kit Microgen Bioproducts limited, United Kingdom). Latex particles in Rotascreen® Test reagent is coated with rabbit antibodies raised against a pool of different *Rotavirus* isolates. When a fecal extract is mixed with test reagent any *Rotavirus* antigens present will react with sensitizing antibodies, resulting in visible agglutination of latex particles. A Control Reagent, latex particles coated with normal rabbit globulin, was included to identify non-specific reactions which may occur with some fecal specimens. The test was carried out based on the manufacturer’s guidelines.

Serological detection of *Rotavirus* antigen by ICA (Rotascreen® dipstick strip/fastest strip and *Rotavirus* rapid test device)

Eighty-two fecal samples were subjected to ICA for detection of *Rotavirus* antigen by a commercial kit (Rotascreen® Dipstick M580, Microgen Bioproducts limited, United Kingdom and Atlas Medical, United Kingdom). This test is a qualitative ICA for detection of *Rotavirus* antigen in fecal samples. The membrane is pre-coated with mouse monoclonal antibodies, on the test band region, against viral antigens. During testing, the sample is allowed to react with the colored conjugate (Anti-*Rotavirus* mouse monoclonal antibodies-red microspheres) which was pre-dried on the test. The mixture then moves upward on membrane by capillary action. As the sample flows through test membrane, colored particles migrate. In the case of positive result, specific antibodies present on membrane capture colored conjugate, producing red band. The mixture continues to move across membrane to immobilized antibody placed in control band region, colored band always appears. The test was done based on the manufacturer’s instructions.

Results

Clinical findings

Clinical findings of the examined enteric calves revealed signs of severe enteritis with variable degree of diarrhea. The most cases came to VTH are dehydrated. Thriftiness, weakness and recumbences of the enteric cases were also noticed. The examined calves

were emaciated. Straining with arched back and raised tail was obvious (Figure 1a). The eyes were sunken in most cases (65/83). Range of body temperature respiratory of the enteric cases was 37.6 to 39.8 °C. The fecal consistency of the enteric calves was varying from pasty to profuse watery. Eight (50%), 4 (25%) and 4 (25%) were suffering from profuse watery diarrhea, pasty to watery diarrhea and pasty diarrhea, respectively. Eleven (68.75%) cases had yellowish semi-pasty diarrhea (Figure 1b). Four (25%) cases with greenish diarrhea and one case had yellowish brown diarrhea. Odor of fecal samples was varying from unpleasant to offensive. Seven (43.75%) cases had unpleasant odor diarrhea and 9 (56.25%) cases have offensive diarrhea. Fourteen (87.50%) and one (6.25%) fecal sample contained mucus and mucus with clotted blood, respectively.



Figure 1: A: Enteric emaciated calf with straining, arched back and lateral deviation of tail during evacuation of intestinal contents, B: Pale yellowish pasty diarrhea of the same calf.

Serological diagnosis

Serological diagnosis of *Rotavirus* antigen by using LAT

Nine (9.68%) of the tested fecal samples of the enteric calves were serologically positive (Figure 2 and Table 1).

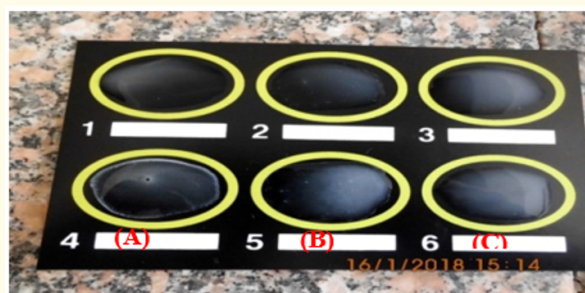


Figure 2: LAT for detection of *Rotavirus* antigen. (A1,4) Positive result (agglutination or sandy like). (B2,5) and (C3,6) Negative results (milky suspension).

Serological test	No.	Positive	%
LAT	93	9	9.68
ICA	82	7	8.54

Table 1: Serological detection of *Rotavirus* in the examined enteric calves by LAT and ICA (n = 175).

Serological detection of *Rotavirus* antigen by ICA

Seven (8.54%) of the serotested fecal samples of diarrheic calves were positive (Figure 3,4)

Discussion

Neonatal enteritis is a multifactorial problem due to interaction between immune status of calves, environment, management, nutrition beside enteropathogenes. The later plays an important role in neonatal enteritis particularly in herds with sublevel hygienic measures. *Rotavirus* is frequently implicated as a one of the major pathogens of neonatal calf diarrhea [1,7]. The current work indicated that *Rotavirus* infection was serologically detected in 16 (9.14%) cases of the investigated enteric calves (n = 175) with characteristic clinical features of enteritis with variable degree of diarrhea. Mostly the enteric discharge of diseased calves was yellowish watery feces contained mucus, followed by anorexia, emaciation, dehydration and weakness. Some enteric calves had straining with or without arched back. The systemic reactions of the seropositive cases were varied from case to another; however, tachycardia and polypnea were prominent. Similar findings were reported by [2,8]. Detailed pathophysiological changes of the intestinal tracts associated with *Rotavirus* infection are described by [9]. The triple protein coat of the *Rotavirus* helps them to escape unaffected from acidic pH of stomach and digestive enzymes in the gut. VP7 and VP4 of outer layer of capsid of *Rotavirus* bind to host cell through sialic acid receptor. After contact with cellular receptor; VP4 spikes undergo conformational change through cleavage to VP5 and VP8 by protease enzyme (trypsin). Furthermore, *Rotavirus* invades the surface epithelial cells of the small intestinal villi inducing stunts and exfoliates enterocytes' villi of small intestine which become covered by immature cells arising from the villous crypt. This immature cell causing disorder in intestinal mucosal barriers in association with spectacular reduction in absorption capacity and in secretion of digestive enzymes that lead to profuse viscous fluid containing undigested and unabsorbed nutrients in intestinal lumen. The nonstructural protein, NSP4 acts as a viral enterotoxin has both secretory and subsequent anti-secretory actions which result in loss of Cl⁻ into intestinal lumen that increasing of microbial activity and osmotic imbalance causing draw more water into gut lumen. Subsequently diarrhea, weakness, dehydration, acidosis and hypoglycemia lead to respiration and heart work could be changed, depression and all physiological function may be altered [9,10].

Serologically, LAT and ICA are feasible rapid tests for detection of *Rotavirus* in feces of animals [1,11]. The current work suggests that LAT and ICA are possible methods for examining fecal samples



Figure 3: Rotascreen® Dipstick strip/FASTest strip for detection of *Rotavirus* antigen. A: Negative result (One pink line in the Control region). B: Positive result (pink line in the Test region in addition to the Control line).

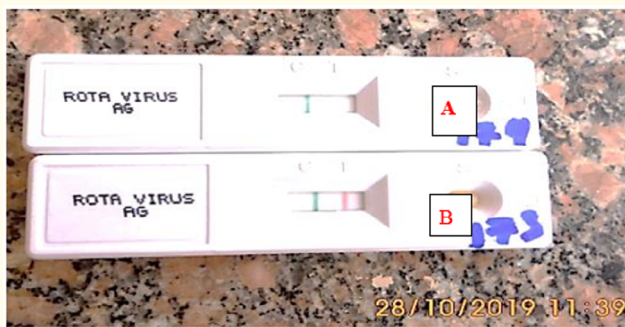


Figure 4: Rapid Test device for detection of *Rotavirus* antigen. A: Negative result (only one green band (Control line) appears in the white central zone of the test (Control region)). B: Positive result (In addition of green band (Control line), a distinguishable red band (Result line) appears in the white central zone of the test (Result region)).

collected from calves with suspected diarrhea that may be infected by *Rotavirus*. The present study indicated that 9.68% of fecal samples of enteric calves were positive by LAT. [12,13] elucidated that the percentage of *Rotavirus* infection in the enteric calves was 9.59% and 6.76%, respectively. Conversely, the highest percentage of infection with *Rotavirus* of the enteric calves were reported by [14-16]. Their results indicated that the percentage of infection with *Rotavirus* was 32.08%, 35.25%, and 65%, respectively.

The current study revealed that 7 (8.54%) of 82 diarrheic fecal samples of calves were positive by ICA; These 7 enteric cases were as follows: 4 (12.12%) of 33 by Rotascreen® Dipstick strip/FAST strip and 3 (6.12%) of 49 by *Rotavirus* Rapid Test device. The obtained result of FAST strip was higher than the result obtained by [8] who indicated that the percentage of positive serological detection of *Rotavirus* of tested enteric calves was 10%. On the contrary, the highest rate of *Rotavirus* infection in enteric calves were concluded by [15,17]. They indicated that the percentage of *Rotavirus* seropositive enteric cases was 35.25%, and 53.6%, respectively. Conversely, [10,18,19] indicated that the rate of *Rotavirus* infection in enteric calves was 36%, 15.63% and 12.50%, respectively. Such variations in the rate of infection of *Rotavirus* in enteric calves may be attributed to geographical variation, difference in the timing of sample collection, clinical phase of the disease is a suitable time for sampling to obtain the optimum result from a test, hygienic measures and environmental conditions.

Conclusion

Rotavirus plays an outstanding role in enteritis of neonatal calves. LAT and ICA are a reliable and rapid method for the detection of *Rotavirus* infection in neonatal diarrheic calves. Attention should be directed toward viruses in any control programs put to overcome enteritis in neonatal calves in Assiut Governorate.

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Not applicable.

Ethics Approval and Consent to Participate

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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