Babylon University
College of Medicine

Incidence of Rotavirus & Other Enteropathogens Causing Acute Diarrhea in Hilla Infants

A Thesis

Submitted to the Council of the College of Medicine in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

By

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علي حسين محمد المرزوكي

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بسم الله الرحمن الرحيم

سَنُرِيهُم آياتنا في ألأفاقِ وفي
أنفُسَهُم حَتى يَتبينَّ لَهُم أنّهُ
ألحَق أوَلَم يَكفِ بربِّكَ أنّهُ
على كُلِّ شيءٍ شَهيد

صدق الله العلي العظيم

سورة فصلت

الآية (53)

We certify this thesis was prepared under our supervision at the University of Babylon as partial requirements for degree of M.Sc. in Microbiology….

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Dedication

To our savior .....
Holy Imam .....
Al-Hujja ..

Mohammad AL-Mahdi

Chapter One

INTRODUCTION

Chapter One

Introduction

Acute diarrheal disease is the commonest single cause of morbidity and mortality worldwide. Infectious diarrhea has been estimated to cause at least 5 million deaths each year in the developing world. Very young children are particularly susceptible to infection and suffer the highest mortality. Rotavirus is the commonest enteric pathogen in young children in both developing countries and the developed world. A striking feature of rotavirus infection in temperate climates is its seasonality. In contrast to other enteric pathogens, which are the commonest during the warm months of the year, the rotavirus "season" is in winter and spring. Typically children under 2 years of age are affected and rotavirus may be responsible for up to 50% of acute admissions to pediatrics units during the winter months *(Cryan, et.al., 2003).*

Rotavirus is the most important etiological agent of serious dehydrating diarrhea among infants and young children, causing an estimated nine million cases of severe disease, and more than 800 000 deaths per year worldwide, rotavirus particles are 65-75 nanometers in diameter, with a double protein shell and II strands of double-stranded RNA *(Kapikian, et.al., 1990).* The rotavirus genome comprises 11 segments of double-stranded

RNA (dsRNA) contained within the core of the mature, triple-layered particle. The 11 dsRNA segments can be separated by using electrophoresis *(Cunliffe, et.al., 2002).* The majority of rotaviruses known to infect humans and animals share a common-group antigen and are termed group A rotaviruses *(Kapikian, et.al., 1990).*

In developing countries this virus accounts for nearly 6% of all diarrheal episodes and for 20% of all diarrhea-associated deaths of children under five *(de Zoysa, et.al., 1985)*. In industrialized countries as well, rotavirus gastroenteritis is a major cause of hospitalization of infants and young children *(Kapikian, et.al., 1982)*.

Rotavirus diarrhea is most prevalent among children aged 6-24 months; it has been estimated that an effective vaccine could reduce diarrheal mortality among this age group *(Clark, 1988).*

Chapter Two

LITERATURE REVIEW

Chapter Two

Literature review

2.1 Acute diarrhea infections

A universal definition of diarrhea does not exist although patients seem to have no difficulty defining their own situation. Although most definitions center on the frequency, consistency, and water content of stools, many authors prefer the definition that diarrheal stools take the shape of their container *(Arthur & January, 2003)*.

Diarrhea is one of the most common reasons patients seek medical care. In the developed world, it is the most common reason for missing work; while in the developing world, it is a leading cause of death. In developing countries, it is a seasonal scourge usually made worse by natural phenomenon as evidenced by monsoon floods in Bangladesh in 1998. An estimated 100 million cases of acute diarrhea occur in any given year in the United States; of these patients, 90% do not seek medical attention and 1-2% requires admission. Diarrheal diseases can

quickly reach epidemic proportions, rapidly overwhelming public health systems in even the most advanced societies *(Atilla & Ertan,. 2001).*

In 1976, John Rohde, highlighting the importance of diarrhea as prime killer of children in the developing world, beckoned the scientific community to "take science where the diarrhea is". The World Health Organization estimates that one billion diarrheal episodes occur in infants annually resulting in 3.3 million deaths, making diarrheal disease a major contributor to infant mortality in developing world *(Bern et. al., 1992).*

Acute diarrhea usually is caused by infectious agents. These agents cause diarrhea by adherence, mucosal invasion, enterotoxin production, and/or cytotoxin production. These mechanisms result in increased fluid secretion and/or decreased absorption. This produces an increased luminal fluid content that can not be adequately reabsorbed, leading to dehydration and loss of electrolytes and nutrients *(Atilla & Ertan,. 2001).*

Diarrheal illnesses also may be classified as follows:

- *Osmotic*, due to an increase in the osmotic load presented to the intestinal lumen, either through excessive intake or diminished absorption.
- *Inflammatory (or mucosal)*, when the mucosal lining of the intestine is inflamed.
- *Secretory*, when increased secretory activity occurs
- *Motile*, caused by intestinal motility disorders *(Arthur & January, 2003).*

Table (I)

Differential diagnosis of diarrhea

TYPE OF DIARRHEA	CAUSES
Acute "Common"	➢ *Gastroenteritis* ➢ *Systemic infection* ➢ *Antibiotic associated* ➢ *Overfeeding*
Acute "Rare"	➢ *Primary disaccharidase deficiency* ➢ *Hirsch sprung toxic colitis* ➢ *Adrenoganetal syndrome*

From (Richard, et.al., 1998)

2.2 General information about causes of infantile diarrhea "Bacterial, Parasitic, Viral, Fungal "

Microorganisms may produce toxins that facilitate infection. Enterotoxins are generated by bacteria (ie, enterotoxigenic *Escherichia coli* and *Vibrio cholera*) which act directly on secretory mechanisms and produce typical copious watery (*rice water*) diarrhea. No mucosal invasion occurs. The small intestines are primarily affected and elevation of the adenosine monophosphate (AMP) levels is the common mechanism *(Richard, et.al., 1998).*

The major causes of diarrhea;

Bacteria that commonly cause gastroenteritis include various types of *E. coli*, *Salmonella*, *Shigella*, and *Campylobacter* species, as well as organisms that cause

cholera and dysentery. Illness may result from the bacteria themselves, or from toxins produced by some bacteria. Some toxins cause intestinal cells to secrete fluid, producing large amounts of watery diarrhea. Others damage the intestinal lining so that blood is visible in the stool. Some bacteria produce proteins that enable themselves to adhere to the intestinal wall and multiply, crowding out the normal beneficial intestinal bacteria. Certain parasites found in food and water also cause gastroenteritis. *Giardia* and amebiasis are two examples of parasitic causes of gastroenteritis. *Giardia* is often contracted by campers who drink directly from streams. Infected individuals often develop a prolonged course of gastrointestinal symptoms. Amebiasis or amoebic dysentery is also caused by a parasite *(Atilla, Ertan,. 2001)* , in general *(Table II)*;

- *Viral (50-70%)*

  - *Rotavirus*: is a leading cause of gastroenteritis in children, but can also be found in adults. May cause severe dehydration *(Villena, C. et.al., 2003), (Schnagl, R.D. et.al., 1978), (Kurugol, Z. et.al., 2003)& (Duffy, L.C. 1986).*
  - The Norwalk virus is the leading cause of viral gastroenteritis in the United States. Norwalk virus belongs to the species of Noroviruses (formerly known as Norwalk-like viruses). Noroviruses, along with the Sapoviruses (formerly known as Sapporo-like viruses) are members of the Caliciviridae family of viruses *(Chiba, et.al., 1979).*

  - Caliciviruses: Various caliciviruses, other than Norwalk, are likely responsible for many outbreaks of previously unidentified viral

gastroenteritis *(Kapikian, 1996), (Bon, et.al., 1999)* and *(Matson, et.al., 1989).*

- Adenovirus, Parvovirus, Astrovirus, Coronavirus, Pestivirus & Torovirus *(Schnagl, R.D. et.al., 1978).*

❖ *Bacterial (15-20%)*

- *Shigella, Salmonella , C jejuni , Yersinia enterocolitica (Sethi, et,al. 1984, Gosh, et.al., 1991) , E coli* - Enterohemorrhagic 0157:H7 (enterotoxigenic, enteroadherent, enteroinvasive) *(Gosh, et.al., 1991, Mandal, 1981, Weindling, et.al. ,1980 & Al-Kelaby, 1999), Campylobacter (Merten, et.al., 1990) , V. cholera , Aeromonas , B. cereus , C. difficile ,Clostridium perfringens , Listeria , Mycobacterium avium-intracellulare , (MAI), immunocompromised , Providencia , Vibrio parahaemolyticus ,* & *Vibrio vulnificus (McIver, C.J. et.al., 2001).*

❖ *Parasitic (10-15%)*

- *Giardia (Jay, 1994), Amebiasis (Abbas, 1986), Cryptosporidium, Cyclospora (McIver, et.al., 2001).*

❖ *Food-borne toxigenic diarrhea*

- Preformed toxin; *S. aureus, B. cereus*
- Post colonization; *V. cholera, C. perfringens, enterotoxigenic E. coli, Aeromonas(McIver, et.al., 2001).*

❖ *Drug-associated diarrhea*

- Antibiotics due to alteration of normal flora
- Laxatives, including magnesium-containing antacids
- Colchicine , Quinidine , Cholinergics , Sorbitol *(Arthur & January, 2003)*.

❖ *Pseudomembranous colitis*

- Overgrowth of *C. difficile*

❖ *Other causes*

- Unknown agents, especially in developing countries
- Ischemic colitis, Ulcerative colitis, Crohn disease, Carcinoid tumor or, vasoactive intestinal peptide tumor (VIPoma) , AIDS , Dumping or short bowel syndrome , Radiation or Chemotherapy *(Arthur & January, 2003)*.

Table (II); Virulence characteristics of enteropathogens (Richard, et.al., 1998)

ORGANISMS	VIRULENCE PROPERTIES
Campylobacter jejuni	*Invasive ; enterotoxin*
Clostridium difficile	*Cytotoxin ; enterotoxin*
Cryptosporidium	*Adherence*
Cyclospora	*Inflammation*
Entamoeba histolytica	*Cyst resistance to physical destruction ; invasion ; enzyme & Cytotoxin production*
Enteric Adenovirus	*Mucosal lesion*
o *Escherichia coli; Enteropathogenic*	*Unknown ,possibly Cytotoxin or adherence*
o *Enterotoxigenic*	*Enterotoxin (Heat Stable or Labile)*
o *Enteroinvasive*	*Invasion*
o *Enterohemorrhagic 0157:H7*	*Cytotoxin*
Giardia lamblia	*Cyst resistance to physical destruction ; adherence to mucosa*
Norwalk - like virus	*Mucosal lesion*
Rotavirus	*Damage to microvilli*
Shigella	*Invasion ; Enterotoxin ; Cytotoxin*
Salmonella	*Invasion ; Enterotoxin*
Vibrio cholera	*Enterotoxin*
Vibrio parahaemolyticus	*Invasion ; Cytotoxin*
Yersinia enterocolitica	*Invasion ; Enterotoxin*

Cytotoxin production by bacteria (ie, *Shigella dysenteriae*, *Vibrio parahaemolyticus*, *Clostridium difficile*, enterohemorrhagic *E. coli*) results in mucosal cell destruction leading to bloody stools with inflammatory cells. A resulting decreased absorptive ability occurs. Enterocyte invasion is the preferred method by which microbes such as *Shigella* and *Campylobacter* organisms and enteroinvasive *E. coli* cause destruction and inflammatory diarrhea. Similarly, *Salmonella* and *Yersinia* species also invade cells but do not cause cell death. Hence, dysentery does not usually occur. These bacteria will, however, invade the blood stream across the lamina propria and cause enteric fever such as typhoid *(Arthur & January, 2003).*

2-2-1 Rotavirus

In 1973, Bishop and colleagues using electron microscope, in the duodenal epithelium of children with diarrhea, observed a 70-nm virus, subsequently designated rotavirus (Latin, Rota = wheel) because of its appearance *(Figure I & III) (Bishop, et.al., 1973*). Before this discovery, a bacterial, viral, or parasitic etiologic agent could be detected in only 10% to 30% of children with diarrhea. Within 5 years, rotavirus was recognized as the most common cause of diarrhea in infants and young children worldwide, accounting for approximately one third of cases of severe diarrhea requiring hospitalization *(de Zoysa, et,al. 1985).*

Group A rotaviruses are the most important agents of severe diarrhea in children and infants worldwide *(Joana, et.al., 2002).* Human rotavirus (HRV) is the leading cause of severe gastroenteritis in infants and young children *(Kapikian, 1996).* HRV also infects neonates within some hospital nurseries, where infection is nosocomially acquired and is characteristically asymptomatic *(Cicirello, et.al., 1994)* and *(Kilgore, et.al., 1996)* . Despite the lack of associated symptoms,

neonatal rotavirus infections have gained a considerable attention because they protect against severe rotavirus diarrhea in later infancy *(Bishop, et. al., 1983)* and *(Bhan, et. al., 1993)*. A number of rotaviruses that have been recovered from neonates (so-called nursery strains) have been tested as rotavirus vaccine candidates *(Vesikari, et. al., 1991)* and *(Barnes, et. al. , 1997).*

Group A rotaviruses are the most common causative agents of acute gastroenteritis in children under 2 years of age and are also associated with diarrhea in the young avian (chicken, turkey, and pigeon) and many mammalian (simian, porcine, bovine, ovine, caprine, equine, canine, feline, lapine, and murine) species *(Max Ciarlet, et.al., 2002)*.

Rotavirus infection is primarily restricted to the villus epithelium of the small intestine, and the outcome of infection is age restricted. The impact of rotavirus disease in humans includes over 600,000 annual associated deaths in developing countries . In the United States, annual economic losses due to rotavirus infections have been conservatively estimated at $1 billion *(Glass, et.al., 1996)* , *(LeBaron, et.al., 1990)* , *(Matson, 1990)* and *(Pérez-Schael, et.al., 1997)*. Therefore, development of a safe and effective rotavirus vaccine is a global priority.

2-2-1-1 Nomenclature & Taxonomy;

Rotavirus, an icosahedral virus in the family Reoviridae, has a distinct morphologic appearance by negative-stain electron microscopy *(Figure I & III) (Kapikian, et. al., 1996).*

2-2-1-2 Morphology, Physical characters, Antigenic Structure, Multiplication;

The viral capsid is triple-layered; the inner layer (core) contains the virus genome, which comprises 11 segments of double-stranded RNA, each coding for products that are either structural viral proteins (VP) or nonstructural proteins (NSP) *(Figure III)*. The segmented genome of rotavirus readily re-assorts during coinfection, a property that has been used in developing vaccines and undoubtedly plays a role in virus evolution. The major antigenic properties of rotaviruses group, subgroup, and serotype are determined by the viral capsid proteins. Rotavirus has seven major groups (A-G); most human strains belong to group A, although groups B and C have occasionally been associated with human illness *(Maria, et.al., 2002)*.

Rotaviruses are distinct serologically from the three Reovirus serotypes and from all Orbiviruses with which they have been tested. Most human rotaviruses share a common group antigen and are designated group A rotaviruses, but other antigens separate the group A rotaviruses into serotypes and subgroups. Ten human rotavirus serotypes have been defined by neutralization of one of the outer capsid proteins, VP7. Group A rotaviruses can also be separated into two distinct subgroups by various assays. The neutralization and subgroup specificities are encoded by different genes. Rotaviruses also have been detected in stools of the young of numerous animals with diarrhea. Rotaviruses of humans and animals characteristically share a common group antigen, but strains may differ in serotype specificity by neutralization. However, many animal rotavirus strains (simian, canine, feline, equine, murine, porcine, and lapine strains) share serotype specificity with human rotavirus. Additionally, a few human and animal rotavirus

strains have been detected (by electron microscopy) that do not share the common group antigen; these have been designated as non-group A rotaviruses. The non-group A viruses are divided into groups B, C, D, E, F, and G on the basis of distinct group antigens. The group A rotaviruses are the most important agents of severe diarrhea in infants and young children and are prevalent worldwide. The group B and C rotaviruses have a more limited distribution and are not considered to be an important cause of infantile diarrhea. Group B rotavirus has been responsible for large outbreaks of severe gastroenteritis in China, which predominantly involved adults, but the importance of the group B rotaviruses outside China has not been defined. Groups D, E, F, and G have not been detected in humans (*Gentsch, et.al., 1996*).

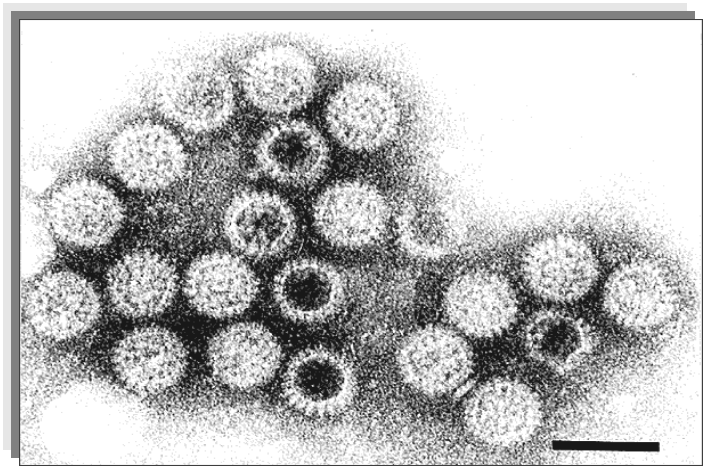

Figure (I)

Rotavirus particles visualized by immune electron microscopy in stool filtrate from child with acute gastroenteritis. 70-nm particles possess distinctive double-shelled outer capsid. Bar = 100 nm. (Gentsch, et.al., 1996).

The product of the 6th gene of group A rotaviruses encodes VP6, the most abundant viral protein, which is the major determinant of group reactivity, the

target of common diagnostic assays, and contains the antigen used to further classify rotaviruses into subgroups I and II. The outer capsid proteins, VP7, the glycoprotein or G-protein (encoded by gene 7, 8, or 9, depending on the strain), and VP4, the protease-cleaved or P-protein (encoded by gene segment 4), determine the serotype specificity and form the basis of the binary classification (G and P type) of rotaviruses. Both G and P proteins induce neutralizing antibodies and may be involved in protective immunity *(Arguelles, et,al. 2000)*.

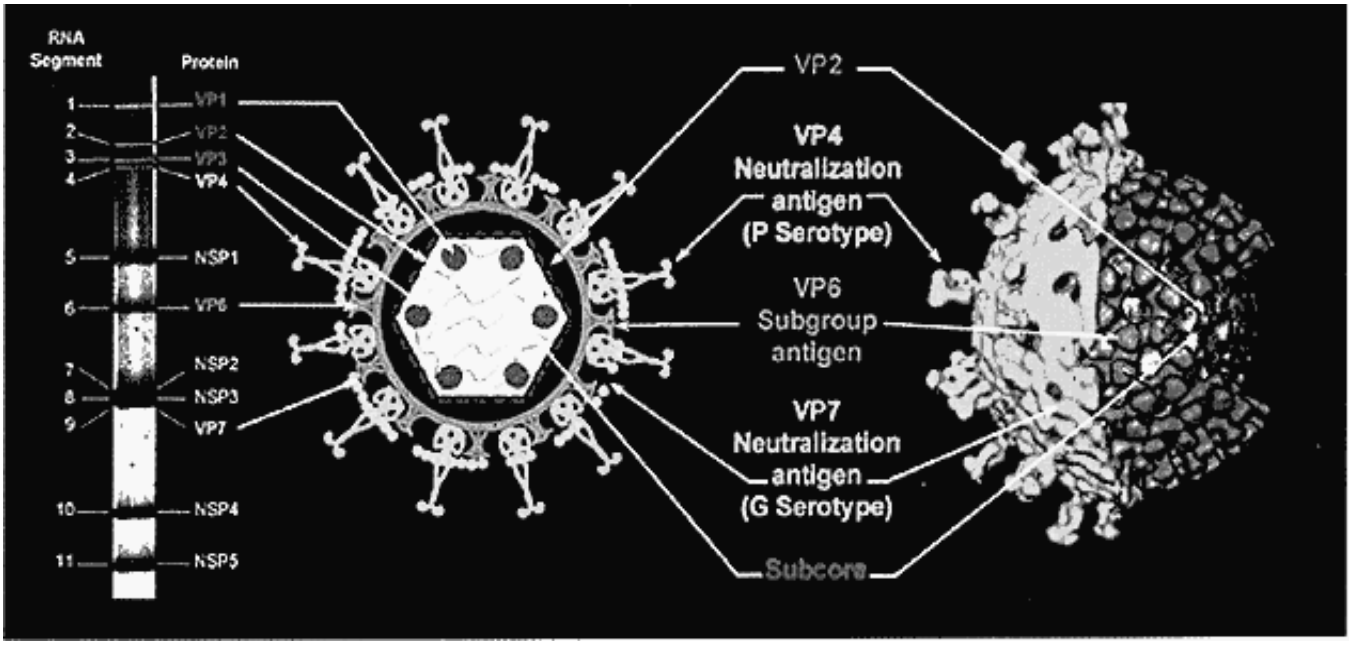

Figure (II)

Gene coding assignments and three-dimensional structure of rotavirus particles. Double-stranded RNA segments separated on polyacrylamide gel (left) code for individual proteins, which are localized in the schematic of virus particle (center) or in different protein shells of virus (right). Outer capsid proteins VP4 and VP7 are neutralization antigens, which induce neutralizing antibody; protein that makes up intermediate protein shell, VP6, is the subgroup antigen. Adapted from (Gentsch, et.al., 1996).

Fourteen G serotypes of rotavirus, 10 of which occur in humans, have been defined by cross-neutralization studies with polyclonal animal serum samples;

these serotypes correlate with antigenic specificities of the VP7 glycoprotein. The characterization of P serotypes has been difficult because adequate reagents are not available. Eight P serotypes of human rotaviruses have been characterized. Additional VP4 gene variants have been identified, so ultimately the number of P serotypes may exceed 20. Theoretically, 80 different strains of rotavirus could result from various combinations of the known 10 G and 8 P serotypes of human rotaviruses. For vaccine development purposes, it is fortunate that only four common strains (P[8]G1; P[8]G3; P[8]G4; and P[4]G2) of rotavirus predominate globally *(Figure II) (Gentsch, et.al., 1996.).*

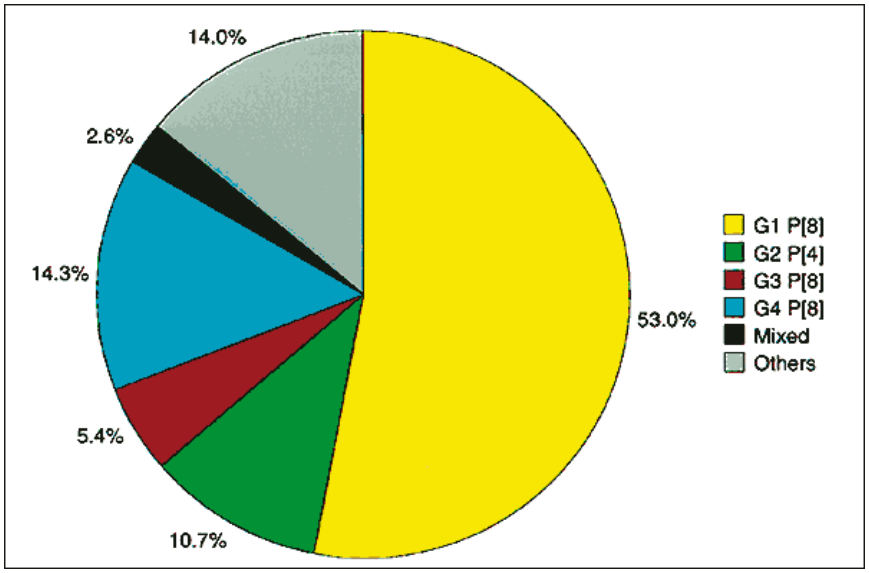

Figure (III) Distribution of rotavirus strains from a global collection of 2,748 strains. "Others" includes strains that were not typable. Adapted from (Gentsch, et.al., 1996).

However, the prevalence of rotavirus strains varies considerably from one geographic area to another, and unusual strains are common in several developing countries (e.g., unusual P[6] strains, including those with serotype G9 specificity, accounted for 9.5% of all rotaviruses from a multicenter collection in India)

(Ramachandran et.al., 1996). Multiplication of Rotaviruses: HRV replicate exclusively in the cytoplasm. The virion enters the cell by endocytosis (or direct membrane penetration if activated by protease), and the outer shell of the double capsid is removed in lysosomes with the liberation of 50-nm sub viral particles, thus activating the viral RNA polymerase (transcriptase). RNA positive-sense transcripts induce the production of proteins and are also a template for the production of anti-sense strands, which remain associated with the positive-sense strand. About 8 hours after infection, viroplasmic inclusions of dense granular material, representing newly synthesized proteins and RNA, accumulate in the cytoplasm. Viral RNA is packaged into core particles, and viral capsid proteins assemble around the cores. These particles accumulate in vesicles of the endoplasmic reticulum and leave the viroplasm by budding through the membrane of the endoplasmic reticulum, where they acquire the outer capsid protein. The budding process (plus transient acquisition of an envelope) is unique to rotaviruses among members of the family Reoviridae. Particles are released by cell lysis *(Gentsch, et.al., 1996)*.

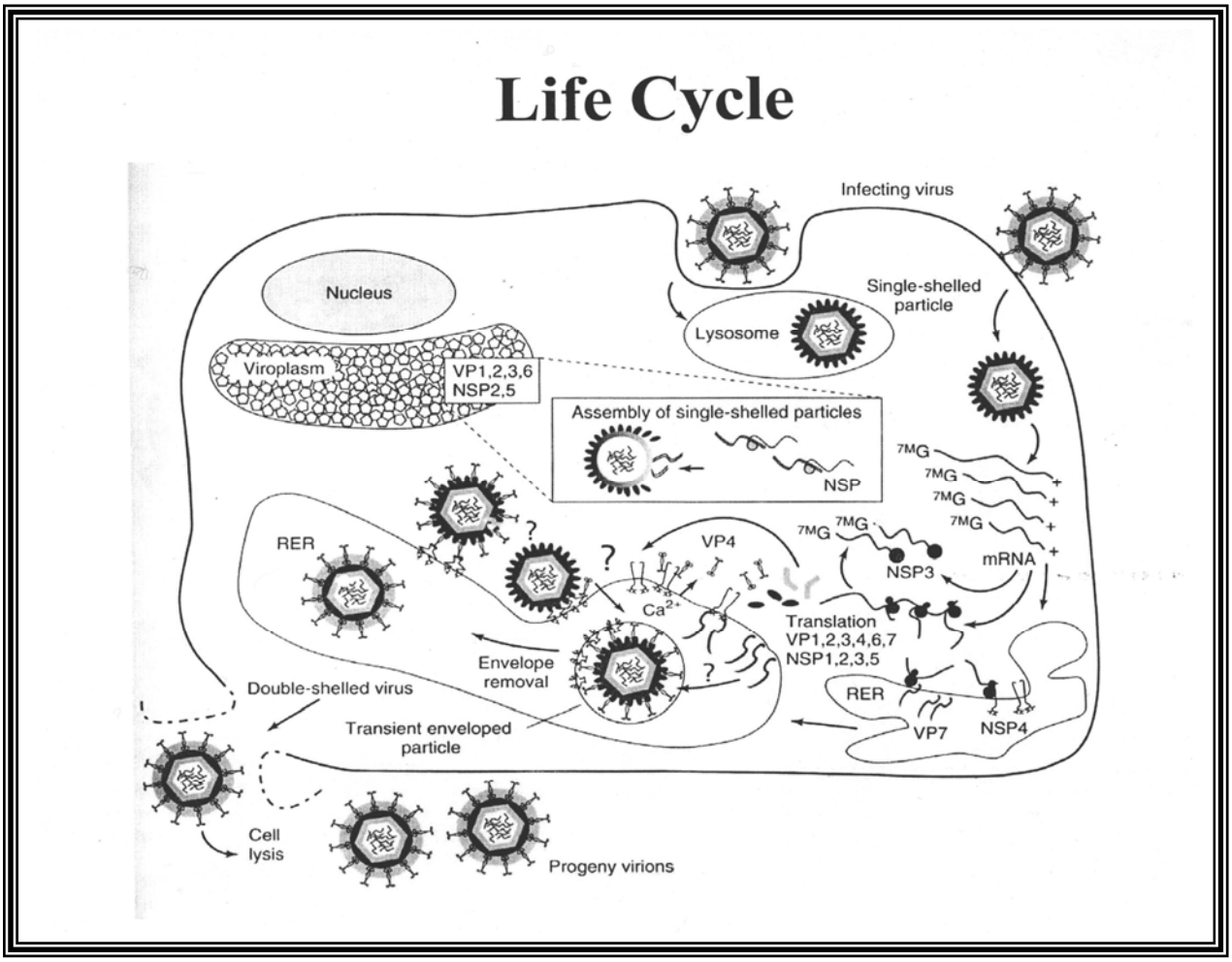

Figure (IV) HRV life Cycle & Multiplication

(Gentsch, et.al., 1996).

2-2-1-3 Epidemiology

2-2-1-3-1 Geographical distribution;

Rotaviruses are ubiquitous; 95% of children worldwide are infected by 3 to 5 years of age. The prevalence of rotavirus infection in neonates has not been

systematically examined, but high infection rates were documented in newborns in six hospitals in India *(Cicirello et.al., 1994).*

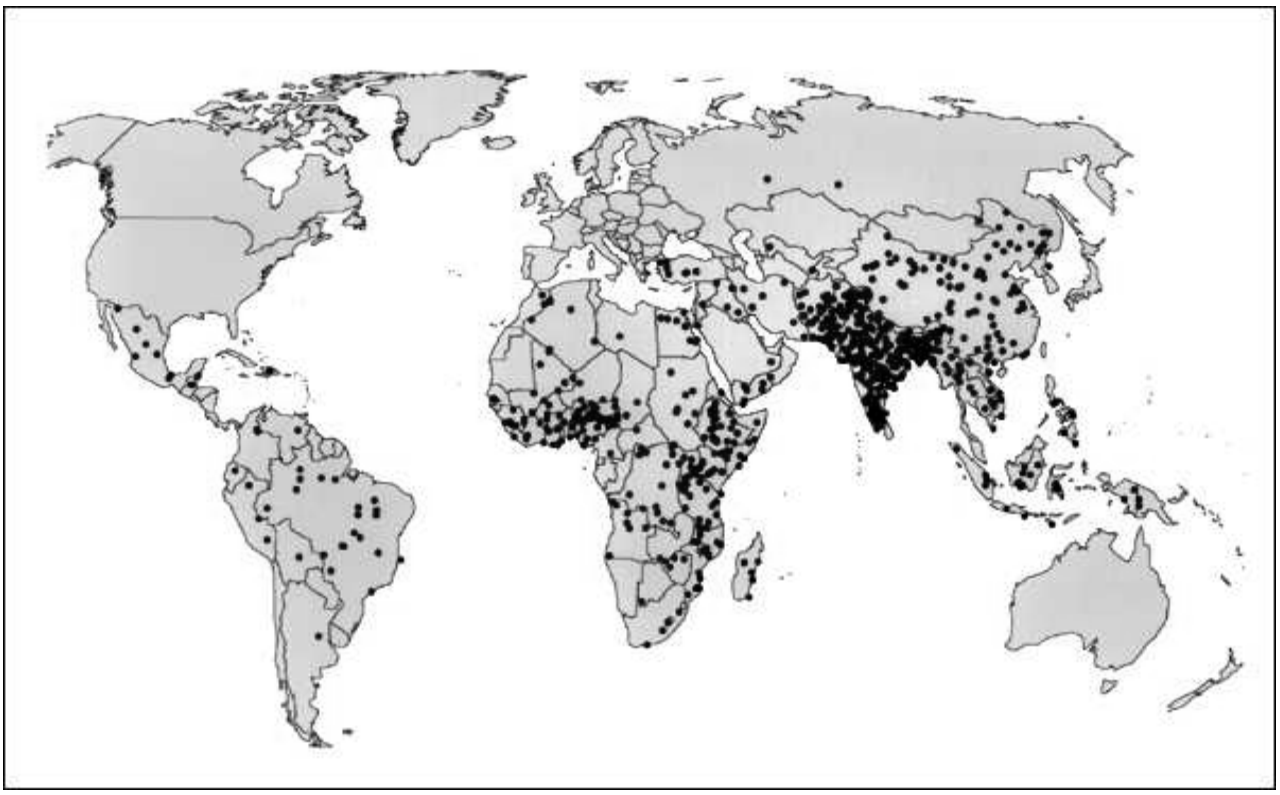

Figure (V)

Estimated global distribution of the 800,000 annual deaths caused by rotavirus diarrhea. Adapted from (Gentsch, et.al., 1996).

In our country , Iraq, there are some study that work on the same subject "HRV" , the first one done by (*Abbas, 1986*), by using ELISA technique , which revealed that the percent of the virus was nearly to 40.17%, the other one , was done by Al – (*Kelaby, 1999*), which revealed that the percent of the virus was nearly to 41.05%.

In ***Japan***, the rate of HRV type A was (65%) *(Imamura, et.al., 1992)*. Also, HRV was detected in Russia by using (EM), enzyme immunoassay & rotavirus neutralization in cell culture, in ratio (34.9 %) *(Khaustov,. et.al., 1989)*.

In Santiago, ***Chile***, they studied the transmission of rotavirus (RV) in 950 patients under 2 years of age hospitalized for diarrhea. Stool samples were collected every other day from all patients during their entire hospital stay. The ratio was 81%. It was concluded that the infants admitted shedding RV are the major source of nosocomially transmission and there was not a RV strain that was particularly transmissible *(Gaggero, et.al., 1992).*

In ***Bulgaria***, they tested by electron microscopy 7,530 samples from children admitted to different hospitals in Sofia between February 1981 and February 1986; from these, rotaviruses were found in 725 (9.6%) faecal samples *(Shindarov, et.al., 1988).* The prevalence of rotavirus infection in hospitalized ***Venezuelan*** children with gastroenteritis was studied during the period November 1975 to December 1976. Rotaviruses were found cause (41.3%) *(Torres, et.al., 1978).*

Excretion patterns of fecal viruses were studied in 51 rural ***Costa Rican*** children. They found presence of rotavirus, *(Simhon, 1985)*. At booth Hall Children's Hospital, ***Manchester, England***, they study episodes of rotavirus gastroenteritis diagnosed by electron or immune electron microscopy .They insured that HRV infection constitute (78.3%) *(Bates, et.al., 1993)*.

In ***Western Australia***, The children were under six years of age and came from all parts of Western Australia, Rotaviruses, were detected as well as the usual potentially pathogenic bacteria and parasites *(Schnagl, et.al., 1978)*.

In ***Barcelona, Spain***, 2000, the epidemiological system 'Alert' of the Public Health Institute in Tirane reported an outbreak of acute gastroenteritis with rotavirus. And ratio was (56.4%) *(Villena, et.al., 2003)*.

In ***Izmir, Turkey***, 920 children less than five years of age with acute gastroenteritis admitted to three pediatric hospitals in Izmir were studied. Rotavirus was identified in 39.8% of the children. *(Kurugol, et.al., 2003)*.

In ***Chicago, USA***. Rotavirus has been recognized for 30 years as the most common cause of infectious gastroenteritis in infants and young children *(Anderson, et.al., 2004)*. In ***Delhi, India*** rotavirus is the most common cause of diarrhea in children older than 3 months of age *(Cicirello, et.al.,1994)*.

In the Prince of Wales Hospital, Randwick, ***Australia*** there was a study to determine the isolation trends of common and emerging pathogens in children over a 12-month period. Pathogens were detected in (33%) samples, with rotavirus most common (40%) *(McIver, et.al., 2001)*.

In ***Brazil***, they detected rotavirus 557 feces samples from hospitalized children (0-5 years of age) concerning rotavirus which ranged to 29.2% *(Cardoso, et.al. , 1992)*.

In ***Botswana*** they detected, for the first time, of human rotavirus in stools of children .They collected 249 stool samples between 1999 and 2001 from children with diarrhoea .Group A rotavirus antigen was detected in 43 of 249 (17%) of the samples tested by enzyme-linked immunosorbent assay. *(Kasule, et.al., 2003)*.

In ***Hong Kong***, stool specimens were obtained from each of 371 neonates. Seventy neonates excreted human rotavirus (HRV) while they were in the hospital, *(Tam, et.al., 1990)*.

In ***Bangladeshi*** children, they detect HRV in 60% (*Bern, et.al.,1992*). In ***Shanghai, China***. Stool samples were collected from 1230 hospitalized children ,493 (40.1%) were group A rotavirus. No group B or group C rotavirus was found. *(Zeng, et.al., 2004).* In ***France***, they determined the rotavirus in healthcare-associated infection (HAI). The attack rate and the incidence of healthcare-associated acquired rotavirus infection were 6.6% during a winter outbreak *(Piednoir, et.al., 2003)*. In Children's Medical Centers and one general hospital in ***Tehran, Iran.*** Stool specimens from 704 children less than 5 years of age suffering from diarrhea were tested for the presence of rotaviruses by a monoclonal antibody-based enzyme immunoassay. Rotavirus antigen was detected in 15.3% of patients. *(Zarnani, et.al., 2004).* In India, one of every 250 children or about 100-150,000 children dies of rotavirus diarrhoea each year *(Broor, et.al., 2003).*

In ***Georgia, USA*** the primary site of rotavirus infection is the small intestine. Pathologic investigations of patients who died of rotavirus infection are limited to data from a few reported autopsies, and dehydration with electrolyte imbalance is believed to be the major cause of death. Several recent reports suggest that children who died during a rotavirus illness were viremic before death, because rotavirus was detected at several extra intestinal sites. They report 3 rotavirus-associated deaths among children, 2 of whom had evidence of rotavirus genome in extra intestinal tissues detected by use of novel molecular diagnostic methods *(Lynch, et.al., 2003).*

In Pune, ***Maharashtra*** State, ***India***, the stool samples of 426 children were investigated by electron microscopy and ELISA for the presence of viruses Rotavirus was detected in 28.6% *(Singh, et.al., 1989).*

Between March 1982 and March 1983 rotaviruses were detected in faecal specimens from 193 (19%) of 1020 ***Jamaican*** children under 5 years old with acute gastroenteritis. *(Dowe, et.al., 1988).*

Between December 1976 and January 1978, infection with rotavirus was detected by electron microscopy in 61 (25%) of 242 infants and young children hospitalized with acute gastroenteritis at two hospitals in ***Mexico City*** *(Espejo, et.al.,1977).* In ***Rouen, France***, the study assessed the epidemiologic characteristics of acute viral gastroenteritis in hospitalized children. Virologic tests revealed rotavirus in 17.3% of samples *(Marie-Cardine, et.al., 2002).*

Detection of rotavirus by electron microscopy was conducted on fecal specimens from 1,722 infants and young children with acute diarrhea. During a 41-month survey from April 1978 through December 1981 in ***Guayaquil, Ecuador***; 376 of these specimens (21.8%) were positive *(Suzuki, H. et.al.,1986)* and *(Suzuki, H. et.al., 1981)*

In ***China*** between April 1998 and April 2000, they screened for rotavirus in children. Rotavirus was detected in 41% *(Zhao-Yin, et.al., 2002).*

In Blantyre, ***Malawi*** , group A rotaviruses were detected by enzyme-linked immunosorbent assay in 35 (25%) of 142 specimens *(Cunliffe, et.al., 2002).* In ***Germany***, acute infantile gastroenteritis was caused by reoviruses. Rotavirus positive stool specimens were found in 46% of the material sent in by paediatric clinics *(Dennin, 1978).*

2-2-1-3-2 Age distribution;

Most studies referred that age of infected people with HRV was below 5 years. In Santiago, Chile, they studied the transmission of rotavirus (HRV) revealed that patients age was under 2 years of age *(Gaggero, et.al., 1992).*

Broor, et.al., 2003, found Rotaviruses cause an estimated 140 million cases of gastroenteritis and 800,000 deaths in children between the ages of 6 months to 2 years in developing countries.

In other study done by *(Torres, 1978)* Rotaviruses were readily detected in children of (0-5 years) who were susceptible to rotavirus infection. The frequency of infection was slightly higher in the age group 13-24 months, and significantly lower in children younger than 6 months old. Spain study revealed that the age group with the highest morbidity with HRV was 0-5 years (89.7%), followed by the 6-9 (6.2%) and 10-15 years age groups (4.1%) *(Villena, et.al., 2003).*

All these infection with HRV infected children belonged to the group A Rotavirus, Most children with rotavirus gastroenteritis (80.7%) were younger than two years of age and that was compatible with *Kurugol, 2003.* In ***Brazil***, they detected rotavirus from hospitalized children (0-5 years of age), Rotavirus were more prevalent amongst children whose age ranged between 1 to 11 months *(Cardoso, et.al., 1992).* In ***Bangladeshi*** children, HRV diarrheal episodes among children younger than 2 years of age caused dehydration *(Bern, et.al., 1992).* Iranian study showed that children less than 5 years of age were suffering from HRV diarrheal infection, and Infants between 6 and 12 months of age were most frequently affected. Also they revealed that Rotavirus could be regarded as a major etiologic agent of acute diarrhea in infants and children up to 5-years-old in Iran *(Zarnani, et.al., 2004).*

Kelkar, et.al., 1999, found out that the age group of infected children with HRV was 6-24 months , and it was the most susceptible age to infection. *Milaat , et.al., 1995,* in their study revealed that the mean age for all cases of HRV was 20.2 months and HRV cases showed a steady rise from the neonatal period onward, reaching a peak between 6-14 months, this result was certified by *(Dutta, et.al.,1990).*

2-2-1-3-3 Sex distribution;

The infection with HRV in female was higher than males. The ratio was 1.3:1, with 86 percent of reports for children in the 1-4 year age group *(Communicable Diseases Intelligence)*.

Rotavirus infection with ages ranging from 1 day to 16 years with a median of 13 months revealed that the male-to-female ratio was 1.2:1 *(Chiu, 2000).*

Rotavirus was most frequently detected in the age group 6-11 months (26.6%). and it was not detected at all above 24 months of age *(Dutta, 1990)*. Females accounted for a higher percentage of all HRV diarrheal cases *(Milaat, 1995)*, HRV disease was more predominant in females *(Kelkar, 1999).*

2-2-1-4 Transmission , Occurrence & Clinical features;

Rotaviruses induce a clinical illness characterized by vomiting, diarrhea, abdominal discomfort, fever, and dehydration (or a combination of some of these symptoms) that occurs primarily in infants and young children and may lead to hospitalization for rehydration therapy.

Fever and vomiting frequently precede the onset of diarrhea. Although milder gastroenteric illnesses that do not require hospitalization are also common, most studies of clinical manifestations of rotavirus-induced gastroenteritis rely on data from hospitalized patients. The duration of hospitalization ranges from 2 to 14 days with a mean of 4 days. The highest attack rate is usually among infants and young children 6 to 24 months old, and the next highest attack is in infants less than 6 months old. Normal neonates infected with rotavirus do not usually develop

clinical manifestations. Deaths from rotavirus gastroenteritis may occur from dehydration and electrolyte imbalance. In older children and adults, rotavirus gastroenteritis occurs infrequently despite the fact that subclinical infections are common *(Dutta, 1990)*.

Rotaviruses also induce chronic symptomatic diarrhea in immunodeficient children, with an occasional fatal outcome. In addition, rotavirus infections can be especially severe and sometimes fatal in individuals of any age who are immunosuppressed for bone marrow transplantation. Rotavirus infections have also been associated with necrotizing enterocolitis and hemorrhagic gastroenteritis in neonates in special-care units. Rotaviruses have also been found in stools of patients with a variety of other conditions, but the association appears to be temporal rather than etiologic *(Kapikian, 1996)*.

2-2-1-5 Pathogenesis & Immunology of HRV

Rotaviruses infect the mature absorptive villous epithelium of the upper two thirds of the small intestine. After replication in the upper small intestine, infectious particles are released into the intestinal lumen and undergo further replication in the distal areas of the small intestine. Infection is generally confined to the intestinal mucosa. Although rotaviruses can be found in the lamina propria and regional lymphatic, replication at these sites and systemic spread usually do not occur in immunocompetent persons *(Kapikian, 1996).*

Rotaviruses infect intestinal enterocytes, and the early events in infection are mediated by virus-epithelial cell interactions. Diarrhoea may be caused by several mechanisms including ***(i)*** malabsorption that occurs secondary to the destruction of enterocytes, ***(ii)*** villus ischaemia and activation of the enteric nervous system that may be evoked by release of a vasoactive agent from infected epithelial cells in the

absence of significant pathologic lesions or enterocyte damage, and ***(iii)*** intestinal secretion stimulated by the intracellular or extracellular action of the rotavirus non-structural protein, NSP4, a novel enterotoxin and secretory agonist with pleiotropic properties *(Estes, et,al. 2001)* .

Despite the superficial nature of mucosal infection, rotaviruses induce both local intestinal and systemic immune responses *(Ward, 1996 & Offit, 1996).*

Early animal studies suggested that the presence of rotavirus antibodies in the intestinal lumen (but not in the serum) was correlated with protection against disease. Oral administration of preparations containing rotavirus antibodies has successfully treated chronic rotavirus infection and diarrhea in immunocompromised children *(Ebina, et.al., 1983 & Hilpert, et.al., 1987).* In a randomized clinical trial, a single oral dose of gamma globulin reduced the duration of illness and the shedding of virus in infants hospitalized with rotavirus diarrhea. These observations indicate that intestinal immunity protects against rotavirus diarrhea and that the success of a rotavirus vaccine will depend, in part, upon its ability to induce mucosal immune responses.

In infants and young children, neutralizing antibodies directed primarily against the G serotype of the infecting strain (homotypic response) develop after primary infection with rotavirus *(Offit, 1996).*

Repeated rotavirus infections elicit both a homotypic and heterotypic (against strains with different G serotypes) antibody response. Protection against rotavirus diarrhea correlates with the serum antibody titers following natural infection of young children, and infected children are more protected against reinfection with similar rather than different G serotypes. A protective role of placentally

transferred maternal antibody among infants < 3 months of age has also been speculated since rotavirus disease is uncommon in this age group. However, serum neutralizing antibody responses among vaccine recipients have sometimes correlated poorly with the protection from disease; therefore, the exact role of serum antibody in protection against disease remains unclear *(Rennels, et.al., 1986).*

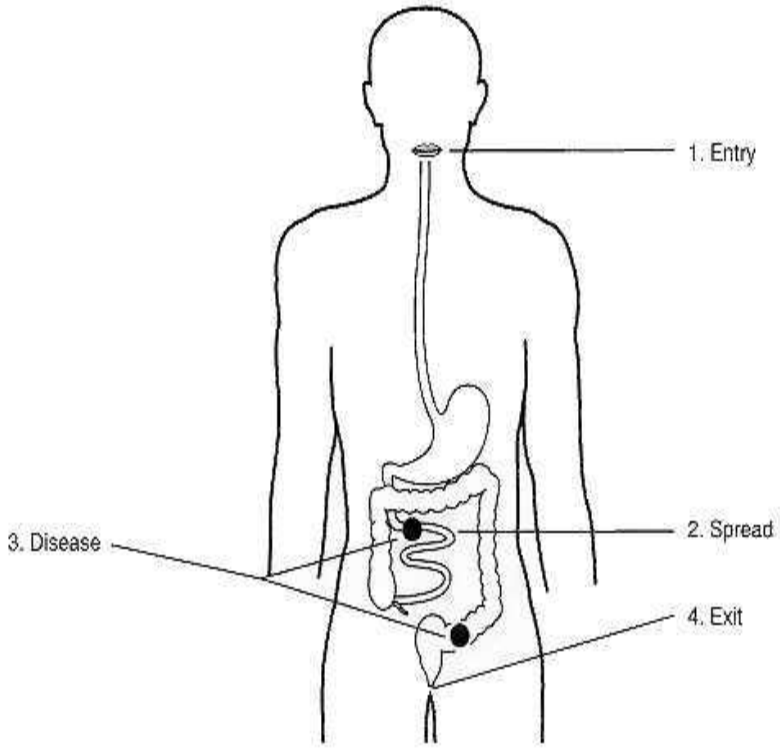

Figure (VI)

Pathogenesis of rotavirus infection.

Virus entry is via the oral route, with virus replication and pathology in proximal small intestine, resulting in diarrhea and/or vomiting (Kapikian, 1996).

2-2-1-6 Laboratory diagnosis of HRV

Rotaviruses are shed in large numbers during episodes of diarrhea, and usually are detectable by antigen enzyme immunoassays (EIA) up to 1 week after infection or for more than 30 days in immunocompromised patients. A recent study has shown that as many as 30% of immunocompetent infants with severe rotavirus diarrhea may have virus detectable by polymerase chain reaction (PCR) for more than 25 days after hospital admission *(Nakagomi, et. al., 1991)*.

In Russia by using (EM), enzyme immunoassay & rotavirus neutralization in cell culture, they detected that sporadic gastroenteritis cases were caused by rotavirus *(Khaustov, et.al., 1989).*

Because the clinical manifestations of rotavirus gastroenteritis are not distinct enough to permit a specific diagnosis, specimens must be examined in the laboratory. This is necessary even in temperate climates during the cooler months of the year when more than 50 percent of hospitalizations due to diarrhea may be associated with rotavirus *(Kapikian, 1996).*

Laboratory diagnosis of rotavirus infections requires identifying the virus in feces or rectal swab specimens or demonstrating a fourfold or greater increase in antibody to a rotavirus antigen between acute- and convalescent-phase sera. Numerous methods to detect rotavirus in stool and rectal swab specimens have been described. they include:

electron microscopy, radioimmunoassay, counterimmunoelectro-osmophoresis, centrifuging of clinical material onto tissue culture cells followed by immunofluorescence, inoculation of tissue cultures, latex agglutination, reverse passive hemagglutination assay, polyacrylamide gel

electrophoresis, dot hybridization, polymerase chain reaction, and *enzyme-linked immunosorbent assay (ELISA) (Carlson,et.al., 1978).*

ELISA has now become the mainstay in most laboratories, because it is practical, rapid, and efficient and does not require sophisticated laboratory equipment. Commercial ELISA kits are now available: certain ones should be used with caution if confirmatory reagents (i.e., positive and negative controls) are not included in the kit since nonspecific reactions may yield false-positive results. When the number of specimens is limited, the most rapid method of rotavirus diagnosis in a hospital setting is obtained by examination of a stool specimen by negative-stain electron microscopy. This can be accomplished in a few minutes. Non-group A human rotaviruses that do not share the common group antigen cannot be detected by conventional serologic assays; however, they can be detected by electron microscopy because they are morphologically identical to conventional rotaviruses *(Kapikian & Chanock, 1990).*

Serologic evidence of rotavirus infection can be detected by various techniques, such as ELISA immunofluorescence, neutralization, and complement fixation *(Kapikian, 1996).*

2-2-1-7 Vaccination

Monovalent "Jennerian" Vaccines

Initial development of rotavirus vaccines was based on the Jennerian approach, which involved the use of a live, attenuated, antigenically related virus derived from a nonhuman host *(Kapikian, 1994.).* This approach was prompted by studies indicating that animal and human rotaviruses shared a common group antigen and

that experimental animals immunized with animal strains of rotavirus had a significantly lower risk for illness and viral shedding when subsequently challenged with human rotaviruses. Furthermore, neutralizing antibodies to human rotavirus serotypes in the animal models indicated the potential for cross-protection.

Bovine Vaccines

The first two Jennerian vaccines were developed with bovine rotavirus strains RIT4237 and WC3. The WC3 strain was passage in cell culture less than RIT4237 and was developed because of concern that excessive passaging of the RIT vaccine might cause over attenuation and diminished efficacy. RIT4237 and WC3 were non reactogenic and immunogenic when administered to infants of 2 to 18 months of age. However, the protection conferred by both vaccines varied greatly in efficacy studies, 0% to 76% against any rotavirus diarrhea and 0% to 100% against severe disease *(Vesikari, et.al., 1984 , Vesikari , et.al., 1985. & Rennels, et.al., 1986.).* A well-defined correlate of protection was not identified, and reasons for the variable efficacy were unknown, although late age at vaccination, timing of vaccination with respect to the onset of the rotavirus season, and variations in the strength and number of doses of the vaccine were proposed as contributing factors. Both vaccines performed less well in developing than in industrialized countries, possibly because of interference by other enteropathogens or inadequate surveillance during follow-up.

Rhesus Vaccine

The third Jennerian vaccine was developed with rhesus rotavirus strain MMU18006, which shares neutralization specificity with human rotavirus G3 strains. Besides sharing antigenic specificity with an epidemiologically important

human rotavirus serotype, MMU18006 was suitable for vaccine development because it grew efficiently in cell culture. As in the bovine rotavirus-based vaccines, MMU18006 was safe and immunogenic, although in some trials, one third of infants became febrile for 3 to 4 days after vaccination. The reactogenicity of MMU18006 was particularly high in two studies in Finland and Sweden in which 64% and 79% of infants, respectively, became febrile. Most children with febrile responses were >5 months of age; lack of passively transferred maternal antibody might have contributed to the high reactogenicity of the vaccine. As in the RIT4237 and WC3 vaccines, the protective efficacy of MMU18006 in field trials was quite variable, 0% to 60% against any rotavirus diarrhea and 0% to 85% against severe rotavirus diarrhea *(Rennels, M.B. Losonsky, G.A. et. al., 1986. , Gothefors, et. al., 1989. Madore , et. al.,1992).*

Reassortant "Modified Jennerian" Vaccines

The greatest efficacy of MMU18006 was observed in a Venezuelan trial in which the rotavirus strain circulating in the community (G3) was the same serotype as the vaccine strain, which suggested that serotype-specific immunity against each of the epidemiologically important strains of human rotaviruses may be required for maximum protection. Similar observations in vaccine challenge cross-protection studies in animals initiated the development of vaccines that used a modified Jennerian approach in which animal-human reassortants expressing VP7 proteins of serotypes 1 through 4 were used as the immunogens *(Madore , et. al.,1992).*

Rhesus-Human Reassortant Vaccines

Rhesus-human reassortants were generated by co-infecting cell cultures with rhesus rotavirus (RRV) strain MMU18006 (G serotype 3) and human rotavirus strains D (G serotype 1), DS-1 (G serotype 2), and ST3 (G serotype 4). Selection

pressure (induced by the addition of neutralizing antibody to VP7 of RRV) produced reassortant strains D x RRV, DS-1 x RRV, and ST3 x RRV, each of which possessed the VP7 gene from HRV serotype 1, 2, or 4 and the other 10 genes from RRV *(Kapikian, et.al., 1996).* Because vaccines made from the individual reassortants were safe and immunogenic, RRV-TV was developed incorporating each of the three reassortants and MMU18006 to provide coverage against the four common VP7 serotypes of rotavirus.

Chapter Three

MATERIALS & METHODS

Chapter Three

Material & Methods

a. Materials:

1- Specimens

In this study, we used 315 stool specimens collected from infants below 24 months with diarrheal case in Babylon maternity and children hospital, in clean container, between November/2003 to May /2004.

2- Culture Media ;

The fowling culture media were used according to the requirements;

- *Nutrient agar medium* *(Oxiod-U.K.)*

To isolate, cultivate and to save bacterial strains.

- *MacConkey's agar medium* *(Mast-U.K.)*

To isolate Gram-negative bacilli and to differentiate lactose fermented from non lactose fermented bacteria.

- *Salmonella – Shigella agar medium* *(Mast-U.K.)*

For secondary specific isolation of Salmonella and Shigella species and to differentiate lactose fermented from non lactose fermented bacteria.

- ***Tetrathionate broth medium*** *(Mast-U.K.)*

This medium was prepared according to Manufacturing Company to distribute the broth in test tubes "10 ml for each ". Heating to boiling degree, then 0.2 ml iodine solution was added to for each tube. This medium was used as a selection and enrichment medium for secondary isolation of pathogenic bacteria especially Salmonella & Shigella spp.

- ***Blood agar medium*** *(Mast-U.K.)*

Blood agar base was prepared according to manufacturing company, it was autoclaved at (121^{o}C \ for 15 minutes), then cooled to 50 ^{o}C, and % of human blood were added. This medium was used to cultivate bacterial strains and to see their ability of blood haemolysis.

- ***Simmon's Citrate medium*** *(Oxiod-U.K.)*

This medium was used to discover the bacterial ability to exhaust Citrate as only source of carbon.

- ***Christenen's urea medium*** *(Biolife – Italy)*

Basal medium was prepared according to manufacturing company, it was autoclaved at (121^{o}C \ for 15 minutes), cooled to 50 ^{o}C, then added to 1 litter of the medium fifty ml of 20% urea (50 ml urea to 950ml media) solution "autoclaved" from (Mast-U.K.) company and this medium insured that their pH was (6.8 – 6.9). This medium was used for detecting bacterial ability to produce Urease Enzyme.

- ***(MR-VP) medium*** *(Oxiod-U.K.)*

This medium was used to detect the ability of bacteria to complete and make partial analysis of glucose.

- ***Peptone water media*** *(Mast-U.K.)*

This medium was used to detect the ability of bacteria to produce Indole.

- ***Motility medium***

This medium was prepared by adding 0.4 gm of nutrient agar to the nutrient broth , then it was distributed in tubes , autoclaved at (121^{o}C \ for 15 minutes), it was use to detect the ability of bacterial motility .

- ***Kligler iron agar medium*** *(Biolife – Italy)*

This medium was used to detect the bacteria ability to produce (H2S), fermented sugars with producing acid and gas.

- ***Gelatin agar medium***

This medium was prepared by adding (4.4 gm) of gelatin (Oxiod-U.K. Company) to nutrient agar medium. It was used to detect bacteria ability to make hydrolysis gelatin.

- ***Eosin methylen blue (EMB) medium***

This medium was used to cultivate specific Enterobacteriaceae (*E. coli & Klebsiella*), which produced special color "Green metallic shine for *E. coli*".

- ***Brielent green***

This medium was used to detect the *Salmonella spp.*

3- Reagents & solutions ;

- ***Methyl red reagent***

This reagent was prepared by dissolving 0.1gm of methyl red reagent in 300 ml of 95% ethyl alcohol, filling the volume to 500 ml with distal water, and using this indicator for complete glucose hydrolysis.

- ***Voges – Proskauer reagent***

It contained:

1. (α- naphthol); indicator; which was prepared by dissolving 5 gm of α-naphthol in 100 ml of absolute ethylic alcohol.

2. (NaOH solution, sodium hydroxide solution); prepared by dissolving 40 gm of NaOH in 100 ml of distil water; it was used for partial glucose hydrolysis. *(Macfaddin, 1979).*

➢ *Kovac's reagent*

This reagent was prepared by dissolving 5 gm from (Dimethylamin Benzylaldehyde) in 75 ml amyl alcohol, and 35 ml of concentrate HCL acid which was added .This reagent was used for the detection of Indole *(Macfaddin, 1979).*

➢ *Catalase reagent*

This reagent was prepared in 30 % concentration using H_2O_2 as dilute and stored in dark bottle, it was used to study bacterial ability to produce Catalase. *(Macfaddin, 1979).*

Oxidase reagent

This reagent was prepared by dissolving 1 gm (Tetra-Methyl-Phenylnene-Diamine-Dihydrochloride) in 100 ml distil water. *(Finegold & Baron, 1986).*

➢ *Frazier's reagent*

This reagent was prepared by dissolving 5 gm $HgCl_2$ in 20 ml of concentrated HCL (98%), with the addition of 100 ml distil water. It was used to detect the ability of bacteria to analyses gelatin. *(Bisson & Cabili, 1979).*

➢ *Iodine solution*

This reagent was prepared by dissolving 6 gm iodine crystals and 5 gm of potassium iodine in 20 ml distil water, then heating the solution with shaking to dissolve iodine crystals, from this solution 0.2 ml was added for each 10 ml of tetrathionate broth medium before cultivation. *(Finegold & Baron, 1986).*

➢ *Dilution solution*

Physiological saline solution (PSS) was prepared by dissolving 9 gm of NaCl in 1000 ml distil water, & distributing it in tubes and autoclaving at (121^{o}C \ for 15 minutes), then use it for isolation, as diluent's and for direct examination of stool specimens.

➢ *Gram stain solutions*

The solutions were prepared according to the required microbiological methods *(Talib, 1996).*

* *Methyl Violet Solution*;

This solution was prepared by dissolving 0.5 gm of Methyl Violet in 100 ml distil water.

* *Iodine solution;*

This solution was prepared by crushing 1 gm of iodine crystals and 2 gm Potassium iodine (KI) , dissolving them in 20 ml distil water, and then completing the volume to 100 ml and storing it in a dark bottle . One ml of this solution was added to 5 ml distil water to prepare the working solution.

* Decolorizer solution;

Acetone was used as decolorizer in 95 % concentration.

* Safranine solution;

This solution was prepared by crushing 0.5 gm of the Safranine stain in 10 ml of alcohol then completing to 100 ml with distil water and stored it in dark bottle.

3. *Serological diagnostic kits*

• *Rotavirus diagnostic kit;*

Slidex Rota kits which were produced by (Bio-Kit) were used to diagnose Rotavirus in stool specimens, the kit contained:

I- Reagent 1 (R1)

Represented by latex molecules which was sensitive with monoclonal antibodies to connected with capsid antigens belong to Rotavirus.

II- Reagent 2 (R2)

Negative control latex.

III- Reagent 3 (R3)

Positive control latex, which was antigenic suspension of rotavirus.

IV- Reagent 4 (R4)

Representing the buffer (pH=7.2)

V- Disposable test cards

Ten cards, every card was divided to six black fields which were called (reaction field).

- ***Enteropathgenic E. coli diagnostic kits (EPEC)***

Specific agglutination sera which was produced by (Behring - Germany) was used to diagnosis pathogenic group of EPEC which caused disease for stomach & intestine (EPEC = Entero - Pathogenic - *Escherichia Coli*), the kit include the following antisera; Anti 026, Anti 044, Anti 0158, Anti 0144, Anti 0125, Anti 0124

- ***Salmonella group diagnostic kits***

Represented by two groups of antisera Somatic & Flagellate antisera (polyvalent O & Polyvalent H Agglutinating sera) produced by (Murex-U.K.)

- ***Shigella flexneri polyvalent antisera,***

  Produced by (Murex-U.K.)

b. Methods:

I- Collection of data:-

We got the information from the parents of 315 infants (below 2 years) consulting hospital (outpatients and inpatients) who had diarrhea and were admitted to Babylon maternity & children hospital between (novembar/2003 To May\2004) by questionnaire papers including information about;

** Name of the patient (infant).*

** Age, must be below 2 years.*

** Sex.*

** Mother's age.*

** Mother's education.*

** Residence, Rural or Urban.*

** Vaccine received.*

** Type of feeding.*

** type of water supply.*

** Family income.*

** No. of sibling.*

** Previous diarrhoea.*

** Clinical features, like: Fever, Vomiting, Dehydration, Abdominal colic.*

**Duration of diarrhoea.*

** Type of diarrhoea.*

** Antibiotic treatment, the patient must not have taken any antibiotics to avoid the negative false results.*

II- Collection of specimens:-

We collected 315 stool specimens from infants suffering from gastroenteritis (one sample for each case). We collect (5-8 gm.) of stool in clean sterile container used for this purpose, then we take the specimens at once to the laboratory to do the necessary tests, like;

- test for Rotavirus.
- test for bacteria (Stool cultures).
- test for parasites (Direct stool examination).

III- Laboratory tests:-

The laboratory diagnostic tests on the collected stool specimens included:

1) Diagnosis of Human Rotavirus "HRV"

Rotavirus diagnostic kit, produced by (Bio-kit) was used to diagnose the presence of rotavirus in the stool specimens collected as follow;

a. Two ml of buffer (R4) were added to stool specimens in the tube which contained one gm of stool.
b. The mixture was shaken with vortex for (2 min.).
c. The solution was left on bench for (5 min) to stand down.
d. We centrifuged the solution (800 rpm\ 10 min in room temperature), the supernatant was aspirated and the precipitate was rejected.
e. The supernatant was taken to do the test by using (Precision micropipette), we used two field on cards to do the test for each specimens, we mixed 0.05 ml of supernatant with equivalent volume of reagent (R1) in the first field, and in the second field we mixed 0.05 ml of supernatant with equivalent volume of negative control (R2), then mixed the solution with different woody stick.

***The result*;**

After (2 min.) from suspension, the agglutination appeared in the first field (specimen+ Buffer + R1) which meant positive result (presence of virus), Otherwise, the milky color & no agglutination meant negative result (absence of virus).

2) Direct examination of parasites

We proceeded the direct stool; and examination on the patient specimens, this test including wet preparation

Wet preparation

0.5 gm of stool specimen was placed on slides and mixed with two drops of (Physiological saline) & (Lugel's iodine), then covered with cover slide, and examined with light microscope using (10X & 40X power), to determine the presence of cyst or other forms of parasites.

3) Bacteriological examinations

1)Stool Culture:

a. 0.2 gm of stool specimen was diluted with 2 ml of physiological (pH=7.3) final diluting equaled 1:10.
b. From each diluted specimen a loop full was streaked on Blood agar & MacConkey's agar, cultivate the enriched media with 1 gm of stool specimens. Incubate the cultures at (37^{o}C) for (24 h.) for primary isolation. Take loop full from Tetrationate broth "cultivate with stool specimen & incubate for 24 h." and cultivated it by streaking method on Shigella – Salmonella agar, incubate the cultures at (37^{o}C) for (24 h.).

The specific identification scheme were performed according to Bergy's Manual for each pathogenic bacteria, and as follow;

I. The colonies that ferment lactose, which grow on MacConkey's agar for E. coli isolation.

II. The colonies that do not ferment lactose which grow on MacConkey's agar with "Yellow Pale Colonies" which grow on (S-S agar) for Shigella & Salmonella isolation.

III. The colonies that do not ferment lactose which grow on MacConkey's agar and appear with Beta - haemolysis on blood agar for Aeromonas hydrophila isolation.

After selecting the bacterial colonies depending on morphological appearance & staining with Gram stain, the selected colonies were processed for the following biochemical tests:

❖ *Biochemical tests*

a. Oxidase test

This was done by streaking the nutrient agar medium with the selected bacterial colonies which are incubated at (37^{o}C) for (24 h.) then adding to the growing colonies few drops of Oxidase reagent, when the colonies were colored with violet color the result positive *(Macfaddin, 1979).*

b. Catalase test

By streaking the nutrient agar medium with the selected bacterial colonies which were incubated at (37^{o}C) for (24 h.) then transferring the growth by the loop to a clean slide & adding one drop of (30% H_2O_2). The result was positive when the gas bubbles appear.*(Macfaddin, 1979).*

c. Indole production test

Cultivate the tubes that contain the peptone water with the selected bacterial colonies which were incubated at (37^{o}C) for (24 h.) .Then few drops of Kovac's reagent were added . The appearance of pink ring color meant positive result.*(Macfaddin, 1979).*

d. Methyl red test

Cultivate the tubes which contains the (MR-VP broth) with the selected bacterial colonies and incubated at (37^{o}C) for (24 h.) then the result was reading by adding (5 drops) of methyl red reagent. The appearance and observation appear & observed of red color meant a positive result and a complete analysis of glucose. *(Macfaddin, 1979).*

e. Voges – Proskauer test

The tubes that contained the (MR-VP broth) were seeded with the specific bacterial culture and were incubated at (37^{o}C) for (48 h.) then we read the result by adding (0.6 ml of α- naphthol reagent) and (0.2 ml of 40% NaOH solution) , appear once of red color after (15 min.) means positive result due to partial analysis of glucose, which produce acetone or (Acetyl methyl-carbinol) .*(Macfaddin, 1979).*

f. Hydrogen sulfide production test

The tubes that contained a slant of (Kligler iron agar medium) were seeded with the specific bacterial culture by sticking to the bottom of the medium & striking on the slant , incubating it at (37^{o}C) for (24h.) then reading the result, appear once of black color in the bottom means positive result. *(Macfaddin, 1979).*

g. Urease production

The tubes that contained a slant of (Urea agar) were seeded with the specific bacterial culture which was incubated at (37^{o}C) for (24 h.) then read the result, appear once of red color meant positive result. *(Macfaddin, 1979).*

h. Motility test

The tubes that contained the medium specific for motility by the sticking with the specific bacterial culture which was incubated at (37^{o}C) for (24h.) the distribution of growth outer of sticking region meant the positive result. *(Macfaddin, 1979).*

I. Gelatin hydrolysis test

The gelatin medium plates were seeded with the specific bacterial inoculums by streaking method, incubated at (22 oC) for (2-3 days), the plates were treated with Frazier's reagent for (5-10 min.), the positive result was the pale colonies.*(Macfaddin, 1979).*

j. Citrate utilize test

The citrate agar tubes which contained the slant of (citrate agar) were stuck to the bottom and striking on the slant. Then incubate it with (37 oC) to (24-48 h.) then the change of the medium from green to blue color meant positive result.*(Macfaddin, 1979).*

❖ *Serological tests*

A specific anti sera Produced by (Behring- Germany), was used according the manufacture manuals to diagnose pathogenic group for *E. coli* which caused

disease for stomach & intestine (EPEC = Entero-Pathogenic- *Escherichia - Coli*), which was represented by antisera;

Anti 026, Anti 044, Anti 0158, Anti 0144, Anti 0125, Anti 0124

For *Salmonella*; Somatic & Flagellate antisera (polyvalent O & Polyvalent H Agglutinating sera) produced by (Murex-U.K.) were used, according to the manufacture manuals.

For *Shigella*; we used *Shigella flexneri* polyvalent antisera, produced by (Murex-U.K.), according to the manufacture manuals.

While other bacteria were diagnosed depending on the Biochemical tests only.

We use the morphological and staining characteristic to determine the specific bacterial genus.

Statistical analysis:

We used the student's ***Chi-Square test*** and ***Hypothesis test*** of the difference between two-proportions.

الخلاصة

إن من أهم أسباب الامراضية والوفيات بين الأطفال الرضع بعمر عامين فاقل في اغلب بلدان العالم هو الإسهال الحاد. وضعت هذه الدراسة البحثية لتقدير مدى انتشار الممرضات المعوية المسببة للإسهال الحاد ومقدار وبائيتها وانتشارها وعلاقتها مع العمر، الجنس، نوع التغذية، الإقامة، الحالة التعليمية للام وبمساعدة الكادر الطبي في مستشفى بابل للولادة والأطفال.

أخضعت 315 عينة خروج لمرضى الإسهال الحاد ، و شملت 225 من المرضى الراقدين و 90 عينة من المرضى الخارجيين للفحوصات التشخيصية الخاصة بالممرضات ذات الاحتمالية بارتباطها بالمرض، ومنها الفيروس الدوار المصيب للإنسان (*Human Rotavirus*) والذي تم التقصي عنة باستخدام تقنية اللاتكس (*Latex agglutination*) ، كما استخدمت الاختبارات التشخيصية و القياسية الخاصة بالبكتريا و الطفيليات ، وقد أظهرت نتائج الفحوص النتائج التالية:

1. الممرضات المعوية المسببة للإسهال الحاد (فيروس ، بكتريا، طفيليات) شكلت 219 (75.5%) حالة.
2. الإصابات المشتركة المتسببة عن الممرضات المعوية شكلت 30 (13.6%).
3. المسببات الأكثر شيوعا للإسهال الحاد هي الفيروس الدوار 41.8% ، الإصابات البكتيرية مختلفة حسب نوع البكتريا؛ EPEC شكلت 4.65%، *Salmonella spp.* 13.17%، *Shigella spp.* 7.75%، *Proteus* 3.87%، *Klebsiella* 0.775% ، *Enterobacter* 0.775%، *Giardia lamblia* 5.42%، *Entamoeba histolytica* 12.40 %، *Candida* 9.30%.
4. معظم حالات الإسهال الحاد تم تشخيصها في المرضى الراقدين في المستشفى وتعد الممرضات (*Rotavirus, Entamoeba histolytica, EPEC, Salmonella, Shigella*) هي من أكثر المسببات لحالات الرقود. وهذه الممرضات تسبب حالة الجفاف و التي تستدعي الرقود في المستشفى للعلاج.

5. نسبة الإصابة الفيروسية والبكتيرية تشكل 36.7% وهي الأعلى نسبة بين حالات الإصابات المشتركة، نسبة الإصابة الفيروسية و الطفيلية تشكل 20% بينما الإصابات البكتيرية الطفيلية تشكل 26.6%، والإصابات البكتيرية المشتركة تشكل 13.3% بينما الإصابات الطفيلية المشتركة تشكل 3.3%.
6. معظم الإصابات بالإسهال الحاد تحدث في الأطفال الرضع بعمر(1-12) شهرا، هذا العمر يعتبر مرحلة حرجة من عمر الطفل تستدعي اهتماما من الوالدين.
7. هنالك فرق في حالات الإصابة بالإسهال الحاد بين كل من الذكور والإناث.
8. نوع الرضاعة و التغذية مؤثر على حالات الإصابة بالفيروس الدوار، الأطفال الفطيمين هم أكثر عرضة للإصابة بالفيروس الدوار من الرضع الذين يعتمدون رضاعة طبيعية.
9. هنالك فرق معنوي بين الرضع الساكنين للمناطق الريفية من الذين يسكنون المدينة في حالات الإصابة بالفيروس الدوار.

Thesis Contents

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Chapter Four

RESULTS

Results

From the 315 diarrhea specimens collected during the period of this study, only 219 (75.5%) were identified as in table (1), while (24.5%) were not identified their causative agent as shown in figure (1).

Table (1) shows that viral infection had caused 71 (32.4%) acute diarrhea, while the enteric bacterial infection constituted 67 (30.5%), and the enteric parasite (Protozoal) caused 51 (23.2%) and there is non-significant difference between pathogens except mixed infection is significance from the others $p<0.05$.

Table (1)

Distribution of pathogens (in groups) that cause infants diarrhea

Group	*No. of cases*	*%*
Viral infection	*71*	*32.4*
Bacterial infections	*67*	*30.5*
Parasitic infections	*51*	*23.2*
Mixed infections	*30*	*13.6*
Total	*219*	*99.7*

The results in table (2) reveal that the percentage of HRV “Human Rotavirus” cases in inpatients “Hospitalized” was higher than outpatients who were treated in the out clinical. The ratio was 54 (24%) in the Hospitalized, while it was 17 (18.9%) for outpatients and there is significant difference between hospitalized and outpatients $p<0.05$.

Table (2)

Incidence of HRV in infants with acute Gastroenteritis (GIT).

Gastroenteritis patients	*No. of examinations*	*No. of HRV positive (%)*
Hospitalized group	*225*	*54 (24%)*
Outpatient group	*90*	*17 (18.9%)*
Total	*315*	*71 (22.53)*

Table (3) shows that viral infections constituted 37.55% from all acute diarrheal cases and it was represented by HRV. It is considered the major cause of acute diarrhea in infants. Enteric Bacterial infection was different according to the type of bacteria; Enteropathogenic *Escherichia coli* (EPEC) constitute 13.2%, *Salmonella spp*. 11.11%, *Shigella spp*. 5.29%, *Proteus* 3.17%, *Klebsiella* 1.58% and *Enterobacter* 1.05%.

Enteric parasitic infection included two major species *Giardia lamblia* 5.29%, *Entamoeba histolytica* 12.1% and *Candida* constitute 9.5%

Table (3)

Percentage of each pathogens causing acute infantile diarrhea

Pathogens	*No. of cases*	*%*
HRV.	*71*	*37.5*
EPEC	*25*	*13.20*
Salmonella spp.	*21*	*11.11*
Shigella spp.	*10*	*5.29*
Proteus	*6*	*3.17*
Klebsiella	*3*	*1.58*
Enterobacter	*2*	*1.05*
Candida	*18*	*9.50*
Giardia lamblia	*10*	*5.29*
Entamoeba histolytica	*23*	*12.10*
Total	*189*	*99.79*

In table (4), the percentage of the HRV infection and Bacterial infection constitute 36.7% and it was the highest ratio among the mixed infection, other types of mixed infection like HRV infection and parasitic infection constitute 20%. While the Bacterial infection associated with Parasitic infection formed 26.6%, mixed bacterial infection formed 13.3%, and there is non significance between the type of mixed infections.

Table (4)

Distribution of mixed infections for multiple pathogens caused acute infantile diarrhea

MIXED INFECTIONS	*TYPE OF MIXED INFECTIONS*	*NO.*	*SUM. OF EVERY TYPE MIXED INFECTIONS*	*%*
HRV + B.	*HRV.+ EPEC*	*8*	*11*	*36.7*
	HRV.+ Salmonella spp.	*1*		
	HRV.+ Shigella spp.	*0*		
	HRV.+ Proteus	*1*		
	HRV.+ Klebsiella	*1*		
	HRV.+ Enterobacter	*0*		
B. + P.	*EPEC+ Entamoeba histolytica*	*2*	*8*	*26.6*
	EPEC+ Giardia lamblia	*1*		
	Enterobacter + Entamoeba histolytica	*1*		
	EPEC+ Candida	*4*		
HRV + P.	*HRV + Entamoeba histolytica*	*4*	*6*	*20*
	HRV + Giardia lamblia	*1*		
	HRV + Candida	*1*		
B.+ B.	*EPEC+ Salmonella spp.*	*3*	*4*	*13.3*
	EPEC+ Klebsiella	*1*		
P. + F.	*Giardia lamblia+ Candida*	*1*	*1*	*3.3*
Total			*30*	*100*

*HRV. \Human Rotavirus *B. \Bacteria *P. \Parasite*

Table (5) reveals that the ratio of each pathogen that caused acute diarrhea alone without other causative agents. We see that HRV 41.08%, Enteric Bacterial infection was different according to the type of bacteria; EPEC constituted 4.65%, *S. spp*. 13.17%, *Sh*. *Spp*. 7.75%, *Proteus* 3.87%, *Klebsiella* 0.775% and *Enterobacter* 0.775%. Enteric parasitic infection included two major species *G. lamblia* 5.42%, *E. histolytica* 12.40%. In addition, *Candida* constituted 9.30% .

Table (5)

Percentage of each pathogen (a lone) causing acute infantile diarrhea

PATHOGENS	*NO. OF CASES*	*%*
HRV.	*54*	*41.08*
EPEC	*6*	*4.65*
Salmonella spp.	*17*	*13.17*
Shigella spp.	*10*	*7.75*
Proteus	*5*	*3.87*
Klebsiella	*1*	*0.775*
Enterobacter	*1*	*0.775*
Candida	*12*	*9.30*
Giardia lamblia	*7*	*5.42*
Entamoeba histolytica	*16*	*12.40*
Total	*129*	*99.19*

Table (6) shows that the ratio of infection with acute diarrhea in infants depending on the type of the pathogens was statistically higher in the hospitalized infants than outpatient's infants in all types of pathogens except the *Klebsiella* .

Table (6)

Enteropathogens detected in infants with acute diarrhea.

PATHOGENS	*HOSPITALIZED GROUP (%)*	*OUTPATIENT GROUP (%)*	*TOTAL*
HRV.	*56 (78.8%)*	*15 (21.12%)*	*71*
EPEC	*21 (84%)*	*4 (16%)*	*25*
Salmonella spp.	*16 (76.1%)*	*5 (23.8%)*	*21*
Shigella spp.	*7 (70%)*	*3 (30%)*	*10*
Proteus	*6 (100%)*	*0 (0%)*	*6*
Klebsiella	*1 (33.33%)*	*2 (66.66%)*	*3*
Enterobacter	*2 (100%)*	*0 (0%)*	*2*
Candida	*14 (77.77%)*	*4 (22.22%)*	*18*
Giardia lamblia	*6 (60 %)*	*4 (40%)*	*10*
Entamoeba histolytica	*18 (78.2%)*	*5 (21.7%)*	*23*

Table (7) reveal that there was non significant difference in HRV infection between males and females p> 0.05, HRV infection in males 24.69% was higher than females 20.13%, while there is non significance between inpatients and out patients depending on sex p> 0.05.

Table (7)

Sex distribution in infants with acute diarrhea, %

PATIENTS GROUP	*SEX DISTRIBUTION*				*P VALUE*
	Females		*Males*		
	No. of examinations	***No. of HRV positive (%)***	***No. of examinations***	***No. of HRV positive (%)***	
HOSPITALIZED GROUP	*123*	*25 (20.3%)*	*130*	*31 (23.84%)*	*>0.05 n.s*
OUTPATIENT GROUP	*26*	*5 (19.23%)*	*36*	*10 (38.46%)*	*>0.05 n.s*
TOTAL	*149*	*30 (20.13%)*	*166*	*41 (24.69%)*	
P value	*>0.05 n.s*				

n.s Not significance

Table (8) Shows there is significant different in HRV infection between the rural patients and urban patients in each patients group (p< 0.01) .Although, there is non significant different between the tow group of patients (Rural and Urban) according to the admission (Hospitalized and Outpatients) p>0.05 .

Table (8)

Residential distribution in infants with acute diarrhea,%

Patients group	*Residential distribution*				*P value*
	Urban		*Rural*		
	No. of examinations	***No. of HRV positive (%)***	***No. of examinations***	***No. of HRV positive (%)***	
Hospitalized group	*105*	*15 (14.28%)*	*147*	*41 (30.04%)*	*<0.01 **
Outpatient group	*22*	*4 (18.18%)*	*41*	*11 (26.82%)*	*<0.01 **
Total	*127*	*19 (14.96%)*	*188*	*53 (28.19%)*	
P value	*>0.05 n.s*				

*** Highly significant*

Table (9) reveals the percentage of HRV infection among infants with consumes water. The results shows that HRV patient in infants using river water was higher than in those who used tap water 46.6%, 26.24% respectively p<0.05, while there is highly significance difference between Boiled and Unboiled p<0.01

Table (9)

Water consumed by infants including the study

Type of water		*No. of examination*	*HRV positive (%)*	*P value*	*P value Between Tap & River*
Tap	*Boiled*	*51*	*2 (3.9)*	*<0.01**	*P<0.05**
	Unboiled	*94*	*21 (22.34)*		
River	*Boiled*	*60*	*4 (6.6)*	*<0.01**	
	Unboiled	*110*	*44 (40)*		
Total		*315*	*71 (22.53)*		

** Significant* *** Highly significant*

Table (10) reveals the clinical features related to HRV infection. Watery diarrhea constituted 100%, duration of diarrhea below 8 days 87.3%, Fever 88.73%, Vomiting 88.73%, Dehydration 97.18%, Abdominal colic 98.59%.

Table (10)

Clinical features of rotavirus cases

Clinical features			*Rotavirus cases*	*%*
Diarrhea	*Type*	*Watery*	*71*	*100*
		Bloody	*0*	*0*
	Duration	*<8 day*	*62*	*87.3*
		>8 day	*9*	*12.67*
Fever			*63*	*88.73*
Vomiting			*63*	*88.73*
Dehydration			*69*	*97.18*
Abdominal colic			*70*	*98.59*

Table (11) reveals that HRV infection was more in infants whose mothers were illustrate in their education 67.6%, while infants of primary educated mothers had HRV in percent 23.94%, and there was significant difference between mother education according to HRV infections $p<0.01$.

Table (11)

Social characteristics of infants including the Mother Education

EDUCATION			*NO. 0F POSITIVE HRV*	*%*
MOTHER	*TYPE OF EDUCATION*	*ILLITERATE*	*48*	*67.6*
		PRIMARY	*17*	*23.94*
		SECONDARY	*6*	*8.45*
		HIGHER	*0*	*0*
TOTAL			*71*	*100%*

Table (12) shows that most infection with HRV was caused in age between 1-11 months (92.95%), and the greatest ratio was in age 4-7 months 42.25% and it is highly significant difference than age group (12-24) $p<0.01$.

Table (12)

Relation of positive cases with the range of infant's age

Range of Age(Months)	*Positive cases*	*%*
1 - 3	*18*	*25.35%*
4 - 7	*30*	*42.25%*
8 -11	*18*	*25.35%*
12 - 15	*5*	*7.04%*
16 - 19	*0*	*0*
20 - 24	*0*	*0*
Total	*71*	*99.99%*

Table (13) reveals the HRV infection in infants with artificial feeding "Bottle feeding & Mixed feeding" was 35.44%, 26.14%, while breast feeding 13.51% and there was significant difference between Breast feeding and other type of feeding $p<0.01$, but there is non significance between Mixed feeding and Bottle feeding $p>0.05$.

Table (13)

Type of feeding in infants with acute diarrhea, %

Patients group	*Type of feeding*					
	Breast		*Bottle*		*Mixed*	
	No. of examinations	***No. of HRV positive (%)***	***No. of examinations***	***No. of HRV positive (%)***	***No. of examinations***	***No. of HRV positive (%)***
Hospitalized group	*118*	*17 (14.40%)*	*66*	*22 (33.33%)*	*68*	*16 (23.52%)*
Outpatient group	*30*	*3 (10%)*	*13*	*6 (46.15%)*	*20*	*7(35%)*
Total	*148*	*20 (13.51%)*	*79*	*28 (35.44%)*	*88*	*23 (26.14%)*

Table (14)

Age distribution in infants with acute diarrhea showing the incidence of HRV according the seasonality, %.

Patients group	***November***		***December***		***January***		***February***		***March***		***April***	
	No.	***No. of HRV positive (%)***	***No.***	***No. of HRV positive (%)***	***No.***	***No. of HRV positive (%)***	***No.***	***No. of HRV positive (%)***	***No.***	***No. of HRV positive (%)***	***No.***	***No. of HRV positive (%)***
Hospitalized group	*28*	*10 (35.7%)*	*54*	*21 (38.8%)*	*38*	*7 18.42%)*	*20*	*5 (25%)*	*70*	*6 (8.57%)*	*46*	*6 (13.04%)*
Outpatient group	*17*	*3 (17.6%)*	*16*	*8 (50%)*	*3*	*2 (66.6%)*	*4*	*0*	*5*	*2 (40%)*	*14*	*1 (7.14%)*
Total	*45*	*13 (28.8%)*	*70*	*29 (41.4%)*	*41*	*9 (21.9%)*	*24*	*5 (20.8%)*	*75*	*8 (10.7)*	*60*	*7 (11.66%)*

Table (15) shows that the ratio of acute diarrhea infection caused by enteropathogens was higher in the rural area than in the urban area. Viral infection was higher than other type of infection, followed by bacterial infection and parasitic infection and there is non significant difference in enteropathogens infection between rural and urban areas $p>0.05$, except the case with fungal infection , has highly significant difference $p<0.01$.

Table (15)

Distribution of infections in pathogenic groups (Rotavirus, bacteria, parasite & Fungal) according to the residence.

RESIDENCE (TOTAL)	*VIRAL INFECTION (%)*	*BACTERIAL INFECTION (%)*	*PARASITIC INFECTION (%)*	*FUNGAL INFECTION (%)*
Rural (133)	*52 (39.09 %)*	*48 (36.09 %)*	*25 (18.79 %)*	*8 (6.01%)*
Urban (56)	*19 (33.92 %)*	*19 (33.92 %)*	*8 (13.91%)*	*10 (17.8 %)*
Total (189)	*71 (37.56 %)*	*67 (35.44%)*	*33 (17.46%)*	*18 (9.52%)*
P value	*>0.05 n.s*	*>0.05 n.s*	*>0.05 n.s*	*<0.01 ** *

*** Highly significant n.s Non Significant*

Table (16) revealed that Hospitalized infants who were infected with enteropathogens were higher than outpatients. There is non significant difference in (Viral, Bacterial, Parasite and Fungi) infection between inpatients and out patients.

Table (16)

Distribution of diarrheal causes (HRV, Bacteria, Parasite, and Fungi) in Hospitalized infants "inpatients" & outpatients infants

Type of admission (Total)	*Viral infection %*	*Bacterial infection %*	*Parasitic infection %*	*Fungal infection %*
Hospitalized patients (147)	*56 (38.09%)*	*53 (36.05%)*	*24 (16.32 %)*	*14 (9.52 %)*
Outpatients (42)	*15 (35.41 %)*	*14 (33.33 %)*	*9 (21.4 %)*	*4 (9.5 %)*
Total (189)	*71 (37.56 %)*	*67 (35.44%)*	*33 (17.46%)*	*18 (9.52%)*
P value	*>0.05 n.s*	*>0.05 n.s*	*>0.05 n.s*	*>0.05 n.s*

n.s Non Significant

Figure (1)

Distribution of acute diarrhea according to the known & unknown causes in percent

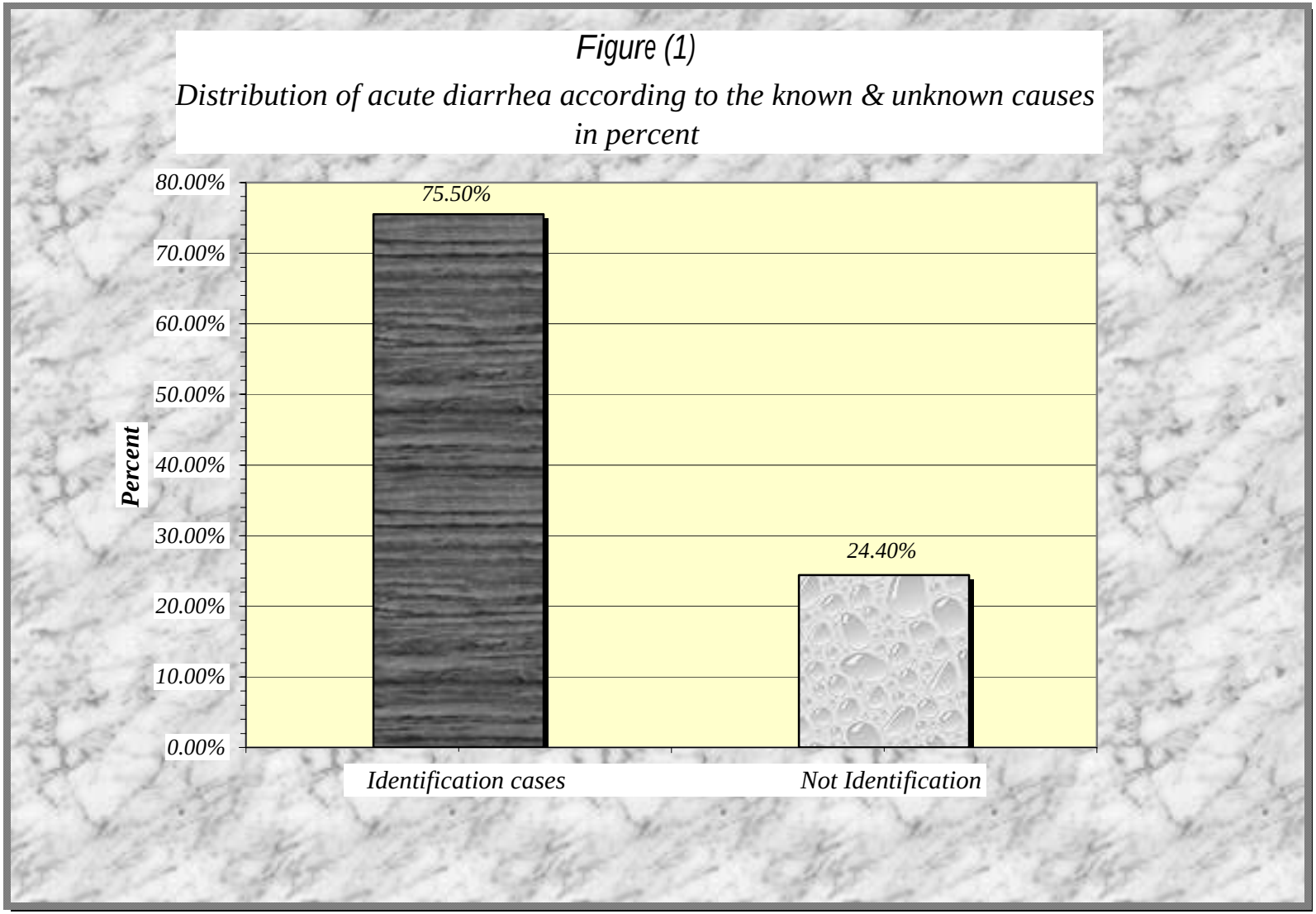


Figure (2)

Relation between the infants age & positave cases

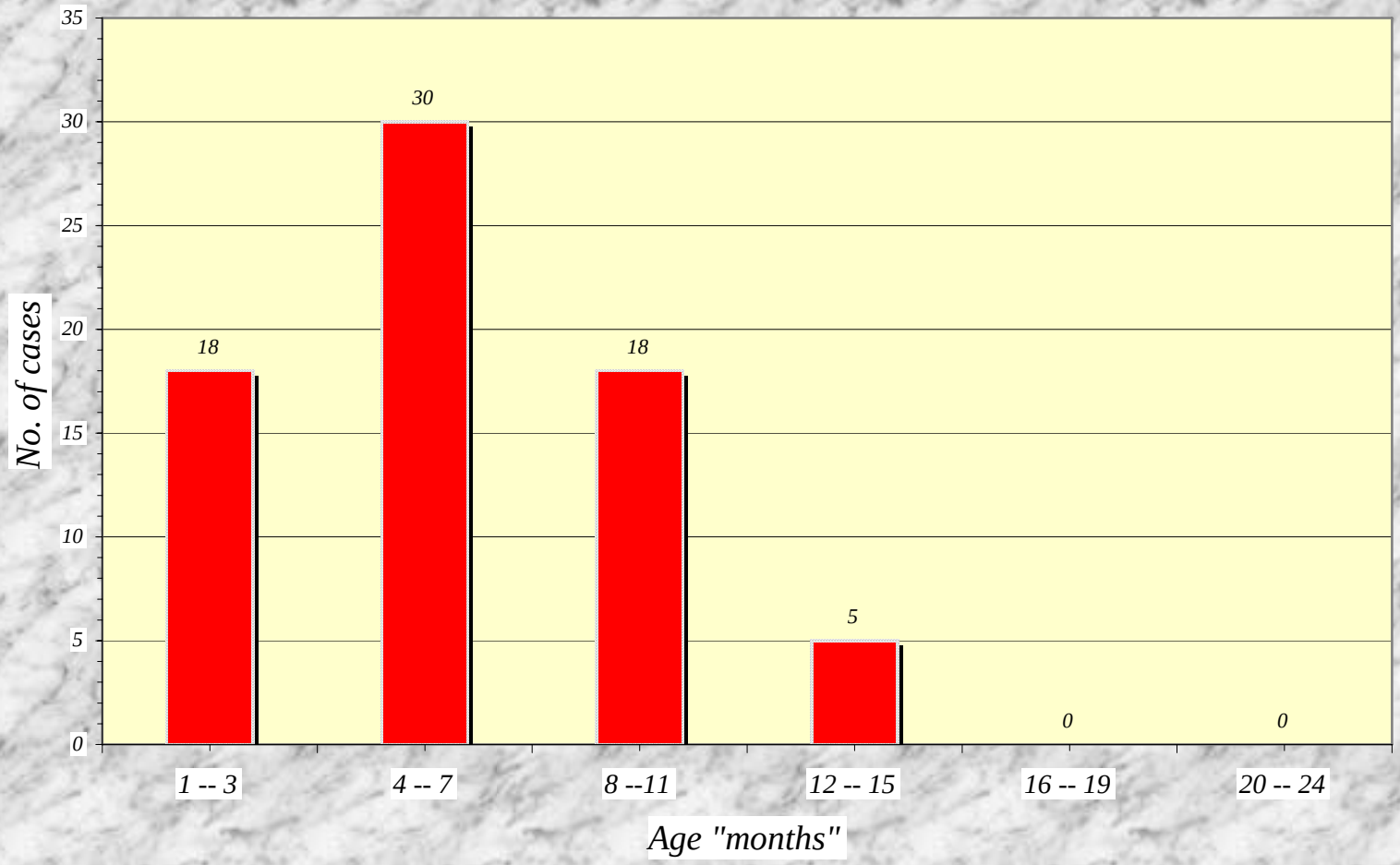


Figure (3)

Relation between months & positive cases

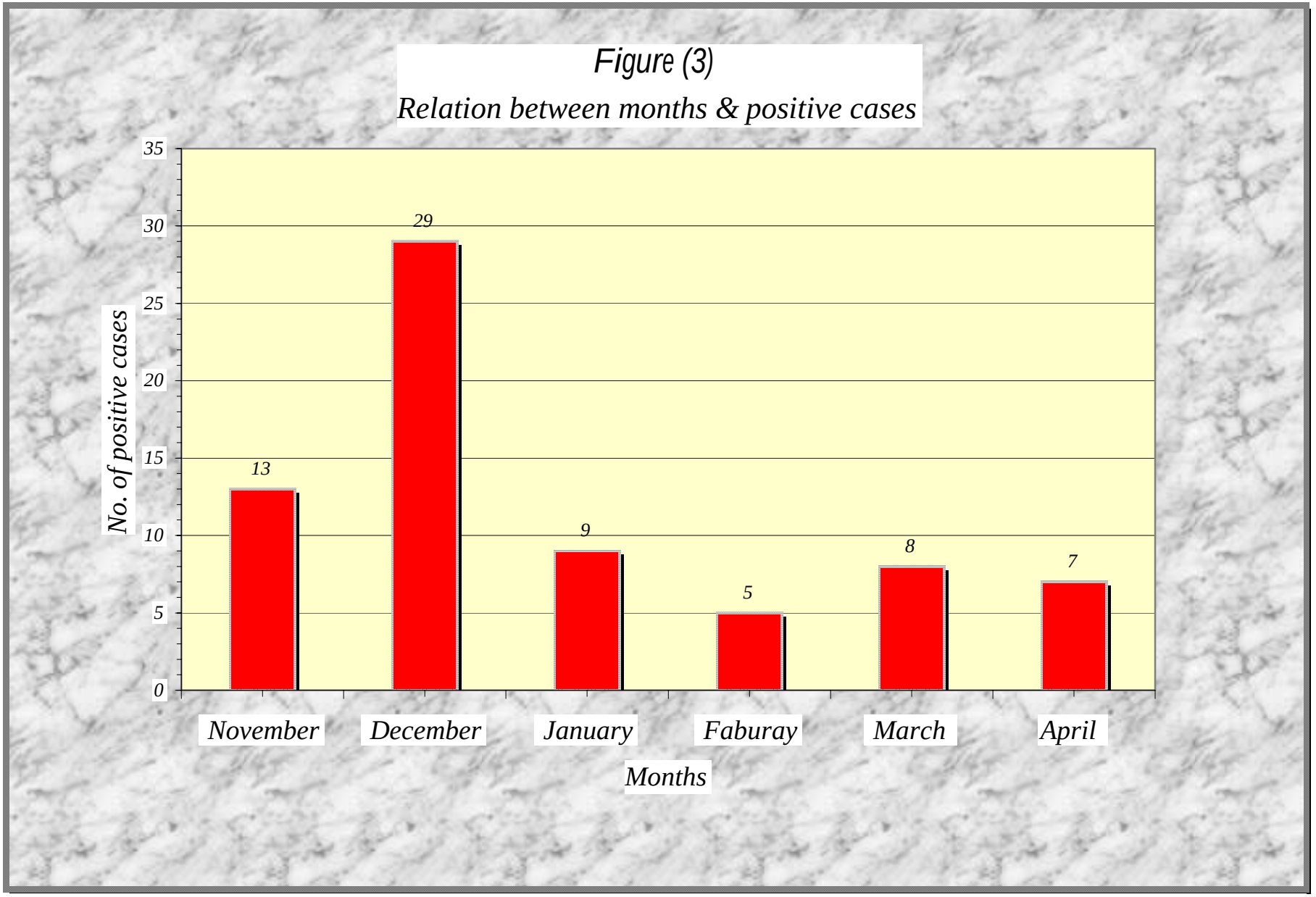


Figure (4)

Relation of positive cases with age of infants infected with HRV

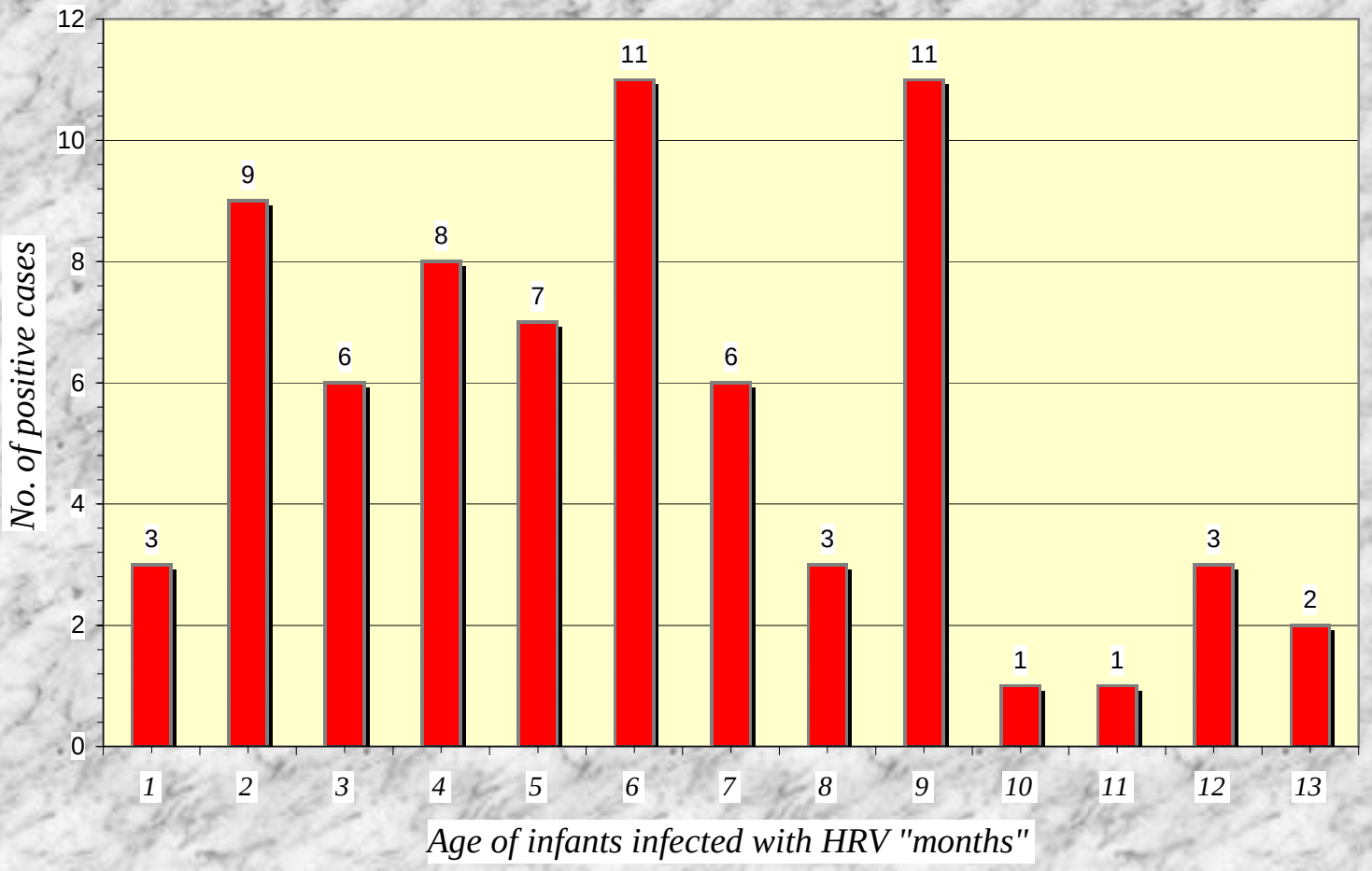


Figure (5)

Prevelance of pathogens causing infantil diarrhea

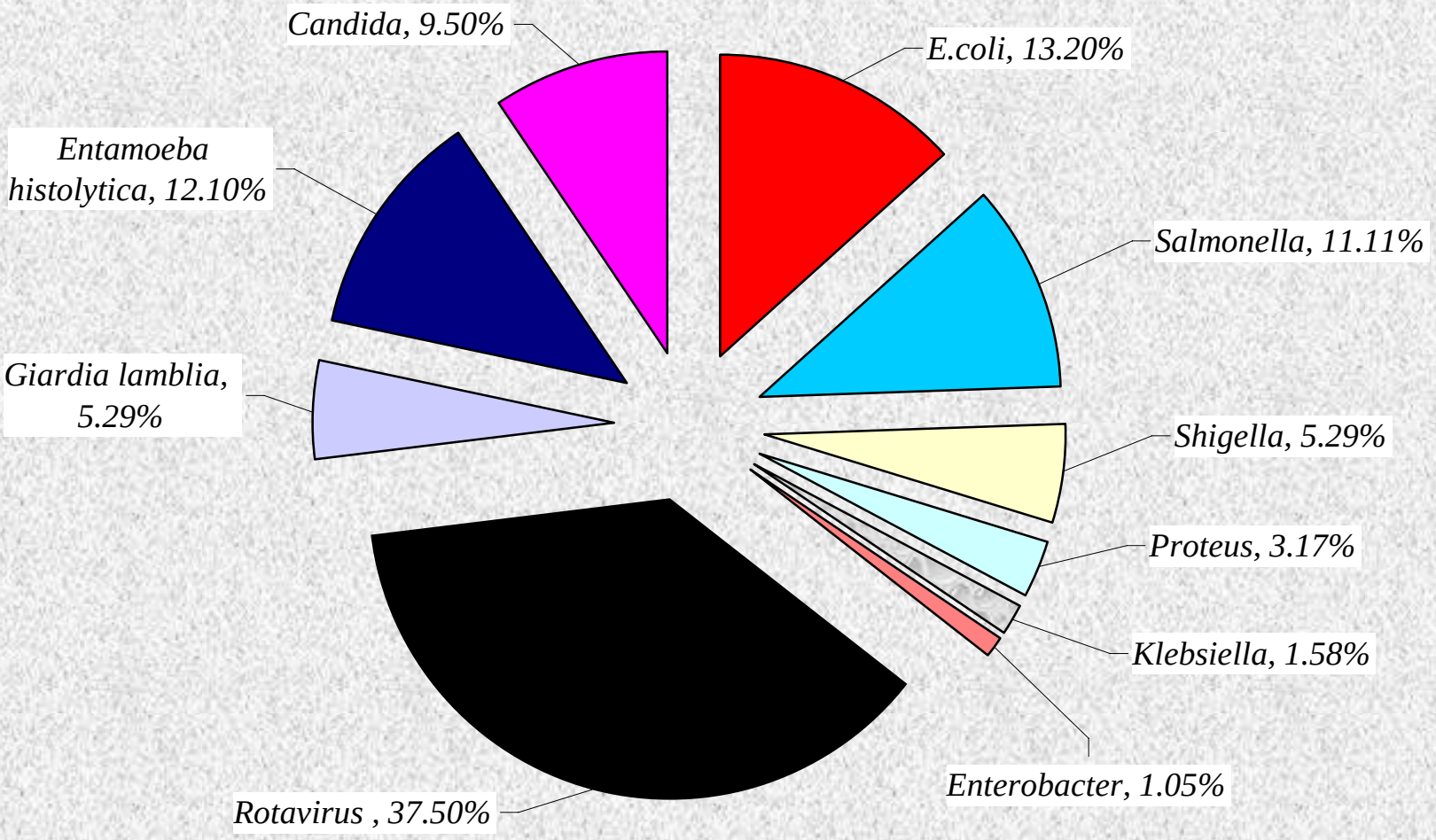


Figure (6)
Distribution of diarrhial causes (HRV, Bacteria, Parasite, Fungi) in Hospitalized infants"inpatients" & out patients infants

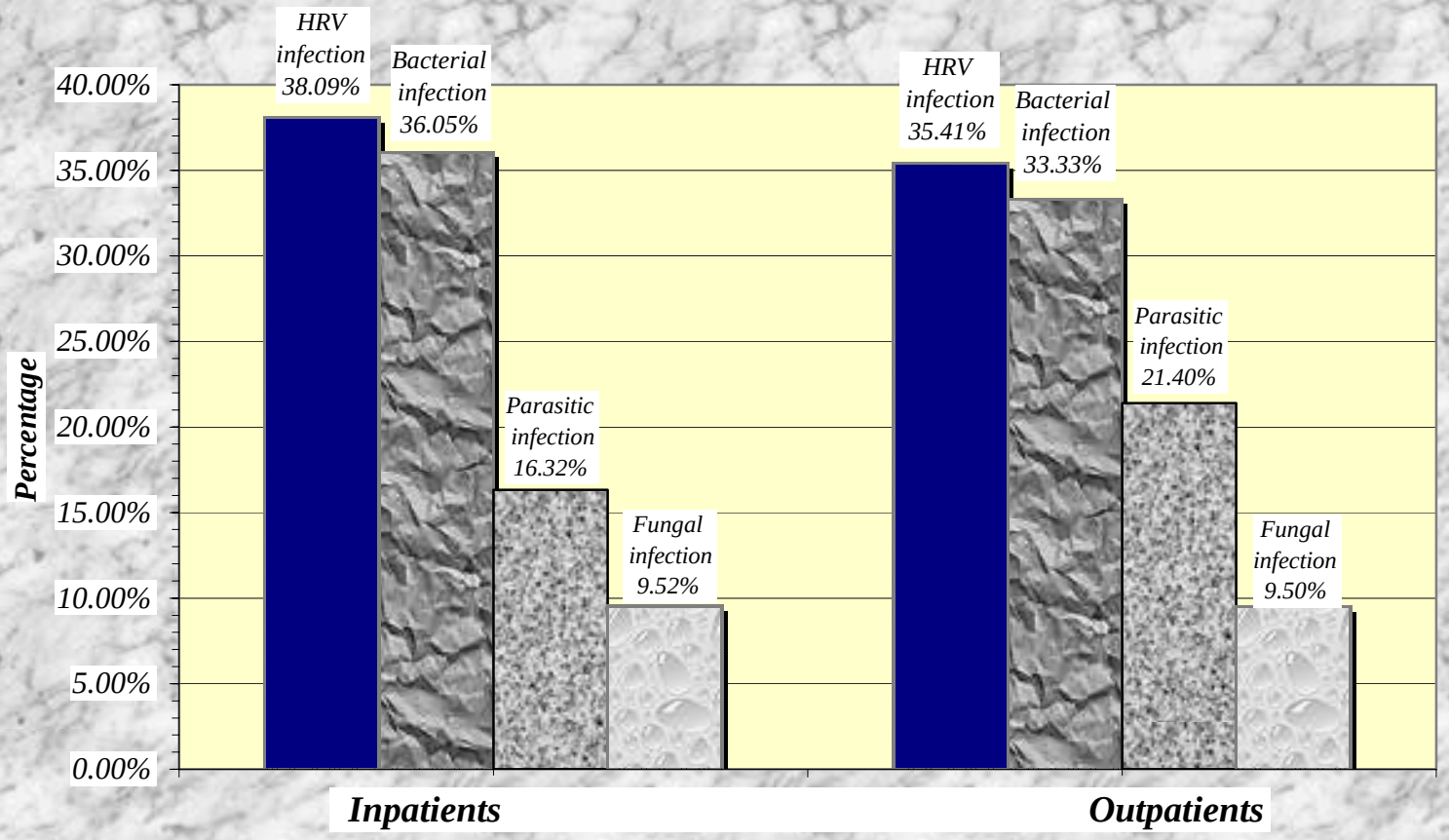


Figure (7)
Distribution of diarrhial causes (HRV, Bacteria, Parasite & Fungi) according to the residance

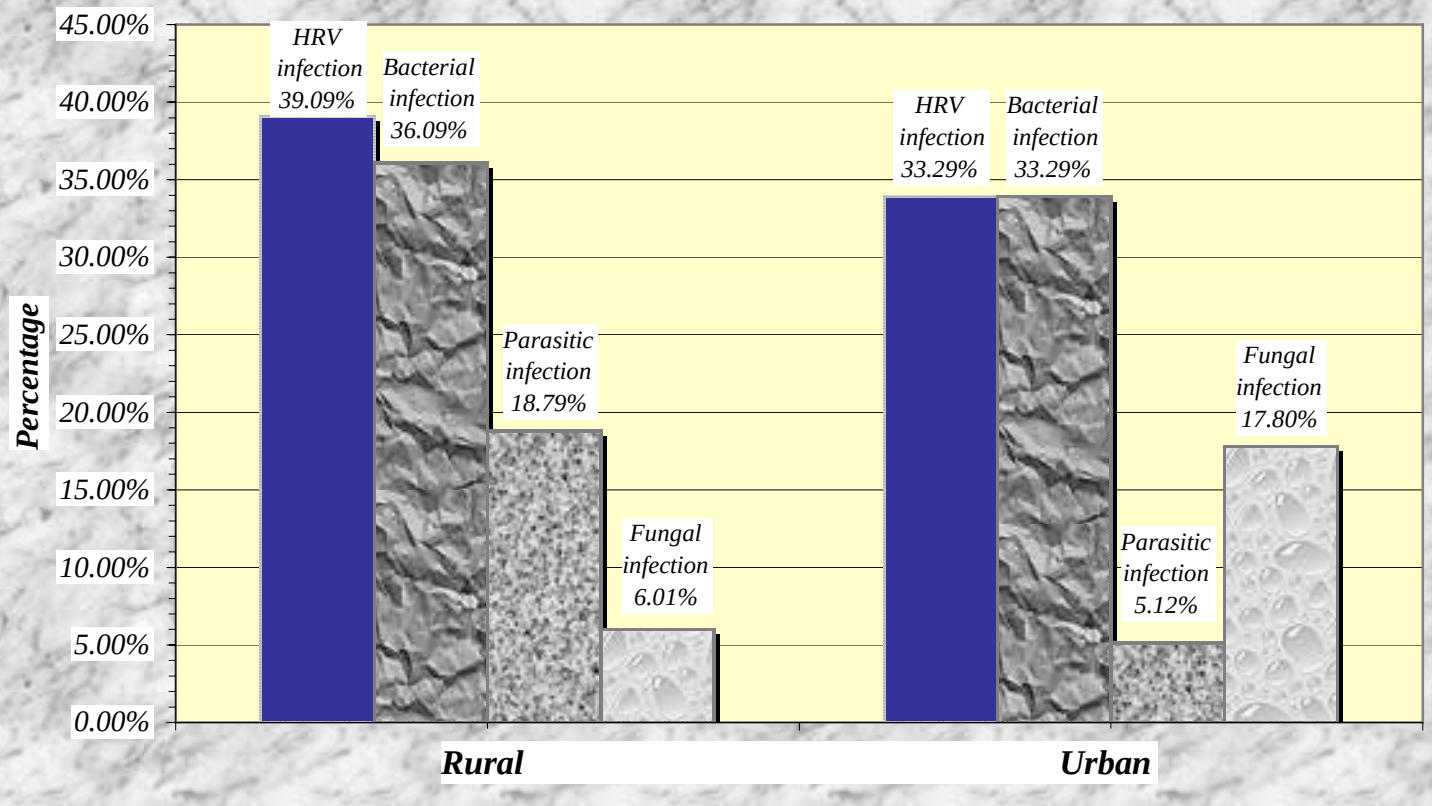


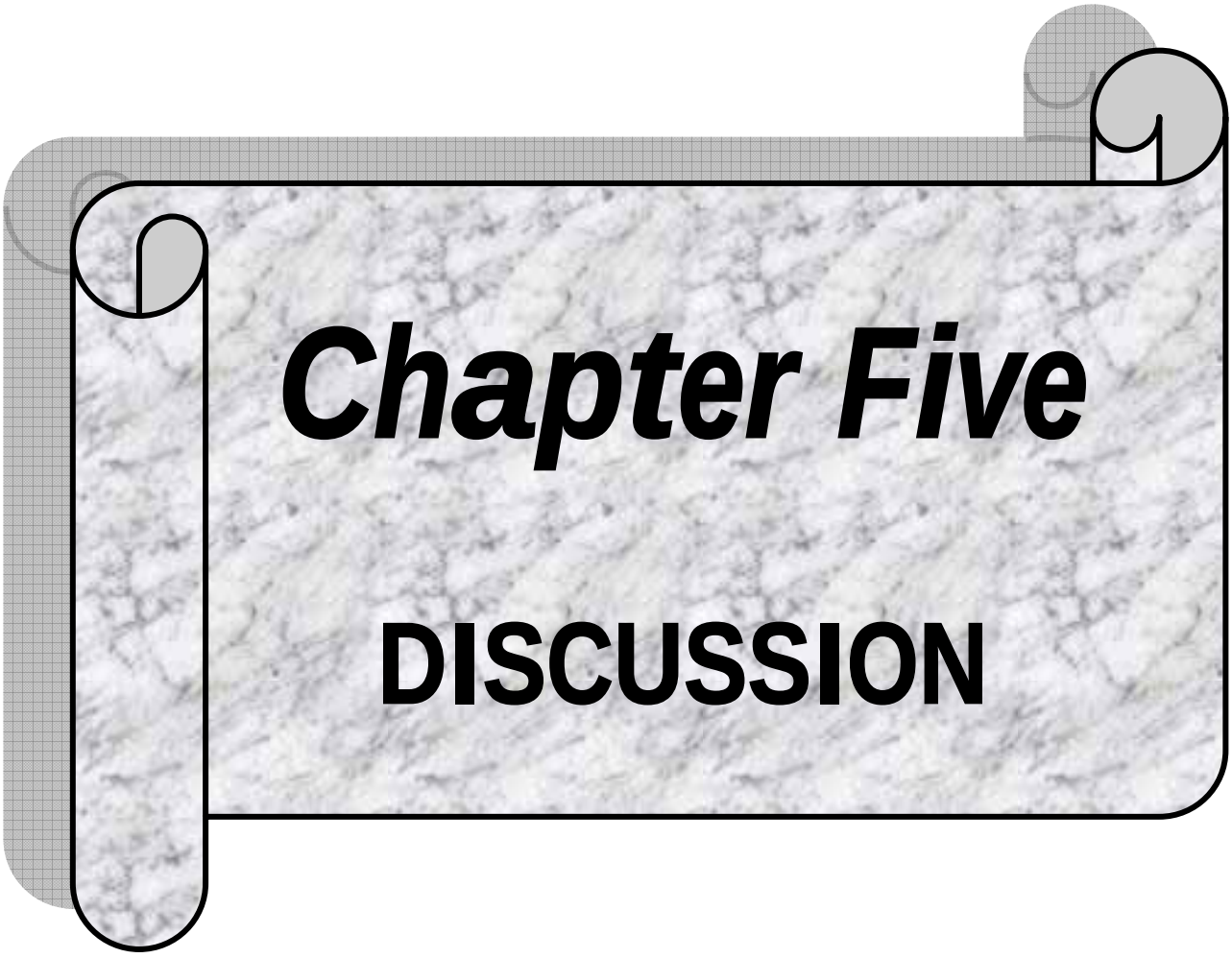
Figure (1) shows that there were 75.5% of the cases were known for the causative agent that causes diarrhea, while 24.4% of all the cases were unknown for the causative agent.

Figure (2) reveals the distribution of infected infants according to their age, the highest ratio was in age group (4-7) which constituted 30 cases, (1-3) 18 cases, (8-11) 18 cases and (12-15) 5 cases. Figure (3) reveals the relation between months & positive cases. It shows that December was the highest month in the HRV cases (29 cases).

Figure (4) reveals the positive case with age. It shows that infants with 6 months and 9 months were of higher incidence than other ages (11 cases). Figure (5), shows the distribution of causative pathogens that cause acute diarrhea, the percentage is like that in table (3).

Figure (6) shows the percentage of causative agents that cause diarrhea in both hospitalized and outpatients, the results were the same as in table (16).

Figure (7) shows the percentage of causative agents that cause diarrhea in both rural and urban; the results were the same as in table (15).


Chapter Five
DISCUSSION

Chapter Five

Discussion

Gastroenteritis is a nonspecific term for a variety of pathological states of the gastrointestinal tract. The primary manifestation is diarrhea, but it may be accompanied by nausea, vomiting, and abdominal pain. A universal definition of diarrhea does not exist. Although most definitions concentrated on the frequency, consistency, and water content of stools, some authors prefers the definition that diarrheal stools take the shape of their container. Acute gastroenteritis is usually caused by infectious agents. These agents cause diarrhea by adherence, mucosal invasion, enterotoxin production, and/or cytotoxin production.

These mechanisms result in increased fluid secretion and/or decreased absorption. This produces an increased luminal fluid content that cannot be adequately reabsorbed, leading to dehydration and loss of electrolytes and nutrients.

The small intestine is the prime absorptive surface. The colon then absorbs additional fluid, transforming a relatively liquid faecal stream in the cecum to well-formed solid stool in the recto sigmoid.

One can infer from these observations that disorders of the small intestine result in increased amounts of diarrheal fluid with concomitantly greater electrolyte and nutrient loss.

Microorganisms may produce toxins that facilitate infection. Enterotoxins are generated by bacteria (ie, enterotoxigenic *Escherichia coli, Vibrio cholera*) that act directly on secretory mechanisms and produce typical copious watery (rice water) diarrhea. No mucosal invasion occurs. The small intestine primarily affected with elevation of the adenosine monophosphate (AMP) levels is the common mechanism.

Cytotoxin production by bacteria (ie, *Shigella dysenteriae, Vibrio parahaemolyticus, Clostridium difficile*, enterohemorrhagic *E. coli*) results in mucosal cell destruction leading to bloody stools with inflammatory cells, resulting in decreased absorptive ability.

Enterocyte invasion is the preferred method by which microbes such as *Shigella* and *Campylobacter* organisms and enteroinvasive *E coli* cause destruction and inflammatory diarrhea. Similarly, *Salmonella* and *Yersinia* species also invade cells but do not cause cell death. Hence, dysentery does not usually occur. These bacteria will, however, invade the blood stream across the lamina propria and cause enteric fever such as typhoid *(Arthur, 2003)*.

Rotaviruses are the major causes of sever dehydrating diarrhea in infants and young children throughout the world. Rotavirus is estimated to cause 480,000 – 640,000 deaths in children each year (approximately 20% of the estimated 2.4 - 3.2 million deaths from diarrhea) *(Stanly, A.P. 1999)*.

5.1 The ratio of acute diarrhea

In this study, we found out that 75.5% of diarrhea cases in infants were caused by enteropathogens (Fig.1), which is nearly the same as that of *(Al-*

Kelaby, 1999) ; study done in Najaf, who found out that enteropathogens causing diarrhea in infants under two years formed (65.73%).

Another study done in Baghdad by *(Abbas, 1986)* on children bellow five years, found that enteropathogens were causing acute diarrhea (53.09%).

This difference between that study and our study may be related to the procedure used to detect the virus, Abbas used the ELISA technique in his study while we used the latex agglutination technique, and the range of age was up to 5 years in his study, the children over 2 years had higher immunity than the infants, because the previous infection with the virus gave high titer of immunoglobulin, which decreased the influence of disease *(Christianes, 1989).* In addition to that, during the past decade there was a shortage in all aspects regarding the diagnostic procedures.

5.2 The role of enteropathogens in causation of acute diarrhea

Of the 315 infant patients with acute diarrhea the causative agent was detected in 189 patients only (75.5%) (*Fig.1*). Human Rotavirus "***HRV***" was the major pathogen (71 patients) 37.5% (*Table 3 & Fig.5*), our results were the same as other studies done in Iraq and other countries for example, a Russian study done by *(Khaustov, V.I. et.al., 1989)* 34.9% ,which was so near to our results, in Izmir, Turkey, where HRV was identified in 39.8% of the children *(Kurugle, et.al., 2003)*. In Iraq, a study done by *(Abbas, 1986)* 39.15% and *(Al-Kelaby, 1999)* 41.03%. In Australia a study done by *(McIver, 2001)* 40%,

This increment in HRV infection mainly in inpatients cases could be explained as a result of the unhygienic environment in the pediatric hospitals wards, which resulted in increase transmission of HRV, and because the route of transmission to the HRV was faecal-oral pathway, and those viruses were

discharged in high value in the faeces of the patients *(Kapikian, 1985)*, and small inoculums were enough to cause the infection *(Clark, 1988).*

Bacterial infections that caused acute diarrhea was formed 34.39%, and were mostly caused by Enteropathogenic ***E. coli*** (EPEC) 13.2% (*Fig.5, Table 3*). Other authors like *(Gosh, et.al., 1991, Mandal, 1981, Weindling, et.al., 1980 & Al-Kelaby, 1999)* stated that { *EPEC playing a major role in the cases of the acute diarrhea throughout the first 2 years of infants age*}. In the Prince of Wales Hospital, Randwick, NSW, Australia, ***Salmonella spp.*** constitute 10% *(McIver, 2001). Patrecia, 2001* referred to the role of the ***Salmonella*** and ***Shigella*** as common causes of acute diarrhea. Also *(Weindling, et.al., 1980)* referred to the role of ***Salmonella*** in causing diarrhea .

In our study, we noticed appearance of two genuses of pathogens; ***Proteus*** and ***Klebsiella,*** which were not mentioned by other studies which could be a causative agent of acute diarrhea. So, the appearance of these genuses might be explained by contamination of the stool specimens with the urine. ***Enterobacter*** had formed very low ratio in this study, and this was due to the type of bacteria and the age of infected children whose age was (> 2 years). ***Candida*** were isolated from 9.5%, and were considered as normal flora.

Parasitic infections were detected as a cause for acute diarrhea, the common one was ***Entamoeba histolytica*** 12.1% then ***Giardia lamblia*** 5.29% (*Fig.5, table 3*). These results are similar to those referred to by *(Periodical information on nutrition nutrinews. Gastroenteritis reality & treatment)* ***Entamoeba histolytica*** constituted 2-15%, while ***Giardia lamblia*** was 4-20%. Also *(Richard, 1998)* referred to those two causative agents of diarrhea, and *(Jay, 1994 & McIver, 2001)* pointed out that ***Giardia*** was considered as the enteropathogens causing diarrhea.

In this study, we found cases of acute diarrhea which were caused by Mixed pathogens (Virus, Bacteria, Parasite & Fungi). It formed about 13.6% of the acute diarrhea cases (*Table 1*). Most of these mixed pathogens were between the

HRV and bacteria; 11 cases were (36.7%), and the most common bacteria was EPEC, it formed 72.7% (*Table 4*). These results are nearly similar to that of (*Veregara, et.al., 1992)* and *(Al-Kelaby, 1999)*. The ratio of the mixed pathogens between HRV and the parasite was 20%, and mostly with ***Entamoeba histolytica*** it formed 66.6% (*Table 3*).

The combination role between the HRV and the other enteropathogens was referred to by *(Schnagl, et.al., 1978), , (Abbas, 1986) & (Al-Kelaby, 1999)*

It is thought that the infection with the HRV was accelerating the infection with other enteropathogens and supported their growth. Moreover, it was correlated with mechanism action of HRV through its ability to invade the epithelial layer in the intestine and destroy the villi which is the main the absorption site. In addition, the villi contains important enzymes, and its destruction causes gaps that may become suitable sites to be colonized by other bacteria and parasites *(Versikari, et.al., 1981)*.

There was a mixed infection between bacteria and parasite in 8 cases (26.6%) of 30 cases of mixed pathogens (*Table 4*). This combination between the bacteria and parasite could be explained by the role of bacteria in supporting the pathogenisity of parasite especially ***E. histolytica***, and this association was connected with the development of intestinal amebiasis *(Phillip, et.al., 1958)*.

Other mixed pathogens were seen between EPEC and ***G. lamblia*** in one case. ***G. lamblia*** infection was correlated with bacteria that lived in the intestine, which worked together with the ***G. lamblia*** in destroying mucosal layer and might decrease B_{12} absorption which was correlated with the Giardiasis *(Tomkins,1979)*.

There was an association between ***Salmonella*** and EPEC in 3 cases (*Table 4*), which was not recorded by other studies like *(Al-Kelaby, 1999)* and *(Abbas, 1986).*

Association between other bacterial genuses belonged to the difficulty in isolating other bacteria because of their needs to specific growth requirements,

like the anaerobic condition, or they required a specific technique to isolate them like other groups of ***E. coli***.

The causative agents of acute diarrheal cases were unknown in 24.5% (*Fig.1*), which could be explained by the fact that the causative agent might belong to other pathogens that we could not identify with our techniques. There were several pathogens which had the ability to cause diarrhea like ETEC "Enterotoxigenic ***E. coli***" and EIEC "Enteroinvasive ***E. coli***" , ***Yersinia***, ***Campylobacter*** which were mentioned by *(Patrecia, 2001),* Astrovirus and Adenovirus referred to by *(McIver, 2001 , Cardoso, et.al., 1992 & Bates, et.al., 1993)*, Calcivirus referred to by *(Marie-Cardine, et.al., 2002),* Coronavirus referred by *(Singh, et.al., 1989 & Simhon, et.al., 1985)*, ***Clostridium spp.*** *(Merobol, et.al., 1997),* Norwalk and Norwalk –like viruses played an important role in causing diarrhea *(Jiang, et.al., 1995),* ***Cryptosporidium*** , ***Isospora***, were related to acute diarrhea *(Synder, 1996),* also the cases that took antibiotics were influence by the culture result *(Merobol, et.al., 1997),*.

5.3 Incidence of Enteropathogens according to age of patients

Our study revealed that HRV infection was common in infants between 1-12 months (*Fig.2, Table 12*). This result is nearly the same as that proved by *(Al-Kelaby, 1999),* who found that the range of infants age was under 12 months and these results are the same as that of *(Cicirello, H.G. Das, B.K. et.al., 1994.)* in India who found the incidence of clinical illness peaks of diarrhea among children was between (4 months to two years) , while *(Torres, B.V. et.al., 1978)* in Venezuela found that the frequency of infection with HRV was common in children under 5 years. But the highest ratio was under 2 years old and it was slightly higher in the age group (13-24 months old), and was significantly lower in children younger than 6 months old. This difference from

our results could be explained by the type of feeding , because most infants in our study depended on the mixed and bottle feeding especially in the first year of their life, and this made HRV infection higher in the first year of infants ages than the second one . Additionally the early exposure to HRV infection reduced the danger repeated infections.

Also *(Kurugol, et.al., 2003)* in Turkey found out that 80.7% of diarrheal cases that were caused by HRV were under 2 years old. *(Georgescu, et.al., 1985 & Sierra, et.al., 1982)* found out that infants infected with HRV were ranging between (4 months-3 years). In Iran *(Zarnani, et.al., 2004)* proved that HRV was most frequently in children under one year and was slightly higher between 6-12 months. *(Carlson, et.al., 1978)* mentioned that most HRV infection was approximately less than one year old.

Our results are the same as those of the *(Cardoso, D. et.al. , 1992)* results which revealed that Rotavirus was more prevalent among infants between (1 to 11 months of age).

This increase in the number of HRV infections at this age groups (< 2 years) was explained as they resulted from using solid food beside or instead of breast feeding or bottle feeding, and these foods might contain many enteropathogens as well as HRV because there was faecal-oral transmission *(Nimri & Hijazi, 1996)*.

Decrease in the HRV infection in children over one year old, indicated that immunity was acquired from the several infections in the first year of their life , in addition to that , the children who consumed solid food might be infected with the other pathogens like ETEC, ***Salmonella***, ***Shigella***, ***Giardia***….. etc, *(Young, et,al. 1987)* .

The low numbers of infections with HRV in infants who depended exclusively on breast feeding belonged to the passive immunity that infant received from his mother during this period, which could protect him against

the common infections disease like the HRV infections, and decrease of the possible transmission from contaminated food *(Nimri & Hijazi, 1996)*.

5.4 Incidence of Enteropathogens according to the residence

Diarrheal disease was found a year round, in both urban and rural settings *(Ellen, et.al., 2003)*.

Our results revealed that the percentages of the HRV infections were more in infants living in rural area than in urban area 28.19%, 14.96% respectively (*Table 8*) and this result approached that of *(Simon, et.al., 1985)*. Bacterial infection in rural infants was higher than in urban area (36.09%, 33.92% respectively) (*Table 15, Fig.7*) and there was increase in the ratio of ***Salmonella***, ***ETEC, Shigella*** which was closely the same as that of *(Black, et.al., 1980)* .

The difference in distribution could be explained as a result of the nature of life style in our rural area and the peoples dependency on using river water without sterilization (boiling or chemical) as shown in (*Table 9*). And, as we know, most of these pathogens have resistance to most of the disinfected agent, so the enteropathogens will be distributed through out the water *(Nimri & Hijazi, 1996)*.

The percentage of the parasitic infection in the rural area was higher than that in the urban area (25)18.79%, (8)13.91% respectively (*Table 15, Fig.7*). This could be explained by the fact that many families used stored water in their tanks or directly from river which was more prone to contaminate, and this increase the possibility of infection .

However, data from rural areas in Egypt, Guatemala and Bangladesh demonstrated higher rates in their rural area of 3, 14 and 50%, respectively *(Ângelo, et.al., 2001)*. Rural America has a 20 percent rate higher than urban

areas, according to a paper from the National Rural Health Association (*NRHA*) *(American family physician. 1999)*.

5.5 Incidence of Enteropathogens according to the feeding pattern

In our study the ratio of the HRV infection among infants who were on bottle feeding and mixed was higher than infants who were on breast feeding only 35.44%, 26.14% and 13.51% respectively (*Table 13*), this result is close to the *(Kurugol, Z. et.al., 2003)* who found out that the infants who were not exclusively breast-fed were at a two-fold greater risk of rotavirus diarrhea than those who were exclusively breast fed.

(Duffy, L.C. 1986) found that out the attack rate of rotavirus gastroenteritis on breast-fed and bottle-fed infants was (20 %, 17 %, respectively); however, the clinical course of rotavirus gastroenteritis was quite different. Infants who were breast-fed had illnesses that were characterized by milder symptoms of shorter duration while bottle-fed infants who acquired rotavirus gastroenteritis were classified as having acute illnesses with longer duration. These data suggest that factors associated with breast-feeding, although not affecting rotavirus infection rates, may moderate the clinical course of rotavirus gastroenteritis. *(Simon, et.al., 1985)* stated that prolonged breast feeding may explain the reduced of the pathogenisity of viral gastroenteritis.

(Zarnani, et.al., 2004.) found out that Rotavirus infection was significantly less frequent in breast-fed than among bottle-fed babies. This result may indicate that breast-fed infants, besides acquiring a passive immunity from the mothers, may also acquire resistance to infection with rotavirus as is implied here by the low frequency of breast-fed infants among patients.

Dr. David Newburg, 1998 from the Shriver Center for Mental Retardation in Massachusetts, and his team, studied milk samples from breast-feeding mothers which were taken and analysed weekly until four weeks after birth, then monthly. Those samples were assayed for a collection of substances, including lactadherin. Newburg and colleagues found out that the highest anti-rotavirus activity was due to lactadherin and they considered that this research might eventually lead to the development of a new therapeutic agent that could be taken as a tablet by new mothers to protect their babies from childhood diseases. Lactadherin is one of the classes of molecules known as human-milk glycoconjugates. In addition to that, *Dr. David* improved that human milk contains mucin-associated glycoprotein, lactadherin, which binds specifically to rotavirus and inhibits its replication and lactadherin protects against symptoms of HRV infection.

5.6 Incidence of Enteropathogens between the outpatient & inpatient

In our study we noticed that the percentage of the hospitalized infants with HRV was more than outpatient infants 38.9% and 35.41% respectively (*Table 16, Fig.6*). This difference in the ratio was related to the fact that HRV infection caused sever dehydration *(Bern, et.al., 1992)*, and patient needed the rehydration therapy by intravenous because the long time dehydration in infants may be lethal for them. Our result is the same as that of the *(Al-Kelaby, 1999)* 44.4% in hospitalized and 28.2%in outpatients, and *(Abbas, 1986),* in addition to that, HRV infection was caused nosocomially in the nursing departments and children wards *(Gaggero, et.al., 1992)* . Also EPEC, ***Salmonella*** and ***Shigella*** infections were higher in hospitalized infants than in the outpatient infants, because of their dehydrating state .

The parasitic infections were also higher in the hospitalized infants *(Plevris, 1996)* (*Table 16, Fig.6*), because this type of infection might cause great damage to the tissue and we notice that those patients were in need of intravenous management .

5.7 Incidence of Enteropathogens according to the sex

In our study we noticed that the ratio of the HRV infection among the males was higher than female (27.51%, 20.13% respectively) in ratio 1.35:1(*Table 7*). Also the male to female ratio was 1.3/1, with 86 per cent of reports for children in the 1-4 year age group *(Communicable Diseases Intelligence)*. The male to female ratio was 1.2/1 *(Chiu, 2000)*

This difference may be correlated with the anatomical and physiological factors; also the culture of our community . All these factors may be effective on the ratio of infection. Rotavirus was most frequently detected in the age group 6-11 months (26.6%). Rotavirus was not detected at all above 24 months of age *(Dutta, 1990)*.Males accounted for a higher percentage of all diarrheal cases *(Milaat, 1995)*; HRV disease was more predominant in Males *(Kelkar, 1999).*

CLINICAL AND EPIDEMIOLOGIC FEATURES OF ROTAVIRUS DISEASE

Rotavirus is the most common cause of severe gastroenteritis in infants and young children in the United States. Worldwide, rotavirus is a major cause of childhood death. The spectrum of rotavirus illness ranges from mild, watery diarrhea of limited duration to severe, dehydrating diarrhea with vomiting and fever, which results in death (1–5). Virtually all children become infected in the first 3–5 years of life, but severe diarrhea and dehydration occur primarily among children aged 3–35 months. Rotaviruses are shed in high concentrations in the stools of infected children and are transmitted by the fecal-oral route, both through close person-to-person contact and through fomites (6). Rotaviruses also might be transmitted by other modes, such as respiratory droplets (7). In the United States, rotavirus causes seasonal peaks of gastroenteritis from November to May each year, with activity beginning in the Southwest United States and spreading to the Northeast (8–10).

Rotavirus appears to be responsible for approximately 5%–10% of all diarrheal episodes among children aged <5 years in the United States, and for a much higher proportion of severe diarrheal episodes (2,11). Although rotavirus gastroenteritis results in relatively few deaths in the United States (approximately 20 per year among children aged <5 years) (12), it accounts for more than 500,000 physician visits (13,14) and approximately 50,000 hospitalizations each year among children aged <5 years (4,9,15). Rotavirus is responsible for 30%–50% of all hospitalizations for diarrheal disease among children aged <5 years, and more than 50% of hospitalizations for diarrheal disease during the seasonal

peaks (11,16–18). Among children aged <5 years in the United States, 72% of rotavirus hospitalizations occur during the first 2 years of life, and 90% occur by age 3 years (15).

In the first 5 years of life, four out of five children in the United States will develop rotavirus diarrhea (2,19); one in seven will require a clinic or emergency room visit; one in 78 will require hospitalization; and one in 200,000 will die from rotavirus diarrhea (4,14). The risk for rotavirus diarrhea and its outcomes do not appear to vary by geographic region within the United States. Limited data suggest that children from disadvantaged socioeconomic backgrounds and premature infants have an increased risk for hospitalization from diarrheal disease, including rotavirus diarrhea (20). In addition, some children and adults who are immunocompromised because of congenital immunodeficiency, hematopoetic transplantation, or solid organ transplantation experience severe, prolonged, and sometimes fatal rotavirus diarrhea (21–23). Rotavirus is also an important cause of nosocomial gastroenteritis (1,11,16,24,25). Among adults in the United States, rotavirus infection infrequently causes diarrhea in travelers, persons caring for children with rotavirus diarrhea, and the elderly (26). Each year in the United States, rotavirus diarrhea results in $264 million in direct medical costs and more than $1 billion in total costs to society (14). Direct medical costs are primarily the result of hospitalizations for severe diarrhea and dehydration, and societal costs are attributable primarily to loss of work time amongparents and other caregivers. Several reasons exist to adopt immunization of infants as the primary public health intervention to prevent rotavirus disease in the United States. First, similar rates of illness among children in industrialized and less developed countries indicate that ***2 MMWR March 19, 1999*** *clean water supplies and good hygiene have not decreased the incidence of rotavirus diarrhea*

in developed countries, so further improvements in water or hygiene are unlikely to have a substantial impact (2,27–31). Second, in the United States, a high level of rotavirus morbidity continues to occur despite currently available therapies. For example, hospitalizations for diarrhea in young children declined only 16% from 1979 to 1992 (9), despite the widespread availability of oral rehydration solutions and recommendations by experts, including the American Academy of Pediatrics, for the use of oral rehydration solutions in the treatment of dehydrating gastroenteritis (32– 34). Third, studies of natural rotavirus infection indicate that initial infection protects against subsequent severe diarrheal disease, although subsequent asymptomatic infections and mild disease might still occur (30,35). Thus, immunization early in life, which mimics a child's first natural infection, will not prevent all subsequent disease but should prevent most cases of severe rotavirus diarrhea and its sequelae (e.g., dehydration, physician visits, and hospitalizations).

Laboratory Testing for Rotavirus

Because the clinical features of rotavirus gastroenteritis are nonspecific, confirmation of rotavirus infection in children with gastroenteritis by laboratory testing of fecal specimens will be necessary for reliable rotavirus surveillance and could be useful in clinical settings (1,36). The most available method is antigen detection by enzyme immunoassay directed at a group antigen common to all Group A rotaviruses. Several commercial enzyme immunoassay test kits are available that are inexpensive, easy to use, rapid, and highly sensitive (approximately 90% compared with detection by electron microscopy); these properties make rapid antigen detection kits suitable for use in rotavirus surveillance systems. Other techniques — including electron microscopy, reverse

transcription-polymerase chain reaction, nucleic acid hybridization, polyacrylamide gel electrophoresis, and culture — are used primarily in research settings. Serologic methods that detect a rise in serum antibodies, primarily enzyme immunoassay for rotavirus serum immunogloblulin G (IgG) and immunogloblulin A (IgA) antibodies, have been used to confirm recent infections. In vaccine trials, detection of rotavirus-specific IgA and neutralizing antibodies to vaccine strains have been used to study the immunogenicity of rotavirus vaccines (37).

Morphology, Antigen Composition, and Immune Response

Rotaviruses are 70-nm nonenveloped RNA viruses in the family Reoviridae. The viral nucleocapsid is composed of three concentric shells that enclose 11 segments of double-stranded RNA. The outermost layer contains two structural proteins: VP7, the glycoprotein (G protein), and VP4, the protease-cleaved protein (P protein). These two proteins define the serotype of the virus and are considered critical to vaccine development because they are targets for neutralizing antibodies that might be important for protection (38,39). Because the two gene segments that encode these proteins can, in theory, segregate independently, a typing system has been developed to specify each protein; 14 VP7 (G) serotypes and 20 VP4 (P) genotypes have been described. Only viruses containing four distinct combinations of G and P proteins are known to commonly circulate in the United States — G1P1A, G2P1B, G3P1A, G4P1A (40); these strains are generally designated by their G serotype specificity (serotypes 1–4). In Vol. 48 / No. RR-2 MMWR 3 some areas of the United States, recent surveillance has detected strains with additional combinations — G9P6 and G9P8 (serotype 9) (41). In addition to these human strains, animal strains of rotavirus that are

antigenically distinguishable are found in many species of mammals; these strains only rarely appear to cause infection in humans.

Although children can be infected with rotavirus several times during their lives, initial infection after age 3 months is most likely to cause severe diarrhea and dehydration (30,42,43). After a single natural infection, 40% of children are protected against any subsequent infection with rotavirus, 75% are protected against diarrhea from a subsequent rotavirus infection, and 88% are protected against severe diarrhea. Second, third, and fourth infections confer progressively greater protection (30). The immune correlates of protection from rotavirus infection and disease are not completely understood. Both serum and mucosal antibodies are probably associated with protection from disease, and in some studies, serum antibodies against VP7 and VP4 have correlated with protection. However, in other studies, including vaccine studies, correlation between serum antibody and protection has been poor (44). The first infection with rotavirus elicits a predominantly homotypic, serum-neutralizing antibody response to the virus, and subsequent infections elicit a broader, heterotypic response (1,45). The influence of cell-mediated immunity is less clearly understood, but likely is related both to recovery from infection and to protection against subsequent disease (44, 46).

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Conclusion

1. The pathogens were detected in 75.5% of the 315 infants who had acute diarrhea.
2. The common cause of acute diarrhea in this study was HRV, followed by enteric bacterial infection, EPEC, *Salmonella spp*., *Shigella spp*., *Proteus, Klebsiella* and *Enterobacter* and parasitic infection *Giardia lamblia, Entamoeba histolytica*.
3. The pathogens were detected mostly in the hospitalized patients, which were caused by (*Rotavirus*, *Entamoeba histolytica, EPEC, Salmonella, Shigella*).
4. There was mixed infection between HRV and bacterial infection which was higher than other mixed infection.
5. Most infections with acute diarrhea occurred in infants whose ages were under one year, this range of age was critical and it required more care from the parents.
6. There was non signifies difference between male and female in Rotavirus infection.
7. There was a role for breast feeding in decreasing the incidence of Rotavirus infections.
8. There was a significant difference between the rural and urban patients with Rotavirus.
9. Mother education was effective on the infant's infection.

Recommendations

1. It is recommended to prepare laboratory test including full test for all stool specimens for infants who suffer from acute diarrhea caused by viruses, bacteria and parasite, because the full test gives a clear picture about the patient state and help the doctor to give the suitable drug or treatments.
2. It is recommended to treat the water supply in rural and urban area to reduce the risk of infections.
3. We should encourage mothers to feed their infants naturally (Breast-feeding), and give them nutrient solutions.
4. We should insure taking the specimens from the patient before taking any antibiotics, because all bacteria which are sensitive to antibiotics will never be isolated.
5. We should encourage the researches to be done for detection the other enteropathogens, which have the ability to cause the death to infants.
6. Latex agglutination test was a simple easy and fast way to detect the HRV, it did not require specific complicated equipments and it was high sensitive and specific

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The common sub-groups of the Enterobacteriaceae

	Escherichia* *Shigella	***Salmonella*** *Citrobacter*	***Proteus*** *Morganella*	***Klebsiella* *Enterobacter*** *Serratia*
Mixed acid fermentation (MR)	+	+	+	-
Butylene glycol fermentation (VP)	-	-	-	+
Indole *from tryptophan*	*V*	-	+(-)	-
Citrate *as sole carbon source*	-	+	*V*	+
H_2S *from amino acids*	*V*	+	*V*	-
Urease *production*	-	-	+	-(+)

Clinical features in children with and without positive viral detection

Symptom	***Diarrhea***	***Bloody diarrhea***	***Vomiting***	***Fever***	***Abdominal pain***
No. of samples/total *No. of positive samples (%)*	*230/256* *(89.8)*	*4/256* *(1.6)*	*175/256* *(68.3)*	*169/256* *(66.0)*	*62/256* *(24.2)*
No. of samples/ total *No. of negative samples (%)*	*76/92* *(82.6)*	*10/92* *(10.9)*	*41/92* *(44.6)*	*53/92* *(57.6)*	*35/92* *(38.0)*
P[b]	*0.100*	*<0.001*	*<0.001*	*0.189*	*0.016*

[a] *Positive samples contained at least one of the four viruses: rotavirus, human caliciviruses, astrovirus, or adenovirus type 40 or 41.*

[b] *A P value of ≤0.05 (Yates corrected χ^2 test) was considered significant for differences between children with and without positive viral detection.*

Summary

Acute diarrhea is considered as the major cause of morbidity and mortality disease to infants in the world. This research is done to reveal the causative pathogens that cause diarrhea with their epidemiology. It is conducted with assistance from medical staff of Babylon maternity and children hospital and the purpose is to reveal the major causes of acute diarrhea, their spreading according to age, sex, type of feeding, residence and mother education .

The incidence of rotavirus infection was studied in 315 children less than two years of age who were suffering from acute diarrhea, between November 2003 and April 2004 in Babylon governorate including 225 hospitalized patients and 90 outpatients. Rotavirus antigen was detected by latex agglutination technique in 41.8% in the stool samples examined as alone causative. The frequency of rotavirus infection was significantly higher among patients under 12 months of age (99%) than among children who were two years old or more (1%).

Breast-feeding has a protective action against rotavirus infection. This study reveals that rotavirus is an important etiological agent of acute gastroenteritis among children in Babylon.

Standard tests that identified bacterial and parasitic pathogens that cause acute diarrhea were done to reveal the single and combination role of these pathogens. The identification tests shows the following results:

1. Enteropathogens that cause acute diarrhea (Rotavirus, Bacteria and Parasite) constituted 219 (75.5%).
2. The mixed infection with the enteropathogens formed 30 (13.6%) from acute diarrhea cases.
3. The common causes of acute diarrhea in infants was HRV 41.08%; Enteric Bacterial infection was different according to the type of bacteria. EPEC constituted 4.65%, *Salmonella spp*. 13.17%, *Shigella spp*. 7.75%, *Proteus* 3.87%, *Klebsiella* 0.775% and *Enterobacter* 0.775%. Enteric parasitic infection included two major species *Giardia lamblia* 5.42%, *Entamoeba histolytica* 12.40%. and *Candida* constituted 9.30%.
4. Most of the diarrheal cases were detected in the hospitalized patients which were caused by (*Rotavirus 37.5*, *Entamoeba histolytica 12.10*, *EPEC 13.20*, *Salmonella 11.11*, *Shigella 5.29*) and these cases were suffering from dehydration which required rehydration.
5. The percentage of the HRV infection and Bacterial infection constituted 36.7% and it was the highest ratio among the mixed infection; HRV infection and parasitic infection constituted 20%. While the Bacterial infection associated with Parasitic infection formed 26.6%, mixed bacterial infection formed 13.3% and Parasitic mixed infection was 3.3%.
6. Most infection with acute diarrhea occurred in infants whose ages were between (1-12) months. This range of age was critical and it required more care by the parents.

7. There was a difference between male 27.51% and female 20.13%in the infection with Rotavirus.
8. Type of feeding was effective on Rotavirus infection, weaned {not exclusive breast fed} infants were more effective to infected with Rotavirus {51.5%}than the exclusively breast-feed infants {13.51%}.
9. There was difference between the rural {28.19%}and urban patients {14.96%} with Rotavirus infection.

Aims of the study

- Estimate The incidence of rotavirus infection in infants in Babylon maternity and children hospital.
- Reveal the causative pathogens that cause diarrhea with their epidemiology.
- Reveal the major causes of acute diarrhea, their spreading according to age, sex, type of feeding, residence and mother education .

Prevalence of Rotavirus & Other Enteropathogens Causing Acute Diarrhea in Hilla Infants

By\

Ali Hussein Mohammad AL-Marzoqi

SEPTEMBER/ 2004

SHA'BAN/ 1425

Aims of the Study

- Estimate the prevalence of rotavirus infection in Hilla infants.
- Reveal the causative pathogens that cause diarrhea with their epidemiology.
- Reveal the major causes of acute diarrhea & their spreading according age, sex, feeding pattern, residence and mother education .

Introduction

- Acute diarrheal disease is the commonest single cause of morbidity and mortality worldwide.
- Infectious diarrhea has been estimated to cause at least 5 million deaths each year in the developing world.
- Very young children are particularly susceptible to infection and suffer the highest mortality.
- Rotavirus is the commonest enteric pathogen in young children in both developing countries and the developed world.
- A striking feature of rotavirus infection in temperate climates is its seasonality. In contrast to other enteric pathogens, which are the commonest during the warm months of the year, the rotavirus “season” is in winter and spring.
- Typically children under 2 years of age are affected and rotavirus may be responsible for up to 50% of acute admissions to pediatrics units during the winter months

Introduction…. Continued

- Rotavirus is the most important etiological agent of serious dehydrating diarrhea among infants and young children, causing an estimated nine million cases of severe disease, and more than 800 000 deaths per year worldwide.
- Rotavirus particles are 65-75 nanometers in diameter, with a double protein shell and II strands of RNA .
- The rotavirus genome comprises 11 segments of double-stranded RNA (dsRNA) contained within the core of the mature, triple-layered particle. The 11 dsRNA segments can be separated by using electrophoresis .
- The majority of rotaviruses known to infect humans and animals share a common-group antigen and are termed group A rotaviruses .
- Outbreaks of rotavirus infection are common among infants and young children in hospitals, day-care centers and schools. Such outbreaks result in both clinical and sub clinical cases, and premature and underweight babies are most likely to develop serious infections .

Introduction.... Continued

- The virus is usually shed in the feces for five to seven days. In severe cases, rapid dehydration can lead to renal shutdown and death .
- Rotavirus diarrhea is most prevalent among children aged 6-24 months; it has been estimated that an effective vaccine could reduce diarrheal mortality among this age group .

Procedure

Stool specimens

Bacterial Examination

Viral Examination

Parasitical Examination

Stool culture then;
Diagnose pathogenic Bacteria;

1. Biochemical tests
2. Serological tests

By using
Latex agglutination
Technique

By
Direct examination
“((Wet preparation)) “

Summary

- The prevalence of rotavirus infection was studied in **315 infants** less than two years of age who were suffering from acute diarrhea, between November 2003 and April 2004 in Babylon governorate including **225 hospitalized** patients and **90 outpatients**. Rotavirus antigen was detected by latex agglutination technique in **41.8%** in the stool samples examined as alone causative. The frequency of rotavirus infection was significantly higher among patients under **12 months** of age than among children who were two years old or more .
- Breast-feeding has a **protective action** against rotavirus infection. This study reveals that rotavirus is an important etiological agent of acute gastroenteritis among children in Babylon.
- Standard tests that identified bacterial and parasitic pathogens that cause acute diarrhea were done to reveal the single and combination role of these pathogens. The identification tests shows the following results:

Summary… Continued

- Enteropathogens that cause acute diarrhea (Rotavirus, Bacteria and Parasite) constituted **219 (75.5%).**
- The mixed infection with the enteropathogens formed **30 (13.6%)** from acute diarrhea cases.
- The common causes of acute diarrhea in infants was HRV 41.08%; Enteric Bacterial infection was different according to the type of bacteria. EPEC constituted 4.65%, *Salmonella spp*. 13.17%, *Shigella spp*. 7.75%, *Proteus* 3.87%, *Klebsiella* 0.775% and *Enterobacter* 0.775%. Enteric parasitic infection included two major species *Giardia lamblia* 5.42%, *Entamoeba histolytica* 12.40%. and *Candida* constituted 9.30%.
- Most of the diarrheal cases were detected in the hospitalized patients which were caused by (Rotavirus, *Entamoeba histolytica*, EPEC, *Salmonella*, *Shigella*) and those patients were suffering from dehydration which required the rehydration.

Summary… Continued

- The percentage of the HRV infection and Bacterial infection constituted 36.7% and it was the highest ratio among the mixed infection
- Most infection with acute diarrhea occurred in infants whose ages were between (1-12) months. This range of age was critical and it required more care by the parents.
- There was difference between male and female in the infection with Rotavirus.
- Type of feeding were effective on the Rotavirus infection, weaning infants were more effective to infect with Rotavirus than the exclusive breast-feeding infants.
- There was significant difference between the rural and urban patients with Rotavirus .

Results

Table (1)

Distribution of pathogens (in groups) that causing infantile diarrhea

Group	*No. of cases*	*%*
Viral infection	*71*	*32.4*
Bacterial infections	*67*	*30.5*
Parasitic infections	*51*	*23.2*
Mixed infections	*30*	*13.6*
Total	*219*	*99.7*

Table (2)

Distribution of mixed infections for multiple pathogens caused acute infantile diarrhea

MIXED INFECTIONS	TYPE OF MIXED INFECTIONS	NO.	SUM. OF EVERY TYPE MIXED INFECTIONS	%
HRV + B.	HRV.+ EPEC	8	11	36.7
	HRV.+ Salmonella spp.	1		
	HRV.+ Shigella spp.	0		
	HRV.+ Proteus	1		
	HRV.+ Klebsiella	1		
	HRV.+ Enterobacter	0		
B. + P.	EPEC+ Entamoeba histolytica	2	8	26.6
	EPEC+ Giardia lamblia	1		
	Enterobacter + Entamoeba histolytica	1		
	EPEC+ Candida	4		
HRV + P.	HRV + Entamoeba histolytica	4	6	20
	HRV + Giardia lamblia	1		
	HRV + Candida	1		
B.+ B.	EPEC+ Salmonella spp.	3	4	13.3
	EPEC+ Klebsiella	1		
P. + F.	Giardia lamblia+ Candida	1	1	3.3
Total			30	100

*HRV. \Human Rotavirus *B. \Bacteria *P. \Parasite*

Table (3)

Percentage of each pathogen (a lone) causing acute infantile diarrhea

PATHOGENS	*NO. OF CASES*	*%*
HRV.	*54*	*41.08*
EPEC	*6*	*4.65*
Salmonella spp.	*17*	*13.17*
Shigella spp.	*10*	*7.75*
Proteus	*5*	*3.87*
Klebsiella	*1*	*0.775*
Enterobacter	*1*	*0.775*
Candida	*12*	*9.30*
Giardia lamblia	*7*	*5.42*
Entamoeba histolytica	*16*	*12.40*
Total	*129*	*99.19*

Table (4)

Enteropathogens detected in infants with acute diarrhea.

PATHOGENS	*HOSPITALIZED GROUP (%)*	*OUTPATIENT GROUP (%)*	*TOTAL*
HRV.	*56 (78.8%)*	*15 (21.12%)*	*71*
EPEC	*21 (84%)*	*4 (16%)*	*25*
Salmonella spp.	*16 (76.1%)*	*5 (23.8%)*	*21*
Shigella spp.	*7 (70%)*	*3 (30%)*	*10*
Proteus	*6 (100%)*	*0 (0%)*	*6*
Klebsiella	*1 (33.33%)*	*2 (66.66%)*	*3*
Enterobacter	*2 (100%)*	*0 (0%)*	*2*
Candida	*14 (77.77%)*	*4 (22.22%)*	*18*
Giardia lamblia	*6 (60 %)*	*4 (40%)*	*10*
Entamoeba histolytica	*18 (78.2%)*	*5 (21.7%)*	*23*

Table (5)

Sex distribution in infants with acute diarrhea, %

PATIENTS GROUP	SEX DISTRIBUTION			
	Females		Males	
	No. of examinations	No. of HRV positive (%)	No. of examinations	No. of HRV positive (%)
HOSPITALIZED GROUP	123	25 (20.3%)	130	31 (25.20%)
OUTPATIENT GROUP	26	5 (19.23%)	36	10 (38.46%)
TOTAL	149	30 (20.13%)	166	41 (27.51%)
X^2	0.46 *		0.41*	

Table (6)

Residential distribution in infants with acute diarrhea,%

PATIENTS GROUP	RESIDENTIAL DISTRIBUTION			
	Urban		Rural	
	No. of examinations	No. of HRV positive (%)	No. of examinations	No. of HRV positive (%)
Hospitalized group	105	15 (14.28%)	147	41 (30.04%)
Outpatient group	22	4 (18.18%)	41	11 (50%)
Total	127	19 (14.96%)	188	53 (28.19%)
X2	127 *		188 *	

* Significant

** Insignificant

Table (7)

Water consumed by infants including the study

Type of water		No. of examination	HRV positive (%)	X^2
Tap	Boiled	51	2 (3.9)	2.77 *
	Unboiled	94	21 (22.34)	2.95 *
River	Boiled	60	4 (6.6)	4.46 *
	Unboiled	110	44 (40)	12.4 *
Total		315	71 (22.53)	27.58 *

Table (8)

Clinical features of rotavirus cases

Clinical features			Rotavirus cases	%	X^2
Diarrhea	Type	Watery	71	100	8.03*
		Bloody	0	0	50.8*
	Duration	> 8 day	62	87.3	2.46*
		< 8 day	9	12.67	34.3*
Fever			63	88.73	2.92*
Vomiting			63	88.73	2.92*
Dehydration			69	97.18	6.52*
Abdominal colic			70	98.59	7.25*

* Significant
** Insignificant

Table (9)

Social characteristics of infants including the study

Mother Education		No. 0f positive HRV	%	
Mother Education	Illiterate	48	**67.6**	
	Primary	17	23.94	
	Secondary	6	8.45	
	Higher	0	0	
Total	69.68			

Table (10)

Type of feeding in infants with acute Gastroenteritis, %

Patients group	Type of feeding					
	Breast		Bottle		Mixed	
	No. of examinations	No. of HRV positive (%)	No. of examinations	No. of HRV positive (%)	No. of examinations	No. of HRV positive (%)
Hospitalized group	118	17 (14.40%)	66	22 (33.33%)	68	16 (23.52%)
Outpatient group	30	3 (10%)	13	6 (46.15%)	20	7(35%)
Total	148	**20 (13.51%)**	79	**28 (35.44%)**	88	**23 (26.14%)**

Table (11)

Clinical features of Rotavirus patients

Clinical features			Rotavirus cases	%	X^2
Diarrhea	Type	Watery	71	100	8.03*
		Bloody	0	0	50.8*
	Duration	> 8 day	62	87.3	2.46*
		< 8 day	9	12.67	34.3*
Fever			63	88.73	2.92*
Vomiting			63	88.73	2.92*
Dehydration			69	97.18	6.52*
Abdominal colic			70	98.59	7.25*

** Significant* *** Insignificant*

Figure (1)

Distribution of acute diarrhea according to the known & unknown causes in percent

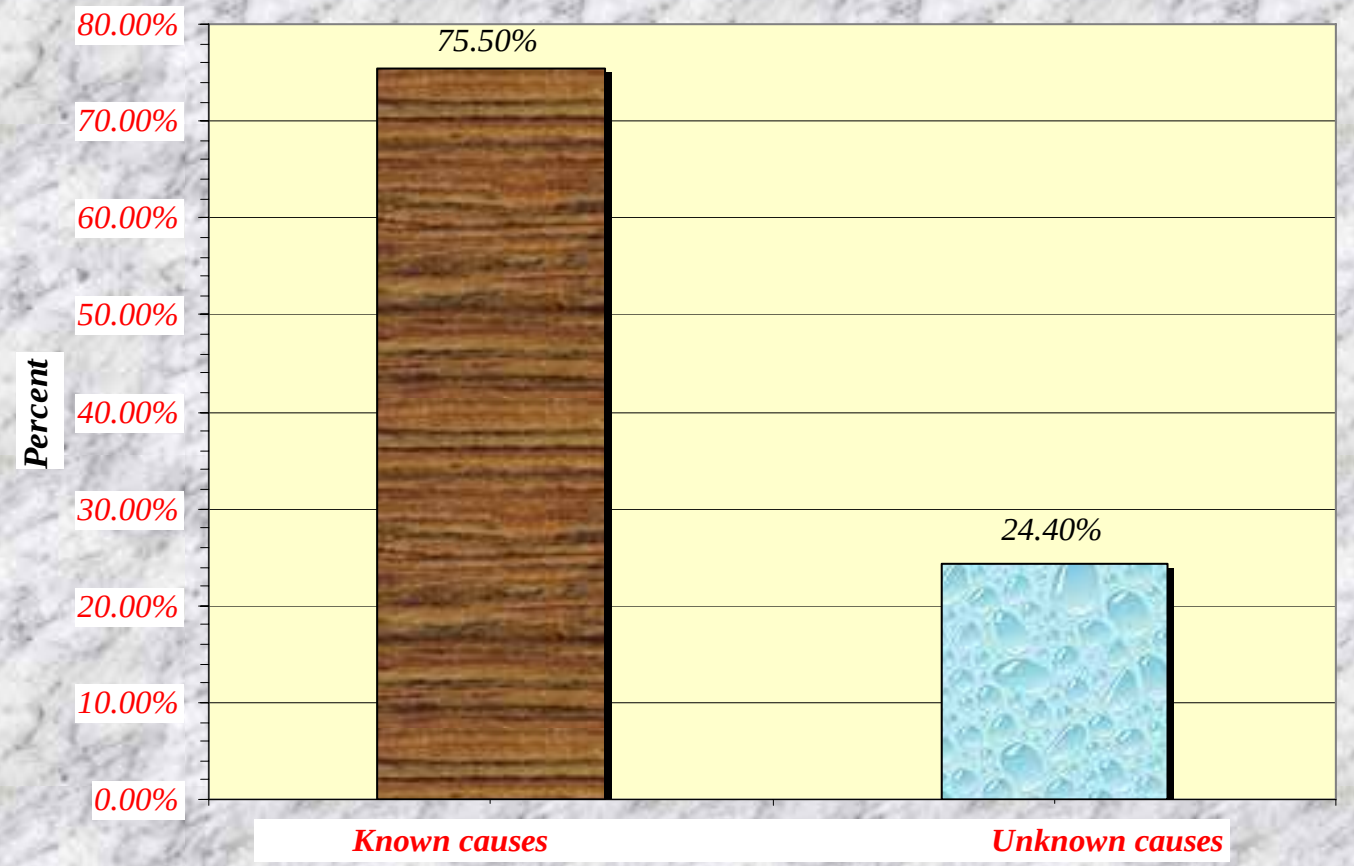


Figure (2)

Relation between the infants age & positave cases

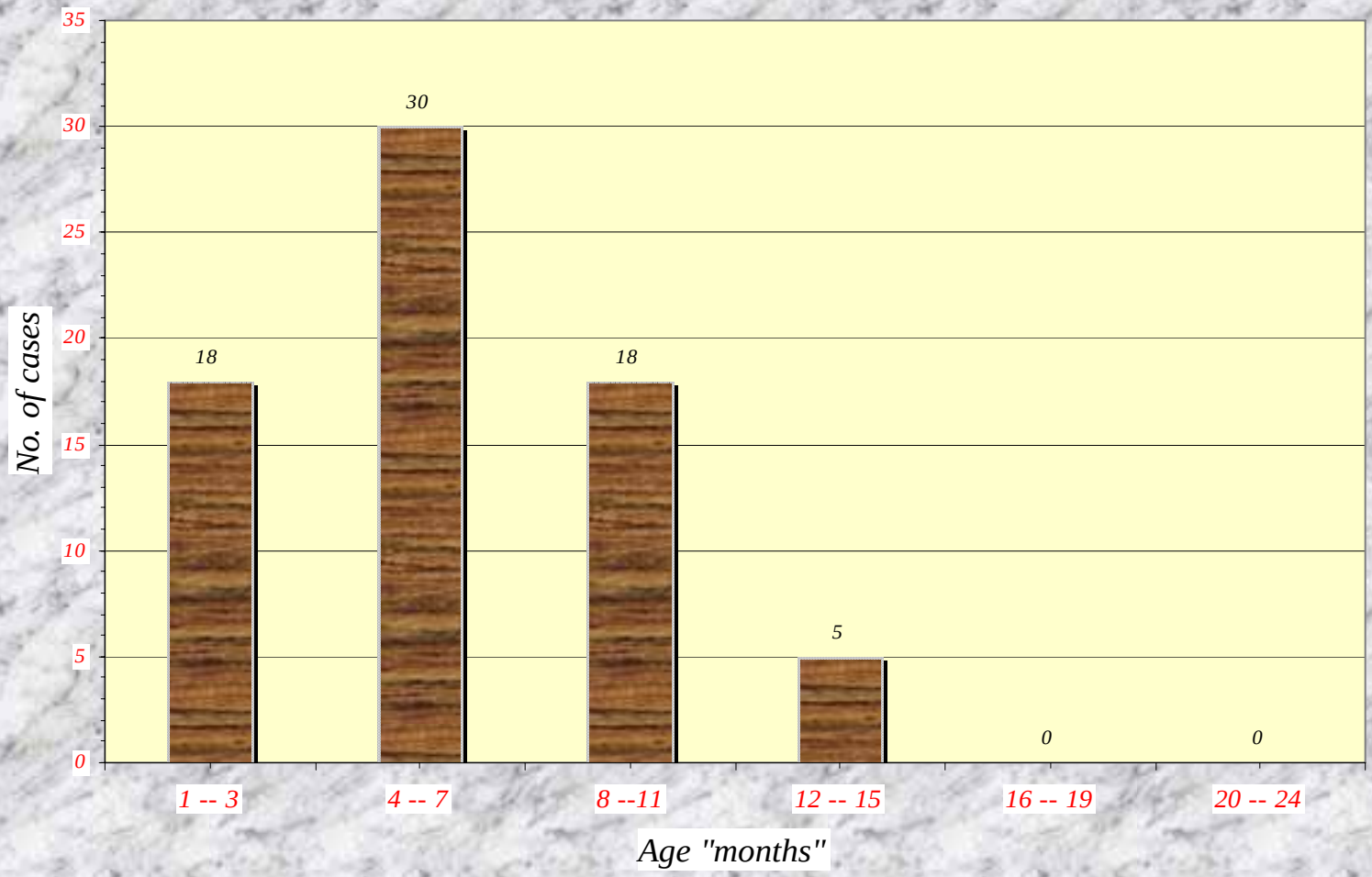


Figure (3)

Prevelance of pathogens causing infantil diarrhea

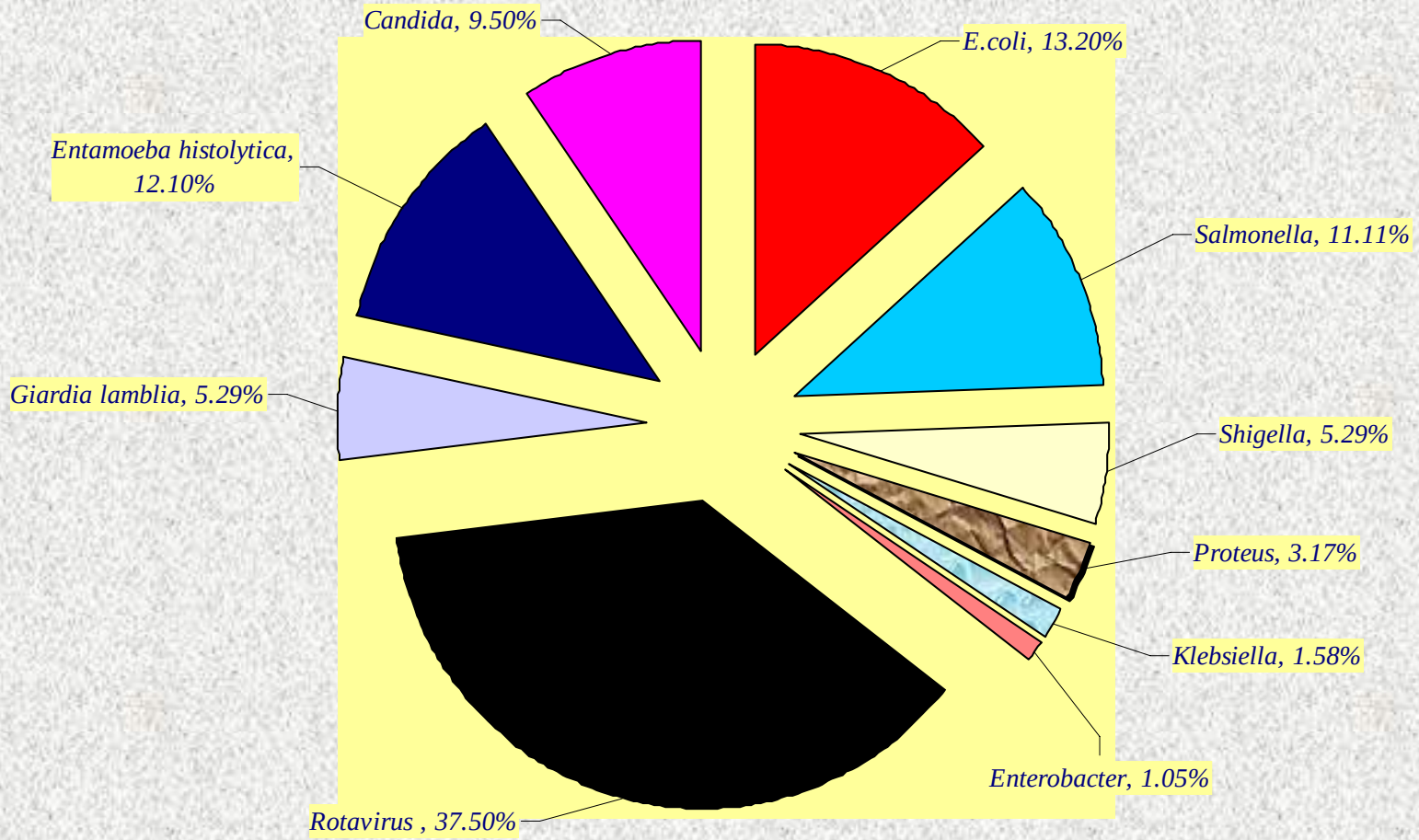


Figure (4)

Distribution of diarrhial causes (HRV, Bacteria, Parasite, Fungi) in Hospitalized infants"inpatients" & out patients infants

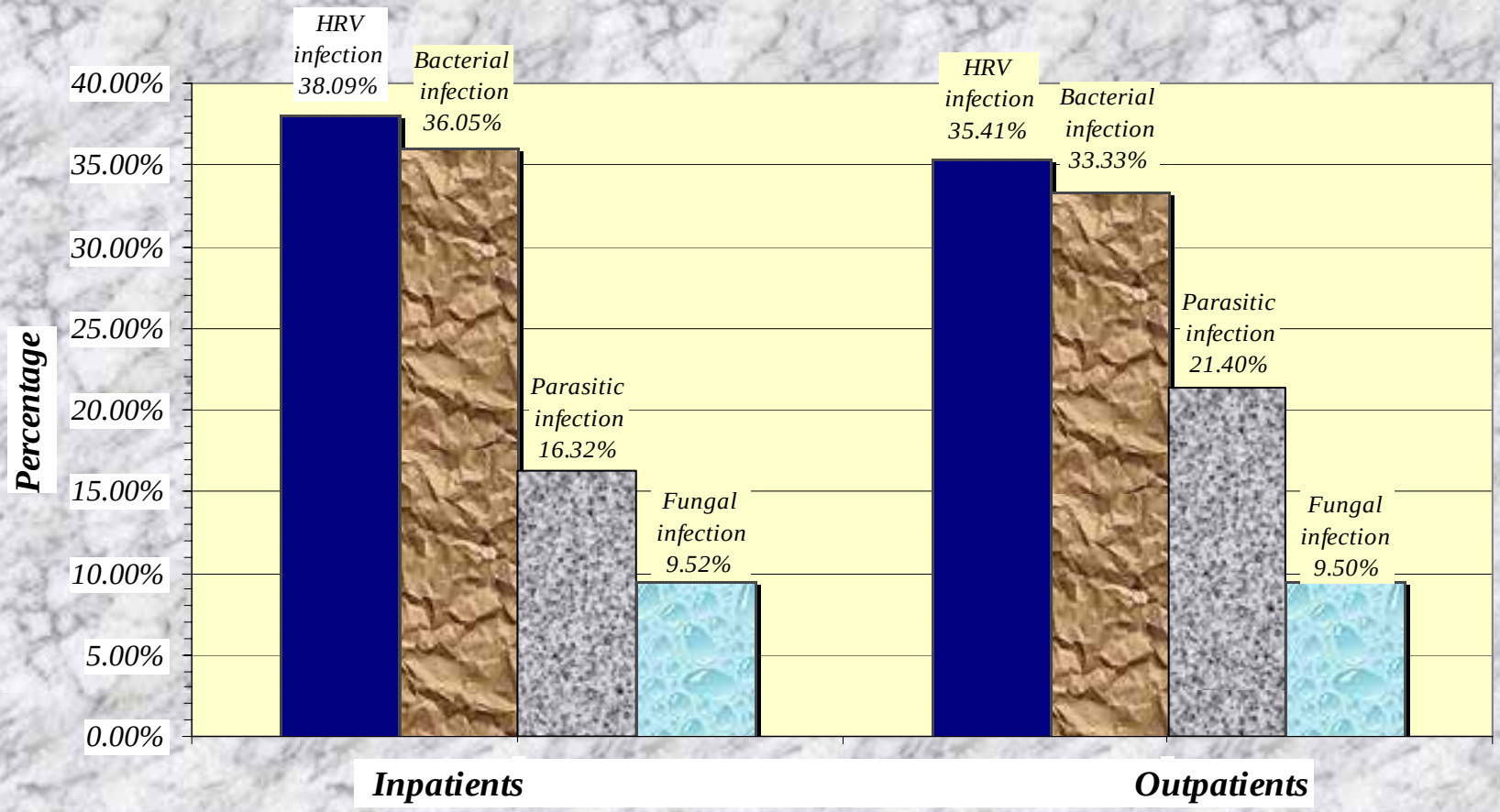
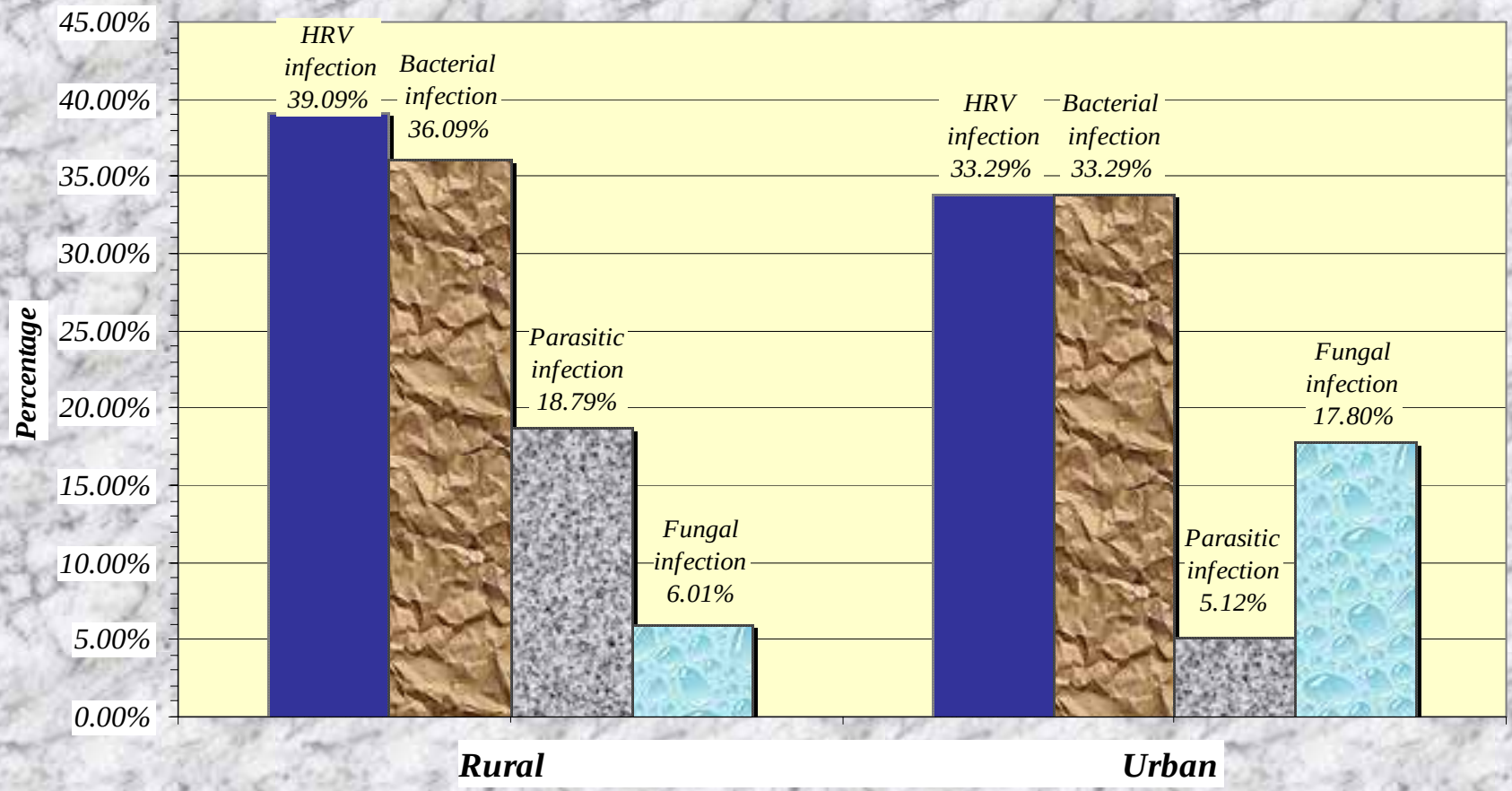


Figure (5)

Distribution of diarrhial causes (HRV, Bacteria, Parasite & Fungi) according to the residance



Thank You

Figure (1)

Distribution of acute diarrhea according to the known & unknown causes in percent