Virology & Mycology
ISSN: 2161-0517

Abdulazeez et al,, Virol Mycol 2018, 7:1
DOI: 10.4172/2161-0517.1000172

Research Article Open Access

The Histopathological Effect of Rotavirus in Small Intestine of Mice Isolated from Different Area of Iraq

Abdulazeez AA[1*], Al-Rawi AS[1], Abdullah WZ[2], Abed MN[2], Al hamadani F[1] and Aufi IM[3]

[1]*Department of microbiology, College of Veterinary Medicine, University of Baghdad, Iraq*

[2]*Institute of Medical Technology Al-Mansor, Iraq*

[3]*Ministry of Health (CPHL), Uganda*

[*]**Corresponding author**: Abdulazeez AA, Department of microbiology, College of Veterinary Medicine, University of Baghdad, Iraq; Tel: +96417787066; E-mail: aaldouri96@yahoo.com

Received date: December 05, 2017; **Accepted date:** January 18, 2018; **Published date:** January 25, 2017

Abstract

A total of 125 fecal samples of infants with acute gastroenteritis were collected randomly from November 2015 to March 2016. Their ages ranged from two weeks to six months. Rotavirus was diagnosed with three detection kits as well as tissue culture. The present study results revealed that 59 (47.2%), 51(40.8%), and 44(35.2%) were positive using chromatographic immunoassay, ELISA, and PCR techniques, respectively. The histopathological changes in intestine at 2 days post infection showed congested diluted blood vessels with inflammatory cells particularly neutrophils and mononuclear cells and edema in sub mucosa in addition to necrosis of crypt and hypertrophy of goblet cells.

Keywords: Rotavirus; Histopathology; Diagnostic assay; Acute gastroenteritis; Neutrophils

Introduction

Rotaviruses are significant enteric virus and cause of acute diarrhea in infants and young children of many mammalian species particularly human and calves [1]. Enteritis linked to rotavirus is a main trouble in domestic animals, particularly in young calves [2]. Rotaviruses belong to the Reoviridae family; which is subdivided into the sub-families of the Sedoreovirinae and the Spinareovirinae in which Rotavirus is one of 15 genera and have a genome including eleven segments of double-stranded RNA enclosed in capsid of three layers [3]. Several techniques have been developed for diagnosing rotavirus in feces. The detection of the viral agent can be performed by electronic microscopy, polyacrylamide gel electrophoresis (PAGE), immunofluorescence, radioimmune assay, reverse passive hemagglutination, enzyme immunoassays (EIA), chromatographic immunoassay, and reverse transcriptase with polymerase chain reaction. Among these assays, chromatographic immunoassay was reported as a being easy to perform in a short time, for diagnosis and control of the disease caused by rotavirus in humans [4]. The infection percentage of rotavirus in Iraq in the period between 2012 to 2017 were, at 2012 the positive cases 550 from 977 (56%), 2013 were 482 positive from 1159 (41%), 2014 were 271 from 726 (37%), 2015 were 464 from 638 (72.7%), 2016 were 497 from 569 (87%) and 2017 128 positive cases from 222 (57.6%), According to statistic from public health directorate done at Central Public Health Laboratory (CPHL) with Control Disease Center in Iraq Baghdad from 11 centers sites in Iraq.

The aim of this research is to study the histopathological effect of rotavirus that isolated from infants in small intestine of mice.

Material and Methods

A total of 125 fecal samples of infants with acute gastroenteritis were collected randomly from November 2015 to March 2016 from five Iraqi governorates (Babil, Kerbala, Missan, Qadissiya, and Wasit) by rectal stimulation with age ranging from two weeks to six months. The feces were collected directly into sterile disposable plastic containers then stored in a cool box and transported to the laboratory. Each sample was transferred to specimen collection tube with extraction buffer that was used in chromatographic immunoassay. After centrifugation for 5 minute at 6000 rpm, the supernatant was stored at -20°C until the assay was conducted.

Rotavirus identification

Three diagnostic kits were used for the detection of rotavirus in fecal samples. The qualitative Rotavirus assay was performed with chromatography one step rotavirus test device kits (Abon Biopharm, Germany). In this method, one step rotavirus test was used; this test was a qualitative lateral flow immunoassay for the detection of rotavirus in feces specimen.

Tissue culture and virus titration

Tissue culture were used for isolating rotavirus by using fetal bovine kidney cells (FBK) with growth media which was Minimum Essential Medium (MEM) (Gibco-USA) prepared by mixing 93 ml of media with fetal calf serum (Gibico-USA) 10 ml, strepotomycin and penicillin (Biowest) 1 ml, Fungizone (Biowest) 0.5 ml and 7% $NaHCO_2$(BDH) 1 ml [5].

For each sample, 2 g of feces were put into tube containing 6 ml of PBS. Samples were then clarified by centrifugation at 2500 rpm for 30 minutes. The supernatants from the homogenates were filtered through 0.22 µm filter and store at -80°C until use. 500 µl of human

filtrate was treated with trypsin for 1 hour in a 37°C water bath after a brief vortex to mix [6].

Virus titration was performed using Spearman-Karbers method. Cells were grown in 96 wells tissue culture plates. About 0.1 ml of the virus from tissue culture made tenfold dilution ranging from 10^{-1} to 10^{-8} was used to infect the cells in each well of the 96 wells tissue culture plate. TCID50 value was calculated according to the equation method [7].

Enzyme linked immunosorbent assay (ELISA kit from Cusabio Biotech Co., Ltd, China) method was done to confirm the result obtained from tissue culture.

The quantitative enzyme immunoassay technique was employed in this test; a micro titer plate was pre-coated with specific antibody for rotavirus antigen. The calculation of result was compared with control according to the following equation:

Cut-off value=the average value of OD negative+0.01

Then we took the positive result from ELISA test and made molecular detection by polymerase chain reaction (PCR) using (AccuPower PCR Premix of Bioneer Corporation, Republic of Korea).

Genomic viral RNA isolation

The viral RNA was isolated from the tissue cultures samples by using QIAamp viral RNA mini extraction kit. All assays were performed according to the manufacturers' instructions.

Estimation RNA concentration and purity

The viral nucleic acid concentration and purity of the sample extracted from feces were estimated by using Nano drop (Acta/USA) where 1 µl of the extracted RNA was put in the machine. The purity was (~2.0) that means RNA is high purity.

Convert the viral RNA to cDNA

Reverse transcriptase kit/ RT-PCR kit (Accupower Rocketscript RT premix) was used to convert the viral RNA which have been extracted to cDNA in this study

The primers used in PCR reaction were from Manual of rotavirus detection [8].

Experimental design

Albino male mice supplied by the Biotechnology Research Centre at Alnahrain University were employed in carrying out the experiments of the study. The mice were of the age of 6 weeks, and their weight was between 23-27 g. They were divided into 2 groups, and each group was containing 5 mice each and was kept in a separate plastic cages, the total number was 10 mice. First group was control to which normal saline was administrated only, while the second group was infected with (0.5 ml) virus particles through oral route with stomach tubes that obtained from positive tissue culture fecal samples and then they were left in their cages for one to two days until the symptom were appeared. The animals were kept at a temperature of 20-30°C, and they had free excess of food (standard pellets) and water throughout the experimental work.

Histopathological examination

After the clinical signs have appeared to all mice, 3 of them were dead and the other was killed by the inhalation of concentrated formalin in closed jar. The samples were collected from vital organs of experimental animals which included intestine that stored in 10% formalin solution for fixation. The Histopathological examination was conducted in the Biotechnology Research Center according to the recommended procedure of the Histopathology Laboratory [9].

Results

Rotavirus diagnosis

A total of 125 fecal samples were collected from infected infants their ages ranged from two weeks to six months. Chromatographic Immunoassay method was done for detection of rotavirus antigen in feces. Our results showed that 59 (47%) positive specimens.

Virus isolation in cell culture

After the first detection by chromatographic immunoassay, fetal bovine kidney cell culture (FBK) were infected with positive fecal samples, after 3 passages the effect appeared confirming the presence of cytopathic effect (CPE) of the virus in the test samples and give us 50 (40%).

Titration of isolated virus

TCID50 value was calculated according to the Reed and Muench equation method [10], thus the titration of rotavirus was $1\times 10^{3.5}$ virus/0.1 ml.

Enzyme linked immunosorbent assay

In current study all samples we get it from tissue culture were confirmed by ELISA tests and showed the presence of rotavirus antigen and gave us 51(41%) positive results.

Polymerase chain reaction

In this study, PCR technique was used for detection the two outer layers' proteins VP4 and VP7.

All samples which had been tested by ELISA that gave positive results, tested by using PCR and the results revealed 44 (35%) positive specimens as it shown in Figure 1.

The histopathological effect

After the clinical signs appeared, and the death of all mice after 2 days, the samples were collected from small intestine of experimental animals which included intestine that stored in 10% formalin solution for fixation.

Our observation for each group showed:

In group 1 the mice which infected with normal saline as a control showed no lesion was found in mice intestine as it shown in Figures 2 and 3.

While in group 2 the mice infected with rotavirus and results shown the histopathological changes in intestine at 2 days post infection showed congested diluted blood vessels with inflammatory cells particularly neutrophils and mononuclear cells and edema in sub

mucosa in addition to necrosis of crypt and hypertrophy of goblet cells as it shown in Figures 4-7 and compared the results with negative control.

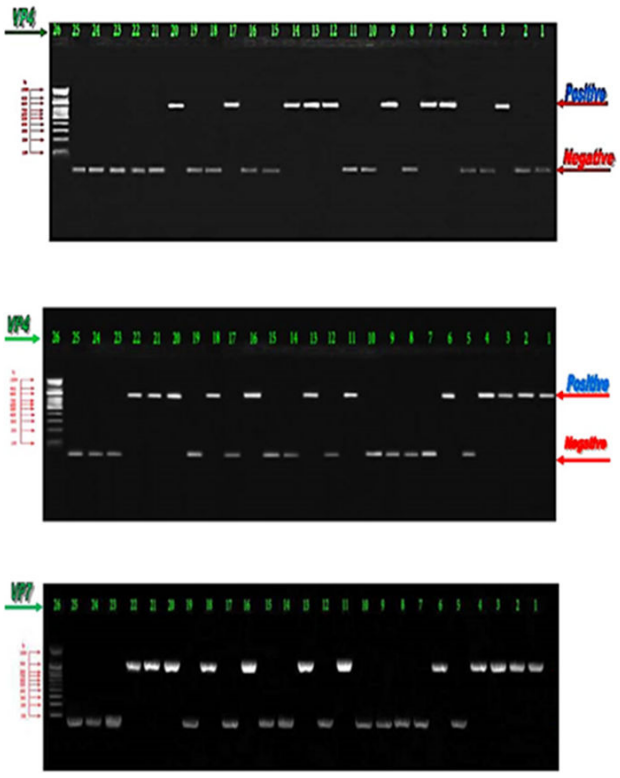

Figure 1: The obtained results by using PCR method for Rotavirus in tissue culture samples.

Group 1: the mice infected with only normal saline as a negative control. The histopathological changes demonstrated that no lesion was found in mice intestine (Figures 2 and 3).

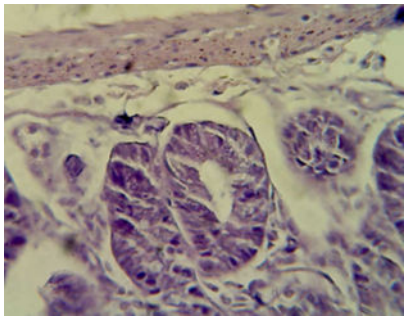

Figure 2: Section in the intestine of normal animal shows no lesion (H&E stain 400X).

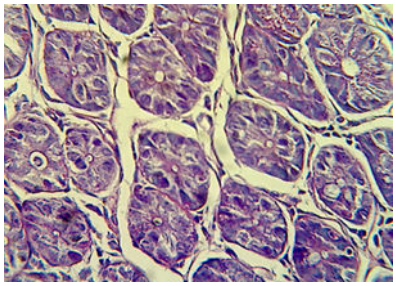

Figure 3: Section in the intestine of normal animal shows no lesion (H&E stain 400X).

Group 2: the mice infected with rotavirus that isolated from stool samples (Figures 4-7).

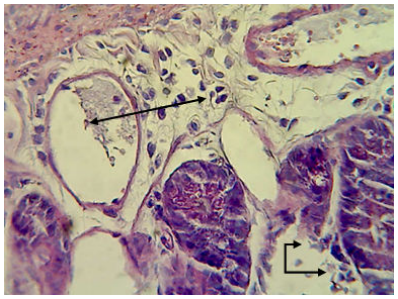

Figure 4: Section in the intestine of animal at 2 days post infection with rotavirus shows congested dilated blood vessels with inflammatory cells particularly neutrophils, mononuclear cells, edema in sub mucosa, in addition to necrosis of crypt (H&E stain 400X).

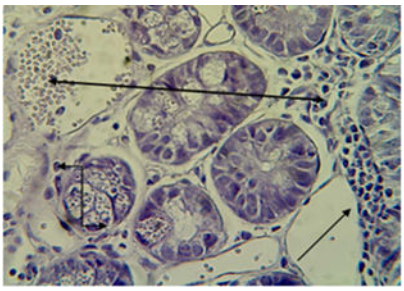

Figure 5: Section in the intestine of animal at 2 days post infection with rotavirus shows inflammatory in congested dilated blood vessels with inflammatory cells particularly neutrophils, mononuclear cells, edema between mucosal glands, in addition to necrosis and degeneration of intestinal glands and dilated of lymphatic vessels(H&E stain 400X).

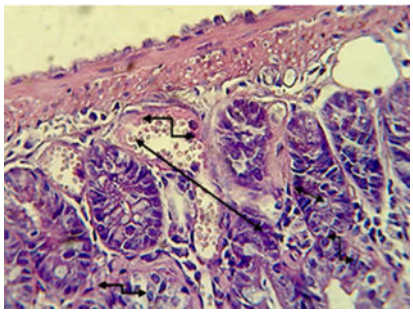

Figure 6: Section in the intestine of animal at 2 days post infection with rotavirus shows congested dilated blood vessels, with inflammatory cells particularly neutrophils, mononuclear cells and edema in sub mucosa, in addition to necrosis of crypt of Lieberkuhn and hypertrophy of goblet cells (H&E stain 400X).

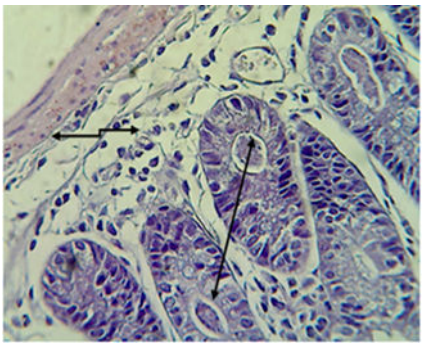

Figure 7: Section in the intestine of animal at 2 days post infection with rotavirus shows congested dilated blood vessels with inflammatory cells particularly neutrophils, mononuclear cells and edema in sub mucosa, in addition to cellular debris in the lumen of crypt (H&E stain 400X).

Discussion

Diarrhea affecting the neonates is an important condition, which affect the herd health, farm profitability and as a whole the economy of the country.

In the present study five mice died with the history of diarrhea revealed mild catarrhal changes in the small intestine with congestion of both edema in sub mucosa layers and these changes were in agreement with earlier report [11].

The presence of rotavirus antigen in the fecal samples was demonstrated by one step chromatographic immunoassay, and confirming rotavirus infection by ELISA. Earlier reports were controversial for the susceptibility of different small intestinal section to rotavirus infection [12].

Chromatographic Immunoassay and ELISA are the simple and good standard methods for detection of rotavirus. These methods however require low cost equipment and simple experience, which is available in many laboratories. Some researchers for detecting rotavirus infection have used ELISA and PCR [13].

The presence of rotavirus antigen in the intestinal tissues by ELISA and chromatographic immunoassay, thus confirming rotavirus infection in infants under investigation; mainly confined to the small intestinal mucosa, earlier reports were controversial for the susceptibility of different small intestinal sections to rotavirus infection [12].

Rotavirus infection alters the function of the small intestinal epithelium, resulting in diarrhea. The diarrhea was generally considered to be malabsorptive, secondary to enterocyte destruction [14].

In animal models, rotaviruses have also been documented to spread beyond the intestine after oral infection. In the mouse model, sites of spread include the lamina propria, Peyer's patches, mesenteric lymph nodes, lung, liver, kidney, and bile duct [15]. Group A rotaviruses have been shown to induce biliary atresia in mice, but this system requires intraperitoneal not oral inoculation of virus [16]. The most detailed studies of extra intestinal spread in the mouse examined spread to the liver following oral inoculation [8,17].

The current study demonstrated that rotavirus induced pathological lesion in the examined organ of 2nd group of mice, thus, this virus was highly virulent and have ability to produce toxic nonstructural protein and overcome of the host defense mechanism. This result match the observation who investigated the functional NSP4 enterotoxin peptide secreted from rotavirus-infected cells where they found that the NSP4, a secreted fragment of NSP4, or appropriate NSP4 peptides have an activity similar to that of toxin and stimulate diarrhea when injected into mice [18].

The enterocytes lining the small intestine are generally divided into two types: enterocytes and crypt cells. Villus enterocytes are mature, non-proliferating cells covering the villi that are differentiated to digestive and absorptive functions. The absorptive enterocytes synthesize a number of disaccharidases, peptidases, and other enzymes that are expressed on the apical surface, where they carry out their digestive functions. Absorption across the enterocyte barrier occurs both by passive diffusion of solutes along electrochemical or osmotic gradients and by active transport. While the majority of water transport is passive along osmotic gradients, transporters such as the sodium-glucose co-transporter 1 (SGLT1) transport water along with solute [19]. The crypt epithelium lines the crypts and is the progenitor of the villus enterocytes. Crypt cells lack the well-defined microvilli and absorptive functions of the enterocyte and actively secrete Cl^- ions into the intestinal lumen. In the normal animal, the combined activity of the enterocytes and crypt cells results in a constant bidirectional flux of electrolytes and water across the epithelium. On the villi, the balance is toward absorption, and in the crypts, the balance favors secretion [20].

The histological changes in ileum characterized by desquamation and necrosis of crypts with villous atrophy, infiltration of lymphocytes in inter villous areas and decreased population of lymphocytes in Peyer's patches and mesenteric lymph node were in agreement with earlier reports [21,22].

Acknowledgments

We are grateful to the many friends and colleagues who generously contributed their time and effort to help us make this paper as accurate and useful as possible. The support of our other colleagues at the

ministry of health (CPHL) Dr. Faisal Al Hamadani and Dr. Iman M Aufi who help us to use the lap for our research.

References

1. Estes MK, Greenberg HB (2013) Rotaviruses. 6th edn. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia pp: 1347-1401.
2. Martella V, Banyai K, Matthijnssens J, Buonavoglia C, Ciarlet M, et al. (2010) Zoonotic aspects of rotaviruses. Vet Microbiol 140: 246-255.
3. Estes MK (2001) Rotaviruses and their replication. 4th edn. Lippincott Williams and Wilkins, Philadelphia pp: 1747-1785.
4. Ferreira TL, Becho MC, Bernardo AR, Chaves TCB, Ribeiro RS, et al. (2006) Performance of latex agglutination test in the diagnosis of acute gastroenteritis by rotavirus. Braz. J. Microbiol 37: 587-589.
5. AL-Rawi AS (2013) Serological and molecular identification of food and mouth disease from different Area in Iraq. PhD. Thesis. Department of microbiology. College of veterinary medicine, Baghdad University.
6. Ennima I, Sebbar G, Harif B, Amzazi S, Loutfi C, et al. (2016) Isolation and identification of group A rotaviruses among neonatal diarrheic calves, Morocco. BMC Res Notes 9: 261.
7. Villages P, Purchase HG (1989) A laboratory manual for the isolation and identification avian pathogens. 3th ed. New Bolton center Kennett, Pennsylvania pp: 187-188.
8. World Health Organization (WHO) (2009). Manual of rotavirus detection and characterization method.
9. Boshuizen JA, Reimerink JH, Korteland-van Male AM, van Ham VJ, Koopmans MP, et al. (2003) Changes in small intestinal homeostasis, morphology and gene expression during rotavirus infection of infant mice. J Virol 77: 13005-13016.
10. White DO, Frank JF (1986) Medical Virology. Academic Press Inc, Orlando pp: 49-50.
11. Chauhan RS, Singh NP (1992) Assessment of intestinal damage in rotavirus infected calves by D-xylose malabsorption test. J Vet Pathol 16: 13-16.
12. Vanamayya PR, Mohanty GC (1993) Experimental rotavirus infection in colostrum deprived neonatal calves: Clinical and pathological changes. J. Vet. Pathol 17: 1-6.
13. Guandalini S, Vaziri H (2011) Diarrhea: Diagnostic and therapeutic advances. Humana Press, Springer Science Business Media, USA.
14. Kapikian AZ, Hoshino Y, Chanock RM (2001) Rotaviruses 4th ed. Lippincott Williams & Wilkins, Philadelphia pp: 1787-1833
15. Conner ME, Ramig RF (1997)Viral enteric diseases Lippincott-Raven Publishers, Philadelphia pp: 713-743
16. Czech-Schmidt G, Verhagen W, Szavay P, Leonhardt J, Petersen C, et al. (2001) Immunological gap in the infectious animal model for biliary atresia. J Surg Res 101: 62-67.
17. Notle T, Brander-Weber P, Dangler C, Deschl U, Elwell MR, et al. (2016) Nonproliferative and proliferative lesions of the gastrointestinal tract, pancreas and salivary glands of the rat and mouse. J Toxicol Pathol 29: 1S-125S.
18. Zhang M, Zeng CQ, Morris AP, Estes MK (2000) A functional NSP4 enterotoxin peptide secreted from rotavirus-infected cells. J Virol 74: 11663-11670.
19. Loo DDF, Wright EM, Zeuthen T (2002) Water pumps. J. Physiol 542: 53-60.
20. Moon HW (1994) Pathophysiology of viral diarrhea. Marcel Dekker Inc, New York pp: 27-52
21. Dhama K, Chauhan RS, Mahendran M, Malik SVS (2009) Rotavirus diarrhoea in bovines and other domestic animals. Vet Res Commun 33: 1-23.
22. Chauhan RS, Singh NP (1992) Detection of rotavirus infection in calves employing immunofluorescence. J Applied Anim Res 1: 51-55.