FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

FREQUENCY OF ROTAVIRUS AMONG CHILDREN < 5 YEARS WITH ACUTE DIARRHEA: A HOSPITAL BASED STUDY IN DUHOK / IRAQ

ASHTI ASSIM M. SALIM

Master's Thesis

DEPARTMENT OF BIOLOGY

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DECLARATION

I hereby declare that I wrote this Master's Thesis titled “Frequency Of RotaVirus Among Children < 5 Years With Acute Dıarrhea: A Hospital Based Study In Duhok / Iraq” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

02 February 2022

ASHTI ASSIM M. SALIM

PREFACE

First of all, I am grateful to the most merciful God for giving me strength and health to carry on my study. our research is about prevalence of Rotavirus among children less than five years with acute diarrhea. During the working period in our research we have overcome all problems like dealing with children's parents, storing of samples and analyzing. It is a great pleasure to express my deepest thanks and gratitude my supervisor Prof. Dr. Eyüp BAĞCI Faculty of Science/ Firat University for guiding and helping me in order to complete this study a well achievement. I would like to express my deepest thanks and sincere appreciation to prof. Dr. Samim Al Dabbagh faculty of medicine/ Duhok University for helping me in everything and he didn't skimp on me in his time and knowledge. Also I would like to thanks to Dr. Nadia AL Derzi for her support me always. Also take this opportunity to deeply thanks to Dr. Saad AL Agha to help me to create statistics and tables. Special thanks to Mr. Omer Alasady, Dr. Saman, Dr. Moqdad and Mr. Hakam for their kindness help to complete this thesis. My thanks and my gratitude to my family member for their patience, support and prays. Moreover special and deepest thanks to my lovely wife Mrs. Solin Paydawi for her long term. Actually without her patience and her support I would not have been able to resist obstacles and reach a stage. Finally special thanks to my kids (Dilaram, Adam).

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ELAZIG, 2022

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ABSTRACT

Frequency Of RotaVirus Among Children < 5 Years With Acute Dıarrhea: A Hospital Based Study In Duhok / Iraq

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Background: Rotavirus is the most important cause of diarrhea among children less than 5 years of age causing high morbidity and mortality, particularly in developing countries.

Aims: The aims of the present work is to measure the prevalence of rotavirus diarrhea among children less than 5 years who visited a referral hospital suffering from acute gastroenteritis by ELISA test; and to measure the validity of ELISA test in comparison to PCR confirmatory test.

Patients and Methods: Across sectional study design was adopted. Approval of the ethics committee was obtained and the procedures were carried out according to the ethical roles. The study included 310 children aged less than 5 years of both genders who diagnosed by specialist to be suffering from acute diarrhea; at a secondary care hospital in Duhok /Iraq . Stool samples were collected form those children during the period January–June, 2021, and then ELISA test was conducted for Rotavirus antigen. Information were also collected regarding: age, gender, father and mother education, crowding index in houses, urban/rural residency, feeding practice in the first year of life, vaccination to Rotavirus and attaches of diarrhea suffered in last month. The validity of ELISA test was measured by taking a sub sample of positive and negative cases who were further tested by PCR.

Results: The prevalence of Rotavirus by ELISA test was found as 29%. No significant association was detected regarding age, gender, crowding index and Rotavirus infection. On the other hand there was a significant association between Rotavirus infection and; education family with higher rate among those with illiteracy or lower education and urban residency exclusive bottle feeding in the first year of life, while partial or complete vaccination gave protection to Rotavirus. The results also revealed that children who had more attacks of diarrheas in the month preceding admission had significantly lower prevalence of Rotavirus infection than those who had only one attack of diarrhea. The validity of ELISA test in comparison to PCR as gold standard was measured on 45 positive and 65 negative ELISA samples who were also tested by PCR. The results showed that the sensitivity, specificity, predictive values of a positive and a negative test, likelihood ratios of a positive and a negative were 88.89%, 100%, 100%, 92.86%, unidentified and 0.113 respectively, and the diagnostic accuracy of the ELISA test was determined as 95.45%.

Conclusions: Rotavirus is an important cause of gastroenteritis, particularly among infants, in Duhok, Kurdistan Region, Iraq. All children less than 5 years who are admitted with gastroenteritis should be scanned for Rotavirus with ELISA test; which found to be a valid screening test. Also vaccination was an important measure and recommended for the control of the diseases.

Keywords: *Rotavirus, Diarrhea, Prevalence, ELISA, PCR, Validity*

ÖZET

5 Yaş altı çocuklarda Rotavirüs ün Akut İshal ile Frekansı, Irak/ Duhok' da Hastahane Bazlı bir çalışma

ASHTI ASSIM M. SALIM

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Rotavirus, özellikle gelişmekte olan ülkelerde 5 yaş altı çocukların arasındaki ölüm ve hastalıkların sebebi olan ishalin en önemli etkenidir. Bu çalışmanın amacı 5 yaş altında olan ve barsak enfeksiyonu nedeniyle hastaneye başvuran çocuklarda rotavirüs' ün prevalansını Elisa testityle ölçmek ve Elisa testinin geçerliliğini PCR destekleyici test ile karşılaştırmasını yapmaktır. Hasta ve metot olarak Kesitsel bir çalışma modeli uygulanmıştır. İşlemler için etik komitesinin onayı alınmış ve hastalara işlemler etik kurallar çerçevesinde uygulannıştır. Çalışma, Duhok/ Irak'ta, ikinci basamak bir hastaneye başvuran her iki cinsiyette akut ishalden muzdarip 5 yaş altı 310 çocuğu kapsamaktadır. Dışkı örnekleri bu çocuklardan ocak – haziran ayları (2021) arasında toplanmış ve Rotavirüs antijeni için Elisa testinde Yürütülmüştür. Ayrıca, anne ve baba eğitimi, yaş, Cinsiyet, evlerdeki kalalablık indeksi, kentsel ve kırsal yerleşim, yaşamın ilk yılında beslenme uygulaması, rotavirus aşısı ve geçen ay ishal olma durumuna ait bilgiler toplanmıştır. Elisa testinin geçerliliği PCR ile daha fazla test edilen pozitif ve negative vakaların bir alt örneği alınarak ölçülmüştür. Rotavirüs'ün Elisa testi ile elde edilen prevalansi 29 % olarak saptanmıştır. Yaş, cinsiyet, kalabalık indexi ve rotavirus enfeksiyonu açısından anlamlı bir ilişki saptanmamıştır. Öte yandan rotavirus enfeksiyonu ile; okuma yazma bilmeyen veya daha düşük eğitime sahip olanlar arasında, eğitimli aile oranı daha yüksek ve yaşamın ilk yılında biberonla beslenme, kentsel yerleşme, kısmi veya tam aşılama rotavirüse karşı koruma sağlama arasında önemli bir ilişki olduğu bulunmuştur. Ayrıca Sonuçlar, hastaneye yatıştan önceki ayda daha fazla ishal nöbeti geçiren çocukların yalnızca bir ishal atağı geçirenlere göre rotavirus infeksiyonu prevalansinin önemli ölçüde daha düşük olduğunu ortaya koymuştur. Altın standart olarak PCR 'a kıyasla Elisa testinin geçerliliği, yine PCR ile test edilen 45 pozitif ve 65 negatif Elisa numünesi üzerinde de ölçülmüştür. Sonuçlar, bir pozitif ve bir negative testin duyarlılık, özgünlük , öngörücü değerlerinin, bir pozitif ve bir negatif olasılık oranlarının sırasıyla, %88.89, %100, %100, % 92.86, tanımlanmayan ve 0.113 olduğunu ve tanısal doğruluğunun Elisa testi % 95.45 olarak belirlenmiştir. Sonuçta, Rotavirus Duhok/ Irak' ta özellikle bebeklerde önemli bir barsak enfesksiyonu nedeni olduğu saptanmıştır. 5 yaşından küçük tüm çocukların geçerli bir test olan Elisa testi ile rotavirus taramasından geçirilmelidir. Ayrıca aşılama önemli bir önlem olup hastalıkların kontrolü için tavsiye edilmektedir.

Anahtar Kelimeler: Rotavirüs, İshal, Prevalans, ELISA, PCR, Geçerlilik

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1. INTRODUCTION

Infection by group A rotaviruses; which belong to the Reoviridae family of non-enveloped dsRNA-containing viruses; is considered as the most important cause of acute gastroenteritis among children. The majority of morbidity and mortality caused by Rotavirus(RV) gastroenteritis is experienced by children under five years of age. Diarrhea in general is the main causes of morbidity and mortality among children less than 5 years in the world, particularly in developing countries.The RV represent about 40% of hospitalizations for diarrheal that occur among children under five in developing countries Globally it has been estimated that daily there are, 370,000 episodes of rotavirus diarrhea cases of which, 50,000 cases hospitalized and 2000 deaths [1-4] The disease is characterized by profuse diarrhea, mild fever, and vomiting, with mild to severe dehydration.. The disease generally is resolved by itself and does not need medication, but is responsible for high morbidity and can cause severe dehydration and death [5]. In Iraq and Kurdistan it also the main cause of morbidity and mortality among children. In a study conducted in Erbil in Iraq in 2006 a prevalence of 37% was detected among children less than 5 years suffering from gastroenteritis by PCR. The most common genotypes were G1,G2,G4,G9, P[6] and P[4]. Moreover the commonest G/P combination (about 50%) was P[8]G1 among the 8 combinations detected [6].

Vaccination against the disease has been found to be very effective in the control and prevention of Rotavirus gastroenteritis. Once introduced in 1973 a sharp declines in the morbidity and mortality have been noticed all over the world. The anti RV vaccine has now been part of the vaccination schedule for under-fives children; in several countries including Iraq [7]. The symptoms and signs of Rotavirus diarrheas can varied from completely asymptomatic to sever and even fatal attacks. In general they are relatively similar to those of other causes of gastroenteritis and they alone are not sufficiently distinctive to permit diagnosis. Clinicians will need investigation to establish the diagnosis. Those can varied from rapid screening test for antibodies, or detection of antigen in the stool by ELISA test. The PCR and Electron microscopy have also been used but rather for research or genetic studies [7-8].

1.1. Rotavirus

Rotavirus (RV) is an non enveloped RNA virus of Reoviridae family. The genome composed of 11 segments of double-stranded (ds) RNA surrounded by a triple-layered protein capsid. The RNA segments include. six structural viral proteins (VP1 to VP4, VP6 and VP7) and six nonstructural proteins (NSP1 to NSP6) [9]. There are eight species within the rotavirus genus. Rotavirus A, B, C, D, E. F, G, and H [10].

It is excreted in stools and considered as the commonest cause of diarrhea globally among infants and children less than 5 years of age. Group A (RVA) species are the most significance as prime cause of severe diarrhea in infants and young children [11]. It has been estimated that before the introduction of the vaccine, almost all of them would be infected by rotavirus by the age of 5 years. The virus is very contagious [12]. The RV infects enterocytes and destroyed the absorptive enterocytes, intestinal secretion stimulated by RV non-structural protein 4 and activation of the enteric nervous system [13].

Despite wide researches on rotavirus the virion morphogenesis and the virus cell entry are complex processes, which have not fully understood [1, 14, 15].There are ten species of rotavirus, referred to as A, B, C, D, E, F, G, H, I and J. Humans are primarily infected by the species rotavirus A. Rotaviruses group A are subdivided to several G and P genotypes; based on the molecular characterization of the outer capsid proteins, VP7 (glycoprotein) and VP4 (protease-sensitive, which was cleaved into VP5 and VP8 by trypsin) respectively In rotaviruses A there are at least 51 P genotypes and 36 G genotypes encountered in humans and animals.

A–E species cause disease in other animals, species E and H in pigs, D, F and G in birds, I in cats and J in bats. Within rotavirus A there are different strains, called serotypes. As with influenza virus, a dual classification system is used based on two proteins on the surface of the virus. The glycoprotein VP7 defines the G serotypes and the protease-sensitive protein VP4 defines P serotypes. Different combination of the P-G serotype combinations due to assortment and segregation of the G and P proteins will produce of different strains A whole genome genotyping system has been established for rotavirus A, which has been used to determine the origin of atypical strains. The prevalence of the individual G-types and P-types varies between, and within, countries and years. There are at least 32 G types and 47 P types but in infections of humans only a few combinations of G and P types predominate.

The most common genotypes detected globally include four common human G genotypes (G1, G2, G3, G4) with the most common human P genotypes P[4], P[6], and P[8]. On the other hand; G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] are the most common G/P genotypes combinations infecting humans; globally responsible for almost 90% of rotavirus infections [5, 10, 16-20].

Rotaviruses are generally high species-specific, but cross transmission with animal RV, has been shown in many case. This transmission is relatively uncommon; however, millions of people will be exposed to animal rotaviruses each year; mainly among farmers and visitors to the countryside [17, 21].The RV is known and named for the wheel-like appearance visible under an electron microscope. Picture of the virus and the schematic representation of a rotavirus virion are shown on Figure1.1. and 1.2. below.

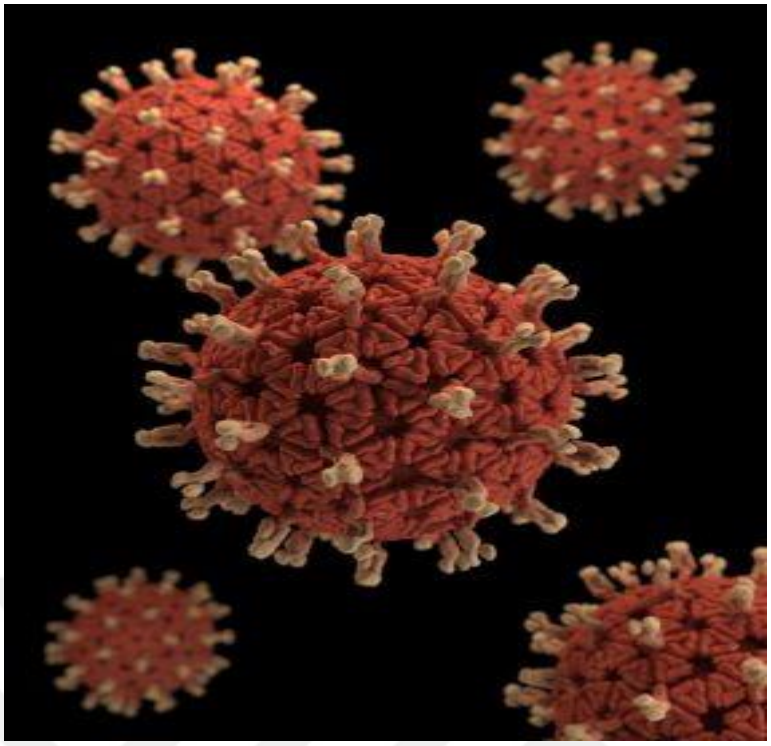


Figure 1.1. 3D graphical representation of Rotavirus virions

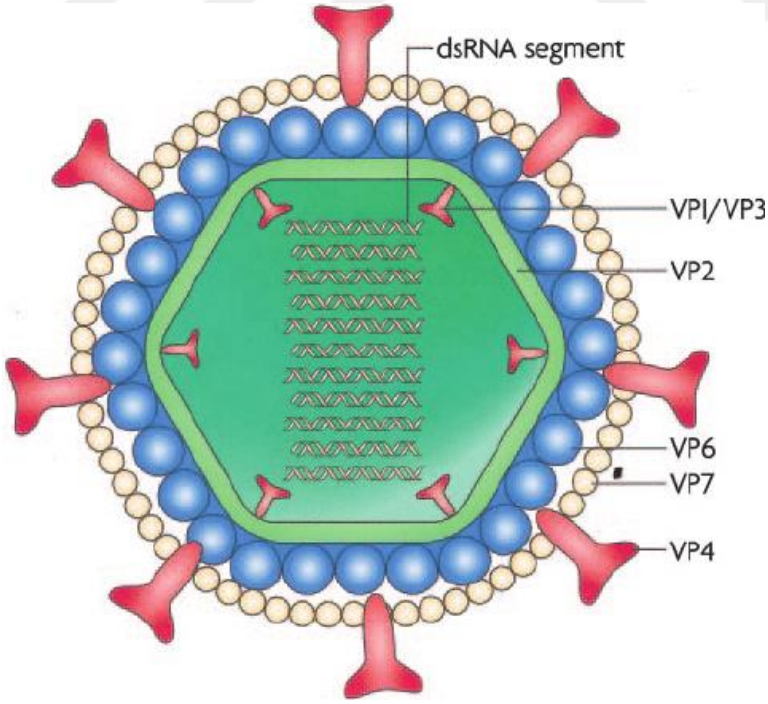


Figure 1.2. Schematic representation of a rotavirus virion (……..)

1.2. Rotavirus infection

The aetiologic agent of infantile gastroenteritis was not identified until 1973 where virus particles were detected in duodenal biopsy and then in fecal samples from children with severe diarrhea. The name rota was given to the newly discovered virus. The name was an adoption from Latin word 'rota', which means wheel. Rotaviruses were soon confirmed as a major cause of gastroenteritis in children <5 years of age worldwide and also in many the young of many mammalian and avian species. The RV is mainly transmitted by feco-oral rote; but the virus can also by transmitted by contaminated fomites and toys. The cycle of RV infection in human is shown in Figure 1.3.

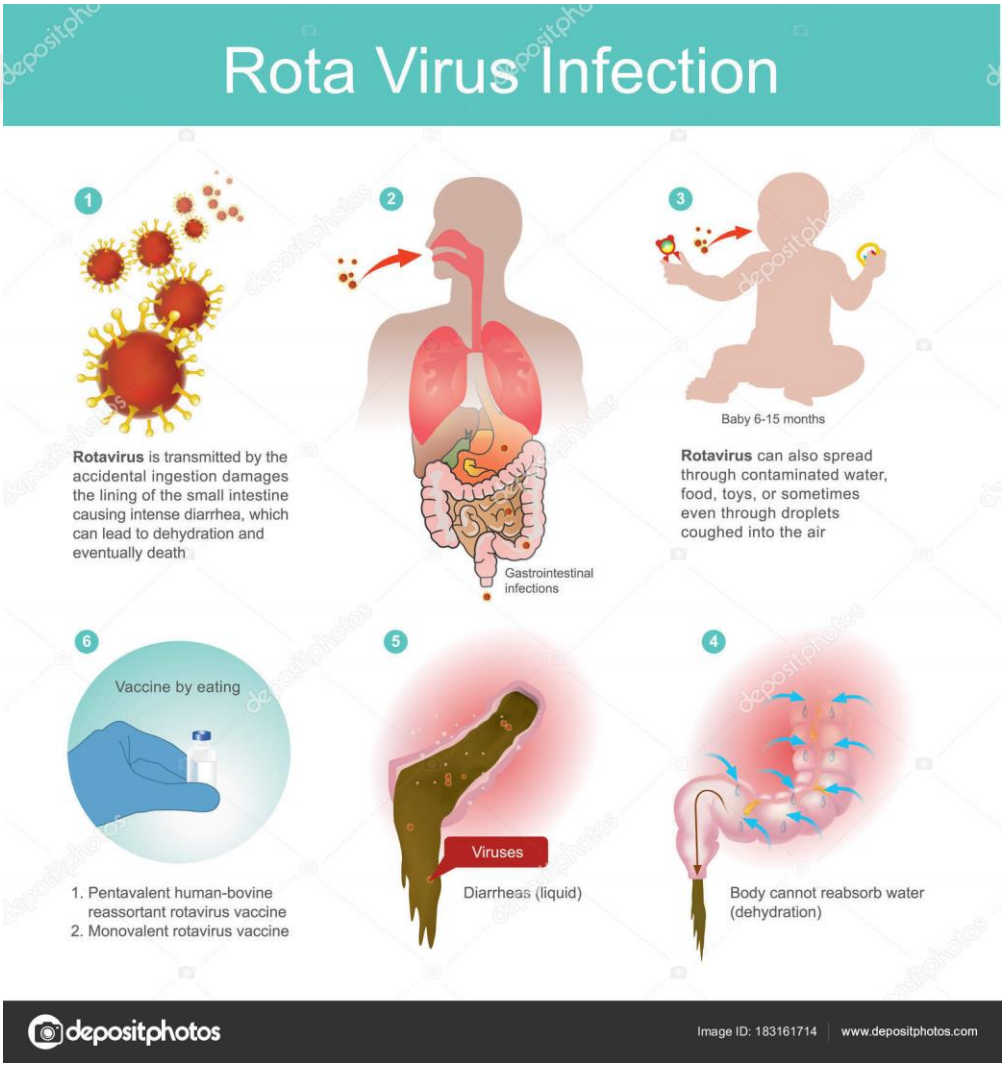


Figure 1.3. The cycle of RV infection in human (by curtsey to deposit photos)

In human the commonest clinical presentation of RV infection is acute gastroenteritis; mainly among children less than 5 years of age In general acute gastroenteritis from different causes

is a leading cause of childhood morbidity and mortality worldwide. It has been estimated that it is responsible for about 15% of total deaths in younger children; mainly in developing countries with low socio-economic standard and where children are suffering from malnourishment [1-3, 22].

The presentation of RV gastroenteritis can include severe diarrhea, vomiting, fever, and dehydration. The dehydration can be varied from mild to severe one. The symptoms are those of dehydration including dry mouth and throat, low urinary output, feeling dizzy and lethargic, loss of skin turgor, and other signs and symptoms depending of the severity of the disease.

The disease is more prevalent in winter and spring. The virus can spread to other children, and even to adults. The incubation period is about 2-3 days. The acute gastroenteritis can remained for up to a week or more depending on the severity of the disease. The disease is usually self-limiting and there is no specific treatment other than correcting dehydration and general other supportive measures. Although the commonest age for RV gastroenteritis is children less than 5 years of age, adults can also be infected but with milder clinical presentation [3, 7, 23, 24].

1.3. Risk factors In Rotavirus Infection

Infections with RV are present globallyin developed and developing countries. There is a wide variation in the mortality and morbidity of the disease between different countries and within different population living in the same country. Several risk factors have been incriminated in the epidemiology of RV gastroenteritis includings.

Age. the RV gastroenteritis was prevalent among children less than 5 years of age. The World Health Organization (WHO) stated that most of such cases are occurring in children aged between 6 and 24 months; with a peak incidence at 9 to 12 months. Moreover a study in A study conducted in 6 hospitals distributed in 6 Indonesian provinces found that children aged <3 months are less likely to have rotavirus gastroenteritis [25]. Another hospital-based surveillance cohort study which was also conducted at Sanglah Indonesia; between April 2009 to December 2011; as a part of the Asian Rotavirus Surveillance Network also found that children aged <3 months are less susceptible to rotavirus, while those 6 - 24 months are highly susceptible[26]. Another study conducted in Kenya found that the mean age of the children infected with RV gastroenteritis was 23 months (SD+16 months); but the majority were in the age group 0-6 months, followed by age-group 7-12 months [27]. In Jordan a study conducted among refugees also found that the prevalence was significantly higher (62%) in children aged less than 24 months in comparison to those of older children [28]. In Mexico the median age of RV diarrhea was 3-26 months[29], while In India, (98%) of cases occurred during the first 2 years of life, with peaking at 9–11 months [30]. In Jordan RV cases were found to be common among children aged < 5 years mainly in the age group < 2 years [31].

In developed countries the disease is still a major cause of morbidity, with the incidence also being common among children less than 5 years of age [32]. Asymptomatic cases were studied in developed countries. A cohort study in America showed that about 30% of children aged 2 years or less annually got asymptomatic rotavirus infection [33]. Similar results have also been reported in England [34].

Gender. several studies have shown that RV diarrhea to be more common in males than females. In one study in Nigeria males. females ratio of 1.8.1 was reported, suggesting that males had a higher rotavirus excretion rate than females [35]. In Indonesia the male to female ratio of rotavirus infection was 1.6.1 [26]. It has also been reported that boys are twice more susceptible to RV infection than girls [36].The WHO scientific group indicates that the affected males was up to 20% higher females in some studies. This might be due to a higher susceptibility of boys to rotavirus exposure or a greater likelihood of affected boys to seek medical care by their parents in comparison to girls [37]. Similarly in a multivariate analysis of RV risk factors showed that RV diarrhea was significantly more frequent in boys than girls; with adjusted RR of 1.4.[25].

General cleanliness. RV is transmitted by direct person-to-person contact. Accordingly hand washing and proper disposal or disinfection of contaminated items are essential measures in the control and prevention of the disease. In one study, mothers practicing hand hygiene during child feeding were found to be significantly less likely to have RV gastroenteritis among their children [27]. In developed countries the disease is less frequent that in developing countries. This might partially be due to higher standards of hygiene and cleanliness. Nevertheless and unlike bacterial gastro enteric RV are shed in the feces in amounts up to 1010 particles per gram of stool. Studies showed that 10 or less particles are infectious. The large virus shed is probably the reason that improvements in hygiene in the developed world have not significantly reduced the incidence of the disease [39- 40].

Breastfeeding. In general breastfeeding has been associated with reduction of gastrointestinal infections. This might be due to combination of several factors. Breast milk contains lactadherin which can protect against RV infection. Several ingredients in breast milk including secretory IgA, Lactadherin, bactericidal lactoferrin, T-and B-lymphocytes, human milk glycans and oligosaccharides will protect the intestinal epithelium against pathogens. Anti-rotavirus antibodies that are present in human milk might also play a role against the RV[41-43]. One of the most important constituents of breast milk is lactadherin. This can bind to RV and inhibit its replication. A study conducted on 200 infants in Mexico, by measuring lactadherin, butyrophilin, mucin, and secretory IgA in mothers milk and associated that with the presence of RV in the stool of the infants. The study found a significant Protection against RV by glycoprotein lactadherin in breast milk and the findings were independent of products of the secretory immune system[44]. The Anti-rotavirus antibodies is exclusively of the IgA type. This showed in vitro neutralization of the infectivity of

RV strains.Nevertheless these antibodies are only partly responsible for the neutralizing activity ; which indicates that other components may be also responsible for the suppressive activity against rotavirus[45]. Breast milk also contain probiotics which were shown, in several randomized control trials, to prevent or reduce the duration and complaints of RV gastroenteritis. Human milk is also rich in oligosaccharides that exert prebiotic. This has also been proven too of possessing an anti-RV activity, by decreasing the replication through the acute stage of RV infection[46-47], Also, human milk mucin was suggested to share in the preventive power of Breast feeding against RV gastroenteritis. The mechanisim was suggested to be by binding to rotavirus and inhibiting its replication. Moreover variations of i milk mucin levels may be associated with different levels of protection against RV[43]. Another substance is lactoferrin . this is a potent inhibitor against several viruses, including RV. It is ecreated in high level in the stool of breast-fed infants .A study found that its mechanisim of action will be will be preventing virus attachment to intestinal cells and by binding to viral particles, and inhibiting a post adsorption[48]. Current WHO guidelines on the management of diarrhea recommend continued breastfeeding during a diarrhea episode [26.41]. Moreover transplacentally acquired IgG and IgM antibodies and in breast milk can protect children aged <3 months against rotavirus infection. A Cohort study by found a lower incidence of rotavirus diarrhea in infants that received breast milk [49]. Similar results have been reported in other studies[27]. Despite that, however, another cohort study [50] found no difference between the breast-fed and bottle-fed infants in the rates of rotavirus. Similar results have also been observed by Wobudeya et al. and by Salim et al [26,41].

Seasonality. RV gastroenteritis is occurring all around the year. Nevertheless there is an apparent increase in the incidence of the disease in the cool, dry season. A study conducted in in India found that about two third of cases occurred in the cooler months. On the other hand a study in Indonesia showed a clear seasonal trend of rotavirus infection in the hot and dry seasons with low humidity [51-52]. On the other hand others found no consistent seasonal variation in cases 2 consecutive years [26].

Education of parents. Studies have shown a negative association between the level of education and several infectious diseases, including gastroenteritis. This has thought to be due to the fact that parents with high education will be more strict in following preventive measures[49]. Similar associations have been observed also with RV gastroenteritis. In a study conducted in Nigeria a study found that the highest prevalence was detected in children whose parents had primary education [54]. Similarly in a study conducted in Sudan among755 children aged less than 5 years showed that the siblings of illiterate parents were more infected with rotavirus than children of educated parents [55]. Similar results have been reported in other studies [56].

Other factors. Other risk factors have also been incriminated in the etiology and severity of RV gastroenteritis including. malnutrition, history of low birth weight, history of low birth weight,

overcrowding, previous diarrheal episodes, and unemployment which were positively associated with RV gastroenteritis. A high prevelance of RV gastroenteritis has been found to be strongly associated with low socio-economic status. Poverty, overcrowding and low education are closely interrelated with each others which determine the socio-economic status of individual. The of links between the social environment and infectious diseases has long been observed, particularly in developing countries,to increase the burden of infectious diseases, especially feco-oral ones. Recent preventive measures have urged the importance of identifying vulnerable groups on an ecological level. Other risk factors include day care centres for infants and nursing homes for the elderly. This facilitate the transmission of the virus, and lead to Rotavirus outbreaks. Nosocomial RV infections are also frequently reported which might be attributed to overcrowding, high load of viral infection cases and overcrowding [2,26,27, 29,32, 38, 49, 54,56].

1.4. Epidemiology of Rotavirus

Rotavirus is the main cause of non-bacterial gastroenteritis ; particularly among those aged less than 5 years of age [4, 30]. Rotavirus gastroenteritis is present in all developed and developing countries. It causes high morbidity globally and high mortality in developing countries. The latter has been reported to cause about 800,000 child deaths each due to the synergetic effects of poor nutrition and low health care in developing countries [35].

Globally diarrhea, in general, is causing about 1.5 million deaths; where about one fifth of those are among children under the age of 5 [41]. In a review of literature on studies published between 1986 and 1999 the RV gastroenteritis has found to be the cause of 440,000 annual deaths among those children [4]. Moreover in another study a total of 527,000 children aged <5 years were reported died from rotavirus infection in 2004, mainly in sub-Saharan Africa and South-East Asia [58].

Several studies have been conducted globally on the prevalence of rotavirus disease. Most of such studies have been on children<5 years old. There has been great variation on prevalence. In a study conducted in Bangladesh on 574 fecal specimens of children with diarrhea between February 2014 to January 2019; 24.4% of cases found positive for RV type A. The peak was during November to February; with one third of cases among infants aged 4-11 months [59]. Similarly in Albania the prevalence of RV among less than five was 21% of tested stool samples with 80% of positive in children under 2 years of age, which accounted for 80.8% of all positive cases.[60]. In Iran a study was done on 259 hospitalized and 245 children less than five years attending primary health departments suffering from acute diarrhea. The prevalence of RV detected was 78% using three methods for detection ELISA, reverse transcription-polymerase chain reaction, and electropherotyping [61]. On the other hand only a 22.9% of the 717 children with acute diarrhea were found positive for RV using ELISA in Nepal [62]. Similar results also found in Kenya where

17% of the 237 children hospitalized with acute gastroenteritis tested positive for group A rotavirus in stool specimens [63]. In China 34.3% of the 767 stool specimens of children with diarrhea were positive for rotavirus ; with the peak incidence was in February and March [64]. In Indonesia 53% of the 1321 with acute gastroenteritis, stool samples were positive for rotavirus [51] A case-control study of 65 patients less than five years old with acute gastroenteritis and 35 healthy age matched control were studied in Egypt using three detection methods. qr RT-PCR, immune-chromatographic test and ELISA . Rotavirus was detected in (76.9%) (69.2%) and (67.7%) of the 65 patients respectively with the highest rate among children within the age group of 6–12 months [65].

On the other hand the RV gastroenteritis still representing a main cause of nonbacterial enteritis in developed countries, despite the significant general decrease of mortality of gastroenteritis. In Portugal, for example, a study conducted on 237 children hospitalized with acute gastroenteritis showed that RV was the main cause of the disease [66]. Similar findings were observed in other developed countries [3, 7.18, 36].

1.5. Control and prevention of Rotavirus

Rotavirus is transmitted by faeco-oral route. The virus shed in stool in large quantities during diarrhea. Accordingly close person-to-person contact, is important and only few virions are needed to cause the disease due to high contagious character of the disease. Contaminated fomites also have a role in the transmission of rotavirus, especially hospitals [67-68] The airborne droplets route has been also incriminated not been proved [63]. Accordingly, like other causes of diarrhea transmitted by faeco-oral route are important in the control and prevention of RV gastroenteritis.

Rotavirus induces diarrhea through the destruction of enterocytes, intestinal secretion stimulated by rotavirus non-structural protein and activation of the nervous system. In addition antigenaemia and viraemia. Reinfections with the virus are common throughout life, although the severity is reduced with repeat infections. The rotavirus-specific immunoglobulin A has a role in the protection against the virus and recovery from infection, but the mechanism is poorly understood [69].

Following the introduction of rotavirus vaccines A reduction in the disease has been observed in many countries. Studies showed that 10 years after the adoption of RV vaccination into the national immunization schedule there have been reductions of the medians of hospitalizations due to diarrhea 38% ,rotavirus disease hospitalizations 67% and all-cause diarrhea deaths decreased by 38%, 67% and 42% respectively. [21, 25] Also there has been herd immunity of unvaccinated children in developed, but not in developing, although the reasons for this are unclear [70]. The vaccination has been effective in 80–90% against severe disease in high-income countries, despite

the fact that -specific antibodies sufficient for virus neutralization are found in only 30–50% of vaccines [71].

Live oral rotavirus (RV) vaccines used worldwide are most effective in reducing diarrheal hospitalizations from RV in high income countries and least effective in low income countries where RV remains a prime cause of death in children. Research has failed to fully explain the reason for this difference of efficacy for RV vaccines. The ultimate control of RV diarrheal will likely require both oral and parenteral vaccines. In the USA there are two licensed vaccines since 2006. RotaTeq® (RV5), which is given in three doses at 2 months, 4 months, and 6 months of age and Rotarix® (RV1), which is given in two doses at 2 months and 4 months of age.. Vaccine effectiveness against hospitalization ranges between 85 and 90%, and herd protection contributes to the overall impact of vaccination [69, 72]. An extended meta-analysis review on the efficacy of RV vacciens was conducted by Soares-Weiser et al in 2012 by reviewing 41 randomized clinical trials included 186263 participants in low and high child mortality settings. They assessed the two licenced vaccines. the monovalent RV vaccine(RV1, Rotarex) and the pentavalent RV vaccine(RV5, RotaTeq). The study found that the prevention of Rotarix in low mortality regions was 86% and 85% of severe gastroenteritis cases among infants and children of 2 years of age, respectively. The RotaTeq showed a prevention rates of 87% and 82% respectively among similar ages. On the otherhand the prevention rates in high child mortality regions for Rotarix and RotaTeq among the same age groups were 63% and 42% and 57% and 41% of severe gastroenteritis respectively. The study also found no significant difference in adverse events noted between the two vaccines, in the number of serious adverse events, and intussusception. Accordingly the efficacy was lower in high-mortality countries. Despite that , however, the benefits will be higher in those settings, due to the higher burden of disease. Several other types of RV vaccines are available in other countries like China, India and Vietnam , but the efficacy is not fully evaluated[73].The swift and striking declines in severe all-cause and rotavirus gastroenteritis have been demonstrated to sustained for several years after introduction[74].

Another finding from a cohort of 10,421 participants over the age of 6 months who had completed the full course of vaccination showed that the efficacy of Rotarix and RotaTeq was lower for infants born during RV season, while no significant difference observed for children above 12 months of age[75].

In 2016, a total of 81 countries introduced Rotarix or RotaTeq vaccines into the national immunization program . Poor nutrition was found to be associated with decreased in the vaccine efficacy , while no such effect was observed for breastfeeding at the time of vaccination [76].

In addition, some of the demographics of rotavirus disease have changed after the introduction of vaccinations into national immunization schedules In Finland, for example, RV infection was most common in children <5 years of age before vaccine introduction, but became

most common in unvaccinated children between 6 and 16 years of age and individuals >70 years of age. Also some changes in the seasonal pattern of RV were observed, including delays in the start of the RV season, a shorter duration of seasons and blunting of seasonal peaks. In the United States, for example, the regular annual seasonal pattern of RV disease which was observed before vaccine introduction has shifted to biennial increases in circulating rotavirus disease which might be due to the accumulation of unvaccinated children over two successive seasons due to moderate levels of vaccine coverage leading to a larger epidemic every alternate year. For review see Crawford et al [69] Moreover differences in the genotypes of circulating rotavirus strains have been described in some countries after the introduction of RV vaccine. An increased in the prevalence of G2P[4] was observed following introduction of the monovalent G1P[8] RV vaccine in several countries. Also, similar increases in the prevalence of this stain strains have also been observed in countries that introduced the pentavalent RV vaccine (which includes the G2 genotype) .Moreover the monovalent G1P[8] RV vaccine was effective at preventing G2P[4]-induced diarrhea. It is not clearly known if the differences in RV genotypes represent natural seasonal variation or vaccine-induced selection pressure. This necessitate continuous surveillance of RV to monitor genotypes, whole genomic characterization of circulating RV strains changes in the epidemiology following the vaccine introduction in order to evaluate if the vaccines are affecting the evolution of the rotavirus genome. [69,77].The effectiveness of rotavirus vaccines is 30–40% lower in low-income countries than in high-income countries, and the causes of this remain incompletely understood. At present, all licensed rotavirus vaccines are live attenuated vaccines [69, 78].

1.6. Diagnosis of Rotavisrus infection

The clinical presentation of RV infection can range from completely asymptomatic to sever diarrhea. Such infections are usually rare in infants less than3 months of age and are more likely to be of the Asymptomatic. RV infection has no pathognomonic signs or symptoms and RV gastroenteritis presentation will be similar to gastroenteritis caused by bacteria or other viruses gastroenteritis such as E.coli, Salmonella spp, noroviruses, enteric adenoviruses, astroviruses. Moreover reinfection is common, but the severity of disease decreases with each repeat infection Accordingly laboratory confirmation is always needed to prove the diagnosis [3, 11, 78].

Diagnosis was originally conducted by using electron microscopy. This method needs special technique and its used is now limited to somecenters and reference labs; where it is available. Routine diagnosis is most commonly performed by detection of the antigen in fecal samples using commercially available, simple, rapid immunochromatographic dipstick kits. Stool specimens collected from the first to fourth days of illness are ideal for detection of RV. The shedding of the virus may continue up to 3 weeks, or even longer depending on the severity of

illness. Viral shedding may stop prior to or after the cessation of diarrhea, but usually coincides with its duration in most cases

Enzyme Linked Immunosorbent Assay (ELISA) has been widely used in the detection of RV gastroenteritis. It was found to be highly sensitive and specific. The Kopatts kit which was developed in Denmark and modified by WHO was evaluated in diagnostic laboratories in different countries and showed a sensitivity of 100%, but the high cost limited its use in developing countries. Other ELISA kits are now widely available and less expensive.

Reverse Transcription PCR (RT-PCR)-based assays, are more sensitive and permit genotyping of virus isolates. The virus can be detected for longer periods by than by other methods. Moreover the RT-PCR has shown to be more sensitive than other methods in the detection of the virus. Typing of rotavirus is based on the genetic and antigenic diversity of the 2 outer capsid proteins, VP4 and VP7. Surveillance of rotavirus G and P genotypes circulating in communities has become important with the recent introduction of vaccines for rotavirus.

Furthermore the PCR test will be valuable for strain characterization, especially in cases occurring in recently vaccinated patients. Viral culture and serology are not routinely available and are used for research and for strain characterisation, and they do not have a role in diagnosis of acute disease [79-83].

1.7. Aim and Objectives

Aim

To estimate the prevalence of rotavirus among children less than 5 years admitted to secondary care hospital suffering of acute diarrhea, In Duhok, Kurdistan Region, Iraq.

Objectives

A-To estimate prevalence of rotavirus among children suffering from acute diarrhea using ELISA methods

B.To measure the prevalence in comparison to socio-demographic factors including.

- Age
- Gender
- Father and Mother education
- Crowding index in the family
- Urban or rural residency
- Attaches of diarrhea suffered last month
- RV vaccination
- Type of feeding in the first year of life

C. To calculate the validity (sensitivity and specificity) of ELISA in comparison to PCR confirmatory test.

2. PATIENTS AND METHODS

2.1. Study Design

Cross sectional study design was adopted to achieve the aim of this study.

2.2. Setting

The study was conducted in Duhok city, which is the center of Duhok governorate. The governorate, which is of over one million populations, is one of the 4 governorates constituting the Iraqi Kurdistan Region. It is a mountainous area in the far North-West of Iraq bordered by Turkey to the North, Syria to the West and Erbil and Nineveh governorates to the East and South respectively.

The collection of the samples was done in Hevi Teaching Hospital. It is the only secondary and tertiary care hospital in the governorate, of a capacity of 200 beds. Patients are referred from all districts of the governorate and from private clinics. Moreover patients can also be admitted directly from the outpatient department of Hevi hospital.

2.3. Study period

The preparation for study was from 2019 to 2021. The collection of samples was from January 1st, 2021 to May,31st, 2021, the analysis of the samples was fromJune,1st, 2021to July, 1st, 2021 and analysis of the results and writing of the thesis was from July, 1st, 2021 to December, 31st, 2021.

2.4. Study population

The study population was children of both genders aged less than 5 years of age who were presented to the specialist at Hevi hospital suffering from acute diarrhea and suspected to have Rotavirus. All available cases were received by the researcher during his stay in the hospital. All available cases which were referred during the period of data collection were included in the study.

2.5. Eligibility

Inclusion criteria. all patients referred to the researcher with provisional diagnosis of Rota virus diarrhea were included in the study

Exclusion criteria. age more than 5 years and those whose parents refused to participate in the study

2.6. Ethical consideration

The study was enforced by the ethical committee at Duhok Directorate General of Health (Appendix I). Before the start of the study approval was also obtained from the manager of Hevi hospital. Moreover the aim of the study was explained to the parent(s) or caregivers of the patients to take their verbal consent to participate in the study.

2.7. Collection of the samples

The patient was received by the researcher who was sitting next door to the specialist. After taking ethical consideration from the parent(s), and/or caregivers, The following information were obtained as shown in the chart following (see also Appendix II).

Full name.	
Age	Months.
Sex	Male. Female.
Father education	Illiterate-Primary-Intermediate-Secondary-College
Mother education	Illiterate-Primary-Intermediate-Secondary-College
Number of children in the family	
Number of rooms in the house.	
Residency	Urban Rural
Attaches of diarrhea suffered last month	Number
Information about RV vaccination	Complete Partial None
Type of feeding in the first year of life.	Exclusive breast Exclusive bottle Mixed feeding
Telephone number.	

The crowding index in the house was calculated by dividing the number of residents, excluding the newborn infants, divided by the total number of rooms in the house. The urban residency was considered wherever there was municipal office in the city otherwise the area was considered to be rural.

2.8. Collection and storage of stool samples

The stool sample were put in sterile special container for stool collection marked with the number and name of the patient. A stool sample was taken from the patient, as part of the routine in the hospital. Each one sample from the selected patients was marked also with my name for follow up. The lab workers were instructed that the sample marked by my name to be kept at -22 centigrade, after conducting their part on it, for the research. Also a phone call was done with the parent to be sure that the patient had given stool sample. The same procedure was also conducted for patients who were admitted directly to the ward, where a visit was done by the researcher. After the end of the day the researcher visited the lab and collected the samples devoted for the study.

After that the samples were transported by Ice box to be frozen and the process of transferring samples takes approximately 10 minutes. All samples were transferred to the laboratory of the preventive medicine department where each sample was subsequently divided into 3 parts marked and put in Eppendorff tube and stored at -22 centigrade until analysis was done.

2.9. Analysis of the samples

2.9.1. ELISA test

The ELISA test was conducted by the researcher at the virology lab in the preventive medicine department. The test was set to detect the antigen. The kit used was EPITOPE DIAGNOSTICS, INC. (EDI) U S Origin Fecal RV ELISA Kit. Enzyme linked immunosorbent assay (ELISA) is a widely established method for detection of antigens in biological samples. This is conducted by using highly specific antibody-antigen interactions to detect the target antigen. The antigen is immobilized to a solid surface; directly or by the use of a capture antibody itself immobilized on the surface. The antigen is then joined to a detection antibody to a molecule amenable for detection.

In general there are 4 methods in ELISA. direct, indirect, competitive and sandwich ELISA. The sandwich ELISA method, or sometime called sandwich immunoassay, was used in this study. The kit is intended for the qualitative detection of of rotavirus antigen in stool samples This is the most commonly used method. It requires two antibodies specific for different epitopes of the antigen; which are referred as matched antibody pairs. The first antibodies is coated on the surface of the multi-well plate to be used as a capture antibody for immobilization of the antigen. The second antibody is conjugated to help for the detection of the antigen.This method is considered to be highly sensitive and specific with high flecability [79,83].

The procedure was according to the kit instruction as follow.

100 microliter of diluted calibrators was added and diluted samples into the designated micro wells and then mix by gently tapping the plate

The plate was then covered by with one plate sealer and aluminum foil and incubated at room temperature (20-25 centrigrade) for 60 minutes

The plate sealer was then removed and the contents were aspirated from each well. Each well was washed 5 times by dispensing 350 microliter of diluted wash solution into it, and then completely aspirate the contents. Alternatively, and automated micro plate washer can be used.

Then 100 microliter of tracer antibody was added to each well and mixed by gently tapping on the plate.

The plate was then covered by one sealer and aluminum foil and incubated at room temperature (20-25 centrigrade) for 30 minutes.

After that the plate sealer was removed and the. contents were aspirated from each well . Each well was washed 5 times by dispensing 350 microliter of diluted wash solution into it, and then the contents were completely aspirated. Alternatively, an automated microplate washer can be used.

100 microliter of ELISA HRP substrate was then added to each well and mixed by gently tapping the plate.

The plate was covered with one plate sealer and aluminum foil and incubated at room temperature (20 – 25 Celsius) for 20 minutes.

The aluminum foil and plate sealer was then removed and 100 microliter of ELISA Stop solution to each of the wells and gently mixed by tapping the plate.

FİGURE2.1.and Figure 2.2. shows the preparation of the stool sample for the analysis of RV antigen by ELISA

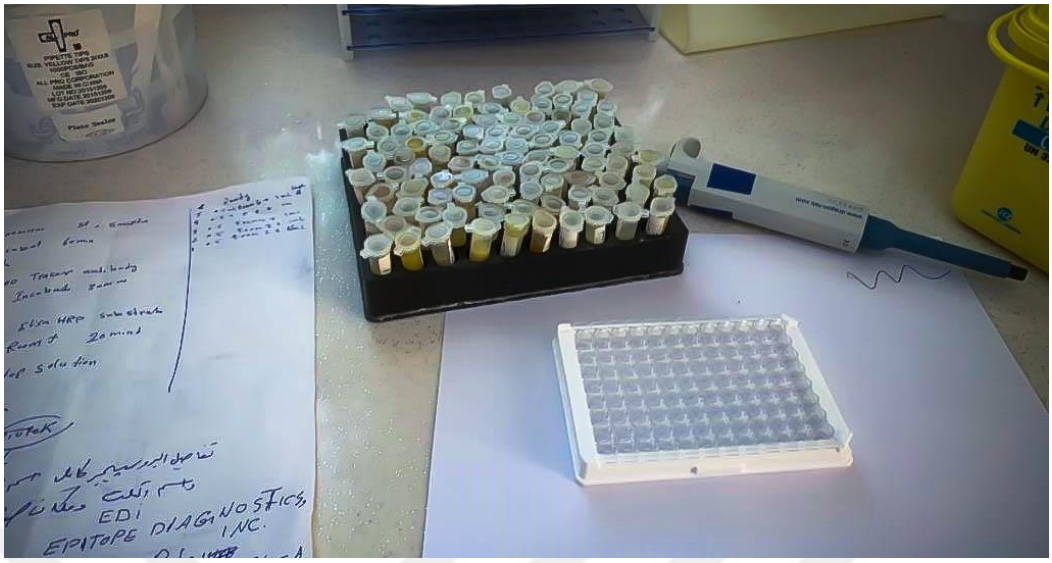


Figure 2.1. The preparation of stool sample for analysis of RV antigen by ELISA

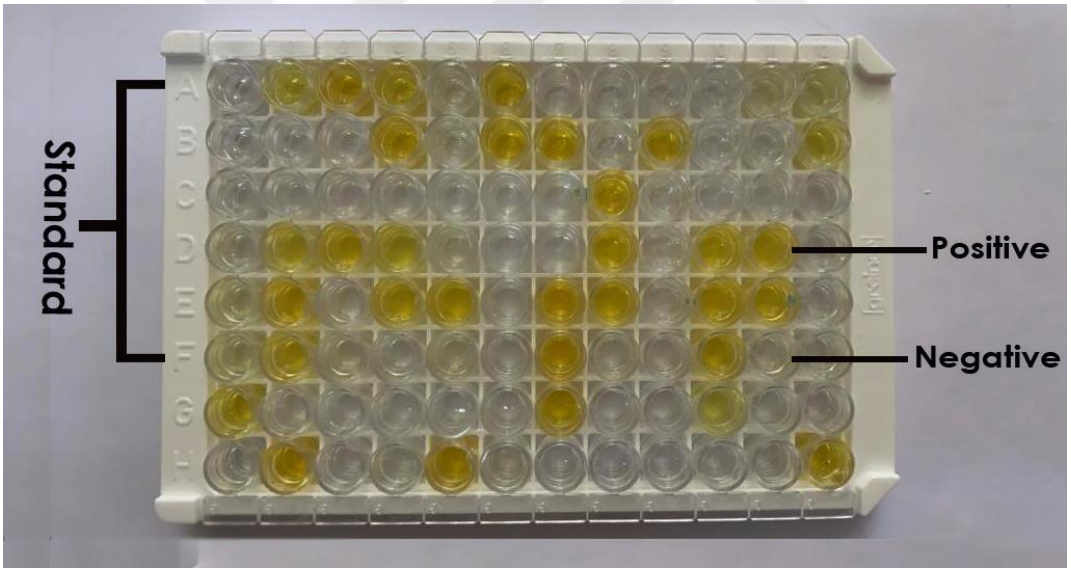


Figure 2.2. ELISA rack (A to F) with positive reaction yellow color) and negative reaction (colorless)

The absorbance was then read at 450 nm within 10 minutes with a micro plate reader using (Biotech ELx 800-Epson/USA), shown in Figure2.3.

Figure 2.3. Biotech ELx 800-Epson/USA used for the detection of RV antigen in stool samples

2.9.2. PCR test

The analysis was done at Vin Private Hospital because the PCR of the central laboratory of Duhok General Directorate of health was devoted totally for Covid-19 tests; using GeneAmp PCR system 9700 USA origin (Figure2.4.).

Figure 2.4. GeneAmp PCR system 9700/USA used for the analysis of the samples

The procedure conducted was according to the as follow.

All positive ELISA samples were transferred 20 – 50 mg in 1.5 ml micro centrifuge tube and dissolved with 250 microliter of pure distilled water and vortex for 10 – 15 seconds; then centrifuged at 13000 rpm for 30 seconds at room temperature. After that 150 – 200 microliter of supernatant was taken for extraction.

The procedure was as follow.

To begin the process the required number of 0.2 (0.5) ml disposable micro centrifuge tubes were prepared then, a reagent mix that is ready to use is prepared for 12 reactions after that, 5 microliters of RT-Gmix-1 was added to the tube that contains the RT-MIX and carefully mixed on vortex for 3 seconds and centrifuged for 5-7 seconds. Having done the previous steps, 6 microliters of revertase (MMIv) was added to the tube with the reagent mix, pipetted 5 times, mixed on vortex for 3 seconds, and centrifuged for 5-7 seconds to remove any drops from the internal surface of the test tubes caps. After that 10 microliters of reagent mix that was ready to be used was disposed into the prepared test tubes. Next, 10 microliters of RNA- samples was added to the appropriate test tube with the mix and mix it using the pipette. After that, the test tubes were placed into the thermocycler and incubated at 37 C for 30 minutes. Finally, each cDNA sample was diluted in to

ratio 1.1 with DNA-buffer. To do that, 20 microliters DNA-buffer was added to each of the used test tubes and mixed carefully for 10 times using the pipette.

PCR protocol. The protocol was cording to GENET BIO Korean origin as shown below

- 12.5 microliters master mix
- 2 microliters primer (forward and reverse)
- 1.5 microliters cDNA
- 4 microliters DNase, RNase free water (total PCR volume 20 microliters)
- 95 Celsius for 5 minutes hold
- 95 Celsius for 30 seconds
- 50 Celsius for 45 seconds
- 72 Celsius for 45 seconds
- 72 Celsius for 7 minutes hold
- primers used for the detection of Rotavirus Con2(ATTTCGGACCATTTATAACC) And Con3 (TGGCTTCGCCATTTATAGACA)

2.9.3. Gel electrophoresis.

Then 50 ml of distilled water was added to a vial containing 1 ml TAE buffer, then 0.5g agarose was added and cooled until all parties reach about 60C , add safe dye and pour double – sided comb until applied it dissolves in the range. Next, the comb was removed and, the sample was loaded by placing it in the Gel tank, with a voltage (100 u 20 min) from negative to positive charge, and then the gel was placed under UV light to visualize the bands.

2.10. Statistical analysis

Data were entered and analyzed using SPSS 26. Frequency and frequency percentages were used to describe categorical variables, and mean, range and standard deviation were used to describe numerical variables. Chi square test (or when not applicable due to low cells frequency, Fisher's exact test) was used to test the relationship between socio-demographic and medical factors, on one hand, and ELISA test result, on the other hand. A p value less than 0.05 was considered statistically significant.

The sensitivity, specificity and predictive values of positive and negative tests and likelihood ratios of positive and negative test results for ELISA were computed considering the PCR test as the gold standard test. For categorical variables, frequency and proportions were computed, The Proportions were tested by chi-squire test, and P value less than 0.05 was considered statistically significant.

2.10.1. Results

A total of 310 children under the age of five suspected to be suffering from RV gastroenteritis were investigated and collected during the study period. The demographic characteristics of the studied population and their characteristics is shown in Table 3.1.

Table 3.1. Demographic characteristics of the study population

Characteristic		Number	%
Age (years)	< 1 year	37	11.9
	1 - < 2 years	123	39.7
	2 - < 3 years	99	31.9
	3 - 4 years	51	16.5
Sex	Male	144	46.5
	Female	166	53.5
Father education	Illiterate	129	41.6
	Primary	49	15.8
	Intermediate	25	8.1
	Secondary	14	4.5
	College	93	30.0
Mother education	Illiterate	128	41.3
	Primary	66	21.3
	Intermediate	21	6.8
	Secondary	8	2.6
	College	87	28.1
Residence	Urban	225	72.6
	Rural	85	27.4
Crowding index	1	164	52.9
	≥ 2	146	47.1
Rota vaccination	Yes	134	43.2
	No	103	33.2
	Partial	4	1.3
	Unknown	69	22.3
Feeding type	Exclusive	28	9.0
	Bottle	20	6.5
	Mixed	262	84.5
Diarrhea during the last month	Yes	291	93.9
	No	19	6.1
Total		310	100.0

Table 1 reveals that about 80% of the patients were in the age group 1-< 3 years; with slightly higher females than males. The illiteracy rate among either or both mother and father exceeded 40%. Table (3.1) also illustrates that about three quarter of the patients were living in urban areas,

and about half of them have a high crowding index. The frequency of diarrheas in the previous month among the studied population was about 94%. Finally Table 3.1. reveals that only 9% of the patients had an exclusive breast feeding in the first year of life while about 85% had mixed type of feeding. Also only 43.2% have had vaccination to Rotavirus.

The descriptive statistics of some patients characteristics is shown in Table 3.2. The means for the age of the sample was 23.9 (+/- 11.11). Months, weight was 9.9 (+/- 3.35) Kg, number of children in the family was 4.5(+/- 1.23), number of rooms in the house was 2.5(+/-0.67) and crowding index rate was 1.9(+/-1.34), as shown in Table 3.2.

Table 3.2. Descriptive statistics of some patients' characteristics

	No.	Minimum	Maximum	Mean	Standard deviation
Age (months)	310	1	48	23.9	11.11
Weight (kg)	309	3.0	17.8	9.9	3.35
No. of children	310	1	10	2.3	1.23
No. of family members	310	3	44	4.5	2.56
No. of rooms	310	1	7	2.5	0.67
Crowding index	310	0.6	22.0	1.9	1.34

The absorbance values of ELISA test was read on wavelength 450 and results of infectivity are shown in table 3.3

Table 3.3 ELISA absorbance values on wavelength 450 showing the results of infectivity test

1	2	3	4	5	6	7	8	9	10	11	12
0.170 STD1	0.101 SMP3	2.145 SMP11	2.259 SMP19	2.109 SMP27	0.166 SMP35	0.220 SMP43	0.957 SMP51	0.201 SMP59	1.315 SMP67	0.171 SMP75	0.173 SMP83
0.192 STD2 4.450	0.170 SMP4	0.177 SMP12	0.431 SMP20	2.088 SMP28	0.145 SMP36	2.144 SMP44	0.152 SMP52	0.218 SMP60	0.162 SMP68	2.628 SMP76	0.220 SMP84
0.208 STD3 10.957	0.139 SMP5	2.799 SMP13	2.608 SMP21	0.163 SMP29	0.200 SMP37	0.166 SMP45	0.144 SMP53	0.284 SMP61	2.590 SMP69	2.103 SMP77	2.614 SMP85
0.258 STD4 22.123	0.183 SMP6	1.857 SMP14	1.675 SMP22	0.211 SMP30	0.332 SMP38	2.141 SMP46	2.368 SMP54	0.144 SMP62	0.211 SMP70	0.162 SMP78	0.195 SMP86
0.350 STD5 55.085	2.712 SMP7	0.312 SMP15	0.167 SMP23	0.187 SMP31	0.279 SMP39	0.149 SMP47	1.519 SMP55	0.152 SMP63	0.145 SMP71	0.198 SMP79	0.314 SMP87
0.610 STD6 147.842	1.314 SMP8	0.320 SMP16	0.340 SMP24	0.557 SMP32	1.995 SMP40	0.256 SMP48	0.424 SMP56	0.275 SMP64	0.346 SMP72	0.700 SMP80	1.312 SMP88
1.276 SMP1	2.781 SMP9	0.205 SMP17	2.834 SMP25	0.177 SMP33	0.199 SMP41	0.215 SMP49	0.412 SMP57	2.130 SMP65	0.198 SMP73	0.221 SMP81	0.261 SMP89
0.576 SMP2	0.320 SMP10	2.333 SMP18	1.899 SMP26	0.339 SMP34	0.410 SMP42	0.191 SMP50	0.434 SMP58	0.267 SMP66	0.263 SMP74	0.430 SMP82	1.682 SMP90

It is found that , 29% of patients gave positive results for RV antigen in the stool, while the rest of 71% of them gave a negative result, as shown in table 3.4 and Figure 3.1.

Table 3.4. ELISA test results for RV antigen among children less than 5 years suffering from gastroenteritis.

	Number	%
Positive	89	29.0
Negative	218	71.0
Total	307	100.0

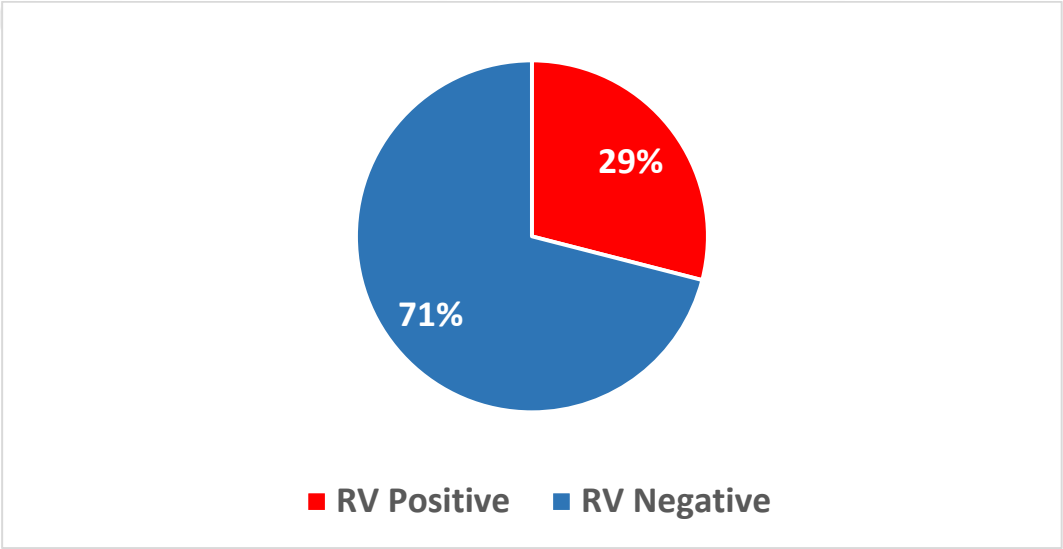


Figure 3.1. The prevalence of RV among among children less than 5 years suffering from gastroenteritis in Duhok, Kurdistan Region, Iraq

The relationship between positivity to RV and different characteristics of the patients is shown in Table 3.4.. No significant relationship was detected between the presence of RV in stool samples by ELISA test and age, gender and crowding index of the studied sample. On the other hand, there was a significant association between the education of fathers and mothers and the rate of Rota virus infection among their children, with higher rate among those with illiteracy or lower education. The rate of RV infection was also significantly higher among rural than urban residents. The study also reveals that all those who received partial or complete vaccination had no attack of Rota virus infection (Table 3.5). The frequency of diarrhea in the last month was significantly associated with Rota virus infection; with higher rates among those having less frequent attacks. Finally the children who had exclusive bottle feeding in the first year of life had significantly higher rates of positivity in comparison to other groups.

Table 3.5. Relationship between ELISA test results and patients' characteristics (n = 307)

Characteristic		ELISA				P value*
		Positive		Negative		
		No.	%	No.	%	
Age (years)	< 1 year	15	40.5	22	59.5	0.099
	1 - <5 years	74	27.4	196	72.6	
Sex	Male	42	29.4	101	70.6	0.891
	Female	47	28.7	117	71.3	
Father education	Illiterate	49	38.6	78	61.4	
	Primary	12	24.5	37	75.5	
	Intermediate	4	16.7	20	83.3	0.038
	Secondary	3	21.4	11	78.6	
	College	21	22.6	72	77.4	
Mother education	Illiterate	50	39.7	76	60.3	
	Primary	14	21.5	51	78.5	
	Intermediate	6	28.6	15	71.4	0.013
	Secondary	1	12.5	7	87.5	
	College	18	20.7	69	79.3	
Residence	Urban	53	23.7	171	76.3	0.001
	Rural	36	43.4	47	56.6	
Crowding index	<2	84	28.9	207	71.1	0.784
	>3	5	31.3	11	68.8	
RV vaccination	Yes	0.0	0.0	133	100.0	
	No	60	58.8	42	41.2	<0.001
	Partial	0.0	0.0	3	100.0	
	Do not know	29	42.0	40	58.0	
Feeding type	Exclusive	10	35.7	18	64.3	
	Bottle	10	52.6	9	47.4	0.038
	Mixed	69	26.5	191	73.5	
Diarrhea during the last month	Yes	77	26.6	212	73.4	<0.001
	No	12	66.7	6	33.3	
Total		89	29.0	218	71.0	

* From Chi square test (except for vaccination schedule, Fisher's exact test).

The PCR test was conducted on 65 negative and 45 positive samples by ELISA, using PCR products of amplified rRNA gene, as shown in Figure3.2.

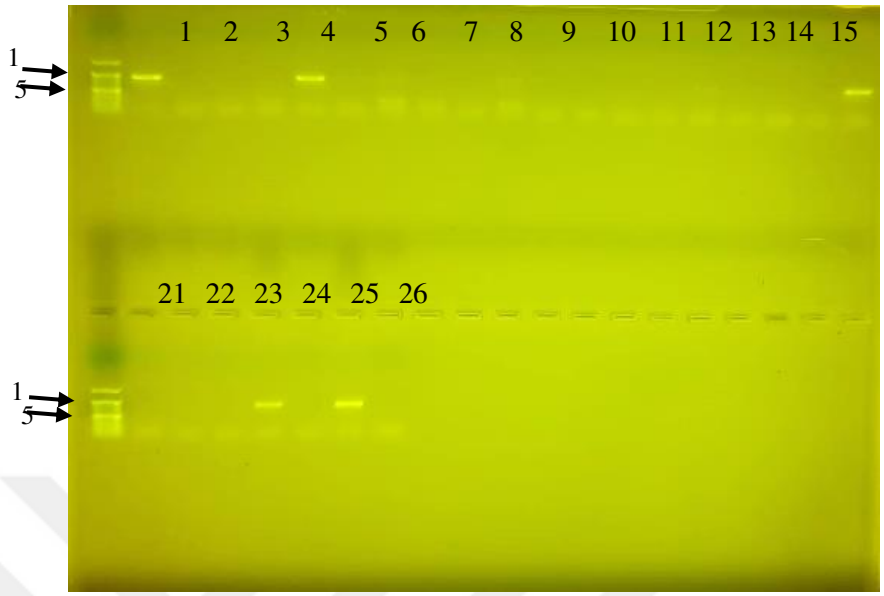


Figure 3.2. PCR products of amplified rRNA gene

The PCR study was used to estimate the validity of ELISA test in diagnosing RV gastroenteritis. The results are presented in table 3.5 below. The table shows that the sensitivity and specificity of ELISA Test were 88.89% and 100% respectively. The predictive values of a positive and a negative test were 100% and 92.86% respectively. Finally, table (3.6) shows that the likelihood ratios of a positive and a negative test were unidentified and 0.113 respectively, and the diagnostic accuracy of the ELISA test was 95.45%.

Table 3.6. The validity of ELISA Test in diagnosing RV infection, by using PCR as gold standared test among a sub sample of positive and negative ELISA test

		PCR		
		Positive	Negative	Total
ELISA		40 (a)	0(b)	40
		5 (c)	65 (d)	70
		45	65	110

Parameter	Estimate
Sensitivity = [a/(a+c)]	88.89%
Specificity = [d/(b+d)]	100.00%
Positive Predictive Value = [a/(a+b)]	100.00%
Negative Predictive Value = [d/(c+d)]	92.86%
Diagnostic Accuracy = sensitivity + specificity	95.45%
Likelihood ration of a Positive Test = [sensitivity/ (1-specificity)]	Undefined
Likelihood ration of a Negative Test = [(1-sensitivity)/ specificity]	0.113

2.10.2. Discussion

To our knowledge this is the first study of the prevalence of RV gastroenteritis in Duhok, Iraq. The sample has been collected from Hevi hospital in Duhok city, the capital of Duhok government of Kurdistan Region of Iraq. This will give strengths to the study as it is the only secondary and tertiary care hospital in the governorate, where all patients needing hospitalization will be transferred to it. Moreover, the hospital also accepts patients coming directly to its outpatient department; or referred by specialist pediatricians. According to the sample is considered relatively representative for the population of Duhok.

Diarrhea is still the main cause of morbidity and mortality among children less than 5 years of age. The prevalence of mortality from diarrhea has decreased in developed countries; but remains a major cause of death among this age group in developing countries [4, 41, 58]. Several causes have been incriminated in the etiology of the disease including several bacteria and viruses. Nevertheless, RV is still considered the major cause of diarrhea among children less than 5 years of age in developed and developing countries [4, 22, 30]

Knowing the prevalence of a disease will usually help in planning for curative and preventive health services in a specified country [1, 2, 8, 33] Few studies are available about prevalence of RV gastroenteritis [6, 86-88].

The current study used EIISA method for the detection of RV antigen in the stool of suspected patients. This method has been shown a high sensitivity, specificity and accuracy rate. This is similar to results reported in other studies and frequently used as a valid screening test while the PCR is commonly used as a reference test for genotyping of R.V [79, 81, 83].

The use of sandwich ELISA system, like the one used in this survey, was found to be effective in the detection of RV antigen in the stool. The method was reported in several studies to be rapid, reliable and sensitive for diagnosing RV infection; where compared with other methods like electron-microscopy, immunoelectro-osmophoresis, and fluorescent antibody techniques. The ELISA was also found to be comparable to PCR in survey, despite the fact that the PCR was relatively more sensitive and specific, but more tedious and needs expert hand and instruments[90-92].

In This study, it is found that the prevalent of RV gastroenteritis by EIISA was 29% of patient presented with gastroenteritis. There have been variations in the prevalences of RV as a cause of diarrhea in difficult countries. Closely similar finding to this study was observed in Bangladesh during a surveillance of circulating rotavirus among children with gastroenteritis for the period 2014-2019; where 24.4% of the 574 examined fecal samples were positive to RV [59]. The finding was also closely similar to the rate of 21% found in a similar hospital based study in Albania for a study period 2007-2010 by ELISA [60] and to the 22.9% detected also by EIISA method among the 717 children with acute diarrhea in Nepal in 2017 [62]. Similarity prevalences of 33% and 37% were found in Jordan in 2000 and in Turkey in 2005 respectively [84-85].

The prevalence in our study was lower than those reported in other countries like Iran in 2004[61], Indonesia in 2009 [47], Eygpt in 2015[65], China in 2013[60] and Diyala in Iraq in 2020[78]. where the positive rates were 78%, 53%, 67.7%, 34.5%, and 47.3% respectively The differences might be due differences in the target population or seasonality. The latter is expected if the samples were collected in the peak incidence months of February and March; as reported in China in 2013 [64]. On the other hand, the rate in the present study was slightly higher than that found in Kenya in 2017 where 17% of the 237 children with gastroenteritis found to had RV infection[63]. Also, the differences might be attributed to the design of the study. In an earlier study in Iran in 2004 for example, a prevalence of 35% was reported [93].

In Erbil the capital of Kurdistan Region a study was conducted on 260 children aged less than 5 years with diarrhea. The study was also used to detect RV antigen in frozen stool samples as a screening test. They found a prevalence rate of 37% by EIISA test. That study was conducted between March and May 2005, and this might explain the slightly higher prevalence observed as RV usually peak on those months [6]. On the other hand, a similar study conducted in Basra, Iraq in 1987 found a prevalence of 24%, which was closely similar to the finding of the study [87], while a recent study in Al-Anbar province in Iraq in 2020 found a prevalence of 32.6% by using

rapid test for stool samples among 150 –children who visited hospitals suffering from gastroenteritis [88]. Also, a prevalence of 33% was found among under-fives admitted to al Masor hospital in Baghdad in 2016 by using Anti-Rotavirus Antibody rapid test [89] It is determined that in this study , about 81% of cases visited the Hevi hospital suffering from diarrhea were in the age group 1-3 years; with a mean age 0f 23.9 months. The study also reveals that the prevalence of RV infection was significantly higher among infants (aged less than 1 year) in comparison with older age groups. Similar results were also found in studies conducted in Iraq in Erbil,Bsrah, AlAnbar, Diyala and Baghdad[6,86-89]Similarly, the WHO found that most cases were among children aged 6-24 months with a peak in 9-12 months [25]. Also, a study conducted in Kenya found that the majority of cases were among children less than 12 months of age [27]. Similar results were also reported in Jordan , India and Turkey [28,30,94]..

It is found that female's rate was slightly lower than males (28.7 vs 29.4) and the difference was not statistically significant. In Baghdad, Iraq a significantly higher rates were reported among females [89] while in Egypt, and similar to this study, both genders showed a relatively similar rates [65]. On the other hand, other studies showed higher rates among males in comparison to females [26, 35, 94]. The WHO stated a 20% excess in male cases and -claimed that might be attributed either to higher susceptibility or more frequent use of health service for male boys than girls. The latter might not be the case in Duhok where both genders are equally seeking health service [37].

This study revealed that siblings of educated parents are significantly less likely to get RV gastroenteritis than those whose parents are illiterate or with primary education. The results were significant for father and / or mother of affected children. There have been several studies which the linked higher prevalence of feco-oral diseases with the education of parents and suspected the cause to be the general cleanliness and observing prevents measures, which are more porn to be conducted by educated parents [-49]. Similarly, higher rates of RV gastroenteritis were reported among the children of illiterate parents in Nigera [35], Sudan [55] and other countries [56].

The current study also found that all vaccinated children had a negative EIISA test of RV in comparison to 41.2% among the unvaccinated children. In Duhok, Kurdistan 2 types of RV vaccines have been used. The current one is Rotarix R, which was in use for last 3 years in the vaccination schedule of under-fives. This vaccine is given in 2 doses orally first in the 2nd month and the second in the 4th month of age, provided that the second dose is not exuding the 6 months of age. Other vaccines were in use before that, starting intermittently since 2011Vaccination against RV has revised the epidemiology the disease, with significant decrease in the mortality and morbidity of RV gastroenteritis. Also, the vaccination stimulate herd immunity of unvaccinated children and has a significant outcome of reducing sever forms of RV gastroenteritis [21, 25, 70-71]. Several studies have also demonstrated the significant effect of RV vaccination in the control

and prevention of the disease. A global analysis of vaccination in 72 countries showed that it can prevent 2.4 million deaths and 83 million disability adjusted life years for the estimated period of 20 years (2011 -2030) [96].

It is found that in this study, about three quarters of negative cases have been from urban areas and the total positive cases was significantly higher in rural than urban children. This might be due to the possibility that urban cases can access the hospital more easily than rural cases; so, they might be suffering from other causes of mild gastroenteritis. Similarly in a study in China about 93% of all deaths from RV had occurred in rural areas [97].

No significant differences were found recoding the crowding in patient's houses. Other studies found that overcrowding was associated with more cases of RV gastro enteritis [2, 26, 38]. The finding in this study might indicate that mothers in all houses are following the general cleanliness regarding of the crowding in the house.

The study showed that children who were on bottle feeding have significantly higher rates of RV than those who were on exclusive breast feeding. Similar results have also been on reported in other studies [27, 49]. This was through to be clue to combination of several factors. Breast milk is more sterile than bottle milk which is exposed to contamination during preparation. Also, it contains several other components including mucin, which inhibit RV replication [42. 43], in addition to the presence of antibodies in breast milk which are the main factors in preventing the disease among infant aged < 3 months [27, 49].

The present study demonstrated that most of the sample had at least one attack of diarrhea, except the present attack, in the month before visiting the hospital. Nevertheless, the prevalence of RV was significantly higher among in children who had no attack of diarrhea, other than the present one, in the previous month. This might be explained by the possibility that those who had frequent attacks of diarrhea might have got some immunity against RV gastroenteritis [98].

Finally, the study revealed that EIISA test has been with a high diagnostic accuracy rate of 95.45%. The sensitivity was about 89% This means that the test can diagnose 89% of true positive cases, and only 11% of true positive case will not be diagnosed by EIISA. This might be suitable for a screening test. On the other hand the specificity was 100%. This means that EIISA test can diagnose all negative cases of RV gastroenteritis. Both positive and negative predicate values were above 92% and 95% respectively, which again will give more faith in the positive and negative results of the test. Similar conclusion will be applied for the likelihood ratios of positive and negative test results. Usually, a likelihood ratio of a positive test >10 is often conclusive for a positive result. The study found that it value is very large (undefined). Furthermore, the like hood ratio of a negative test result was 0.113. This indicates that a negative test result is associated with the absence of the disease, as the smaller the LR- from (1.0) the more informative the negative test [-99]. Generally, EIISA has been widely considered as a good screening test of RV antigen in the

stool [79,81, 83]. This has also been reported in other studies which showed increase detection of RV by using RT-PCR in comparison to EIISA test. In a study in Norway for detection of RV in stool samples where the detection rate by ELISA was 63% in comparison to 72% by PCR among children <5 years of age admitted to hospital with diarrhea for the period 2006-2008. This might be due to the ability of PCR in detecting lower virus load [100-101]. The PCR is more tedious and expensive and needs expert hand. Usually, PCR is not used for screening of RV but rather for research and molecular characterization of RV strain; as with the study conducted in Erbil, Kurdistan Region by in 2006 by Herish et.al. Similar results have also been reported where PCR was used for strain classification; particularly before and after vaccination [16, 17, 18].

3. CONCLUSIONS & RECOMMENDATIONS

3.1. Conclusions

The study showed that RV was very prevalent in the etiology of gastroenteritis in Duhok, Kurdistan region, Iraq detected by ELISA method witch was a valid screening test. The main conclusions drawn from the study are.

1- The RV represent 29% of cases of gastroenteritis among under five children admitted to the only secondary care hospital in Duhok.
2- ELISA method for detecting RV antigen in the stool was an accurate screening test with high sensitivity and complete specificity in comparison to PCR test.
3- Infants < 1 year had significantly higher rate of RV in comparison to other age group.
4- Lower education of father and /or mother was significantly associated with higher positive cases of RV.
5- Exclusive breast feeding was significantly associated with lower -prevalence of RV.
6- No case of RV gastroenteritis was reported among vaccinated children.
7- Rural children are at higher risk of getting RV than urban children.
8- No significant associations were detected between the prevalence of RV infection and gender and crowding index.

3.2. Recommendations

This is the first study on the prevalence of RV among children suffering from gastroenteritis in Duhok. The Rv was an important cause of diarrhea and the main recommendation which can be aken from this study are.

1- RV should always be considered in the etiology of gastroenteritis as it represented an important cause of the disease among under-fives children in Duhok, especially among infants.
2- Encouraging parents to get better education will decrease the prevalence of RV gastroenteritis
3- EIISA test on stool sample was found to be of high accuracy to be recommended for screening tests.
4- A more extended cohort study of children with gastroenteritis is recommended to evaluate the outcome and prognosis of the disease in relation to RV infection.
5- Vaccination is very important tool in the control and prevention of RV, and it should be continued to be included in the routine vaccination program of under-fives.
6- A PCR study of the genotype of RV present in Iraq is recommended to arrange for a proper preventive program in relation to current vaccination.

REFERENCES

[1] Orenstein EW, Fang ZY, Xu J, Liu C, Shen K, Qian Y et al. (2007).1983 to 2005. The epidemiology and burden of rotavirus in China. Vaccine;25(3).406-413.

[2] Yassin K (2000). Morbidity and risk factors of diarrheal diseases among under-five children in rural Upper Egypt. J Trop Pediatr; 46(5). 282-7. doi. 10.1093/tropej/46.5.282.PMID. 11077937

[3] Esona M D, Gautam R (2015). Rotavirus. Clin Lab Med; 35(2).363-91. doi. 10.1016/j.cll.2015 .02.012.PMID. 26004648

[4] Parashar U D, Hummelman E G, Bresee J S, Miller M A , Glass R I(2003). Global illness and deaths caused by rotavirus disease in children. Merg Infect Dis; 9. 565-572

[5] World Health Organization. Editorial. New vaccines (1997). A global vaccine for a global disease - an end to rotavirus diarrhoea? CVI Forum No. 14, WHO, Geneva; p. 2-6.

[6] Herish M A, Coulter J S, Nakagomi O, Hart C A , Jamal M Z, Al-Rabaty A A, et al .(2006). Molecular characterization of rotavirus gastroenteritis strains, Iraqi Kurdistan. Emerg Infect Dis.; 12(5).824-6. DOI. 10.3201/eid1205.051422

[7] Crawford S E, Ramani S, Tate J E, Parashar U D, Svensson L, Hagbom M et al. (2017). Rotavirus infection.Nat Rev Dis Primers. ; 3.17083. doi. 10.1038/nrdp.2017.83.PMID. 29119972.

[8] Solares M Q, Yen C, Richardson V, Esparza-Aguilar M, Parashar U D, Patel MM (2011). Impact of rotavirus vaccination on diarrhea-related hospitalizations among children <5 years of age in Mexico. Pediatr Infect Dis J; 30. S11-S15

[9] Baker M, Prasad B V (2010). Rotavirus cell entry. Curr Top Microbiol Immunol; 343.121-48. doi. 10.1007/82_2010_34.

[10] Attoui H, Mertens P P C, Becnel J, Belaganahalli S, Bergoin M, Brussaard CP, et al. (2012). Family Reoviridae. In. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, et al., editors. Virus taxonomy. Classification and nomenclature. Ninth report of the International Committee on Taxonomy of Viruses. San Diego, California. Elsevier Academic Press; pp. 541–637.

[11] Greenberg H B, Estes M K (2009). Rotaviruses. from pathogenesis to vaccination. Gastroenterology; 136.1939–1951.

[12] Baker M, Prasad B V (2010). Rotavirus cell entry. Curr Top Microbiol Immunol.; 343.121-48. doi. 10.1007/82_2010_34.

[13] Estes M K, Graham D Y, Ramig R F, Ericson B L(1982). Heterogeneity in the structural glycoprotein (VP7) of simian rotavirus SA11. Virology; 122. 8-14

[14] Abdelhakim A H, Salgado E N, Fu X, Pasham M, Nicastro D, Kirchhausen T et al. (2014). Structural correlates of rotavirus cell entry. PLoS Pathog; 10. e1004355. https.//doi.org/ 10.1371 /journal.ppat.1004355

[15] Ruiz M C, Leon T, Diaz Y, Michelangeli F (2009). Molecular biology of rotavirus entry and replication. Scientific World Journal; 9. 1476-97. doi. 10.1100/tsw.158.

[16] Gentsch, Jon & Laird, Ashley & Bielfelt, Brittany & Griffin, Dixie & Banyai, Krisztian & Ramachandran, Madhu & Jain, et at, (2005). Serotype Diversity and Reassortment between Human and Animal Rotavirus Strains. Implications for Rotavirus Vaccine Programs. The Journal of infectious diseases. 192 Suppl 1. S146-59. 10.1086/431499.

[17] Matthijnssens J, Ciarlet M, Mcdonald S M, Attoui H, Bányai K, Brister J R, et al.(2011). Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG) Archives of Virology ;156(8).1397–1413.

[18] Bányai K, László B, Duque J, Steele A D, Nelson E A, Gentsch J R, et al. (2012). Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era. Insights for understanding the impact of rotavirus vaccination programs. Vaccine; 30(1). A122–A130.

[19] Matthijnssens J, Van Ranst M (2012). Genotype constellation and evolution of group A rotaviruses infecting humans. Current Opinion in Virology ;2(4).426–433.

[20] Li D, Gu A Z, Zeng S Y, Yang W, He M, Shi H C (2011). Monitoring and evaluation of infectious rotaviruses in various wastewater effluents and receiving waters revealed correlation and seasonal pattern of occurrences. Journal of Applied Microbiology ;110(5).1129–1137.

[21] Cook N, Bridger J, Kendall K, Gomara M I, El-Attar L, Gray J (2004). The zoonotic potential of rotavirus ;48(4).289-302. doi. 10.1016/j.jinf.2004.01.018.

[22] Black, R. E., Cousens, S., Johnson, H. L., Lawn, J. E., Rudan, I., Bassani, D. G. , et al. Global, regional, and national causes of child mortality in 2008. a systematic analysis. Lancet. 2010; 375(9730).1969-1987. doi.10.1016/S0140-6736(10)60549-1

[23] Parashar, U. D., Burton, A., Lanata, C., Boschi-Pinto, C., Shibuya, K., et al. (2004). Global mortality associated with rotavirus disease among children. J Infect Dis;200. S9–15.

[24] Lin CL, Chen SC, Liu SY, Chen KT. Disease caused by rotavirus infection. Open Virol J. 2014;8.14-19. Published 2014 Dec 11. doi.10.2174/1874357901408010014

[25] World Health Organization (2009). Rotavirus surveillance worldwide. Weekly Epidemiol Record. 2011; 86. 174-176.

[26] Salim H, Karyana P G, Sanjaya-Putra G N, Budiarsa S , Soenarto Y(2014). Risk factors of rotavirus diarrhea in hospitalized children in Sanglah Hospital, Denpasar. a prospective cohort study. BMC Gastroenterology ; 54. DOI. 10.1186/1471-230X-14-54.

[27] Fidhow A M , Samwel A , Ng'ang'a Z , Oundo J, Nyangao J , Wences A (2017). Molecular epidemiology and associated risk factors of rotavirus infection among children < 5 yrs hospitalized for acute gastroenteritis in North Eastern, Kenya, 2012. Pan Afr Med J ; 28(1). 3. doi. 10.11604/ PAMJ.SUPP.2017.28.1.2486

[28] Nimri L F , Hijazi S(1996) . Rotavirus-associated diarrhoea in children in a refugee camp in Jordan. J Diarrhoeal Dis Res ;14(1).1-4.Uribe E S , Aguilar M E , Gastañaduy P A , Desai R, Patel M , Richardson V (2013) . Risk Factors Associated with Rotavirus Gastroenteritis During a Community Outbreak in Chiapas, Mexico During the Postvaccination Era. Journal of the Pediatric Infectious Diseases Society; 2 (1).15–20, https.//doi.org/10.1093/jpids/pis077.

[29] Bahl R , Ray P, Subodh S, Shambharkar P, Saxena M, Parashar U(2005). Incidence of severe rotavirus diarrhea in New Delhi, India, and G and P Types of the infecting rotavirus strains; 192. S114-S119. 10.1086/431497.

[30] Nafi O (2010). Rotavirus gastroenteritis among children aged under 5 years in Al Karak, Jordan. East Mediterr Health J ;16(10).1064-9.

[31] Karsten C , Baumgarte S, Friedrich A W, Eiff C, Becker K, Bockemühl J et al. (2009). Incidence and risk factors for community-acquired acute gastroenteritis in north-west Germany in 2004. Eur J Clin Microbiol Infect Dis ;28(8).935-43.

[32] Bartlett A VIII, Reves R R, Pickering L K (19880 Rotavirus in infant-toddler day care centers. epidemiology relevant to disease control strategies. J Pediat ;1133. 435-414.

[33] Phillips G, Lopman B, Rodrigues L C, Tam C C (2010). Asymptomatic Rotavirus Infections in England. Prevalence, Characteristics, and Risk Factors. American Journal of Epidemiology; 171 (9) ; 1023–1030.

[34] Junaid S A, Umeh C, Olabode A O, Banda J M (2011). Incidence of rotavirus infection in children with gastroenteritis attending Jos university teaching hospital, Nigeria. Virol J; 8. 233 doi. 10.1186/1743-422X-8-233.

[35] Bass C W, Dorsey K N (2004). Rotavirus and other agents of viral gastroenteritis. Nelson Textbook of Pediatrics. Edited by. Richard E, Behrman F.Philadelphia ; 67-110. 16

[36] Borade A, Bais A, Bapat V, Dhongade R(2010). Characteristics of rotavirus gastroenteritis in hospitalized children in Pune. Indian J Med Sci; 64. 210-218.

[37] Harry B. Greenberg M D , Mary K E(2009) . Rotaviruses. from pathogenesis to vaccination. Gastroenterology; 136(6). 1939–1951

[38] Graham D Y, Dufour G R, Estes M K (1987). Minimal infective dose of rotavirus. Arch Virol; 92.261–71.

[39] Dennehy P H (2000) Transmission of rotavirus and other enteric pathogens in the home. Pediatr Infect Dis J ;19(10 Suppl). S103-5.

[40] Wobudeya E, Hanifa B, Karamagi C K, Kalyango J N, Mutebi E, Wamani H (2011). Breastfeeding and the risk of rotavirus diarrhea in hospitalized infants in Uganda. a matched case control study. BMC Pediatr; 11. 17.doi. 10.1186/1471-2431-11-1.

[41] Soenarto Y, Aman A T, Bakri A, Waluya H, Firmansyah A, Kadim M et al . (2009). Burden of severe rotavirus diarrhea in Indonesia. J Infect Dis; 200. S188-S194.

[42] Yolken R H, Peterson J A, Vonderfecht S L, Fouts E T, Midthun K, Newburg D S (1992). Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. J Clin Invest; 90. 1984-1991.

[43] Asensi MT, Martínez-Costa C, Buesa J(2006). Anti-rotavirus antibodies in human milk. quantification and neutralizing activity. J Pediatr Gastroenterol;42(5).560-7. doi. 10.1097/01.mpg. 0000221892.59371.b3..

[44] de Vrese M, Schrezenmeir J(2008). Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol;111.1-66. doi. 10.1007/10_2008_097.

[45] Hester SN, Chen X, Li M, Monaco MH, Comstock SS, Kuhlenschmidt TB, et al(2013). Human milk oligosaccharides inhibit rotavirus infectivity in vitro and in acutely infected piglets. Br J Nutr.;110(7).1233-42. doi. 10.1017/S0007114513000391. Epub 2013 Feb 26.

[46] Seganti L, Di Biase AM, Marchetti M, Pietrantoni A, Tinari A, Superti F(2004). Antiviral activity of lactoferrin towards naked viruses. Biometals.;17(3).295-9. doi. 10.1023/b.biom.0000027 708.27142.bc.

[47] Naficy A B, Abu-Elyazeed R, Holmes J L, Rao M R, Savarino S J, Kim Y et al. (1999). Epidemiology of rotavirus diarrhea in Egyptian children and implications for disease control. Am J Epidemiol; 150. 770-777.

[48] Duffy L C, Byers T E, Riepenhoff T M, La-Scolea L J, Zielezny M, Ogra P L (1986). The effects of infant feeding on rotavirus-induced gastroenteritis. a prospective study. Am J Public Health; 76. 259-263.

[49] Wilopo S A, Soenarto Y, Bresee J S, Tholib A, Aminah S, Cahyono A at al. (2009). Rotavirus surveillance to determine disease burden and epidemiology in Java, Indonesia Vaccine ;27 Suppl 5. F61-6.

[50] Soenarto Y, Aman AT, Bakri A, Waluya H, Firmansyah A, Kadim M, Martiza I at al. (2009). Burden of severe rotavirus diarrhea in Indonesia. J Infect Dis; 200. S188-S194.

[51] Regidor E, De Mateo S, Calle M E, Domínguez V (2002). Educational level and mortality from infectious diseases. J Epidemiol Community Health; 56.682–683.

[52] Mohammed A A, Aminu M, Ado S A, Jatau E D, Esona M D (2016). Prevalence of rotavirus among children under five years of age with diarrhea in Kaduna State, Nigeria. Niger J Paediatr; 43 (4). 264 –268

[53] Magzoub M A, Bilal N E, Bilal J A, Osman O F (2014) .Rotavirus infection among Sudanese children younger than 5 years of age. a cross sectional hospital-based study Pan African Medical Journal. 10; 16.88. doi. 10.11604/pamj.2013.16.88.2519. eCollection 2013

[54] Junaid S A, Umeh C, Olabode A O, Banda J M (2011). Incidence of rotavirus infection in children with gastroenteritis attending Jos university teaching hospital,Nigeria . Virology journal; 8.233. doi. 10.1186/1743-422X-8-233.

[55] Muendo C, Laving A, Kumar R, Osano B, Egondi T (2018). Prevalence of rotavirus infection among children with acute diarrhoea after rotavirus vaccine introduction in Kenya, a hospital cross-sectional study. BMC pediatrics; 18(1). 323.doi. 10.1186/s12887-018-1291-8.

[56] Parashar U D, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, et al.(2009) . Global mortality associated with rotavirus disease among children in 2004. J Infect Dis; 200. 9-15.

[57] Dey S K, Sharif N, Sarkar O S, Sarkar M K, Talukder A A, Phan T, et al. (2020). Molecular epidemiology and surveillance of circulating rotavirus among children with gastroenteritis in Bangladesh during 2014-2019. PLoS One.; 15(11). doi. 10.1371/journal.pone.0242813. eCollection 2020.PMID. 33253257

[58] Kota M, Bino S, Delogu R, Simaku A, Neza B, Ruggeri FM, et al. (2014). Epidemiology of rotavirus diarrhoea in Albania. Arch Virol ;159(9). doi. 10.1007/s00705-014-2093-4. Epub 2014 May 1. PMID. 24788847

[59] Khalili B, Cuevas L E, Reisi N, Dove W, Cunliffe N A, Hart C A (2004). Epidemiology of rotavirus diarrhoea in Iranian children. J Med Virol; 73(2). doi. 10.1002/jmv.20092.PMID. 15122809

[60] Dhital S, Sherchand J B, Pokhrel B M, Parajuli K, Shah N, Mishra SK, et al. (2017). Molecular epidemiology of Rotavirus causing diarrhea among children less than five years of age visiting national level children hospitals, Nepal. BMC Pediatr ;17(1). doi. 10.1186/s12887-017-0858-0.PMID. 28388889

[61] Fidhow A M, Samwel A, Ng'ang'a Z, Oundo J, Nyangao J, Wences A (2012).

[62] Molecular epidemiology and associated risk factors of rotavirus infection among children < 5 yrs hospitalized for acute gastroenteritis in North Eastern, Kenya. Pan Afr Med J ;28(1). doi. 10.11604/PAMJ.SUPP.2017.28.1.2486. eCollection 2017.PMID. 30167031

[63] Sai L, Sun J, Shao L, Chen S, Liu H, Ma L. (2013). Epidemiology and clinical features of rotavirus and norovirus infection among children in Ji'nan, China. Virol J; 10. doi. 10.1186/1743-422X-10-302.PMID. 24099150

[64] Ibrahim S B , El-Bialy A A, Mohammed M S , El-Sheikh A O, Elhewala A , Bahgat S H. (2015). Detection of Rotavirus in children with acute gastroenteritis in Zagazig University Hospitals in Egypt. Electron Physician.; 7(5). Published online. doi. 10.14661/1227

[65] Rodrigues F, Iturriza M, Gray J, Januário L, Lemos L. (2007). Epidemiology of rotavirus in Portugal. G9 as a major cause of diarrhoea in non-hospitalised children. J Clin Virol; 40(3). 214-7

[66] Estes M K, Greenberg H B. In. Field's Virology. Knipe D M, Howley P M, editors. (2013}. Lippincott. Williams, Wilkins.

[67] Butz AM (1993). Prevalence of rotavirus on high-risk fomites in day-care facilities. Pediatrics;92(2).202-5

[68] Crawford S E, Ramani S, Tate J E , Parashar U D , Svensson L, Hagbom M, et al (2017).. Rotavirus infection. Nat Rev Dis Primers; 3.17083. doi. 10.1038/nrdp.2017.83

[69] Burnett E (2017). Global impact of rotavirus vaccination on childhood hospitalizations and mortality from diarrhea. J Infect Dis ;215. 1666-1672

[70] Vesikari T (2006). Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med ;354. 23-33.

[71] Roger I, GlassabBaomingJiangbUmeshParasharb(2018). The future control of rotavirus disease. Can live oral vaccines alone solve the rotavirus problem? Vaccine ; 36 (17). 2233-2236

[72] Soares-Weiser K, MacLehose H, Bergman H, Ben-Aharon I, Nagpal S, Goldberg E,et al (2012). Vaccines for preventing rotavirus diarrhoea. vaccines in use. Cochrane Database Syst Rev.;CD008521 http.//onlinelibrary.wiley.com/doi/10.1002/14651858.CD008521.pub3/full.

[73] Tate JE, Parashar UD(2014). Rotavirus vaccines in routine use. Clin. Infect. Dis. ;59(9).1291–1301

[74] Premkumar PS, Parashar UD, Gastanaduy PA, McCracken JP, de Oliveira LH, Payne DC,et al (2015). Reduced rotavirus vaccine effectiveness among children born during the rotavirus season. a pooled analysis of 5 case-control studies from the Americas. Clin Infect Dis.;60.1075-1078

[75] Burnett E, Yen C, Tate JE, Parashar UD(2016).. Rotavirus vaccines. current global impact and future perspectives. Future Virol. 2016;11.699–708.]

[76] Leshem E (2014). Distribution of rotavirus strains and strain-specific effectiveness of the rotavirus vaccine after its introduction. a systematic review and meta-analysis. Lancet Infect Dis; 14. 1-10

[77] Payne D C (2008). Active, population-based surveillance for severe rotavirus gastroenteritis in children in the United States. Pediatrics; 122.1235–1243

[78] World Health Organization (2009). Manual of Rotavirus detection and characterization methods, The Department of Immunization, Vaccines and Biologicals. Geneva.

[79] Wilhelmi I, Colomina J, Martin-Rodrigo D, Roman E, Sanchez-Fauquier A (2001). New immunochromatographic method for rapid detection of rotaviruses in stool samples compared with standard enzyme immunoassay and latex agglutination techniques. Eur J Clin Microbiol Infect Dis; 20.741-3

[80] Raboni S M, Nogueira M B, Hakim V M, Torrecilha, Lerner H, Tsuchiya L R (2002). Comparison of latex agglutination with enzyme immunoassay for detection of rotavirus in fecal specimens. Am J Clin Pathol; 117.392-394

[81] Pang X L, Lee B, Boroumond N, Leblanc B, Preiksaitis J K, Yulp C C (2004). Increased detection of rotavirus using reverse transcription-polymerase chain reaction (RT-PCR) assay in stools specimens from children with diarrhea. J Med Virol; 72.496-501.

[82] Kelkar S D, Bhide V S, Ranshing S S, Bedekar S S (2004). Rapid ELISA for diagnosis of rotavirus.Indian J Med Res; 119.60-65.

[83] Youssef M, Shurman A, Bougnoux M E, Rawashdeh M, Bretagne S, Strockbine N (2000) Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. FEMS Immunol Med Microbiol; 28.257–63.

[84] Karadag A, Acikgoz Z C, Avci Z, Catal F, Gocer S, Gamberzade S, et al. (2005). Childhood diarrhoea in Ankara, Turkey. epidemiological and clinical features of rotavirus-positive versus rotavirus negative cases. Scand J Infect Dis; 37.269–75

[85] Hameed A R, Mohamed B Y, Abakar A D,Ali K S (2020). Prevalence of Rotavirus among Iraqi children with diarrhea in Diyala province. Iraq Medical Journal, 4 (4), DOI. https.//doi.org/10.22317/imj.v4i4.930

[86] Mahmood D A, Feachem R G (1987). Clinical and epidemiological characteristics of rotavirus- and EPEC-associated hospitalized infantile diarrhoea in Basrah, Iraq. J Trop Pediatr; 33.319–25

[87] Abd-Al Fattah1 S A, Hamad M A, AL-Ani MQ (2020). 80Prevalence and Molecular Detection of Rotavirus in Children in Ramadi City-Iraq. Medico-legal Update. 20(4).2096-2102

[88] Abdulridha A S (2016). Study the effects of age and gender of children on rotavirus infection in Baghdad, Iraq Study the Effects of Age and Gender of Children on Rotavirus Infection in Baghdad, Iraq. Research Journal of Pharmaceutical, Biological and Chemical Sciences 7(4).700-704

[89] Gerna G, Sarasini A, Di Matteo A, Parea M, Torsellini M, Battaglia M(1989). Rapid detection of human rotavirus strains in stools by single-sandwich enzyme-linked immunosorbent assay systems using monoclonal antibodies. J Virol Methods.;24(1-2).43-56.

[90] Grauballe PC, Vestergaard BF, Meyling A, Genner J(1981). Optimized enzyme-linked immunosorbent assay for detection of human and bovine rotavirus in stools. Comparison with electron-microscopy, immunoelectro-osmophoresis, and fluorescent antibody techniques. J Med Virol ;7(1).29-40.

[91] Adlhoch, C., Kaiser, M., Hoehne, M. • Marques AM, Stefas I, Veas F et al. (2011).Highly sensitive detection of the group A Rotavirus using Apolipoprotein H-coated ELISA plates compared to quantitative real-time PCR. Virol J 8, 63 https.//doi.org/10.1186/1743-422X-8-63.

[92] Khalili B, Cuevas L E, Reisi N, Dove W, Cunliffe N A, Hart CA (2004). Epidemiology of rotavirus diarrhoea in Iranian children. J Med Virol; 73.309–12.

[93] Huseyin N, Murat U, Alparslan K, Hale T (2012) .Age Distribution and Seasonal Pattern of Rotavirus Infection in Children Under 5 Years. Jundishapur Journal of Microbiology; 6(1). DOI 10.5812/jjm.4626

[94] Clark A, Black R, Tate J, Roose A, Kotloff K, Lam D, et al (2017). Estimating global, regional and

[95] national rotavirus deaths in children aged & lt;5 years. current approaches, new analyses and proposed improvements. PLoS ONE; 12(9). e0183392.

[96] Atherly D, Dreibelbis R, Parashar U, l Levin, C Wecker J, Rheingans R(2009). Rotavirus vaccination. cost-effectiveness and impact on child mortality in developing countries. J Infect Dis; 2009Suppl1). 28-38.

[97] Zhang J, Duan Z, Payne DC, Yen C, Pan X, Chang Z, et al(2015). Rotavirus-specific and Overall Diarrhea Mortality in Chinese Children Younger than 5 Years. 2003 to 2012Pediatr Infect Dis J; 34(10). e233–e237. doi. 10.1097/INF.0000000000000799.

[98] Velázquez FR (2009). Protective effects of natural rotavirus infection. Pediatr Infect Dis J; 28(Suppl 3). S54–6.

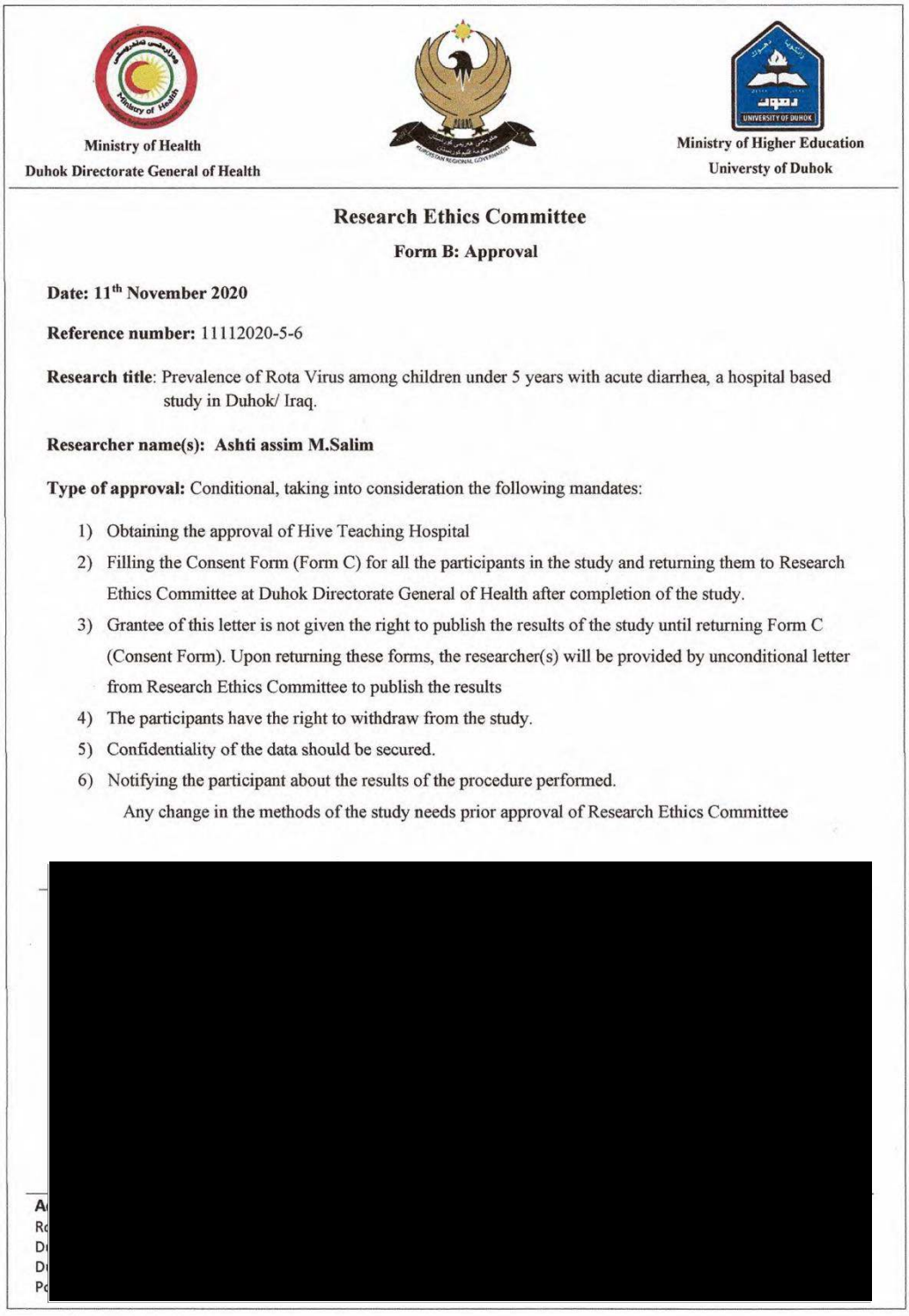
[99] Celentano DD, Szklo M (2018). Gordis Epidemiology. Elsevier publication, 6th edition. Canada, ISBN 978-0323552295.

[100] Slavica Mijatovic-Rustempasica S, Esonaa MD, Williamsb AL, Bowena MD (2016) Sensitive and specific nested PCR assay for detection of rotavirus A in samples with a low viral load. J Virol Methods; 236. 41–46.

[101] Vainio K, Nordbø SA, Njølstad G, Størvold G, Døllner H, Midgaard C, et al.(2009). Detection and characterization of group A rotaviruses in children hospitalized with acute gastroenteritis in Norway, 2006-2008. J Med Virol; 81(10).1839-44. doi. 10.1002/jmv.21576.

APPENDICES

Appendix 1. Approval of Research Ethics Committee letter



Ministry of Health
Duhok Directorate General of Health

Ministry of Higher Education
Universty of Duhok

Research Ethics Committee

Form B: Approval

Date: 11th November 2020

Reference number: 11112020-5-6

Research title: Prevalence of Rota Virus among children under 5 years with acute diarrhea, a hospital based study in Duhok/ Iraq.

Researcher name(s): Ashti assim M.Salim

Type of approval: Conditional, taking into consideration the following mandates:

1) Obtaining the approval of Hive Teaching Hospital
2) Filling the Consent Form (Form C) for all the participants in the study and returning them to Research Ethics Committee at Duhok Directorate General of Health after completion of the study.
3) Grantee of this letter is not given the right to publish the results of the study until returning Form C (Consent Form). Upon returning these forms, the researcher(s) will be provided by unconditional letter from Research Ethics Committee to publish the results
4) The participants have the right to withdraw from the study.
5) Confidentiality of the data should be secured.
6) Notifying the participant about the results of the procedure performed.

Any change in the methods of the study needs prior approval of Research Ethics Committee

[redacted]

Appendix 2. Research Questionnaire

- Name: Date:

- Age: (Months)

- Sex:

- Hight:

- Weight:

- Father education:

Illiterate ☐ Primary ☐ Intermediate ☐ Secondary ☐ Collage ☐

- Mother education:

Illiterate ☐ Primary ☐ Intermediate ☐ Secondary ☐ Collage ☐

- Number of children: ( )

- Residency

Urban ☐ Rural ☐

- Number of Family members:

- Number of rooms in the house:

- Attacks of diarrhea your child had last month: ( )

- Have your child completed the vaccination schedule:

Yes ☐ No ☐ Partial ☐ I don't know ☐

- Type of feeding in the first year:

Exclusive breast-feeding ☐ Bottle ☐ Mixed ☐

Results

ELISA:

PCR:

STOOL EXAM (FROM CASE SHEET):