DIARRHOEAL DISEASES IN PRE-SCHOOL CHILDREN IN THE UNITED ARAB EMIRATES

BY

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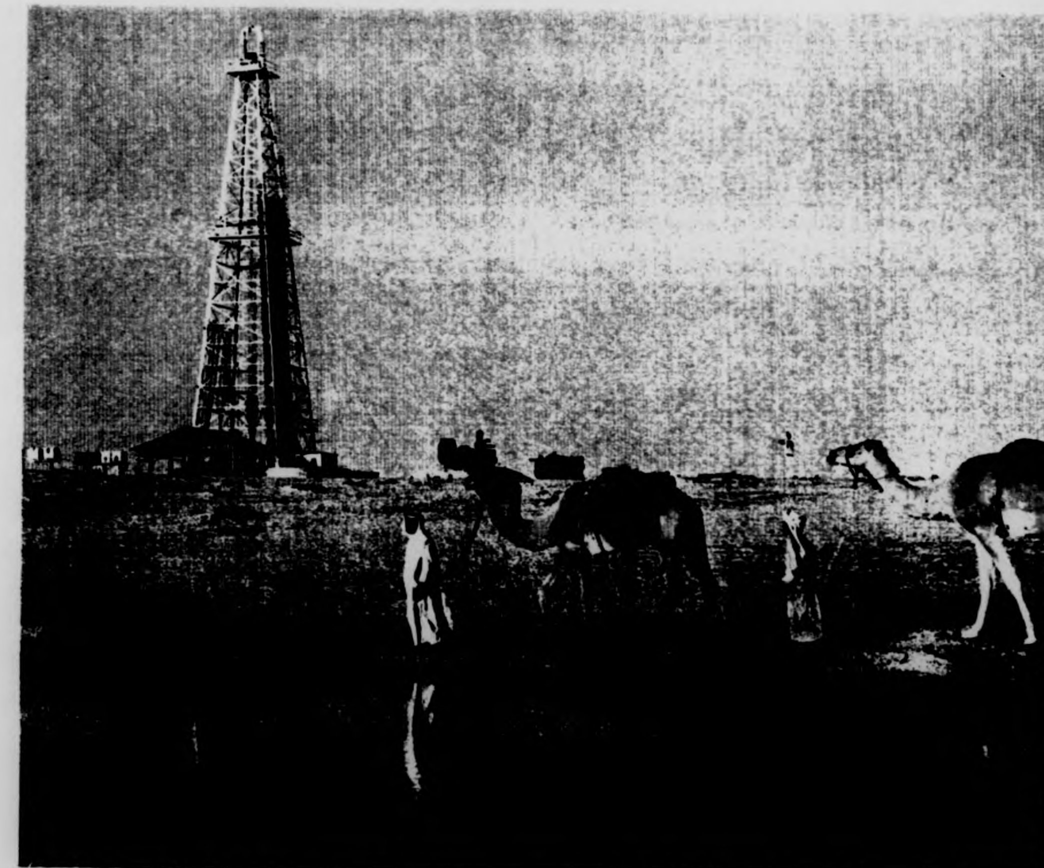
NUMEROUS ORIGINALS IN COLOUR



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DEDICATION

This thesis is dedicated to Safiya who was my pillar of strength during my absences from home. To Yousuf, Suad and Omar who were so understanding of those absences. To my parents for their full and enthusiastic support of my work, each; understandably for their own reasons. And last but not least to Amin for his patience, advice and support.

ABSTRACT

Little information was available on the epidemiology and etiology of diarrhoeal diseases in pre-school children in U.A.E.

Geographically considered as part of the developing world, the U.A.E. could have been expected to have developing world problems - diarrhoea being one of them. This study was conducted to determine why diarrhoea was not at present a leading cause of infantile mortality and morbidity in the study area and to examine the impact of the major health-related interventions on the health-care system.

Various sources of information and approaches to analysis were used:

1. Government statistics-information was obtained from the various government departments that collaborated in the interventions made.
2. Background data for the laboratory studies was obtained from routine clinical laboratories at Jazeira Hospital, Central Hospital and the maternity hospital.
3. Qualitative data was derived from observations made during the home visits to the study families for the six month study period, and included reviews with family members. Historical recollections were collected from older people as to the social conditions prior to the oil boom.
4. Quantitative data were obtained from the questionnaire that was administered to a group of study families and from laboratory analysis of specimens collected from these families over the study period.

Government sponsored interventions at the primary health-care level seemed to have been successful in preventing diarrhoea. This was probably made possible by the explosion of wealth and affluence that came with the oil-boom and the accompanying

change in life-style and attitudes, which made possible the provision of clean water and a sewage system, thus cutting the most important routes of infection.

ACKNOWLEDGEMENTS

I am as grateful to the Ministry of Health and the Ministry of Education for making this study possible as I am grateful to the mothers who allowed me into their homes over the period of 6 months, without them the study would not have been possible. I am deeply indebted to my supervisor Professor Bohumil Drasar for his advice and guidance during this study. His patience and friendly nature always left a reassuring effect.

I am grateful to Professor Abdul-Rahman Salim, my supervisor in Abu-Dhabi for his valuable assistance and advice and for his enthusiastic support of this study. And I am also grateful to Dr. A.M.Nur for his assistance with Ministry of Health data and its interpretations.

I am also grateful to Dr. T.J Harrison for his supervision and patience in what was a challenging subject to me.

My thanks are due to Mr. J. Bokor and Mr. M. Myatt for the invaluable advice and help at computing and without whom I might still be wandering through the amazing labyrinths of Wordperfect and SPSS !

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I would like to extend special thanks to my colleagues at Jazeira Hospital Laboratories for being helpful whenever help was needed.

Special special thanks go to my parents for being so wonderful and supportive; in more ways than one; to my family and myself throughout the duration of this study, not once allowing me to doubt the wisdom of pursuing a higher education alongside the responsibilities of a family. Special thanks also go to my husband and children who have supported and helped me and who have patiently put up with my pre-occupation with my work and who continue to show a pride in my achievements.

Heartfelt thanks go to all my friends who have been a wonderful support to my family and myself throughout the study period- my life is richer for them; may they all be allowed to achieve their dreams as I have.

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Last but not least, my thanks go to my colleagues Mr. A. Tayeh and Dr. E. Al-Khidir for their support, keen interest in this study and their valuable advice and attention.

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List of Abbreviations

° c	Degrees centigrade
B	Bottle feeding
BCG	Bacillus of Calmette and Guerin (Tuberculosis vaccine)
BSA	Bovine Serum Albumin
cpm	Counts per minute
dATP	Dideoxyadenosine 5'- triphosphate
DCA	Deoxycholate Citrate Agar
dCTP	Dideoxycytosine 5'- triphosphate
dGTP	Dideoxyguanosine 5'- triphosphate
DNA	Deoxyribonucleic Acid
DPT	Diptheria, tetanus and pertussis vaccine
DTM	Mixture of deoxynucleotide, tris HCl, Magnesium chloride and mercaptoethanol
E.coli	*Escherichia coli*
EDTA	Ethlene Diamine Tetraacetic Acid
Ent. histolytica	*Entamoeba histolytica*
FCS	Fetal Calf Serum
G.lamblia	*Giardia lamblia*
Hepes	N - 2 - hydroxyethylpiperazine - N - 2
LT	Heat-labile Toxin
M	Molar
MCE	Mercaptoethanol

MCHC	Maternal and Child Health Clinic
ml	Millilitres
mM	Millimolar
MMR	Measles, Mumps and Rubella vaccine
MOH	Ministry of Health
Mos	Months
NOI	No pathogenic Organism Isolated
NS	Not Significant
Oligo/2	Oligonucleotide type 2
ORS	Oral Rehydration Solution
ORT	Oral Rehydration Treatment
PB	Partial breast feeding
SB	Sole breast feeding
SDS	sodium dodecyl sulphate
SDW	Standard Distilled Water
sp	Species (singular)
spp	Species (plural)
SPSS	Student's Package for Social Sciences
SSC	Standard Saline Citrate
ST	Heat-stable Toxin
TEAM	The Evangelical Alliance Mission
UAE	United Arab Emirates
ug	Microgram
ul	microlitre

VT	Vero Toxin
WHO	World Health Organisation

Chapter 1. INTRODUCTION

Contents of Chapter 1

The United Arab Emirates:

Historical Context:

In response to the announcement by Britain that she was pulling out of Suez in 1968, the rulers of 7; then little known Sheikdoms in the south-eastern Arabian peninsula came together and agreed to form a federation known as the United Arab Emirates (U.A.E.) in 1971.

The turn of the Twentieth century found the area going through a period of prosperity due to the demand for natural pearls found in the Gulf waters, which were sold mainly in India. Money from these pearl sales was used in buying goods such as basic food commodities; mainly rice; spices, perfumes, herbal and natural medicines that were brought to the markets. A small trading community developed that gradually attracted some merchants from India and Iran, who came to settle in the Arabian side of the Gulf thereby escaping the oppression and tough taxation system of the of the Kajar Shahs. The naturally harsh environment coupled with the invention of the cultured pearl in Japan in the 1930s saw the area fall into economic hardships (Heard-Bey;1982). The world-wide depression that followed the Second World War did not make matters easier.

As a result of the explosion of wealth that accompanied oil production, the country has undergone a rapid process of economic and social development. The life-style has changed utterly in less than a generation. The main city and capitol of the U.A.E. called Abu-Dhabi has little today to recall the past, simply because before the oil boom there was very little of a town let alone a city. While houses made of palm fronds (Barrasti or Arreshas) or tents (plate 1 and 2), 3 or 4 buildings and a fortress were all that was scattered on the island (plate 3), today the city's skyline is reminiscent of a mini Manhattan when viewed from a distance (plate 4). The past and its difficulties have become mere faded memories in the minds of the older generation.

Geographical Situation:

The U.A.E is situated in the southeastern part of the Arabian peninsula and stretches across an area of 77700 Km sq, with a coastline of about 650 Km along the Gulf (plate 5). Most of the area is desert except for the eastern side of the peninsula which is mountainous.

Climatic Conditions:

The summers are very hot and humid while the winters are mild, followed by a short spring. The highest temperatures are usually reached during the months of July and August and the lowest usually occur during January and February (table 1). Humidity remains almost constant throughout the year (table 2).The annual rainfalls are less than 10 cm and occur in winter. Sandstorms blow in spring from the north and the northwest. Vegetation growth is confined mainly to the oasis and the mountainous areas, although the last 20 years have seen enormous efforts to introduce vegetation and greenery to the country especially Abu-Dhabi earning it the title of "The Garden of the Gulf". (plate 6).

Different types of houses previously found in the U.A.E.

Plate (1): A Barasti House

Plate (2): A Tent House

Plate (3): An Old Aerial View of the Corniche

Plate (4): A Recent Aerial View of the Same Strip of the Corniche

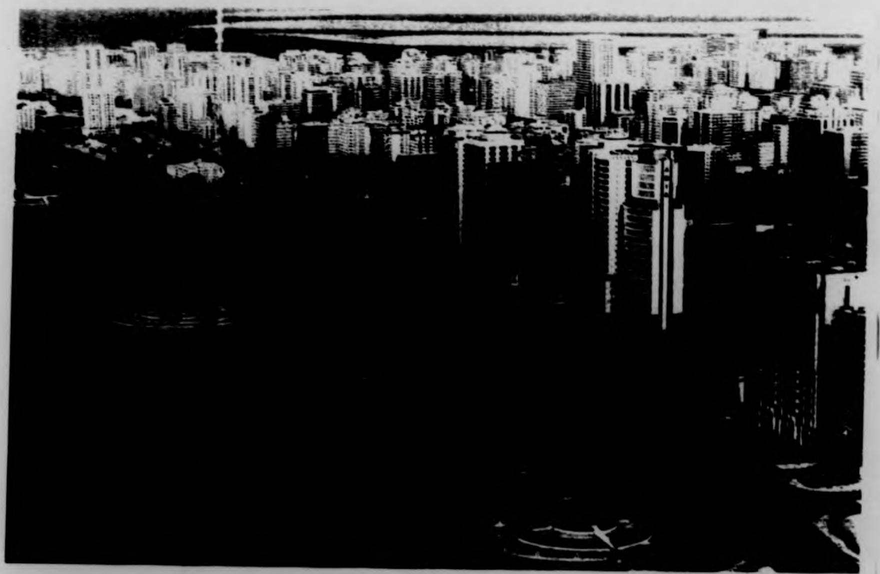


Plate (5): Map of the Gulf Region

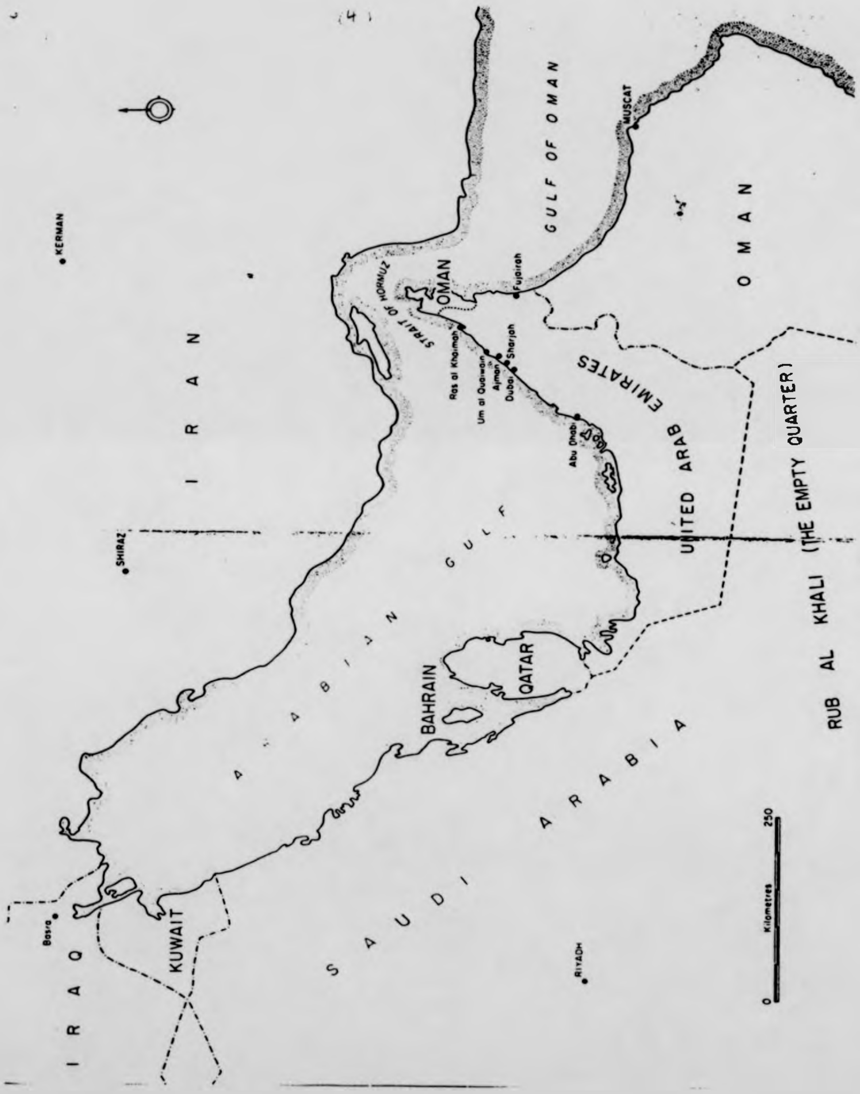


Table 1:

ADIA & BATEEN CLIMATOLOGICAL SUMMARY 1989

DRY BULB TEMPERATURE - DEG. C

ABU DHABI BATEEN AIRPORT - 1989

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	YEAR
Mean daily*	17.3	18.5	21.8	25.0	29.8	32.3	35.0	34.5	32.4	29.0	25.4	20.7	26.8
Mean daily maximum	21.6	23.4	28.1	30.2	37.1	38.4	41.9	41.4	38.9	35.0	31.4	25.4	32.7
Mean daily minimum	12.8	14.0	16.1	20.0	24.0	27.1	29.6	26.7	23.3	19.9	16.0	16.0	21.6
Absolute maximum	24.6	33.8	35.6	35.8	42.0	42.0	46.4	45.2	43.5	38.8	35.6	31.3	46.4
Absolute minimum	8.7	11.5	11.5	15.3	19.5	23.1	24.5	27.6	21.8	19.1	15.4	12.4	8.7

Table 2:

ADIA & BATEEN CLIMATOLOGICAL SUMMARY 1989

RELATIVE HUMIDITY - %

ABU DHABI BATEEN AIRPORT - 1989

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	YEAR
Mean Daily*	66	64	62	64	57	61	56	61	59	65	66	67	62
Mean daily maximum	83	83	87	85	81	83	81	84	82	86	86	84	84
Mean daily minimum	46	41	31	40	27	35	26	29	29	36	41	45	36

Plate (6): Abu-Dhabi, The Garden of the Gulf

Demography:

The UAE - known as the Trucial States before independence in 1971 is made of 7 separate emirates:

Abu-Dhabi, Dubai, Sharjah, Ras al-Khaimah, Fujeira, Ajman and Um al-Quain.

The largest emirate being Abu-Dhabi with an area contributing to 87% of the total area, whilst the smallest being Ajman contributing to an area of 0.33% of the total area or 259 Km sq.

The total population estimate for the UAE according the 1989 census was 1.63 million, showing an increase of 57% since 1980. The UAE nationals are thought to comprise 30% only of the total population and the remaining 70% are made up of the 96 or so nationalities that live in the UAE. 675,100 or 41% of the total population live in the emirate of Abu-Dhabi. Therefore using the 30% estimate the ethnic people of the UAE in the Emirate of Abu-Dhabi number about 202,530 people.

The principle source of income to the country is from the oil revenue and the per capita income is one of the highest in the world.

The ethnic people of the U.A.E. originate from Arab nomadic tribes that roamed the Arabian peninsula setting marked areas for each tribe (Hanthal; undated). Some of these tribes settled around oasis such as Al-Ain (the second major city in the emirate of Abu-Dhabi) or around the market towns such as the market town of Dibba, or in small towns such as Dubai and Abu-Dhabi which had the attraction of being a natural fortress as it is a island and so provided the ruler of the Emirate of Abu-Dhabi with protection from enemies. Settlers in such towns with outlets to the sea became seamen diving for pearls in the seas of the Gulf, or became traders and merchants with India and Iran. The ethnic population also includes families of Iranian or Indian extract that moved into the area during the pearl boom at the turn of the century and whom have become part of this society and have been granted U.A.E. status according to Federal law, but yet kept a separate community identity of their own. The rest of the population or the non-ethnic population is made up of a mixture of 96 nationalities from all over the world, this includes a large number of Arab residents from other Arab countries, a large number of residents from the Indian sub-continent and the far East as well as a sizeable number of Europeans and Americans.

People of U.A.E. status or from countries that are members of the Gulf Cooperation Council are the only people allowed permanent residence in the country. Residence for all other nationalities is related to service contracts and may vary from 1 year to 5 years. At the onset and the expiry of the residency period, all applicants are required to produce a clear health certificate from government health departments. This is in an effort to limit and control the spread of certain infectious diseases and to treat any infectious diseases that are easily treated if diagnosed properly. This is also an effort to protect citizens already living in the country as well as an effort to ensure that workers are healthy to do the work they were contracted to do in their country of origin, for example it is important to ensure that housemaids are not pregnant upon arrival. Such health certificates include blood tests for diseases such as AIDS or Hepatitis, Chest X-rays to check for tuberculosis, urine tests to check for pregnancies especially amongst domestic female workers, stool tests to look for parasitic infections. Failure to provide such a certificate would result in the termination of the residency permit.

Facilities in the U.A.E.:

Medical Facilities:

Within the last 20 years a network of health facilities has been established and funded by the Federal Government of the U.A.E. and local governments of some of the individual emirates making up the U.A.E., throughout the entire country providing hospitals, clinics, Maternal Child Health Centres (MCHC) and public health centres. According to an official at the MOH, although there has been no increase in the budget of the MOH in the past 10 years, the expansion of the health services in terms of quantity and quality could be explained by the fact that other sectors; such as The Dubai Health and Medical Services Department, the petrol companies medical services in addition to the Ministry of Defence; contribute effectively to expenditure. In addition the local government of Abu-Dhabi indirectly supplements the Federal MOH by buying equipment, providing additional funds to overcome any shortages that may arise and by recruiting man power. The increase in salary costs are shown in figure (1) as an example of the increase in expenditure on these health facilities. The country is divided into 8 medical districts (2 being in Abu-Dhabi and one for each other emirate). The services provided by these facilities are easily accessible to all residents of the country. Immunisations and vaccinations are provided free at government clinics.

The health facilities are staffed by qualified expatriates such as doctors, nurses, technicians from a wide range of countries and a steadily increasing number of professional locals of U.A.E. origin. As the ethnic youth of the country are becoming more educated and qualified, these posts filled by expatriates are slowly but gradually being filled by U.A.E. locals.

Transport Facilities:

The entire country is inter-linked through a network of roads enabling movement from city to city and allowing access to the more remote regions of the country, a task that would have involved great effort a mere 2 decades ago. Public transport is available in the forms of taxis and buses, both of which are reasonably cheap.

Water, Sewage and Electricity Facilities:

Piped water reaches the majority of the U.A.E. In some of the remote mountainous areas such as those of Ras-Al-Khaimah or the newly built extensions of some towns the local municipalities provide water in tanks to each and every house, while the permanent systems are being installed. These facilities are again funded by the Federal government ministries of Water and Electricity, but are dispensed by the local authorities according to the needs of each area. Sewage networks as well as electricity reach all the cities of the U.A.E., and these too are funded by the Federal government. In Abu-Dhabi piped water supply reaches the entire city from the local desalination plant, which also provides some of the water supply of Al-Ain, the second city in the emirate of Abu-Dhabi. The desalinated water is treated by chlorination before the distillation step, after which various salts are added and this is followed by another chlorination step. Figure (2) shows the history of increase of water supply in the Emirate of Abu-Dhabi from 1979 - 1989. Over 25 years or so ago water was transported to Abu-Dhabi in tanks from Dubai, or provided by a single boiler; plate (7); which was a single and simple distillation unit and in both cases had to be bought and usually stored in Bitumen lined woven or straw vessels, (Bitumen is a naturally occurring tar in this area that was used for the lining of utensils used for storing liquids - water included. Later a single water line from Al-Ain provided water for the entire island and water was taken by the locals from a single pump (plate 8). At that time that was considered a vast improvement since the only way water could be obtained was by digging shallow wells in areas a little distant from the sea, as this was considered less salty and therefore more acceptable for human consumption, the sand presumably acting as a crude form of filter. These areas were known to the locals as places were rain water and dew collected and floated on top of a bed of salty water, the fresh water presumably being lighter than salty water. When the fresh water from one shallow well was used up another was dug (Heard - Bey; personal communication). Obviously there would have been a variation in the degree of salinity of such water with the variation in rainfall from year to year and in the depletion of the fresh water layer between rainy seasons.

Fig (1): Annual Salary Cost in Abu-Dhabi

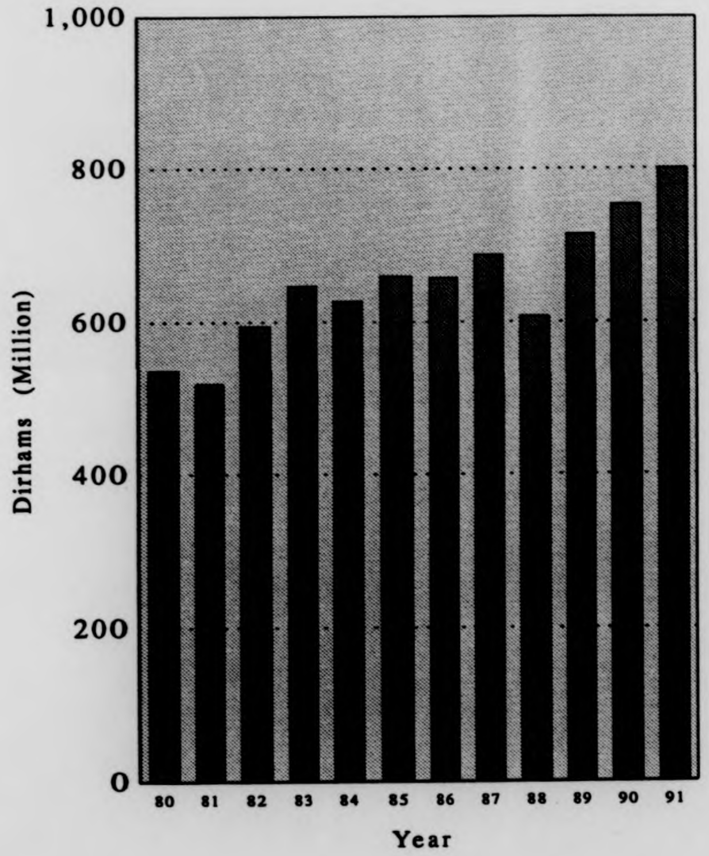


Fig (1): Annual Salary Cost in Abu-Dhabi

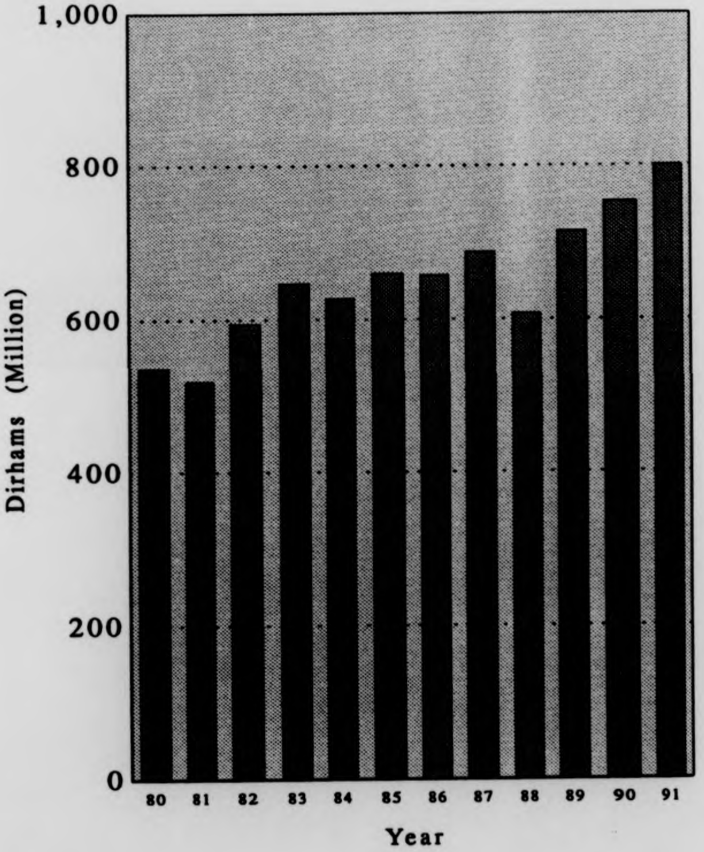
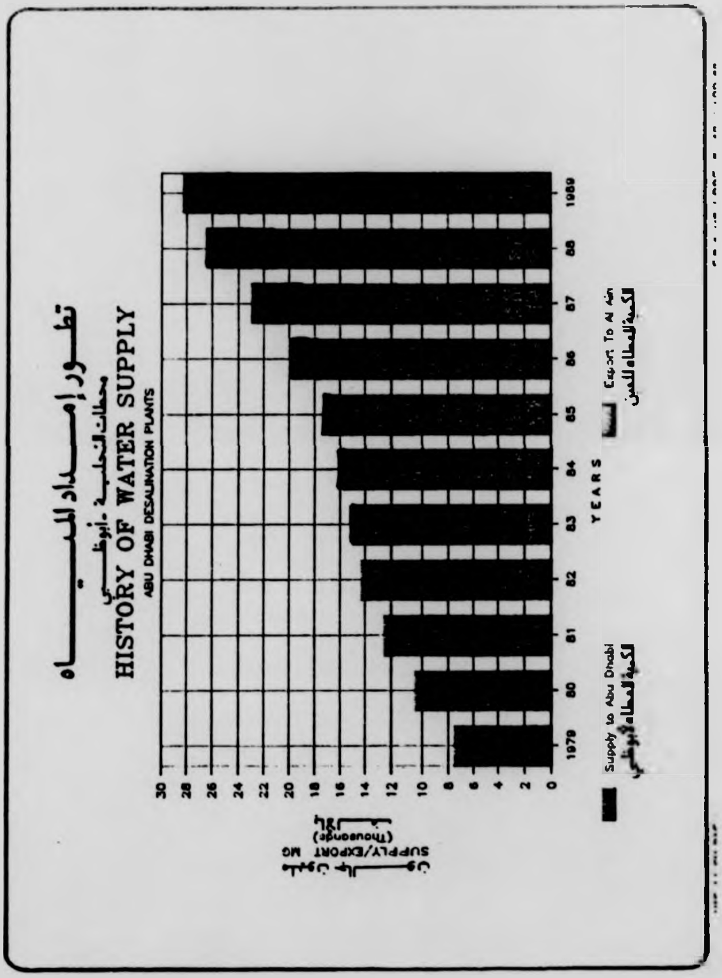


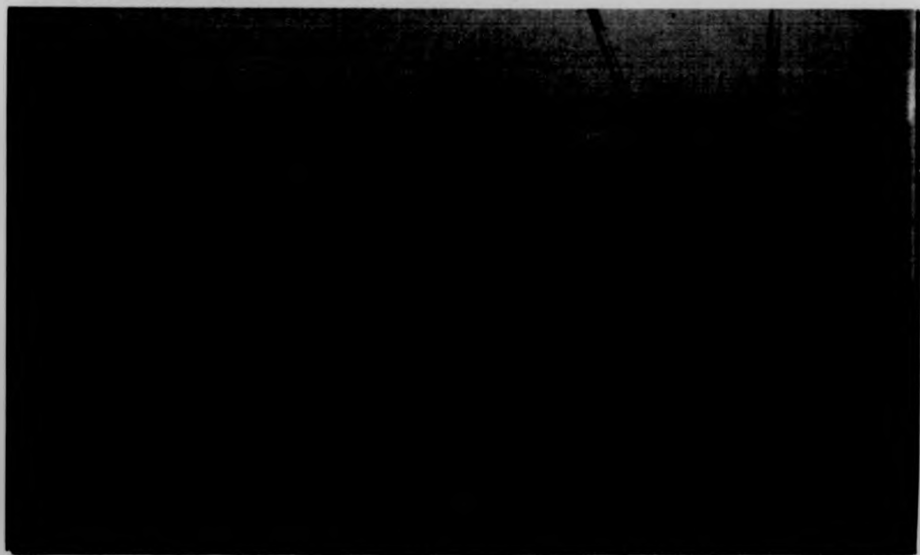
Fig (2): History of Increase of Water Supply in Abu-Dhabi



Methods of obtaining water over 25 years ago

Plate (7): Obtaining Water From the Boiler on the Corniche

Plate (8): Obtaining Water at the Water Pump



A sewage network system reaches the whole city, all toilets benefit from flush systems, another vast improvement from almost 25 years ago when the sea front was used for excretory purposes, the waves provided a means of cleansing the seafront. Sewage is transported via pipelines to a sewage plant 32 kilometres out of Abu-Dhabi city where it is treated chemically and biologically. The chemical treatment is mainly for the disinfection of the effluent which is then returned along a pipeline for distribution in the city where it is used for irrigation purposes. The biological treatment which is a lengthy and extensive process, is for the treatment of the sludge, which ends up as fertilizer for local use. The entire city has electricity supply.

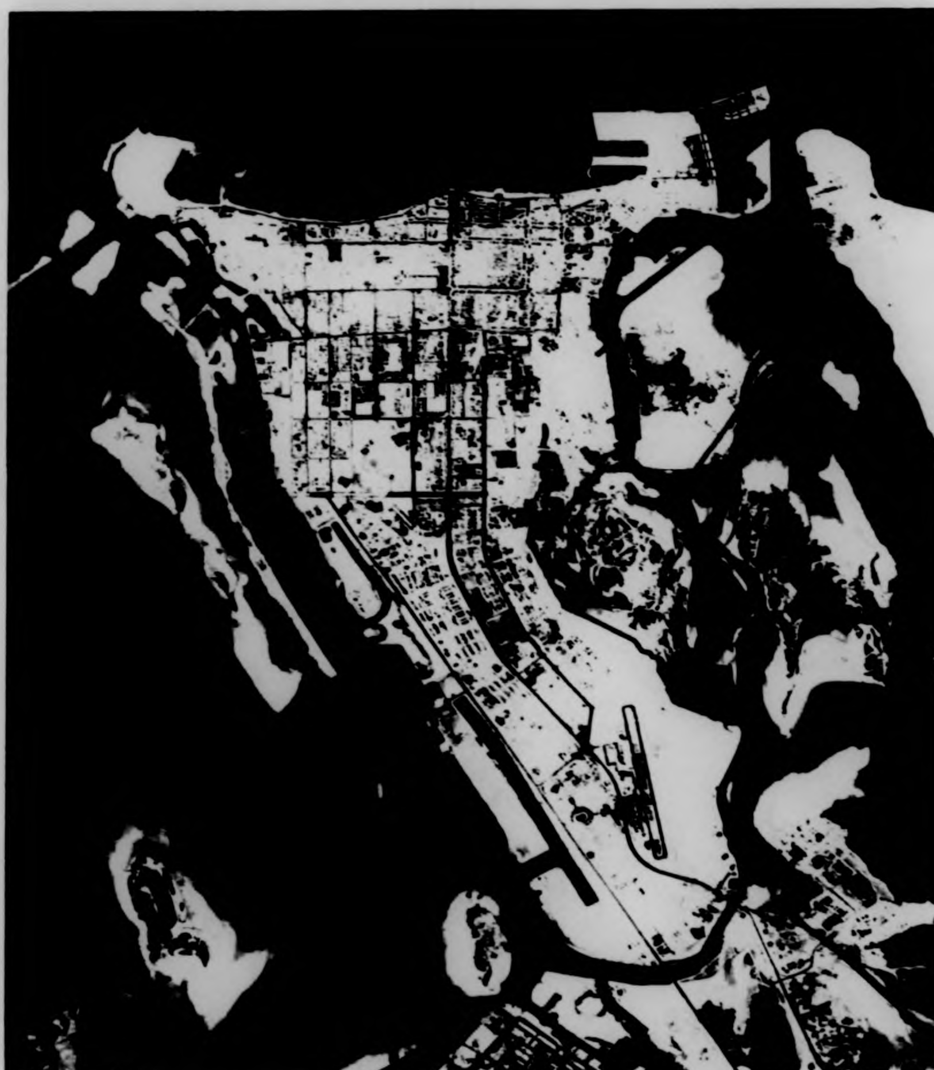
Abu-Dhabi:

Abu-Dhabi city itself is the best example of how "petrodollars" can be used to the best advantage of both city and citizens. Once a desert island connected to the mainland by a narrow strip of land, the island now connected to the mainland by 2 bridges (plate 9), is considered the " Garden of the Gulf " because of the abundance of greenery, achieved at great expense and effort.

The markets are of good quality and hygienic levels (plates 10, 11 and 12), and are subject to frequent inspection by the health departments of the municipalities of each emirate. Most vegetables and fruits are available the whole year through as they are almost all imported from all parts of the world.

With the affluence and the higher standard of living that the last 25 years has seen, the levels of hygiene have vastly improved. The abundance of water as shown by figure (3) of the per capita in water consumption and the availability of basic commodities, previously regarded as luxuries; such as soap, have made being clean easier and more in "vogue". In the absence of soap, sand was previously used as a detergent for washing hands, cooking utensils etc., clothes were washed in sea water and allowed to dry on the sand in the sun. Data obtained from the Chamber of Trade and Commerce and illustrated in Figure (4) show the amount of soap imported to the emirate of Abu-Dhabi from 1980 - 1990. Records from the last 10 years only are available, but they show the trend that has been occurring for these past 2 decades. From talking to ladies who lived during those difficult years and whom now are the grandmothers of this generation, the diet of the people of this area seemed to be mainly made up of rice; imported from India and Iran; fish and dates, fruits and vegetables were not much demanded, the availability and expenses having a lot to do with the acquisition of the habits of eating those foodstuffs. A description of the trading activities and commodities available in the markets at the time is given by Heard-Bey; 19882

Plate (9): Aerial View of Abu-Dhabi



Reduction of a photo mosaic published at scale 1:20,000, produced from photography taken at an altitude of 6,000m.

ABU DHABI ISLAND 1990

Plate (10): Selling Vegetables in Recent Times

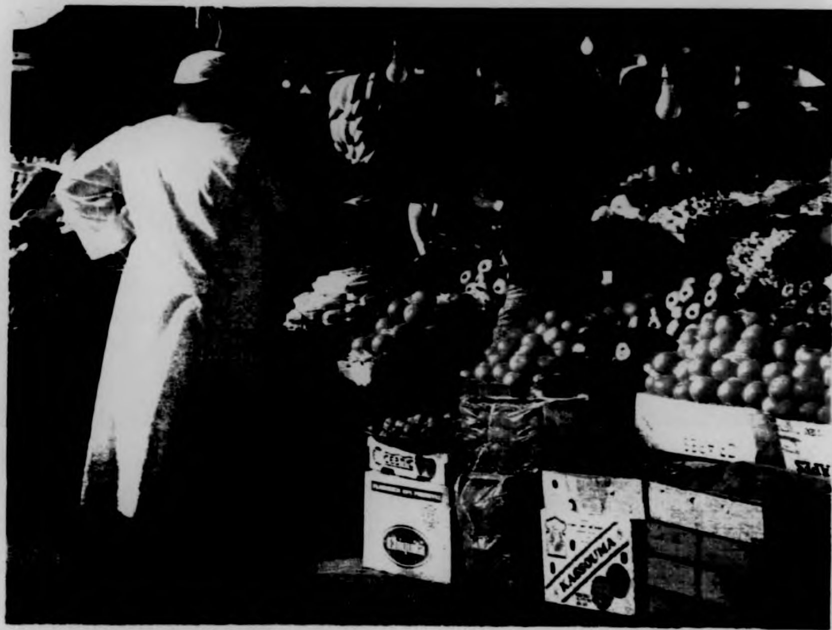


Plate (11): Selling Vegetables About 25 years Ago

Plate (12): Selling Fish About

25 Years Ago

Fig (3): Per Capita Annual Consumption of Water in Abu-Dhabi

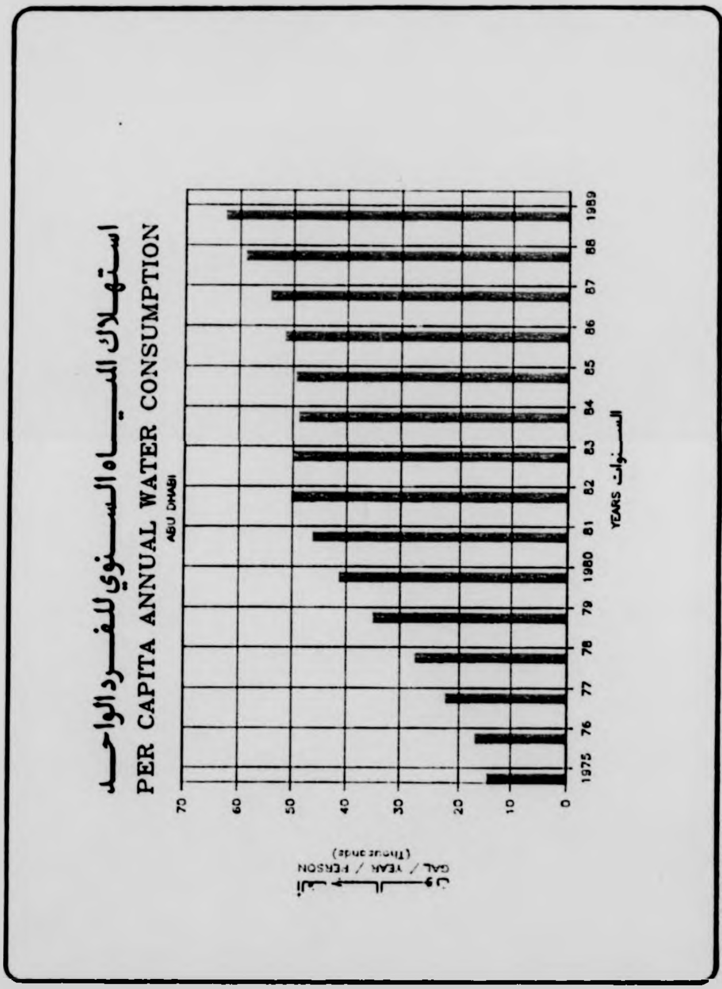
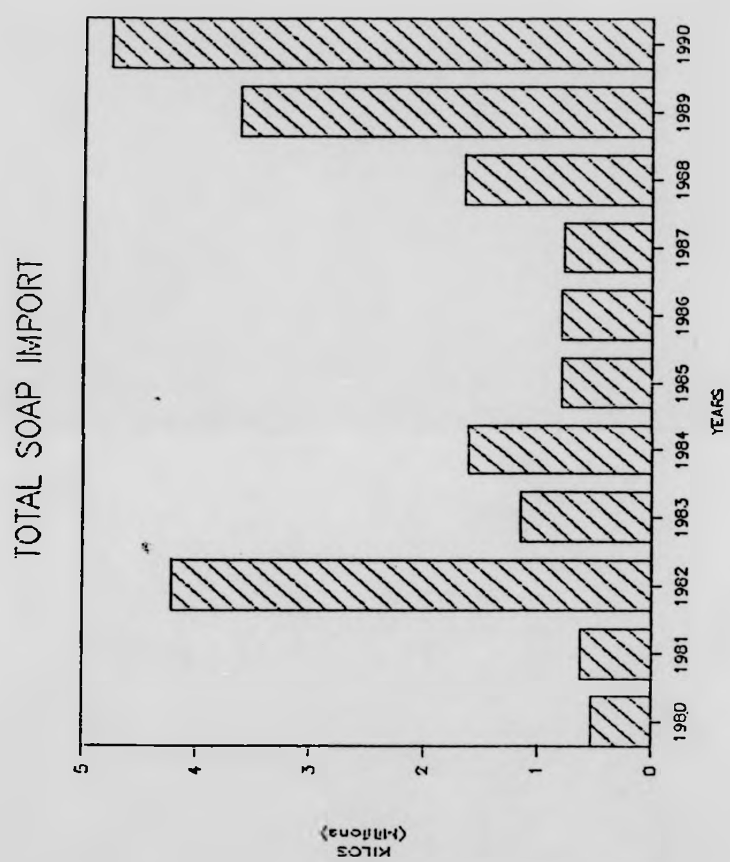


Fig (4): Soap Import Data for Abu-Dhabi



Definition of Diarrhoea:

" Diarrhoea maybe defined as the passing of liquid or watery stools. The liquid stools are usually passed at least 3 times in a 24 hour period; however it is the consistency rather than the number of stools that is the most important feature".

WHO / CDD / 80.2 REV.2 (1990)

From data from 24 published studies, Snyder and Merson (1982) found 8 different definitions of diarrhoea from only 10 of the 24 studies that cited a definition of diarrhoea (Table 3). In the course of this study 10 additional definitions were found from various reviews and studies in various countries. Although similar they had slightly different wordings (Table 4).

The World Health Organization (WHO) document cited above continues :

" In most societies, mothers will know when their children have diarrhoea and may themselves provide a useful working definition for the local situation ".

It is difficult to standardize a definition for a condition which at best may cause a slight inconvenience and at worse may lead to mortality. The statistics of the disease in 1980 according to Snyder and Merson (1982) showed that the annual number of deaths from acute diarrhoeal disease in children under 5 years of age in Latin America, Africa and Asia (excluding China) was estimated to be 4.6 million and the number of acute diarrhoeal episodes in the same age-group was estimated to be 744 million.

Table 3

Definition of diarrhoea used in studies of morbidity rates

(adapted from Snyder and Merson; 1982)

Country	Definition
Bangladesh	more than 2 watery or loose motions in 24 hours.
Cost Rica/Kenya	mother's definition.
Egypt	5 or more stools in one day preceded and followed by 1 week of normal stools.
Ethiopia	4 loose stools/day or 1 watery or bloody stool/day.
Guatemala	< 1 year of age: 5 or more liquid or semi-liquid stools/24 hours.
India	3 or more liquid stools in 12 hours with or without blood or mucus.
India	3 or more loose stools of altered consistency or without blood or mucous in 24 hours.
Indonesia	more than 4 stools in one day.

Table 4 - Definitions of Diarrhoea From Various Studies

Country	Reference	Definition
Australia	Bishop et al; 1979	Change in frequency and/or character of stool (> 6 passages/day)
CAR*	Georges et al; 1984	Emission of at least three times/day of stools taking form of container
India	Khan and Yunus; 1990	One or more loose stools/day
Kuwait	Al-Nakib et al; 1980	Alterations in frequency and character of stools with or without vomiting
Kuwait	Sethi et al; 1984	5 or more loose or watery stools accompanied by symptoms usually associated with gastroenteritis
Mexico	Craviotto et al; 1988	4 or more liquid movements in 24 hours with a liquid or semi-liquid consistency of stool
Nigeria	Huttley et al; 1987	3 or more stools of a consistency < normal in a 24 hour period
NE Brazil	Guerrant et al; 1983	A significant change in bowel habit toward decreased consistency or increased frequency
Peru	Black et al; 1989	A day with liquid stools totalling at least 6 for infants < 1 month, 5 for infants 1 month and 4 for infants > 1 month
Taiwan	Echeverria et al;1977	stools that assume the shape of the container and occur with more than the usual frequency
S.Korea	Echeverria et al;1979	3 or more unformed stools or 2 unformed stools accompanied by tenesmus or vomiting within a 24 hour period.

As seen from table (4) there is a variation in the definitions of diarrhoea given by workers from different countries or even different workers in the same country. Some definitions are very precise on what should be considered a diarrhoea while others are not so precise. This causes difficulties when attempts are made to compare the findings of various studies as the standard applied to begin with may not always be the same. Although the definitions may vary slightly, there is no disagreement about the hazards diarrhoea causes. It leads to dehydration through the loss of water and electrolytes that are not replaced. It also leads to malnutrition which in turn predisposes to diarrhoea, thereby forming a reciprocal relationship. While malnutrition is a determining factor in diarrhoeal duration, it was not found to be a determining factor in the incidence of diarrhoea amongst children (Black et al; 1981)

Factors Associated with Diarrhoea:

Diarrhoea in children is determined by the combination and interaction of 2 types of risk factors:

1. Environmental risk factors .
2. Host risk factors .

Environmental Risk Factors:

The environmental risk factors that contribute to diarrhoea often prevail under conditions of poverty and over-crowdedness (Huttley et al, 1987). There is little doubt that the improvement of sanitary systems (Daniels et al; 1990) and water supply systems (Mertens et al; 1990) and food hygiene (Esrey and Feachem; 1989) would go a long way to controlling infectious diseases especially diarrhoeal diseases.

An analysis of the effect of water supply and sanitation facilities on diarrhoeal incidence showed that improvements in water availability and sanitation disposal facilities had a greater impact in decreasing diarrhoea than improvements in the quality of water (Esrey et al; 1985). The introduction of piped water did not decrease the reported incidence of diarrhoea unless accompanied by an increase in water usage (Feachem et al; 1977).

Hygienic methods in food preparation are also necessary in decreasing the incidence of diarrhoea. Studies from the Gambia (Barrell and Rowland; 1979) and Bangladesh (Black et al; 1982b) have shown that traditional weaning foods as well as commercial bottle formulae are equally hazardous in increasing the risk of diarrhoea when prepared unhygienically using contaminated cooking utensils and water. When stored at ambient household temperatures until consumption, they provide a suitable medium for bacterial multiplication. This is important since it was estimated that a large dosage of 10^{8}-10^{10} bacteria are necessary to produce gastroenteritis (DuPont et al; 1971, Tomkins et al; 1978) and the long storage time accompanied by favourable temperatures allow for such high dosage numbers of diarrhoea causing agents to be reached. However even if the prevailing conditions (such as temperature or standing time) were not suitable for multiplication of the bacteria to occur and if all the food were contaminated as a result of bad hygiene, occasional attacks of diarrhoea are inevitable.

It should be noted that the estimated dose quoted above was obtained from human adult volunteer studies. Making allowance for a smaller body size and the less maturity of the immune system, the dosage number necessary to produce illness in children is likely to be less than that quoted above.

Host Risk Factors:

The host risk factors include age, personal and domestic hygiene, education of the mothers, supplementary feeding and socio-economic status.

Age was found to be closely related to diarrhoeal incidence, the most afflicted group being the under two years old (Snyder and Merson; 1982). In India Khan and Yunus (1990) attributed this to "an immunological immaturity, high morbidity due to frequent respiratory illness, low immunisation coverage, lack of personal hygiene and the greater incidence of malnutrition among them". Personal hygiene and education of the mother, maid, cook, siblings or anyone who comes in close contact with the children are also closely related to diarrhoeal illness. Most pathogenic organisms that cause diarrhoea are transmitted primarily or exclusively via the faeco-oral route. For some enteric organisms such as E.coli, Shigella spp., and possibly G.lamblia and Ent histolytica, man is the main reservoir and transmission originates from human faeces. For other enteric organisms such as C.jejuni, Salmonella spp., Y.enterocolitica, animals can be reservoirs of infection and transmission originates from human and animal faeces. There is evidence that hygiene education can improve hygiene standards and reduce diarrhoeal morbidity rates by 14-48% (Feachem; 1984). The most studied aspect of hygiene has been hand-washing. A study from Bangladesh showed that the use of bar soap and water lowered the risk of diarrhoea by 37% (Khan; 1982). The inability of bar soaps to support the growth of bacteria and to transmit bacteria from user to user was shown by Bannan and Judge (1965).

Diarrhoea and malnutrition are closely associated (Scrimshaw et al; 1968). Both exist in similar socio-economic situations. Malnutrition of children with diarrhoea prolongs the duration of diarrhoea and increases its severity and recurrence (Black et al; 1984), while diarrhoea often aided by the wrongful withdrawal of food during diarrhoeal episodes aggravates the problem of malnutrition in the patient. The socio-economic status of the families is a complex factor. It's evaluation may differ from community to community and from country to country. Generally the low socio-economic status of a family is usually associated with poverty, over-crowded conditions, poor sanitary and waste disposal conditions, inadequate water supply, lack of education. In Nigeria households living in premises with unrendered mud walls and mud floors were classified as "poor housing status" while those that had made improvements to either were classified "improved housing status". A significant reduction in risk to diarrhoea was found in people living in "improved" housing (Huttley et al; 1987). In Iraq methods of cleaning infant feeding bottles were more hygienic in higher socio-economic status families than lower socio-economic status families (Mahmood and Feachem; 1987b).

The introduction of supplementary feeding to the children, be it in the form of formula milk or traditional weaning foods to maintain optimal growth is often accompanied by an increase in diarrhoeal episodes (Guerrant et al; 1983), resulting in what is known as the "weanling dilemma". Whilst it has been observed that in many traditional communities breast-feeding is the only mode of feeding (Rowland et al; 1978), in others there is an increased tendency towards supplementary feeding (WHO; 1981). The evidence that breast-feeding protects from diarrhoea is strong (Feachem and Koblinsky; 1984). The immense number of immunologically active agents found in breast milk (Welsh and May; 1979, Chandra; 1980, Guerrant et al; 1983) along with the fact that breast-feeding eliminates the microbial contamination factor of food and water, have been an important reason in the suggestion by many workers to prolong breast-feeding as a means of controlling diarrhoea (Feachem and Koblinsky; 1984). While a study from Bangladesh has shown breast-feeding alone to be an inadequate source of nutrition in infants of under - nourished mothers, after 3 months of age (Black et al; 1982b), a Chilean study has shown that breast milk if produced sufficiently can maintain normal growth up to the sixth month of life (Juez et al; 1983). During the first half year of life exclusive breast-feeding was found to be more protective against diarrhoea than partial breast-feeding which in turn was more protective than no breast-feeding (Feachem and Koblinsky; 1984). Black et al (1989) found that infants during the first 6 months of life who consumed water, milk and other food in addition to breast-milk had an increased risk of diarrhoea of 40% more than exclusively breast-fed infants. A similar finding was reported by Ruiz-Palacios (1990). However it was found that beyond 6 months exclusive breast-feeding is not nutritionally recommended and in fact maybe a risk factor for, rather than a protective one against diarrhoea. It is therefore recommended that food should be introduced no earlier than the fourth month in order to minimise bacterial infections and no later than the sixth month of life in order to meet growth and nutritional requirements of the infants (Underwood and Hofvander; 1982).

Overview of Global Diarrhoea:

Acute diarrhoeal diseases, have been recognized as a major cause of mortality and morbidity in infants and young children in the developing world,(Walsh and Warren; 1979, Snyder and Merson; 1982, Guerrant et al; 1983). The extent to which these diseases are a problem is shown as a rate of episode / child / year; in table (5). The variations in the rates depends on the definition of diarrhoea being applied by the researchers, the duration of the study- the longer the study the more reliable the results-, the place in which the study was undertaken- an area much researched would have more data to compare with. Therefore the level of accuracy of these rates cannot be standardised. This data is collected from studies made from various countries, ranging from the northern hemisphere to the southern hemisphere, most of the countries could be termed developing countries. The geographical location is responsible for climatic differences and the availability of different types of agricultural products that are used as food which is then available for both children and adults to consume. The differences between these countries other than diarrhoeal rates and geographical location lays in differences in religion and customs and traditions which result in behavioral and ideological differences.

Data collected from 24 studies in 18 countries in Asia, Africa and Latin America were analyzed (Snyder and Merson; 1982) and maybe summarized as follows:

In the first 5 years of life morbidity was highest in the 6-11 month group (fig 5). The median incidence of diarrhoea was 2.2 episodes / child / year and less than one episode / person / year for the above 5 year group. In smaller populations or those that were more frequently surveyed the median incidence for the under 5 year age group was 3.0 episodes / child / year.

The highest mortality rates were in children below the age of 2 years with a median mortality of 20 deaths / 1000 population (fig 6). The estimated case-fatality ratio in children below the age of 5 was 0.6 deaths / 1000 illnesses.

So based on the above calculated global median morbidity rates and on 1980 population figures, the number of acute diarrhoeal episodes in 1980 in children less than 5 years old in Africa, Asia (excluding China) and Latin America was estimated to be 744 million; when the median morbidity rates obtained in studies with smallest populations and frequent surveillance were used, the numbers rose to little over a billion. The annual number of deaths from acute diarrhoeal illness in children under 5 years of age was estimated to be 4.6 million in 1980; 80% of those deaths were in children less than 2 years old.

Table 5

Diarrhoeal Attack Rates From Various Studies

Country	Reference	Rate
Australia	Ratnaike et al; 1989	1.02 episode/child/year (1985) 0.09 episode/child/year (1986)
Bangladesh	Black et al; 1981	4.0 episode/child/year (9-11 months group) 3.0 episodes/child/year (up to 4 years old)
Bangladesh	Khan MU; 1984	3.2 episodes/child/year
Central African Republic	Georges - Courbott et al. 1990	1.6 episodes/child/ year.
Egypt	Higgins et al; 1954	2.8 episodes/child/year
Ethiopia	Jiwa et al; 1981	1.0 - 3.0 episodes/child/year
Guatemala	Mata et al; 1978	7.2 episodes/child/year (18-24 month group)
Ghana	Afari et al; 1988	4.6 episodes/child/year
Indonesia	Soernato et al; 1983	1.0-2.0 episodes /child/year
Mexico	Craviotto et al; 1988	3.0 episodes/child/year
Nigeria	Huttley et al; 1987	14.0 episodes/ child/year
NE Brazil	Guerrant et al; 1983	7.0 episodes/child/year (6-11) month group) 2.0 episodes/ child/group (6 months - 4 years in a poor urban household)
Peru	Black et al; 1989	9.0 episodes/child/year

Fig (5): Estimated Median Diarrhoeal Morbidity Rates for Children <5

(Adapted from Snyder and Merson; 1982)

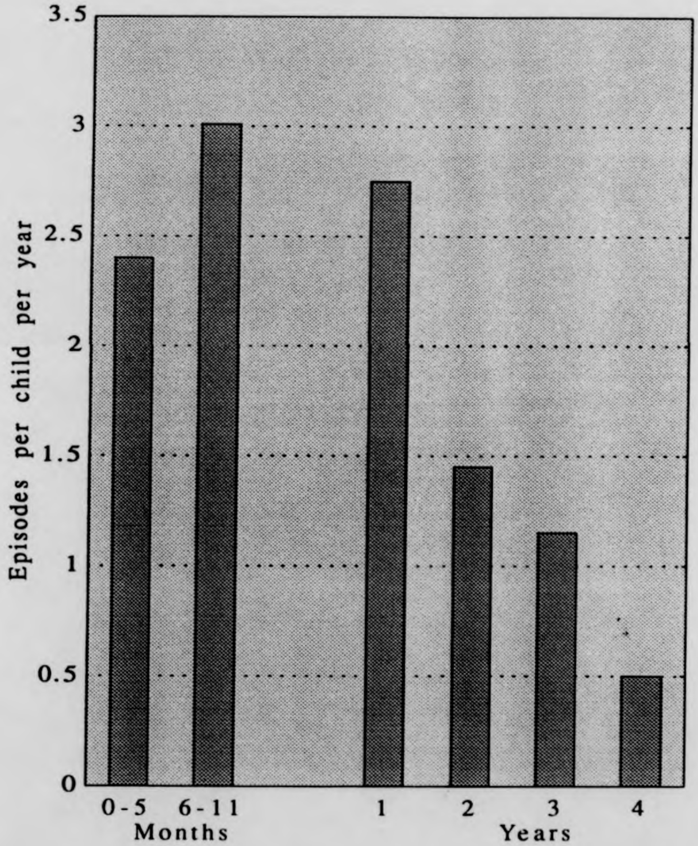
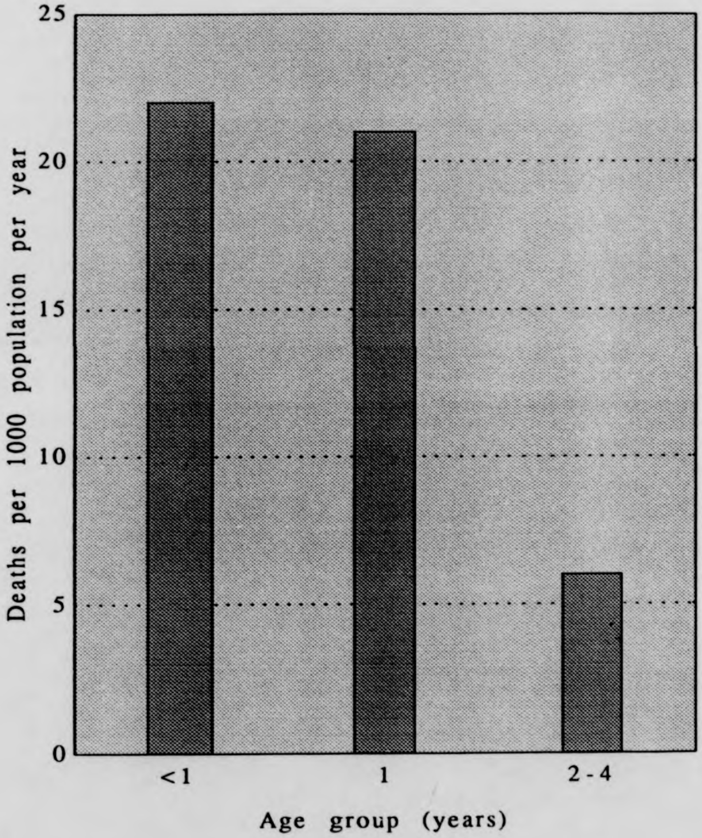


Fig (6): Median Diarrhoeal Mortality Rates for Children <5

(Adapted from Snyder and Merson; 1982)



In Egypt diarrhoea was listed as the most common cause of infant mortality in children under one year old, accounting for 54% of the total mortality (Higgins et al; 1955). In Kuwait gastroenteritis is the second major cause of mortality amongst children (Sethi et al; 1984), and accounted for 25% of admissions into the paediatric department of one regional hospital in the country (Khuffash et al; 1982). In Tunisia acute diarrhoea in children accounted for 24% of all medical institution visits and before Oral Rehydration Treatment (ORT), accounted for 30% of the mortality in children below 2 years of age (El- Zouki AY and Vesikari T; 1985). Despite a decline in the rate of mortality due to gastroenteritis in North America, the illness remains a major cause of infant morbidity (Gurwith and Williams; 1977). Early childhood diarrhoea in North East Brazil is among the highest in Latin America and more than 25% of all recorded listed diarrhoea as either the primary or associated cause of death (Guerrant et al; 1983). In Trinidad and Tobago "enteritis" and other diarrhoeal disease was the 2nd cause of death in children under 1 year and the first cause in those 1-4 years old (Hull et al; 1982).

These and many more examples the world over serve to stress the magnitude of global diarrhoea. However the coverage here has been limited to illustrate:

1. A few typical developing countries which have a developmental history situation resembling that of the U.A.E.
2. Nearby or neighbouring countries to illustrate:
   a) regional variation.
   b) difference in health care policies.

Agents of Diarrhoea:

Diarrhoea is caused by many agents. It is now possible under excellent laboratory conditions to identify the causal agent in 70-80% of cases of acute diarrhoea in comparison to only 20% almost 2 decades ago (Pickering et al; 1978, Vadivelu; 1985, Qadri et al; 1990). Enterotoxigenic E.coli (ETEC) and Rotavirus have been identified as the most common agents of severe watery diarrhoea in the first 2 years of life in developing countries (Black et al; 1981). ETEC have also been implicated in travellers diarrhoea. Enteropathogenic E.coli (EPEC) have been implicated in numerous well-documented outbreaks of infantile diarrhoea. More recently Campylobacter spp and Cryptosporidium parvum have been receiving a lot of attention for their role in causing diarrhoea. Table (6) shows a list of these new agents along with the previously known and long accepted pathogens that cause diarrhoea such as Salmonella spp., Shigella spp. and V.cholerae. Diarrhoea may be caused by one or more of these agents.

The "long accepted pathogens" and the parasites are not reviewed in this study in any depth because:

i they are already well-documented and microbiological data is readily available in standard texts eg. Salmonella, or

ii they were not found to be of any relevance to the population studied in the U.A.E. eg. the parasite group.

Table 6

List of Agents of Diarrhoea

Bacteria	Parasites	Viruses
Clostridium perfringens	*Giardia lamblia*	Rotaviruses
Staphylococcus aureus	*Entamoeba histolytica*	Norwalk Viruses
Shigella spp	*Ascaris lumbricoides*	Adenoviruses
Salmonella spp	*Trichuris trichiura*	Astrovirus
Campylobacter jejuni	*Cryptosporidium*	Calicivirus
Escherichia coli	*Strongyloides stercoralis*	Coronavirus
Vibrio cholerae		Reovirus
Yersinia enterocolitica		Breda-like virus
Baccilus cereus		
Vibrio parahaemolyticus		
Bacillus fragilis		
Clostridium difficile		
Aeromonas hydrophilus		

Escherichia coli (E.coli):

As the most ubiquitous species of the facultative anaerobes in the intestine E.coli are usually considered as normal flora of the intestine where they play an important role in maintaining intestinal physiology (Drasar and Hill; 1974) unless there is a manifestation of disease. This occurs when some strains express virulence factors that enhance the organism's ability to cause diarrhoea in humans as well as animals. E.coli was first isolated from the stools of infants with enteritis by Escherich in 1885, but it was soon realised that the organism maybe isolated from stools of healthy infants and adults. Intensive research has led to the widespread recognition of 4 categories of E.coli as important diarrhoeal pathogens.

The last two categories will be reviewed in this study as they are the most associated of the categories with infantile diarrhoea.

Enteroinvasive E.coli (EIEC):

These have serotypes distinct from ETEC and EPEC and closely resemble Shigellae in many ways. Like Shigella they are able to invade and proliferate within the epithelial cells, this property is dependent on the presence of large plasmid involved in coding for the proteins that are involved in the invasive action within the cell.

Enterohaemorrhagic E.coli (EHEC):

Attention was drawn to these strains in 1983 (Riley et al; 1982). Clinically they cause a bloody but copious diarrhoea unaccompanied by faecal leucocytes and was seen in afebrile patients. These features distinguish it from classic dysentery due to Shigella or EIEC that are characterised by fever and scanty stools of blood and mucous containing faecal leucocytes.

Enterotoxigenic E.coli (ETEC):

The name enterotoxigenic E.coli (ETEC) is reserved for E.coli that cause diarrhoea through the production of enterotoxins. The genetic material that controls the production of these toxins is plasmid borne and so may be easily transferred to recipient cells. There is a lot of evidence that ETEC are an important cause of diarrhoea in all age groups in tropical and developing countries and among travellers to those areas.

Infantile Diarrhoea Due to ETEC:

A study that involved frequent house-hold surveillance of children between the ages of 2 - 60 months showed that ETEC were the most isolated pathogens and that their isolation was strongly associated with diarrhoeal illness (Black et al; 1982a). Another study showed Etec to be the main bacterial pathogen causing dehydrating infantile diarrhoea (Black et al; 1981). This evidence would also suggest that in developed countries with good standards of hygiene, water supply systems, waste disposal systems and temperate climates, these bacteria are of little importance. However several outbreaks of enteritis due to ETEC have been reported in the UK (Rowe et al; 1978, Riorden et al; 1985) and the USA (Ryder et al; 1976, Rosenburg et al; 1977). Since the work carried out by Gorbach and Khurana (1972) in a Chicago hospital, enterotoxigenic E.coli have been accepted as a major cause of infantile and childhood diarrhoea. Numerous subsequent studies have confirmed that. Table (7) shows a selection of these studies in both developed and developing countries. However there are a number of studies also that have reported a non-association of childhood diarrhoea with ETEC (Echeverria et al; 1977, Pickering et al; 1978, Gurwith et al; 1978, Hull et al; 1982), indicating that although they are recognized as a major cause of infantile diarrhoea, ETEC are not necessarily the only one. (Table 7)

Table 7: Selection of Studies on Infantile Diarrhoea

Author	Country	% Isolation Rate	Year
Aguero et al	Chile	31	1985
Black et al	Bangladesh	27	1982
Black et al	Peru	7.4	1989
Basu et al	India	63	1983
Craviotto et al	Mexico	NA	1988
Guerrant et al	Brazil	48	1975
Georges et al	CAR	3.4	1984
Germani et al	Vanuatu	27	1983
Huq et al	Riyadh	4.0	1985
Khuffash et al	Kuwait	12*	1982
Mata et al	Coste Rica	13.4	1983
Pickering et al	Houston	4.0	1975
Pickering et al	Mexico	7.0	1975
Rowe et al	UK	60	1978
Sethi et al	Kuwait	9.0	1989
Soemarto et al	Indonesia	12*	1983
Shukry et al	Egypt	31	1986
Schoub et al	S.Africa	24	1977
Toledo et al	Sao Paolo	NA	1983

* Results include ETEC and EPEC

Traveller's Diarrhoea:

Traveller's diarrhoea occurs world-wide and its duration is usually very brief and characterized by the rapid onset of loose stools. It is sometimes accompanied by a variety of symptoms such as vomiting, nausea and abdominal cramps. Although mild, it is the cause of inconvenience and great personal and economic importance to the traveller. ETEC have been shown to be the most common cause of traveller's diarrhoea in patients travelling to Mexico, Kenya, Morocco and Thailand (Gorbach et al; 1975, Merson et al; 1976, Sack et al; 1979, Echeverria et al; 1981). It is thought that travellers from developed countries are often non-immune and therefore acquire the condition at frequencies varying from 30% - 60% when travelling to countries where the causative organisms abound. Since E.coli involved in childhood diarrhoea seem to be the same as those that produce traveller's diarrhoea then the incidence of traveller's diarrhoea may therefore be taken as an index to the extent of ETEC diarrhoea in any particular geographical location (Sack; 1978, Aguero et al; 1985). In other words in any geographical location the severity and frequency of traveller's diarrhoea maybe taken as an index of the frequency and severity of childhood diarrhoea to be expected since they are both caused by the same pathogen.

Toxins of Enterotoxigenic E.coli:

ETEC are known to produce at least 2 types of toxins : a heat labile toxin (LT) and a heat stable toxin (ST) (Sack; 1975). Some strains are able to produce these singularly or together. Both are plasmid borne on the Ent plasmid (Gyles et al; 1974). The LT is the biggest of the 2 toxins with a molecular weight of 91000 and it is unstable at a temperature of 65°c for 30 minutes. It shares many functional and structural similarities with V.cholerae toxin and can immunogenically cross-react with it (Holland; 1990). The LT toxin causes a slow onset of diarrhoea of a prolonged duration but shorter than that of caused by V.cholerae. ST on the other hand has a low molecular weight of 1800. It is resistant to acid ph and to temperatures of 100°c for 30 minutes and on the basis of its methanol solubility and type of host it infects, maybe further divided into 2 subgroups: STa and STb (Holland; 1990). ST toxin causes a faster onset of diarrhoea and is not immunogenic in character.

Some E.coli culture filtrates have been found to contain a toxin that is cytotoxic for African green-monkey kidney (Vero) cells (Konowalchuk et al; 1977). This has been called Vero-cell toxin (VT), but its role in causing diarrhoeal disease has not been established.

It has been shown by several workers that the ability of E.coli to produce enterotoxin is, by itself, not sufficient to cause diarrhoea; a strain must also be able to proliferate in the small intestine (Gyles et al; 1974, Smith; 1976). And so an enterotoxigenic strain is not necessarily an enteropathogenic one.

The assays for toxin detection are reviewed in Chapter 1 page 53.

Enteropathogenic E.coli (EPEC):

The name EPEC is reserved for E.coli strains that cause diarrhoea by mechanisms distinct from LT, ST and Shigella-like invasiveness and is based on epidemiological observations of these strains (Thoren; 1983). A scheme produced by Kauffmann in the 1940s is used to type E.coli, it enables the groups of isolates to be detected. The classical serogroups are those that belong to the O sero-group. Diarrhoea associated with EPEC is characterised by a high mortality ratio in epidemic outbreaks (Bray;

1945, Giles et al; 1949). But the severity of the symptoms could range from symptomless carriers to critically ill patients making it always not possible to distinguish EPEC diarrhoea from other causes of infantile diarrhoea. EPEC have been incriminated in several outbreaks of infantile diarrhoea and the incidence of diarrhoea caused by EPEC, as that caused by ETEC, is associated with poor sanitary conditions, low hygienic levels, poor water supply, over-crowdedness, bottle-feeding in babies. Although the incidence of reported outbreaks due to EPEC has inexplicably dropped dramatically in the developed world, some serious epidemics with high mortality rates appear now and then (Jacobs et al; 1970). However they still remain the most common bacterial enteropathogen among young children in Canada (Gurwith et al, 1978) and Finland. Studies from South America have shown EPEC to be the first or second most important bacterial cause of infantile gastroenteritis (Toledo et al; 1983, Black et al; 1985)

Epidemiology of ETEC and EPEC:

The gastric barrier of the stomach is usually an effective barrier against pathogenic bacterial invasions, thus as previously mentioned a relatively large inoculum of more than 10^8 may be required to cause illness. The number of organisms necessary to produce illness from ETEC strains was 100,000,000 times higher. The number of organisms necessary to produce illness from dysentery-like strains was 10,000 times higher than that required for <u>Shigella flexneri 2a</u>, where the infectious dose of less than 200 cells were necessary to produce illness in adult volunteers (DuPont et al; 1971). Transmission is through the faeco-oral route and contamination of food and water sources with ETEC is taken as an indication of faecal contamination. This type of contamination when it occurs in developing countries usually does so as a result of overcrowded conditions, poor sanitation, contaminated water supply, inadequate food hygiene. Therefore one of the effective ways to combat diarrhoea would be to tackle the above risk factors.

Food-borne Diarrhoea Due to ETEC and EPEC:

The weaning is the critical stage when the protective breast-feeding ends and the new foods capable of causing disease are introduced. Early observations showed that bottle-fed babies were a higher risk group for contracting EPEC diarrhoea (Bray; 1945, Giles et al; 1949), but since breast-feeding is usually pro-longed in developing countries, this risk is delayed until weaning begins. Following a study in Guatemala, Bruch et al (1963) suggested that food was a significant source of weanling diarrhoea. Weanling food is often a good reservoir for ETEC and EPEC particularly if prepared non-hygienically and / or kept at ambient temperatures for lengths of time (Barrel and Rowland;1979). A significant relationship ($p < 0.05$) was found between the proportion of a child's foods contaminated with E.coli and the attacks of diarrhoea associated with ETEC for that child (Black et al; 1982b). Various workers have suggested promoting and prolonging breast-feeding in an attempt to delay or prevent the onset of diarrhoea (Feachem and Koblinsky; 1984, Black et al; 1982b). Food-borne outbreaks due to EPEC (Costin et al; 1964) and to ETEC (Wood et al; 1983, Riorden et al; 1985) have been reported. Black et al (1982b), found that although specimens of water more frequently contained E.coli than did specimens of food, the number of E.coli in contaminated foods was generally 10 times higher than that in contaminated water. This is possibly due to unhygienic cooking methods that do not

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allow the complete killing of the faecal contaminants that are often found in the water used for cooking and on the cooking utensils and containers themselves. It may also be due to the storing of cooked food at ambient temperatures which provide a suitable milieu for the rapid proliferation of the E.coli. It is this proliferation that is of utmost importance since ETEC and EPEC seem to require a relatively high inoculum to cause diarrhoea (DuPont et al; 1971).

Water-borne Diarrhoea Caused by ETEC and EPEC:

ETEC and have been found to be the causative agents in out-breaks of diarrhoeal illness such as the outbreak in Crater Lake; Oregon, where drinking water was contaminated with sewage water resulting in the infection of more than 200 staff and visitors (Rosenburg et al; 1977). EPEC have also been incriminated in an outbreak at a conference centre in Washington D.C. (Shroeder et al; 1968).

Seasonality of ETEC and EPEC:

ETEC infections seem to be associated with higher temperatures. Black et al (1982a) reported that ETEC diarrhoea occurred most in the hot months from April-September. A study from Western Australia found ETEC isolations to be more prevalent during the wet monsoonal summer than the dry winter (Berry et al; 1983). Guerrant et al (1983) reported a higher rate of infection during the slightly warmer months of October-March.

On the other hand in developed countries, up until the 1940s infections with E.coli; (later recognised as EPEC) were generally regarded as a summer illness. As the standards of hygiene improved, the summer outbreaks were overshadowed by epidemics that occurred mainly during the winter months and affected infants in hospitals and nurseries (Thoren; 1983)

Age-Specificity of ETEC and EPEC:

In contrast to ETEC infections which affect different age-groups in the same population, EPEC infections are usually restricted to children under 2 years of age, (Agbonlahor and Odugbemi; 1982, Craviotto et al; 1988). Thoren (1983) attributes this to the effect of prolonged breast-feeding in developing countries and hence the delayed effect of the weaning food problem. Outbreaks in the adult population are also known to occur. These are usually associated with food (Costin et al; 1964, Riorden et al; 1985) or water-borne (Shroeder et al; 1968, Rosenburg et al; 1977) transmission.

Diagnostic Tests for ETEC and EPEC:

Since E.coli can occur as natural commensal in the gut, it is essential to be able to distinguish between virulent and non-virulent strains, since they do not differ bacteriologically or biochemically from each other. Various tests and assays have been developed for this purpose which involve serotyping, animal models, tissue culture models, immunological assays and DNA hybridisation assays.

Serotyping:

The Kauffmann scheme (Kauffmann; 1947) places E.coli into sero-groups on the basis of antigenic characteristics. The classical serogroups belong to the "O" antigen sero-group, which provides the basis for sero-group and serotype designations. While this has been useful for most EPEC, there is some confusion since the classically designated EPEC strains have been shown on occasion to be avirulent, toxigenic or invasive. It has been of debatable use for ETEC with some workers arguing for and others against it. Sack (1978), suggested that there exists a definable relationship between enterotoxin production and serotype. Other workers (Orskov et al; 1976, Orskov and Orskov; 1977, Merson et al; 1979) have suggested that ETEC fall within a restricted range of serogroups and serotypes regardless of the geographical location and may thus be identified. Gross et al (1976) suggested that E.coli strains responsible for infantile enteritis produce enterotoxins at a level not detectable by the tests they used (those tests being tissue culture using Chinese hamster ovarian cells-CHO and mouse adrenal tumour - Y1 cell lines for LT and infant mouse test for ST) and on that basis they recommended laboratories continue with serotyping for investigating infantile enteritis due to E.coli. Goldschmidt and DuPont (1976) suggested that since the plasmid that control enterotoxin production can be transmitted from one strain to another or to another different species altogether without a change in the serotype of the donor (Smith and Halls; 1968, Gyles et al; 1974), then the validity of serotyping of E.coli becomes questionable. Other workers (Schoub et al; 1977, Basu et al; 1983, Germani et al; 1985, Aguero et al; 1985) did not find a correlation between the enteropathogenicity and serotype described by Merson et al (1979); opening up the possibility that predominant enterotoxigenic serotypes of E.coli in the countries where these studies were conducted are different from those serotypes proposed by Merson et al (1979). The routine use of these antisera should therefore not be recommended until studies in different geographical locations have been carried out to determine the value of antisera in the diagnosis of ETEC diarrhoea and the predominant serotypes in each area (Gurwith et al; 1978, Merson et al; 1980).

Animal Models:

There are a variety of animal models used in the detection and assay of enterotoxins (table 8). Most were adapted from studies originally used for V.cholerae enterotoxin. Enterotoxin presence is indicated by a secretory response in the small intestine of animals eg. dogs, mice or rabbits when a cell free culture supernatant is used in vivo. The type of enterotoxin produced is indicated by the length of the time necessary to produce a response, where ST causes a reaction sooner than LT. As such these models provide conclusive evidence of the changes within the body due to enterotoxin production and activity. They are important in elucidating the pathological and physiological changes that occur in the gut due to enterotoxins. They are not suitable for diagnostic or epidemiological studies where a large number of strains are being investigated.

Table (8): Biological assays for enterotoxins of E.coli and V.cholerae

Assay	E.coli ST	E.coli LT	V.cholerae enterotoxin
Rabbit ileal loop (18 hr) (6-7 hr)	- +	+ +	+ +
Infant rabbit (6-7 hr)	+	+	+
Infant mouse (4 hr)	+	+	±
Rabbit skin	-	+	+
Mouse adrenal cells	-	+	+
CHO cells	-	+	+
Rat epididymal cells	-	±	+
Mouse thymocytes	-	+	+
Dog jejunal thirty vela loops (1-2 hrs) (4-16 hrs)	 + -	 - +	 - +

Adapted from Sack; 1975.

( ) time at which assay is terminated

Tissue Culture Models:

These are in vivo assays carried out in vitro systems to study the morphological effect of the toxins on living cultured cells There are a variety of established cell lines used in these assays eg. Chinese hamster ovary (CHO) cells, Mouse adrenal tumour (YI) cells and African green monkey kidney (Vero) cells. In these methods exposure of the cells to the supernates of cultures containing LT or cholera toxin result in an increase in intracellular cyclic AMP - cAMP - which leads to a morphological response detectable by microscopy. CHO cells respond by elongation and Y1 cells become rounded. Vero cells show a cytotonic or cytotoxic effect (Gross; 1990).

Immunological Assays:

These are possible due to the immunogenic nature of the LT. The toxin molecules are recognised in an in vitro system by specific antibodies and the response maybe read spectrophotometrically by an enzyme-linked immuno-absorbent assay (ELISA) (Yolken; 1977) or radiometrically by a radio-immunoassay (RIA) (Ceska et al; 1978) or by toxin/antitoxin precipitation or agglutination assays such as the Biken test (Honda et al; 1981),or a staphylococcal agglutination test as described by Honda et al; 1983.

The non-immunologic nature of ST is overcome by conjugation of the toxin molecule to carrier protein molecules such as bovine serum albumin (Frantz and Robertson; 1981) or bovine serum albumin before raising the antibodies (de Mol et al; 1985).

DNA-DNA Hybridisation Assays:

These are frequently used to detect the toxin producing genes - "tox" genes -in purified bacterial strains. Radiolabelled specific DNA fragments cloned from enterotoxin genes are used as probes to detect homologous DNA sequences in E.coli strains thus enabling the detection of genes coding for ST and/or LT in stool, food or water specimens.This assay provides conclusive evidence of the presence of the "tox" genes, but it does not follow that the genes are being expressed.

Rotavirus:

The association of rotavirus and non-bacterial diarrhoeal disease was first made by Bishop et al in 1973, in Australia and subsequently confirmed by other workers (Flewett et al; 1973, Konno et al; 1975). The numerous studies that have been conducted have shown that rotaviruses are ubiquitous and occur world-wide. They remain the single most commonly discovered cause of acute diarrhoea (Echeverria et al; 1977, Schoub et al; 1977, Soernarto et al; 1981, Hull et al; 1982, Al-Bwardy et al; 1988). Although rotavirus infections are essentially a disease of children cases of adult infections have been reported (Echeverria et al; 1983) as have geriatric cases (Halvorsrud and Orstavik; 1980, Cubitt and Holzel; 1980, Marrie et al; 1982). There are 5 serogroups of rotavirus designated A-E, group A being the most common in human infections (Flewett and Beards; 1989), although reports of a severe rotavirus diarrhoea occurring in adults of all ages was reported in China (Tao et al; 1984) was subsequently found to be due to infections by group B.

Diarrhoea caused by rotavirus is usually of short duration, often but not always accompanied by either vomiting or fever or both. Asymptomatic carriage of the virus has been reported by some workers (Bishop et al; 1979, Black et al; 1982a, Champsaur et al; 1984, Beards and Brown; 1988), although some differed in reporting that carriage was only symptomatic (Mahmood and Feachem; 1987a, Hull et al; 1982).

Epidemiology:

Route of Transmission of Rotavirus:

Infection occurs when the virus is swallowed. It may be transmitted by dust or infected water supply or just by passive transfer from people's hands. However the observation that in many countries rotavirus infections occur in winter when a lot of respiratory tract infections are common, might support a respiratory route of transmission (Tallett et al; 1977, Huq et al; 1987, Sethi et al; 1989). Unlike bacterial diarrhoea the risk of infection with rotavirus was not found to be related to socioeconomic status of the family or nutritional level of the child (Soernarto et al; 1983, Sethi et al; 1984, Mahmood and Feachem; 1987b).

Seasonality of Rotavirus Infections:

In temperate climates rotaviral diarrhoeal infections are a winter phenomenon. In tropical climates the seasonality is not so well defined and the virus may be isolated throughout the year or in major and minor waves (Soenarto et al; 1981). Studies from various countries showing these seasonality trends are listed in table (9). It is clear from the table that in some tropical countries, different workers have found variations and differences in the seasonality of the infections as is the case in the studies from Kuwait and Saudi-Arabia. It has even been suggested that some years were "rotavirus" years and some were not, when no seasonality infection pattern was found (Beards; personal communication).

Table 9: Seasonality of Rota Virus From Various Studies

Country	Reference	Seasonality
Australia	Walker et al;	Peaks in rainy season
Bangladesh	Black et al; 1981	Constant throughout year with a single peak in December
Brazil	Pickering et al; 1978	Throughout the year
Canada	Gurwith and Williams; 1977	During winter months
Central African Republic	Georges et al; 1984	Peak incidence during dry seasons
Costa Rica	Mata et al;	Major and minor waves
Ethiopia	Stinzing et al; 1981	2 peaks one in rainy season and one in minor season
Gambia	Rowland et al; 1985	Major and minor waves
Houston	Pickering et al; 1978	Throughout year
Kuwait	Al-Nakib et al; 1980	During winter months (Oct - Dec)
Kuwait	Sethi et al; 1984	Throughout year with peaks in months with little rain and low humidity (Nov - Jan and Mar - May)
NE Brazil	Guerrant et al; 1983	Dry winter months from June - Oct
Saudi Arabia	Huq et al; 1987	Throughout year with peaks in winter
Saudi Arabia	Al-Bwardy et al; 1988	Two peaks; one in cold winter months and one in dry hot months
Saudi Arabia	Qadri et al; 1991	Winter months, Dec - Mar
Sweden	Nivenius et al; 1987	Winter and spring months, Feb - May

Age Specificity of Rotavirus Infection:

Although rotavirus is now recognized as a major cause of infantile diarrhoea, outbreaks in older patients have been known to occur (Echeverria et al; 1983, Tao et al; 1984). These have so far only been sporadic cases and the virus remains high on the list of childhood diarrhoea affecting children usually up the age of 2 years old and with a peak usually at 6-11 months of age (Gurwith and Williams; 1977, Al-Nakib et al; 1980, Soernarto et al; 1983, Rowland et al; 1985, Mahmood and Feachem; 1987a, Sethi et al; 1989, Qadri et al; 1990).

Diagnostic Tests for Rotavirus:

Shape of Rotavirus:

The unique shape of rotavirus is used as a diagnostic tool. When negatively stained for electron microscopy, rotaviruses resemble little wheels about 70nm in diameter with a wide hub, short spokes and a clearly defined circular rim (Flewett and Beards; 1989). But electron microscopy is expensive and specialised, it is now possible to use other methods in the laboratory.

Immunological Assays:

Elisa Test:

Since electron microscopy procedures are time consuming and expensive on a large number of samples , other methods have been developed to detect rotavirus or rotavirus antigens (Christensen; 1989). Serology is of little use in rapid diagnosis as a specific antibody response occurs too late in most infections (Beards; 1988). And since it has been difficult to propagate rotaviruses in cell culture (Christensen; 1989), other antigen detection methods have been used. These include Elisa tests and latex agglutination tests and their use has become increasingly popular because of their sensitivity, specificity, safety and low cost. The Elisa test is an antibody / antigen reaction and the results are read spectrophotometrically Most Elisa tests use the 3-layer sandwich technique, where a solid phase enzyme immuno-assay is used to detect rotavirus antigens in faecal material. Anti-rotavirus antibodies are adsorbed to a solid phase. The first antibody is called the primary antibody. After the specimen is added, and if the virus is present, it will bind to the primary antibody. A secondary antibody that is conjugated to an enzyme is then added. The intensity of colour produced is proportional to the enzyme label bound and hence to the antigen present (Christensen; 1989).

Latex Agglutinating Test (LA):

This is a rapid antigen / antibody test where latex particles coated with specific antibody react with rotavirus antigen present in the faecal specimen. Agglutination within 2 minutes is a positive result.

Reports by several workers (Christensen; 1989, Amer et al; 1990) have shown that the Elisa tests to be more sensitive but less specific than the LA test. However the LA test has the advantage of being quick simple and inexpensive. It was found that the sensitivity of the LA test was dependent on the time of collection of stool sample relative to the onset of symptoms (Pai et al; 1985). It was concluded that the LA test was good for screening and definitely positive results were reliable (Julkungen et al; 1985, Amer et al; 1990).

Campylobacter:

In less than a decade C.jejuni has risen from the obscurity as a veterinary pathogen to recognition as an important cause of enteritis in humans (Dekeyser et al; 1972, Blaser and Reller; 1981), and a cause of traveller's diarrhoea in 11.5% of the total number of infections (Skirrow, 1987).

When proper culture techniques were used C.jejuni was isolated from diarrhoeic patients almost as often as Salmonella or Shigella species.

It was found to be the most commonly isolated pathogen in the United Kingdom (Skirrow; 1987).

C.jejuni is a curved, motile, gram negative rod. It is micro-aerophilic, requiring some oxygen for growth, which is best at 42°c in an atmosphere of 5-10% oxygen and 3-5% carbon dioxide. Clinically diarrhoea caused by this organism cannot be distinguished reliably from other acute diarrhoeal infections. The onset of diarrhoea is usually abrupt and in severe cases the faeces may become profuse and watery resembling the toxin mediated secretory diarrhoea due to organisms such as ETEC. Leucocytes are usually present and blood may also appear resembling the invasive actions due to organisms such as Shigella spp. (Ruiz-Palacios et al; 1983, Yeen et al; 1983, Urrestarazu et al; 1987, Haq and Rahman; 1991). The condition usually resolves spontaneously, but the organism continues to be excreted for a long period (about 3 weeks in 50% of patients) Many symptomless infections of C.jejuni have been reported (Blaser et al; 1980, Craviotto et al; 1988, Georges-Courbot et al; 1990,). Calva et al (1988) in Mexico found that early childhood symptomatic infection protected against subsequent symptomatic infections. This finding was confirmed by Georges-Courbot et al (1990).

Epidemiology:

Route of Transmission:

This is through the faeco-oral route by ingesting contaminated food (Blaser et al; 1982) and water (Vogt et al; 1982) or unpasteurised milk (Blaser et al; 1979) or through direct contact with faecal material from infected animals and persons (Blaser and Reller; 1981). Many domestic animals and fowl excrete C.jejuni in their faeces, making them ideal sources of infection to humans in general and infants in particular (Blaser and Reller; 1981, Black et al; 1989). A study from Lima, Peru showed that the most important risk factor for paediatric campylobacter infections was the exposure to C.jejuni infected chickens in the home (Grados et al; 1988). A similar finding was reported in the Central African Republic (Georges-Courbot et al; 1990).

Seasonality of Campylobacter Infections:

In temperate climates eg. Belgium and England (Blaser and Reller; 1981) and the States (Finch and Riley; 1984), the disease has a peak of isolation in the warmer dry summer months. In countries with tropical climates, the disease typically occurs in the rainy months (Blaser and Reller; 1981, Urrestarazu et al; 1987). In Zaire where the temperatures are constant throughout the year, isolation of C.jejuni from patients with diarrhoea was more frequent during the wet season than the dry season (De Mol et al; 1980). Studies from Saudi Arabia and the Central African Republic however did not detect any such seasonality (Al-Bwardy et al; 1988, Georges-Courbott et al; 1990).

Age-Relation of Campylobacter Infections:

In industrialized countries, isolation of campylobacter has always been associated with disease, with the risk of infection being greater in older children than in younger ones (Richardson et al; 1983, Urrestarazu et al; 1987). In developing countries no such association is made and many symptomless infections of C.jejuni have been reported (Blaser et al; 1980, Craviotto et al; 1988, Georges-Courbot et al; 1990). Calva et al (1988) in Mexico found that early symptomatic infection protected against subsequent infections. This finding was confirmed by Georges-Courbot et al (1990).

C.jejuni infections were found to be age-related up to the age of 6 months after that the association was lost (Georges-Courbot et al; 1990). A study from South Africa found the age-relation to end at 9 months (Richardson et al; 1983). The same trend was found in Bangladesh (Blaser et al; 1980, Haq and Rahman; 1991).

Diagnosis:

Culture:

The unique growth requirements that are needed by campylobacter are made use of in their diagnosis. There are a variety of selective agar for the culture of campylobacter that contain different antibiotics that render these media inhibitory to other bacteria. Skirrow's medium - which contains vancomycin, polymyxin B and trimethoprim - is the least inhibitory of those selective media but also the least selective. Most media contain blood, the purpose of which is to neutralise superoxides and free radicals in the basal medium. but a blood-free medium containing charcoal and a single antibiotic -cefoperazone- is also available. The attraction of this medium is that it cuts out the extra step of adding the blood to the media thereby making the media even more selective and lessening the steps for the preparation of the media. Optimum growth is achieved at a temperature of 42 - 43°c and under micro-aerophilic conditions which consist of 5-10% oxygen and 3-5% carbon dioxide. The micro-aerophilic conditions maybe achieved by the use of an anaerobic jar that is partly evacuated and an appropriate gas mixture from a cylinder is introduced into it, or a jar without a catalyst but with the use of a gas-generating kit that is then evacuated or alternatively by the use of a jar with a catalyst and a gas-generating kit (both available commercially from most diagnostic kit manufacturing companies). Microscope preparations from the cultures showing the typical curved, motile, gram-negative rods are a confirmation of the presence of campylobacter. However isolation is not indicative of disease as shedding may continue long after the symptoms disappear.

Serodiagnosis:

This is required for patients thought to have late complications of Campylobacter enteritis (eg. Reactive Arthritis). It is also useful in the investigation of outbreaks due to this organism. Patients infected with C.jejuni develop specific serum antibodies (Skirrow; 1977). Methods used for the detection of these antibodies include tube agglutination (Butzler and Skirrow; 1979), bactericidal assays (Jones et al; 1980). A Complement Fixation test (Jones et al;1980) is sometimes used but it is not as sensitive as the ELISA test (Rautelin and Kosunen; 1983).

Cryptosporidium spp.:

Cryptosporidium has gained recent recognition as a cause of diarrhoea in humans (Fayer and Ungar; 1986). The organism is a small, obligate, intracellular protozoan that can cause infections in the respiratory tract and biliary tree as well as the gastrointestinal tract. Cryptosporidia although not related to Giardia, share a lot of similarities with that group of protozoa in that they are both water transmitted and they can both cause infections in the gastrointestinal tract. Methods originally developed for the isolation of giardia have been adapted for the isolation of Cryptosporidium. It was initially thought that Cryptosporidium species were host specific. But studies carried out in the early 1980s showed little or no host specificity to occur.

Taxonomy:

Levine (1984), proposed 4 species of Cryptosporidium, one for each of those infecting fish (C.nasorum), birds (C.meleagridis), reptiles (C.crotali) and mammals (C.muris). But even this proposition was found unsatisfactory. At least 2 species have been found to infect mammals (C.parvum infecting the small intestine and C.muris infecting the stomach). On the basis of oocyst identification, C.parvum and not C.muris is associated with well documented cases of cryptosporidiosis in mammals (Upton and Current; 1985).

Prior to 1980 infections with C.parvum were considered rare and found in animals. In humans infections were associated with immuno-compromised persons. Since 1982 C.parvum has come to be considered as an important and widespread cause of diarrhoea in humans and domestic animals. In South Africa and Liberia, the prevalence rate of Cryptosporidium was found to be 14.9% in infants with gastroenteritis (Smith and van den Ende; 1986, Hojlyng et al; 1986). It is also now recognised as a cause of traveller's diarrhoea. (Jokipii et al; 1983, Jokipii et al; 1985, Soave and Ma; 1985, Sterling et al; 1986).

Diarrhoea due to Cryptosporidium is characteristically profuse and watery, it may contain mucous but rarely blood or leucocytes and is often accompanied by weight loss. It may also be accompanied by abdominal pain, nausea, vomiting and low grade fever. In immuno-competent people it is of short duration and resolves spontaneously.

In immuno-compromised people the diarrhoea is a life threatening prolonged cholera-like illness and its finding usually carries an ominous prognosis (Current and Garcia; 1991).

The method of action by which diarrhoea is produced is little understood. No toxins have been discovered as yet, and no invasive actions have been noted either.

Epidemiology:

Transmission:

Cryptosporidium oocytes are very resistant to most common disinfectants and can survive for months when kept cold and moist (Smith et al; 1988). Recent data document a water-borne transmission route (Gallaher et al; 1989) and a study showed that routine chlorination or ozonation used for killing most water-borne organisms eg. G.lamblia have little effect on C. parvum (Korich et al; 1990).

Transmission of C.parvum is thought to be a zoonotic one (Current et al; 1983), although there is accumulating evidence that person-to-person transmission is common

(Current; 1983), especially in day-care centres (Walters et al; 1988, Gallaher et al; 1989).

The first documented water-borne outbreak of Cryptosporidiosis occurred in San Antonio, Texas and was associated with sewage leakage into well water (D'Antonio et al; 1985). Several other reports followed (Rush et al; 1987, Smith et al; 1988, Gallaher et al; 1989, Hayes et al; 1989). Such studies coupled with the emergent fact of the ubiquity of C.parvum confirm a likelihood that it's presence as a water pathogen occurs especially where sanitation standards and water technology are low and where a large number of potential hosts (domestic mammals) are in close proximity to humans.

Seasonality and Age Specificity:

From various studies and surveys it appears that there is a greater prevalence of crytosporidial infection in children than adults (Hojlyng et al; 1986, Smith and van den Ende; 1986, Corbett-Feeney; 1987, Rahman et al; 1990) and a higher prevalence in children under 2 years of age. A seasonality was also observed with a higher prevalence during the warmer and wetter months (Cruz et al; 1988, Laxer et al; 1990, Current and Garcia; 1991)

Diagnosis:

This by any of the following 3 methods:

Histopathology:

Prior to 1980 human cryptosporidiosis was diagnosed by histopathologic means of the intestinal mucosa. But these methods are of an invasive, expensive, time-consuming nature. They are no longer required for diagnosis since a variety of techniques have been developed to identify C.parvum in faecal specimens, sputum and bile.

Parasitology:

Oocyte concentration techniques or oocyte staining techniques are followed by appropriate microscopy.

Concentration Techniques:

These include :

1. Flotation of oocysts in Sheather's sugar solution or in zinc sulphate or in saturated sodium chloride.
2. Sedimentation of oocysts using formalin ether or formalin-ethyl acetate.

The sedimentation techniques were originally designed for the diagnosis of helminth eggs and protozoan cysts (eg. G.lamblia and Entamoeba spp.) but have been adapted for the small C.parvum oocysts.

Bright field or phase contrast microscopy are used to view the prepared oocytes (Garcia et al; 1983).

Staining Techniques:

These techniques include a modified acid-fast Ziehl-Nielsen stain which is usually the method of choice in clinical laboratories (Casemore et al; 1984, Garcia et al; 1983, Ma and Soave; 1983), Negative staining (Current; 1983) and Sheather's sugar solution (Current et al; 1983). Fluorescent stains have also been used, these include auramine rhodamine (Garcia et al; 1983, Ma and Soave; 1983, Payne et al; 1984), auramine carbol fuschin (Casemore et al; 1984, Corbett-Feeney; 1987). All stained preparations are viewed under high dry or oil immersion lenses.

Serodiagnosis:

Immuno-fluorescence assay (IFA) (Tzipori and Campbell; 1981) and ELISA (Ungar et al; 1986, Casemore; 1987, Laxer et al; 1990) have been used to a limited extent in a few laboratories. Sporozoite antigens from disrupted purified Cryptosporidium oocysts were used for these procedures. From the limited sero-prevalence data available, it is suggested that cryptosporidial infections, many of which may be asymptomatic (Tzipori and Campbell; 1981, Ungar et al; 1988), are more common than infection rates based on detection of faecal oocytes. Therefore additional evaluations are needed to confirm the use of the serologic procedures for diagnosing and monitoring infection and to determine whether there is any correlation between the presence of Cryptosporidium-specific serum antibodies and resistance to further infections (Current and Garcia; 1991).

Other Agents of Diarrhoea:

These include the remaining agents of diarrhoea mentioned in table (6). Their roles in causing diarrhoea are well documented in standard texts but they are not considered as the main etiologic agents of infantile diarrhoea as is the case with the Salmonella group, or they were not found to be of significance in the study population in the study area which is the United Arab Emirates, as was the case with the parasite group and as such are not included in this study although they maybe referred to when necessary.

This group would also include bacteria that are not usually associated with diarrhoea and are therefore not looked for, but when tested in some studies have been shown to produce enterotoxins. Since the Ent plasmid that control the enterotoxin production may be transmitted between species (Smith; 1976, Goldschmidt and Dupont; 1976), then it would follow that under certain environmental conditions any bacterium is capable of harbouring the plasmid. The stability of these plasmid in certain species may not be high. Non-E.coli enterotoxigenic bacteria were shown to lose the LT production property more rapidly than Etec E.coli (Jiwa et al, 1981), which exhibit a varying degrees of tendency to lose the ability to produce LT (Evans et al; 1977). A range of enterotoxigenic, non-E.coli. bacteria were isolated in a study in Ethiopia. These even included LT-producing strains of Salmonella and Shigella (Jiwa et al; 1981). Similar findings concerning Klebsiella were found in N.E. Brazil (Guerrant et al; 1983) and in Venezuela (Urrestarazu et al; 1987). There have also been a number of studies associating Aeromonas hydrophila with diarrhoea (Sanyal et al; 1974, Agger et al; 1985).

Breast-Feeding and Diarrhoea:

The advantage of breast-feeding and its role in preventing diarrhoea has been widely advocated. Few alternatives have been shown to be as effective in preventing diarrhoea among infants as breast-feeding. The evidence that this mode of infant feeding protects from infant diarrhoea is strong. The World Health Organisation (WHO) has estimated that the promotion of breast-feeding could lead to a 25% reduction in diarrhoeal mortality in the first 6 months of life (Feachem and Koblinsky; 1984, Feachem; 1986).

The protective property of breast milk is thought to be due to the delay in the introduction of weaning foods; which have been associated with diarrhoeal infections; and the immunologically active agents found in breast milk (Welsh and May; 1979, Chandra; 1980, Guerrant et al; 1983).

A relationship between breast-feeding and protection against diarrhoea was noted by many workers, where exclusive breast-feeding during the first 6 months of life was found to be more protective against diarrhoea than partial breast-feeding, which in turn was found to be more protective than non-breast-feeding (Feachem and Koblinsky; 1984, WHO; 1988). A study from Kuwait showed that breast-feeding was more protective if no extra water is given (Portoian-Shubair; 1986). Black et al (1989), found that there was a 40% increase in risk of diarrhoea in infants below the age of 6 months who were partially breast-fed. A case-study in Brazil showed that infants aged 0-2 months who received no breast milk were 25 times more at risk of dying from diarrhoea than exclusively breast-fed infants (Victora et al; 1987). A longitudinal study in an urban slum area of Lima, Peru showed breast-feeding to reduce diarrhoeal morbidity (Brown et al; 1989). Ruiz-Palacios, (1990) found there was a 2.3 times greater risk of contracting campylobacter caused diarrhoea in children who were non-breast fed and that breast-fed children remained free of any diarrhoeal episode for a longer time than non-breast-fed children. This protective effect of human milk; also found by Georges-Courbott et al (1990) and the similar observations by other workers would suggest protection by maternal antibodies from invasive infections.

It has been suggested that breast-feeding alone is insufficient in providing the necessary nutritional requirements of infants beyond the age of 3 months (Waterslow and Thomson; 1979, Black et al; 1982b). But a study from Chile (Juez et al; 1983) has shown that breast-milk if produced sufficiently, can maintain normal growth up to 6 months of life.

From a nutritional point of view the duration of exclusive breast-feeding has been recommended at 4-6 months (Underwood and Hofvander; 1982, Huffman and Combest; 1990). Studies have shown that mothers in developed countries such as, the U.S.A. (Butte et al; 1984) and mothers who were nutritionally poor (in comparison with international reference populations) as in Bangladesh (Brown et al; 1986) had an almost similar lactation capacity of 733 $\pm$ 89 gm/day and 750 gm/day respectively. Data from 10 studies using 3 month old infants as study subjects have also shown women from developed and developing countries produce similar quantities of milk (Huffman and Combest; 1990). But beyond 6 months exclusive breast-feeding is not nutritionally recommended and may be a risk factor for diarrhoea rather than a protective one.

The holy Koran in Sura number (2) verse (233) mentions that it is advantageous to breast-feed up to the age of 2 years old. In the past and throughout history wet-nurses were employed to ensure the infants were sufficiently breast-fed. It has not been elaborated in the Koran why breast-feeding is beneficial, but most women, those in the study included, are willing to accept this as good advice and use it as an extra form of contraception. So when modern science gives the same recommendation it is accepted as something that has been known for about for 13 centuries and that modern science is only confirming old knowledge. However with the increase in urbanisation and the changing role of women in society, attitudes to breast-feeding have shifted in different directions. Working mothers, as those in this study group - who were mainly teachers - find a difficulty in nursing during work hours as their work would usually take them out of the home away from their children. Affluence sometimes makes the option of using the bottle easier where expenses are concerned. The presence of domestic help in the house enables another person to help out with the feeds, be that in the preparation or the administering of these feeds. It is all these factors that come into play in the society in the U.A.E. The mothers are aware of the traditions and the religious preferences, but are either attracted to the ease of the bottle, being not fully aware of the health hazards it brings with it, or have jobs that do not accommodate their responsibilities regarding breast-feeding. There are no data available at the M.O.H. regarding breast-feeding, its prevalence or duration. A single study on the immunisation system done at Madinat Zayed noted that the majority of mothers breast-fed up to the age of 4 months. This in fact was what most of the mothers in this study and from personal observations seemed to aim to do: breast-feed up to 4 months of age, after which weaning food was introduced.

The policy of the M.O.H. is that of encouraging breast-feeding. Rooming in of babies with their mothers is standard procedure at the maternity hospital and suckling is encouraged immediately upon giving birth.

Plenty of advice is given to encourage the mothers to adopt this form of feeding their babies.

Reasons for the Study

Little if any information on the epidemiology and aetiology of diarrhoeal diseases in pre-school children for this part of the world is available. The U.A.E. can be geographically considered part of the Developing World and loosely speaking was expected to have Developing World problems - childhood diarrhoea being one of them. The little data which has come from Kuwait (which has a living standard similar to that in the U.A.E., and shares many climatic, social, cultural, religious and demographic similarities with the U.A.E.) shows that gastroenteritis is a major cause of mortality and morbidity (Sethi et al; 1984, Khuffash et al; 1982). Apart from rotavirus, the etiologic agents of gastroenteritis in Kuwait and other Middle Eastern countries, the U.A.E. included, remain unknown (Sethi et al; 1989).

Abu-Dhabi city was chosen as the study area because it provides a good example of a modern city in the U.A.E. easily contrastable with the Abu-Dhabi of 25 years ago. The study was undertaken to determine the effect of the major interventions in the health care and infra-structure that have been made in the U.A.E.

Such evidence as is available would suggest that 25 years ago the occurrence of infantile diarrhoea in the U.A.E. would have been similar to that in the developing world.

Evidence from Kuwait would suggest that gastroenteritis there has not been eliminated by a rising standard of living. This study provided the opportunity to examine the health interventions undertaken by the government of the U.A.E.

Study Hypotheses:

1. Diarrhoea; an important disease in developing countries is expected to have some impact on the city of Abu-Dhabi on children between 0 - 5 years of age.
2. The affluence of the Abu-Dhabi society and the life-style that inadvertently includes domestic help in the houses, might contribute to the incidence of diarrhoea, since a lot of the domestic help comes from South East Asia where attitudes to hygiene and child-care may be different.

Chapter 2. Sources of Data

The U.A.E. is a new or young country in terms of progress and advancement. Most of this has accompanied the wealth that happened as a result of oil production. Since this explosion of wealth and affluence has happened only within the past 25 years, it is within the reach of human memory to contrast the past and the present especially since no proper records of any sort were kept then, except those kept by the British Government which were mostly of a political nature concerning the area. There are no medical records, birth or death record, literacy itself was a rarity amongst the inhabitants of the area, any information regarding the social or medical or health aspects are obtained by hearsay from people who lived in those times and were old enough then to be able to provide as accurate an account as possible. This is then confirmed from other people to check the validity and accuracy of the information obtained, as all the information is taken from human memory which may vary from person to person according to experience or age or any other influence. There are some photographs of the area 25 years ago and these serve to visually confirm, for example the state of the country in terms of existence of roads, housing facilities etc., and to mentally extrapolate what the situation must have been like then.

As a consequence of the variation of methods used and described in Chapter 2 data, from all these varied sources is collected, collated and incorporated into the study. The sources of information used in this study in addition to the hearsay ones mentioned above may be divided into 4 types of source:

1. Qualitative data derived from observations made during the home visits to the study families over the study period. These included interviews with other family members and friends as well as historical recollections from the older ladies who lived in prior oil-boom conditions, and interviews with personnel

from the oldest hospital opened in Abu-Dhabi in the town of Al-Ain. This hospital was set up by the Evangelical Alliance Mission, with its headquarters in Illinois, U.S.A. These sources also included photographs which were used in the making visual comparisons between the conditions existing today and those prevailing 25 years ago.

This kind of data was felt to be of great importance since the interview was conducted by the worker in the homes of the families participating, thereby allowing the worker a glimpse at each and every family in it's own and natural surroundings and making observations on the family in those surroundings without causing any great interference.

2. Quantitative data obtained from information in the questionnaire as well as the laboratory analysis of the specimens collected from these families over the study period

3. Background or comparative data for the laboratory studies was obtained from the routine clinical laboratories at Jazeira Hospital, Central Hospital and the maternity hospital. These were used as a pointer of conditions out of the study group and in the same locality and served mainly for comparative purposes.

4. Government Statistics: these were provided from statistical surveys carried out by the government such as the population census or the rate of success of the immunisation system to name but a few. This type of data is the official data. An agreement with this type of data would be a confirmation of it and a deviation would have to be investigated and explained. In the case of this study there was no official data on diarrhoeal diseases in children, but official statistics do not list it as a major cause of morbidity as they do for example

respiratory diseases. Birth and death records are kept and diarrhoeal diseases are not on the list as a major cause of infantile death. There is ample data on the immunisation system, which has been an area of interest and concern at the ministry, and that information was used to discuss the absence of diarrhoeal disease from the list of major causes of mortality and morbidity in Abu-Dhabi.

One of the many facilities available today is the M.C.H.C. (Maternal and Child Health clinics). These clinics are well-baby clinics where mothers bring their children for check-ups, immunisations or visits requiring medical attention. They also have ante-natal services. These clinics are felt by the M.O.H. to have had a great impact on public health and awareness as they are conducted under a more friendly atmosphere which is different to that of a working hospital and no appointment is necessary instead the mothers bring their children when it is suitable for them to do so thereby creating an "at ease" situation. And since there are a number (12) of these clinics scattered all over Abu-Dhabi, there is no over-work pressure on any particular clinic, thereby allowing the no appointment system to function smoothly and quickly. Data, information or observations obtained from these clinics would come under the Government data section.

Chapter 3. Materials and Methods

Contents of Chapter 3

Study Plan:

This study was intended to use a variety of approaches to the study of diarrhoea in the U.A.E. These included:

1. Quantitative scientific methods that relied on information in the questionnaire and on laboratory findings from specimens collected from the study group over the study period.
2. Qualitative interview methods made possible by the identification of the investigator with the local community, thereby allowing interaction and communication with the mothers of the study group and socialising with the other members of the study families in order to get descriptions of the way of life in the pre-oil boom era. Since very little documented material is available, hearsay from many people was the method of choice for getting historical information on the pre-oil boom era, using many references to cross check the information given by separate individuals. Interviews with personnel from the oldest hospital, The Evangelical Alliance Mission, in Al-Ain were also used to confirm what the conditions were like.
3. Comparative or background laboratory data from other working M.O.H. laboratories in other hospitals as well as in Jazeira hospitals.
4. Documented information from various government departments and ministries that have contributed to the solution of the problem of diarrhoeal diseases in Abu-Dhabi city.

The study was divided into 2 stages: a short preliminary study and the longer main study.

The preliminary study was a survey period to examine :

1. the types of pathogen causing diarrhoea in the population.
2. to establish the most suitable methods for carrying out the main study in the hospital laboratories.

The methods used in the pilot study were the standard procedures used at Central Hospital laboratory, with the only addition of rotavirus testing, which was done by the worker. These methods included microscopy, when requested, bacteriology, which involved culturing on standard media followed by biochemical identification. The bacteriology methods did not include methods for the isolation of Campylobacter spp. The technicians at Central Hospital and the local and only maternity hospital had separately in the past tried to isolate campylobacter from patients without much success, although the control strains were cultured. Since Campylobacter have specific and unique growth requirements and since they were not felt to be relevant to the local situation as deduced from the low rate of isolation (personal communication) it was dropped from the hospital routine, unless specifically asked for by the doctor - which didn't happen often.

The same methods as those used in the pilot study were later used in the main study in addition to a DNA-DNA hybridisation assay, half of which was done at the Royal Free Hospital School of Medicine; as well as a tissue culture assay done at the London School of Hygiene and Tropical Medicine.

The Preliminary Study:

A preliminary pilot study that extended for 2 months was carried out at Central Hospital - which caters for the non-national population in Abu-Dhabi. In addition to catering for its own patients, Central Hospital laboratories handled specimens from out-clinics that were referred to it.

The stool of any child 5 years old or below, whose clinical data on the hospital forms stated gastroenteritis, diarrhoea or parasites were investigated. Also included were any loose stool specimens for any under 5 year old whose clinical data on the hospital forms did not list any of the above mentioned conditions. On the days when the work load was light eg. when there no paediatric clinics, stool specimens from children without any of the above complaints were chosen at random and investigated. Altogether 152 stool specimens were investigated. This part of the study was basically a survey that involved using the hospital laboratory data and results in addition to doing rotavirus tests.

The investigations carried out consisted of:

i microscopy for parasites when requested by the doctor and was usually done by one of the laboratory technicians (more on this in the main study).

ii bacteriology on all specimens which involved culturing on standard media (MacConkey and DCA), which was followed by biochemical identification using Roche Enterotube II.

iii Virology on all specimens from all children 2 years old or below using the Wellcome Rotazyme kit and when that was unavailable the Wellcome Latex kit.

The Main Study

Qualitative Data:

Sample Group and Size:

The group selected was made up of children whose parents had local U.A.E status and who were therefore U.A.E. nationals. Non-U.A.E. national families were not included in the study for 2 reasons: firstly, language problems were anticipated with non-Arabic and non-English speaking mothers; secondly, the sample size would have had to be larger in order to get a more representative group and since there was a work force limitation, the study group was limited to only one nationality. The one chosen was felt to be the most stable as all other nationalities were considered as expatriates and therefore transient.

The sample size was chosen as the maximum number of families that could be handled by the worker. It was done in this way as there was no backup team available to help with the collection of the specimens and the processing of these specimens in the laboratory. It also became necessary when the drop-out rate turned out to be such a large number.

Family Recruitment:

Permission was obtained from the Ministry of Health (M.O.H.) and the director of Roda Clinic to interview and recruit mothers and families for this research at the Roda clinic. There are (23) clinics in Abu-Dhabi, 11 of these have MCH units attached to them. Each Maternal and Child Health (M.C.H.) unit that has its own doctor and one or two nurses. Roda clinic is identical in all it's facilities and services to all the other clinics in Abu-Dhabi. The only difference is that it caters for U.A.E. nationals only. Mothers attending the clinic do so for ante-natal purposes, while accompanying their children for vaccinations, when seeking treatment or just check-ups. This study was done as a contrast population study that was done on healthy rather than sick children to get better data related to the population regarding diarrhoeal diseases.

The room designated for the interviewer was adjacent to the doctor's room. Normally mothers would register with the nurse who would weigh the child and/or take it's temperature according to the nature of the visit, and then wait for their turn to see the doctor. It was while waiting for the doctor that the interviewer approached the mothers and asked them to come into her room to answer a few questions. All asked about the nature of the questions and were told they were in relation to infant diarrhoeal episodes and feeding practices. Most mothers obliged but a few (18) declined to participate at that stage. A total of 98 mothers were initially interviewed over a period of 2 months, 36 of whom continued with the whole duration of the study. The main criteria for inclusion in the study was to have U.A.E. status, to have one or more children under 4.5 years of age. Every mother coming to the clinic was approached, the idea being that while one mother was with the doctor, the waiting mother would be with the interviewer - the time spent with the interviewer supposedly being less than the time spent with the doctor and so the mothers were not detained any more than they needed to be.

When the mothers came into the room, the interviewer explained the purpose of the study, the requirements from the mother and the duration of the study. She also assured them that the study was being carried out with the knowledge and permission of the M.O.H., of the confidentiality of the reports and that the only persons making the house visits would be the interviewer herself. When the response was favourable the address and phone number were taken and the mother was told when the first house visit would be made, usually within a couple of weeks from the first meeting. 12 mothers declined to participate at this stage.

The Questionnaire:

The questionnaire (appendix i) contained non-laboratory data and information about each family such as the original nationality of the parents, maternal age and education, number of children in the study, number of children in the family, the immunisation status of the study children, who handled the children, who did the cooking in the house for the study children and for the rest of the household, whether the family lived as a single or extended unit. Feeding methods for unweaned children were divided into 3 groups: sole breast-feeding (SB), partial breast-feeding (PB) and sole bottle-feeding (B).

The Mothers:

The mothers of the children in the study were divided into 2 groups:

1. Those who are ethnically originated from the U.A.E.
2. Those who are not ethnically originated from the U.A.E.

These divisions were made to assess whether the mothers from each group had different domestic, feeding or other habits that might affect the welfare of their children.

Frequencies, Cross-tabulations, t-tests and One-way analysis of variance were done to analyze this information using the Statistical Package for Social Sciences (SPSS). A trend test using Epi. Info. was also used.

The First Visit:

When the interviewer was ready to make the home visit she called the families to make appointments. Much emphasis was laid on this visit the purpose of which was to gather some more information; that was not collected at the first meeting due to lack of time; on the family the children and the household in general using a questionnaire for this purpose (appendix i). The interview was conducted in Arabic and the questionnaire was filled in English. The visits took 45 - 60 minutes each and were very social in nature where the families showed hospitality due to a friend rather than the aloofness expected towards strangers. In addition to the filling of the questionnaire, the visit served the purpose of familiarising interviewer and family with each other and reassuring and re-explaining to the latter any point that was hitherto unclear. There were 8 drop-outs at this stage.

At the end of the visit the mothers were told of an approximate date when the actual collections were to begin and told that a couple of days before the beginning of collection, sterile collection containers would be delivered to the mothers. They were also instructed how to take and store the specimens until collection by the interviewer who from then on becomes the worker.

The 22 remaining mothers who dropped out did so for a variety of reasons which included not being able to get agreement from their husbands to participate, did not think the study was relevant, would not open the door for the first interview although an appointment was given by the mothers for such an interview, other unspecified family reasons, and lastly not being able to locate the homes from the mothers descriptions and no telephone being available to contact the mothers for further instructions accounted for the drop-out of another 5 families. 5 of the families were not pursued by the worker although the mothers agreed to join the study because they lived too far and it would not have been possible to collect the specimens and get on with the rest of the work. Another 2 were dropped by the worker as she did not feel comfortable in their home surroundings.

The Fortnightly Visits:

The families were divided into 3 groups (I, II, III) according to living area, to make collection rounds simpler and faster. The day before the collection day, each mother was telephoned and reminded to prepare the specimen for collection the next day. If no specimen was available for any particular child on their designated day then another visit was made the next day and if still no specimen was available then no specimen was available for that child that week.

The visits themselves varied in length from one family to another. Working mothers were very business-like and simply handed the specimens or in their absence their maids did that for them. In households where the mother barely went out, these mothers were more insistent on the worker coming in for Arabic coffee and a chat. These visits were used by the worker as a break period and were used by the mothers to make various enquiries usually regarding their children's health and usually lasted about 10 - 15 minutes. Some mothers became good friends with the worker gradually taking her into their confidence about personal matters. Specimens were transported in an ice-box to the laboratory usually 2-4 hours after the beginning of collection and work on them began straight away.

Sample Collection:

A maximum of approximately 20 specimens were collected from each of the 36 study families over a period of 9 months from November 1989 - June 1990. The mothers were asked to provide as fresh a stool sample as possible in a sterile container that was provided to them by the researcher and was to be collected on a specified day of the week every week. If, on the designated day the sample was not ready then the mothers were asked to prepare another sample the following day and if no sample was available on that day then no data was obtainable for that child that week.

The mothers were instructed to store the specimens in their containers in the freezer compartments of their fridges until they were collected and transported in an ice box to the laboratory. The request to freeze the specimens was done mainly for aesthetic reasons, but also to ensure specimens were not subjected to many temperature variations due frequent opening and closing of the fridge, especially in large households where more than one person would be using the fridge. The impact of freezing the samples will be discussed later. Work on the specimens was begun approximately 2-4 hours after collection had begun, and upon immediate arrival at the laboratory. At each visit a note was made of the child's well-being that week by asking the mother or the maid whether:

-the child had been ill or not,

-a doctor had seen the child,

-and whether any medication had been prescribed.

Sometimes the mothers would report a diarrhoea to the worker and yet the stool presented was a formed one, or no diarrhoea would be reported and the stool presented would be loose. So to cross check between the mother's report and the specimen appearance, 2 definitions of diarrhoea were used in the study - these were:

1. **The laboratory's definition:**

   A decreases in the consistency of the stool. Consistency being defined as taking the shape of the container.

2. **The mother's definition:**

   Whatever the mother regarded as a deviation from the normal bowel movements of her child.

Work Plan for the Laboratory Investigations:

The work time-table was divided as such:

Saturday: collect specimens from group I. Transport to the laboratory and begin tests - Culture and centrifuging for rota test.

Sunday: collect specimens from group II and any leftovers from group I. Transport to the laboratory. Culture group II specimens and centrifuge for rota test. Start biochemical tests for Group I culture isolates.

Monday: collect specimens from group III and any leftovers from group II. Transport to laboratory. Culture group III specimens and centrifuge for rota test. start biochemical tests on group II culture isolates. Identification of isolates from group I using the biochemical tests.

Tuesday: start biochemical tests on group III culture isolates. Identification of isolates from group II using the biochemical tests. Use supernates prepared the previous 3 days to do rotazyme and/or rota latex tests.

Wednesday: identification of isolates from group III using the biochemical tests. Culture pool isolates from the cultures group I and III in nutrient broth for DNA hybridisation.

Thursday: Pipette from nutrient broth cultures into wells of a 96-well microplate.

Every 8 weeks the microplates were removed from the freezer and the filters for the DNA hybridisation assay were prepared.

Quantitative Data:

Microscopy / Parasitology:

Routine testing was done to look for the trophozoites and cysts of pathogenic parasites, such as G.lamblia, Ent.histolytica, Ent.coli or any others. It was also done to look for faecal leucocytes and red blood cells particularly in specimens expected to harbour infections caused by invasive pathogens such as Shigella spp. (Bailey / Scott; 1974). A saline smear was made from the specimen and examined under an ordinary microscope using low and high powers. For further clarification an iodine smear was made to confirm the finding.

Originally, it was intended to do microscopy on all the specimens collected, but this proved impractical as the microscopes were usually required for the hospital routine work. Therefore microscopy was limited only to the specimens that were diarrhoeic according to either definition.

Saline Smear:

A few milligrams of faecal material were placed by means of a wooden applicator or toothpick beside a drop of saline on a clean glass slide and emulsified using the applicator to obtain an even thin spread. A number 1 cover-glass (22 x 22 mm) was carefully placed over the film which was then viewed under the low power (x 10) magnification and then under the high power (x 100) magnification of a binocular microscope.

Iodine smear:

This was prepared in the same way as the saline smear using iodine instead of saline. Diluted Lugol's solution (1:5) was used. A thin smear (thin enough to see newspaper print through) was made and again looked at under low and high magnifications.

Virology

Immunological tests were done using the Wellcome Latex Kit and the Wellcozyme Rotavirus Kit (Wellcome Diagnostics),to look for Rotavirus.

Principles of the Immunology Tests:

The Wellcozyme Rotavirus test is an enzyme immunoassay test in which the specimens under examination are incubated in micro-wells coated with specific antibodies.It uses the "sandwich" principle of solid phase enzyme immunoassay to detect rotavirus antigens in faecal material. A faecal sample suitably diluted in Sample/Reagent diluent is incubated in the well coated with chicken anti-rotavirus group specific antibody. Antigens present in the sample bind to the antibody on the well during incubation. After washing to remove unwanted materials, bound antigen is detected by the addition of bovine anti-Rotavirus antibody conjugated to horseradish peroxidase. Unbound conjugate is removed by washing and the bound conjugate is revealed by the addition of a sensitive peroxidase substrate. The intensity of the colour produced is proportional to the amount of enzyme label bound and hence to the amount of antigen present in the specimen. Assessment of the colour intensity is achieved by measuring the absorbence using a spectrophotometer at 450 nm. Specimens with an absorbence of greater than or equal to the cut-off value (found by calculating the mean of the negative controls and adding 0.1) are considered to be positive for rotavirus antigens. The Latex test on the other hand is a rapid slide test in which latex particles coated with specific antibody react with rotavirus antigen present in the faecal specimens to give agglutination. A Control Latex preparation was provided with the kit to identify any faecal specimens that might cause non-specific agglutination of coated latex particles. Agglutination of the sample but not the Control Latex indicates the presence of rotavirus in the sample.

Protocol for the Wellcozyme Rotavirus Test;

1. Add a small quantity of faecal material to 0.2 ml of Sample / Reagent Diluent in a test tube (to make a 1:1 dilution). Cap the tube and vortex to homogenize any solid material. Centrifuge to obtain a clear solution.
2. Add 0.1 ml of each supernatant to be tested into the appropriate well; using a new disposable pipette each time. Include 2 wells for the positive control and 3 wells for Sample /Reagent Diluent as the negative control.
3. Cover the wells with the lid provided and incubate at 37°c on a heating block for 30 minutes.
4. Wash the wells using the Wash Fluid 4 times.
5. Add 0.1 ml of Conjugate to each well taking care not to touch the sides of the well or splash the top of the well with the conjugate.
6. Cover the wells with the lid and incubate at 37° c on a heating block for 30 minutes.

7. Immediately dissolve required number of Substrate tablets in freshly distilled water (5 ml / tablet). Immediately before the next washing step add 5 ml Substrate Diluent / tablet to the solution. Mix gently
8. Wash the wells using the Wash Fluid 6 times.
9. Add 0.2 ml of the substrate solution; prepared in step 7; to each well.
10. Incubate for 30 minutes at room temperature away from direct sunlight.
11. Add 50 ul of Stop Solution (2M Sulphuric acid) to each well.
12. Read the absorbence of each well at 450 nm within 30 minutes of Stop Solution addition.

Protocol for the Wellcome Rotavirus Latex Test:

1. Add approximately 0.1 ml of faecal material to 1.0 ml of Extraction Buffer in a centrifuge tube.
2. Cap the tube and vortex to homogenize the pellet. Allow to stand for about 5 minutes before centrifuging at 1000 g for 10 minutes
3. Using a disposable dropper dispense one drop of supernatant to be tested, onto each of two circles on the card.
4. Dispense one volume (40 μl) of Test Latex next to one of the drops of supernatant, and one volume (40 μl) of Control Latex next to the other.
5. Mix the contents of each circle using a separate mixing stick for each sample, spreading the mixture over as much of the circle as possible.
6. Gently rock the card and observe for agglutination for up to 2 minutes.

A positive result is indicated by the visible clumping of the Latex particles within 2 minutes of mixing the Latex with the faecal supernatant.

Electron Microscopy:

This was done at the Department of Electron Microscopy, London School of Hygiene and Tropical Medicine by Mr. G. Tovey using a Jeol 12 EX MK2 at 8 KW photographed onto Ilford EM Film and at a magnification of x 5 K. Two different stains were used:

Ammonium Molybdate (AM) pH 6.8

or Uranyl Acetate (U/A) at a very low pH.

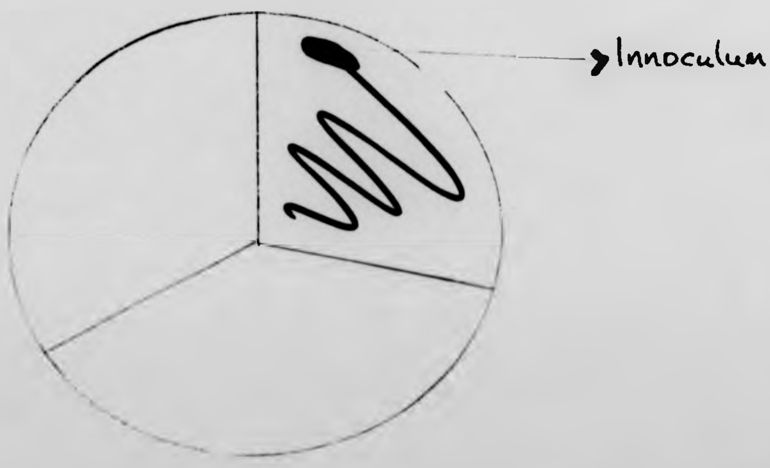
The chosen specimens were frozen at -20°c until they were transported in ice to the London School of Hygiene and Tropical Medicine (LSHTM).

Bacteriology

A variety of methods were used in an attempt to identify bacteria. Each was intended to cross-check the other as a means of confirmation of the interpretation of the tests. Figure (7) shows the sequence of events once the specimens arrived to the laboratory. Each colour denotes the day order on which each tests was done.

Day 1

Upon arrival to the laboratory, a portion of each specimen; approximately 1 mg was inoculated into Selenite-F enrichment broth for the detection of Salmonella and Shigella using a wooden applicator and incubated at 37° c overnight. Another approximately 1 mg portion was emulsified in peptone water and was streak plated onto each of MacConkey agar, Deoxycholate citrate agar (DCA) and Campylobacter blood-free agar, three specimens per plate (see diagram below). The first two media were incubated at 37° c overnight and the Campylobacter media -when done- was incubated at 42° c for 48 hours in an atmosphere with reduced oxygen, increased carbon dioxide and a catalyst in an ordinary candle-jar.



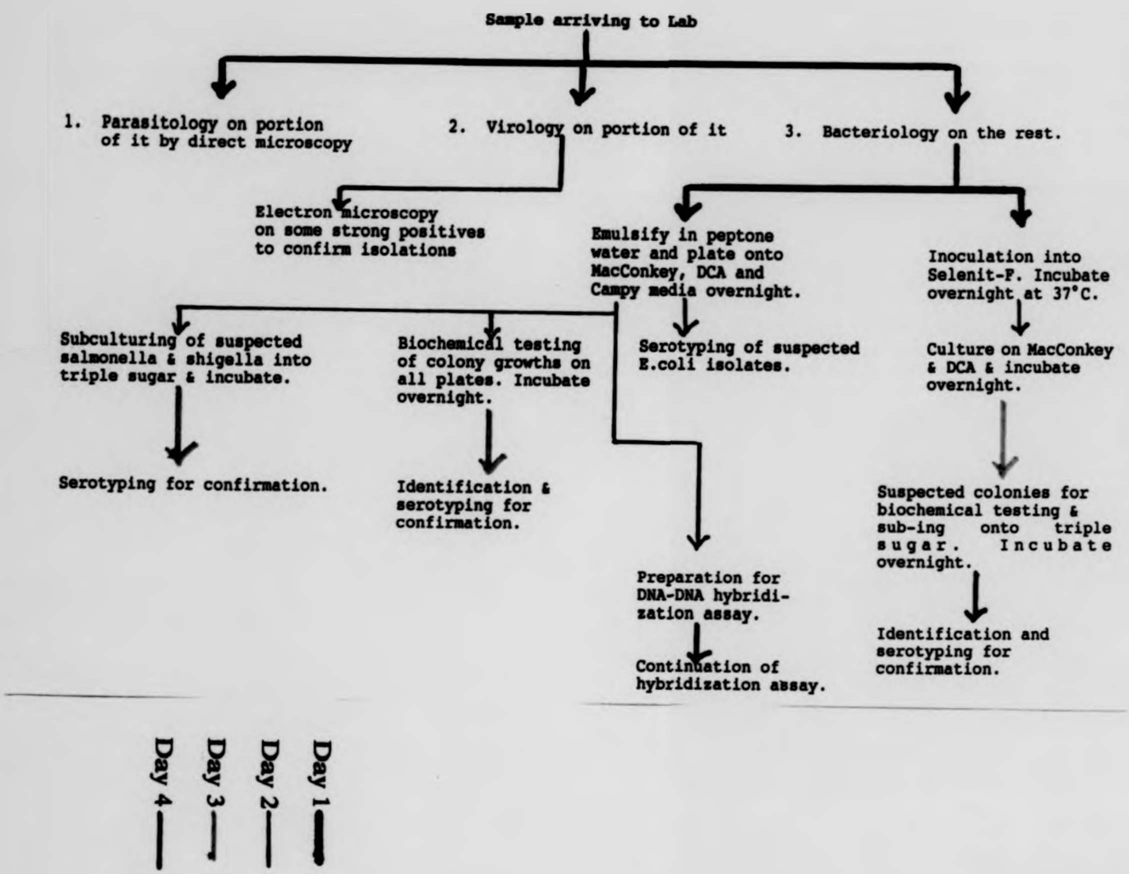


Fig (7): Sequence of Events in The Laboratory

Day 2

The following morning the selenite-F broths from the previous day were sub-cultured onto MacConkey agar and DCA using the streak inoculation method using an ordinary loop. Growth was noted on all plates that were previously incubated, noting non-lactose fermenting colonies, but biochemical testing was done on growths from each specimen inoculated, by choosing a single representative for each colony type and picking it onto a Roche Enterotube II for the identification of Enterobacteriacea. These tubes were then incubated overnight at 37° c.

Colonies that were:

1. suspected of being E.coli and were isolated from diarrhoeic children were sero-typed using the slide agglutinating test with the E.coli polyvalent antisera. If a positive result was obtained with any of the polyvalent antisera, then further sero-typing using mono-sera for that particular group was done to further specify the sero-group of the E.coli. The typing sera used were:

polyserum:	I	II	III
monoserum:	O26	O86	O18 ac
	O55	O114	O44
	O111	O125	O112 ac
	O119	O127	O124
	O126	O128	O142

Sero-typing in both cases was done by emulsifying one pure colony in saline on a clean slide then adding one drop of typing sera and gently mixing with a wooden rod before gently rocking the slide for up to 2 minutes. Agglutination is a positive result for the sero-group being tested.

2. suspected of being Shigella spp or Salmonella spp. were stabbed into Triple sugar iron agar (TSI) slants and incubated at 37° c overnight. The sterile tip of an inoculating needle was dipped into the centre of the colony to be tested. This was then stabbed into the butt of a TSI slant and streaked in a zigzag fashion over the slanted surface. The tube was then closed with a cotton plug to allow the free exchange of air needed to maintain the alkaline condition of the slant. The TSI agar slant is used for determining carbohydrate fermentation and hydrogen sulphide production as a step in the identification of gram negative bacteria. It contains 3 sugars: glucose, lactose and sucrose, it also contains phenol red to indicate fermentation and lastly it contains ferrous sulphate to determine hydrogen sulphide gas production. The glucose concentration is 1:1 of the concentration of the other two sugars to enable the detection of the fermentation of this sugar alone (Scott/Bailey; 1974).

DAY 3

Growths on the incubated plates were noted and again biochemical testing on representative single colonies were made using the same methods mentioned previously and the Enterotubes were incubated overnight at 37° c.

The TSI agar slants were observed for colour changes in the butt and slant of the agar and for gas production. Colonies from positive results from the slants were then subjected to sero-typing using Salmonella and Shigella specific antisera as described by Cruickshank; (1975). This was basically similar to the slide-agglutinating method of E.coli sero-typing with the exception that the antisera appropriate for each pathogen were used.

Day 4

Enterotubes from the previous day were read, any isolates that were identified as Salmonella or Shigella or E.coli (when there was a diarrhoea in the case of E.coli): when there was a diarrhoea; were sero-typed using the previous day's culture, if isolates from that specimen had not already been sero-typed at any of the above stages.

DNA - DNA Hybridisation Assay

Summary:

This assay was used to detect the "LT" gene in purified bacterial isolates. Briefly, bacterial colonies were bound onto Hybond Nylon filters, lysed and then fixed in situ by UV irradiation. This part of the study was carried out in Abu-Dhabi. At a later date, the filters were brought to the Royal Free Hospital School of Medicine, London to complete the assay, which was performed as described by Moseley et al (1980), modified from the method of Grunstein and Hogness (1975). This involved radio-labelling of the probe and hybridisation to the LT sequences released from the bacteria on the filters. After extensive washing, auto-radiography was carried out and used to detect hybridisation - or the presence of the LT genes - by the blackening of the film in areas associated with hybridised probe.

Selection and Isolation of Bacterial Strains:

Bacterial colonies isolated on MacConkey agar from the isolation plates, that had been inoculated with faecal samples as described in Chapter 3 page 104 were pooled (regardless of their identification, 5 - 10 colonies of each type) and inoculated into nutrient agar broth and incubated overnight at 37° c. The following morning 0.25 - 0.30 ml of each culture was transferred into one well of a 96-well round-bottomed sterile tissue-culture microplate. The plates were designated by week of original isolation. They were then stored at -20° c; usually for 8 week intervals after which the lysis and denaturation of the strains were carried out for the 4 week batches.

Transfer of Bacterial DNA onto Hybond-Nylon Filters:

The stored bacterial strains were transferred onto the filters using a sterilized replica-plating template. This template had 48 pinheads attached to a polystyrene base and handle. These pinheads corresponded with the wells of the microplate. After flaming to sterilize, the template was dipped into the micro-wells and then spot inoculated onto the nylon filters. The filters were then placed onto Nutrient Agar plates and incubated overnight at 37° c to allow growth onto the filters.

Lysis, Denaturation and the Fixing of Bacterial DNA:

The following morning the filters were lysed and denatured as described in Membrane transfer and detection methods; Amersham, 1985. Duplicate filters were prepared and stored until further use in London.

Reagents:

Denaturing Solution:

1.5M NaCl

0.5M NaOH

Neutralising Solution:

1.5M NaCl

0.5M Tris HCl pH 7.2

0.1M NA_2 EDTA

SSC Solution :

3.0M NaCl

0.3M NA_3 citrate

Protocol:

1. Inoculate pools of 5 - 10 colonies into nutrient broth and incubate overnight at 37°c.
2. Pipette 0.25 - 0.3 ml of each broth into micro-wells of a sterile 96-well microplate.
3. Dip sterilized template into wells and spot inoculate onto Hybond nylon filter which are then placed on nutrient agar plates and incubated at 37° c overnight.
4. Remove filters from agar surface and place colony-side up on 3 mm filter paper.
5. Place filters colony-side up on a pad of filter paper soaked in denaturing solution. Leave for 7 minutes.
6. Place membranes colony-side up on a pad of filter paper soaked in Neutralizing Solution. Leave for 3 minutes and repeat with a fresh pad soaked in Neutralising Solution.
7. Wash filters in 2 x SSC.
8. Transfer filters to dry paper and allow to dry colony-side up.
9. Wrap filters in Saran wrap and place colony-side down on a standard UV transilluminator for 2 - 5 minutes.
10. Store filters until needed for the hybridisation at The Royal Free Hospital School of Medicine.

The previous stages were repeated with a set of E.coli positive and negative controls on a separate filter that was used as the reference control filter.

Random Oligo-Primed DNA Radio-Labelling of the Probe:

The Heat-labile (LT) probe was derived from the (ampicillin resistant) recombinant plasmid pAT153.H6, comprising of an 800 base pair cloned into the Hind III site of the plasmid vector, pAT 153 (Gennaro et al, 1980). This had already been excised from the plasmid and purified by preparative agarose gel electrophoresis. The fragment was radio-labelled by Dr. T. J. Harrison using ^{32}P dCTP. and the random oligo-primed method of Feinburg and Vogelstein, (1983).

The specific activity of the probe ranged from :

<u>$3.0 - 5.0 \times 10^8$ cpm</u>

Reagents for the Random Oligo-Primed DNA Radio-Labelling:

DTM:	100 μM each dATP, dGTP, dCTP
	in 250 mM Tris HCl pH 8.
	25 mM $MgCl_2$
	50 mM 2-MCE
Oligo/2:	45 /ml pd $(N)_6$ in 1.0 mM Tris HCl
	1.0 mM EDTA pH 7.

For 25 μl Reaction :

5.0	μl DTM
5.0	μl 1M Hepes pH 6.6
1.4	μl Oligo/2 (primer)
1.0	μl Bovine Serum Albumin (B.S.A.)
6.0	μl Distilled water (for a final volume of 25 μl)
3.0	μl ^{32}P dCTP approx 3000 li/mmol
1.0	μl (5 μ) Klenow polymerase

Methods:

A 25 μl mixture for the random oligo-priming of DNA was prepared as above.

3.6 μl of DNA were boiled for 2 minutes (in an eppendorf tube with a punctured cap and cooled immediately to 0°c to prevent the re-naturing of DNA) before being added to the above mixture.

3.0 μl of (^{32}P) dCTP and 1.0 μl of Klenow fragment were also added. The tube was gently tapped to ensure mixing and then centrifuged at room temperature in a microfuge to get all the liquid to the bottom of the tube, then left to react at room temperature for 30 minutes. Percentage label incorporation was checked using trichloroacetate precipitation and a Geiger counter. The unincorporated deoxynucleoside triphosphates was removed using a Sephadex G50 Column, which is an exclusion column and will exclude the DNA which is eluted in the void volume.

Pre-Hybridisation of the Nylon Filters:

Reagents for the Pre-Hybridisation Solution:

To make up a 40 ml solution:

12	ml	20 x SSC
4	ml	5 x Denhardt's solution
2	ml	10% Sodium Dodecyl Sulphate (SDS)
0.8	ml	0.5 mg/ml Herring sperm DNA as carrier DNA (Gene Bloc)
21.2	ml	Distilled water to make required volume of 40 ml

Methods:

The filters were washed in 6 x SSC (standard saline citrate) before pre-hybridising them. Pre-hybridisation was carried out using herring sperm DNA to prevent non-specific binding of the probe to the filter. A 40 ml pre-hybridisation solution was prepared and 5 - 6 filters were placed in each of 2 petri dishes with 20 ml of pre-hybridisation solution and were incubated in a 65° c water-bath overnight, after which they were hybridised.

Hybridisation of the Nylon filters:

Reagents for Hybridisation Solution:

To make up a 10 ml of solution:

3 ml x 2 SSC

1 ml x 5 Denhardt's solution

0.5 ml 1% SDS

0.2 ul Gene Block (Herring sperm DNA)

1.25 ml Gene Probe which was ~ 2.2 x 10^7 cpm

4.05 ml Distilled Water to make up the final volume.

Denhardt's solution (Denhardt, 1966) consists of:

0.2% BSA

0.2% Ficoll

0.2% PVP

Methods:

The gene bloc and probe were boiled for 5 minutes and cooled immediately on ice to prevent the DNA from re-naturing before adding to the rest of the solutions.

5 ml of hybridisation solution were added to each of 2 new petri dishes. The filters were transferred from the pre-hybridisation solutions to the hybridisation solutions, 5 - 6 filters / petri dish DNA side down. The plates were incubated in a 65° c water bath with shaking overnight before washing and auto-radiography.

Washing and Auto-Radiography:

The filters were washed in 2 x SSC allowing 200 ml of solution for every 5 filters and incubated in a 65°c water bath with shaking for 15 minutes. This step was repeated once before being followed by another wash in 2 x SSC + 0.1% Sodium Dodecyl Sulphate (SDS) and again incubated at 65°c with shaking for 50 minutes. The filters were then placed on a sheet of 3 mm filter paper and wrapped in Saran Wrap. Auto-radiography was carried out in the dark at -70° c by placing a Kodak XAR film on the filter papers. After 48 hours, the film was developed using an X-ray developer. Positive hybridisation was detected by blackening of the film over the positive colony.

Tissue-Culture Assay for the Production of Heat-Labile Toxin (LT):

Since there are no bacteriological or biochemical markers that differentiate between toxigenic and non-toxigenic strains, the identification of these strains relies upon the production of LT and/or ST, or VT.

The LT enterotoxin induces morphological changes in African green monkey kidney cells (Vero cells). VT enterotoxin induces cytotoxic effects on Vero cells. No cell lines have been established for the detection of ST, although cells known to be sensitive to STa have been identified.

Bacterial Strains:

At the end of the 6 month period, 55 isolates were sent from Abu-Dhabi on nutrient agar slants. 40 of those isolates came from paediatric patients visiting Jazeira and Central Hospitals. 27.5% of these 40 isolates actually stated gastroenteritis or diarrhoea in their clinical data. The rest of the forms stated routine testing, pallor, otitis media, joint pain, bronchial asthma or croup.

The remaining 15 specimens came from the Quality Food Control Laboratory in Abu-Dhabi. This laboratory investigates cases of food poisoning, the bacteriologic quality of foods in stores and restaurants, among other quality and quantity control functions. The isolates from this laboratory originated from food specimens (frozen chicken, milk or ice-cream) or from stool or water samples. Both sets of isolates had been biochemically identified at the laboratories of origin (table 10a and 10b). No sero-typing had been done to confirm these identifications.

Table (10a): Specimens from Jazeira & Central Hospital laboratories

Specimen no.	Organism isolated	Clinical observation	Age
1	klebsiella E.coli	Routine	1 month
2	E.coli	Gastroent.	6 months
3	E.coli		7 months
4	Proteus		Child
5	E.coli	Gastroent.	2.5 months
6	E.coli	Bronchitis	3 months
7	E.coli	Gastroent.	17 months
8	E.coli	Gastroent.	5 months
9	Coliform	Gastroent.	10 months
10	E.coli	Gastroent.	4 months
11	E.coli		
12	E.coli	Br. Asthma	12 months
13	E.coli	Otitis Media	8 months
14	Klebsiella	Malaria	36 months
15	E.coli	Gastroent.	12 months
16	E.coli	Croup	1 month
17	Coliform	Routine	5 months
18	E.coli	Routine	4 months

19	Klebsiella	Routine	1 month
20	E.coli	Diarrhoea	12 month
21	E.coli	Routine	12 months
22	E.coli	Routine	1 month
23	Klebsiella	Routine	
24	E.coli	Routine	36 months
25	E.coli	Routine	12 months
26	Proteus	Routine	36 months
27	E.coli	Routine	2 months
28	Klebsiella	Routine	48 months
29	E.coli	Routine	60 months
30	E.coli	Pallor	24 months
31	Klebsiella	Gastroent.	24 months
32	E.coli	fever	< 1 month
33	Klebsiella	Diarrhoea	10 months
34	Klebsiella	Diarrhoea	12 months
35	E.coli	Routine	5 months
36	Klebsiella	Routine	6 months
37	E.coli	Routine	6 months
38	E.coli	Routine	36 months
39	Klebsiella	Routine	6 months
40	E.coli	Routine	12 months

Table (10b): Specimens from The Quality Food Control Laboratory

Specimen number	Organism	Source
1	E.coli	Frozen Chicken
2	E.coli	Coconut ice-cream
3	E.coli	Ice-cream
4	E.coli	Ice-cream
5	E.coli	Frozen chicken
6	Klebsiella	Milk
7	E.coli	Urine
8	Pseudomonas	Water
9	Salmonella sp.	Stool
10	Salmonella sp.	Stool
11	Shigella flexneri	Stool
12	Salmonella sp.	Stool
13	Salmonella typhi	Stool
14	Salmonella sp.	Stool
15	Salmonella sp.	Stool

Conditions for the Growth and Toxin Production:

A loopful from each slant was inoculated into 10 ml of Trypticase Soy Broth in sterile disposable containers and grown aerobically at 37°c in a shaking incubator. After 18-20 hours the cultures were centrifuged at 3 g for 2 minutes and then the supernates were filtered aseptically through a membrane filter of pore size

0.22 m. The filtrates were stored at -70° c and used within the next few days for the assay.

Cell Culture:

An established cell line of Vero cells was used as described by Giugliano et al; 1982. The Vero cells were grown in Growth medium that was made up of the following:

10	ml	medium 199
90	ml	S.D.W.
3.5	ml	4.4% sodium bicarbonate
10%	v/v	Fetal-calf serum (F.C.S.)
0.1	ml	Penicillin G 1 units/ml and
		Streptomycin 1 g/ml

The fetal-calf serum was necessary to revive the vero cells from the liquid nitrogen they were stored in. The Na_2 HCO_3 was necessary to supply carbon dioxide for respiration. The antibiotics were added to prevent other bacterial growth. Once the Vero cells became confluent usually after 5-7 days incubation, they were there-after maintained in a glass bottle with a growth medium containing 5% v/v FCS during each passage.

Preparation of the Confluent Monolayer:

The medium in the glass bottle was gently poured away, from the sides of the growth of the monolayer in the bottle. The cells were washed with 10 ml phosphate buffered saline (PBS) by rolling the bottle gently to ensure the cells were all covered with the PBS, this was then again gently poured away, from the side of the monolayer in the bottle. The washing step serves to remove dead cells and old growth medium. The cells were then trypsinised to separate the monolayer from the side of the bottle and to dis-aggregate the cells from each other. Trypsinisation was done by the addition of 10 ml of versene + trypsin (99 ml versene and 10 ml trypsin) and allowed to stand for 3 seconds. The versene was then poured away leaving just a little bit in the bottle to help in the dissociation of the cells from the sides of the bottle. The bottle was then incubated at 37° c for 3 minutes.

The cells which now come away easily from the sides of the bottle are re-suspended in 30 ml of growth medium and an average cell count was made using a haemocytometer. A suspension of concentration of 6×10^4 cells was prepared by diluting the calculated volume of cells in growth medium using the following formula

$$\frac{\text{required volume} \times 6 \times 10^4}{\text{average cell count} \times 10^4} = \text{cell volume}$$

Confluent monolayers were made by seeding 0.1 ml of cell suspension into the wells of a 96-well sterile microplate. The plates were sealed with surgical tape and incubated for 2 days at 37° c in a humidified incubator with an atmosphere of air and 5% carbon dioxide.

Protocol for the Preparation of Confluent Monolayers:

1. Decant the growth medium in the glass bottle, away from the side of the monolayer growth on the bottle.
2. Wash the monolayer with 10 ml PBS and decant away from the side of the monolayer growth on the bottle.
3. Trypsinise cells of monolayer by adding 10 ml of versene + trypsin (99 ml versene + 10 ml trypsin). Allow to stand for 3 seconds. Decant away from the side of the monolayer growth, leaving a little in the bottle.
4. Incubate bottle at 37° c for 3 minutes.
5. Re-suspend cells in 30 ml growth medium.
6. Make an average cell count of cell suspension using an haemocytometer.
7. Dilute cell suspension to a final cell count of 6 x 10^4 cells.
8. Seed 0.1 of cell suspension into wells of a sterile microplate.
9. Seal plates and incubate at 37° c in a humidified incubator with an atmosphere of air and 5% carbon dioxide.

Seeding of Bacterial Filtrates:

After the incubation period the growth medium was removed by the inversion of the plates and replaced by an equal volume (0.1 ml) of maintenance medium that was of the same basic formula as the growth medium but contained FCS 1% v/v.

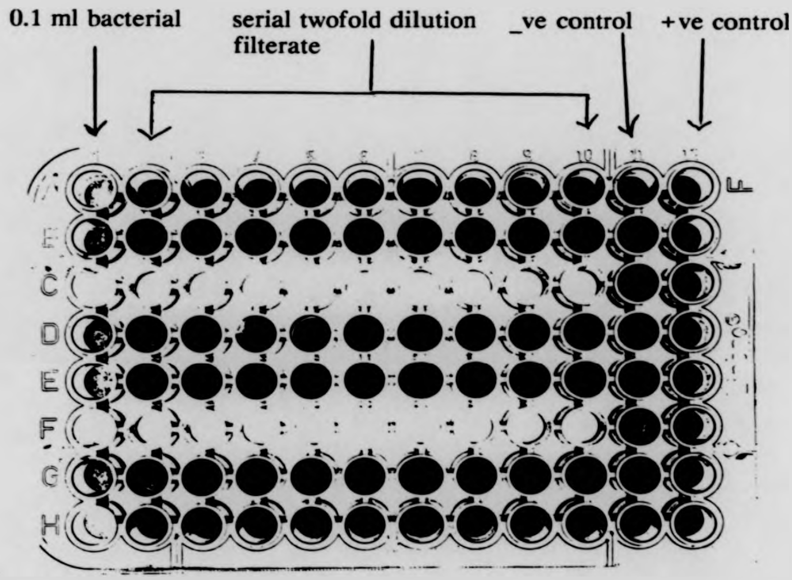
0.1 ml of bacterial filtrates were added to the first well in the rows being used in the microplates and a serial twofold dilution of the filtrates was done (each filtrate was done twice, allowing for 3 filtrates / microplate, as illustrated in plate (13). A negative control containing no filtrate and a positive control using cholera toxin that was serially diluted were included in the assays. The plates were sealed with surgical tape and incubated at 37° c in a humidified incubator with an atmosphere of air and 5% carbon dioxide.

Protocol for Seeding Bacterial Filtrates:

1. Decant growth medium from the microplates by inverting, gently shaking and blotting the plates.
2. Add 0.1 ml of maintenance media to each well.
3. Seed 0.1 ml of bacterial filtrates in the first well of each row, doing duplicates for each filtrate.
4. Serially dilute up to 1:1024.
5. Seal plates and incubate at 37° c in a humidified incubator with an atmosphere of air and 5% carbon dioxide.

Cells were examined microscopically after 24, 48 and 72 hours after the addition of the filtrates to detect any effects on cell morphology or growth. The best results were obtained after 72 hours, so after the first set of plates were done any that followed were examined after a 72 hour incubation period.

Plate (13): Illustration of How Microplates were used



Each sample was done in duplicate

Fixing and Staining of the Cells:

To examine the cells, the reaction had to be stopped and the cells stained in crystal violet stain. The maintenance medium was removed by inverting the microplates, the wells were filled with 70% methanol for 30 minutes. This was removed and the wells were filled with crystal violet stain and again allowed to stand for 30 minutes after which the plates were gently washed with water and allowed to dry before viewing under the microscope.

Protocol for Fixing and Staining the Cells:

1. Decant the maintenance medium by inverting plates and shaking.
2. Fill all wells with 70% methanol and allow to stand for 30 minutes. Decant by inverting plates.
3. Fill all wells with crystal violet and leave to stand for another 30 minutes. Decant by inverting plate.
4. Wash plates gently with tap water. Blot to remove any excess water and leave to air-dry before viewing under the microscope.

Materials

Microscopy/Parasitology:

Reagents/Materials:	Supplier
Diluted Lugol's solution	Trypsin

Virology:

Reagents/Materials:	Supplier
Wellcome Rotazyme kit	Wellcome
Wellcome Latex kit	Wellcome
Ammonium Molybdate	Trypsin
Uranyl Acetate	Trypsin

Bacteriology:

Reagents/Materials:	Supplier
Roche Enterotube II	Roche Products
E.coli polyvalent antisera	Difco labs
E.coli monovalent antisera	Difco labs
Salmonella antisera	Wellcome
Shigella antisera	Wellcome

DNA Hybridisation Assay:

Reagents/Materials	Supplier
pd (N)6	Pharmacia 27-2166-01
^{32} P dCTP3000 Ci/mmol	Amersham
96-well round-bottomed sterile	ICN Biomeds
82 mm Hybond Nylon filters	Amersham
Bovine Serum Albumen (BSA)	Sigma
Gene bloc	Int. Nat. Lab. Sercices
E.coli positive and negative controls	LSHTM stock
Hepes buffer	Sigma
Klenow Fragment 5u/ul	Amersham
Oligo/2 primer	Cambridge Bio Sciences
Sephadex G50 column	Pharmacia

Tissue Culture Assay:

Reagents/Methods	Supplier
African Green Monkey Kidney cells (Vero)	ICN Biomeds
Cholera toxin	Sigma
Fetal Calf Serum (FCS)	Life Techs
Medium 199	Life Techs

Addresses of Suppliers:

Amersham International
Lincoln Place,
Green End
Ayelsbury,
Buckinghamshire HP20 2TP

Cambridge Bio Sciences,
25 Signet Court,
Swans Road,
Cambridge CB5 8LA

Difco Laboratories Ltd,
P.O.Box 14 B,
Central Avenue,
West Molesey,
Surrey KT8 OSE.

Glaxo Group Research Ltd,
Greenford Road,
Greenford,
Middlesex UB6 OHE

ICN Biomedicals Ltd,
Eagle House,
Peregerine Business Park,
Gomm Road,
High Wycombe. HP13 17DL

International Laboratory Services Ltd,
14-15 Newburt Street,
London EC1A 7HY

Life Tecnologies Ltd,
P.O.Box 35,
Washington Road,
Abbotsinch industrial Estate,
Paisley,
Scotland PA3 4EP

Trypsin Ltd,
Hunter Boulevard,
Magna Park,
Lutterworth,
Leics LE17 4XN

Pharmacia (GB) Ltd,
Davy Avenue,
Knowlhill,
Milton Keynes,
Bucks MK5 8PH

Roche Products Ltd,
Diagnostics Division,
P.O.Box 8,
Welwyn Garden City,
Herts AL73 AY.

Sigma,
Chemical Company Ltd,
Fancy Road,
Poole BH17 7NH,
Dorset.

Unipath Ltd,
Wade Road,
Basingstoke,
Hampshire RG24 OPW.

Wellcome Diagnostics Ltd,
Temple Hill,
Dartford,
Kent, DA51 4AH.

Chapter 4. RESULTS

Contents of Chapter 4

Introduction:

Information for the preliminary study was obtained only from laboratory analysis of the specimens arriving during that study period. They involve bacteriological tests as well as tests for rotavirus.

Information for the main study on the other hand was obtained from 4 types of source:

1. Qualitative derived from:

   a. observations of the households visited over a period of 6 months which included interviews with other family members or friends. Since some mothers were reluctant to allow the worker to inspect the areas of their homes that had most relevance to the children such as the kitchen; where their food was prepared; and the bathrooms used by the children, these visits then allowed for the passive observation of these areas without offending the mothers by asking the questions directly.

   b. Historical recollections; from older ladies; of the social conditions prior to the oil-boom and the health situation that prevailed under those conditions. As well as information obtained from personnel at the oldest hospital in the emirate of Abu-Dhabi; in the city of Al-Ain; run by The Evangelical Alliance Mission (TEAM).

2. Quantitative obtained from:

a. The questionnaire; which was filled out during the first home visit. Additional information; such as a new birth, the beginning of weaning, or any other relevant information; was sometimes added later.

b. From the laboratory analysis of the specimens collected from these families over the study period.

3. Background or comparative data from the routine work from neighbouring hospital laboratories. This was used to compare the results obtained from the study group, and was mainly concerned with the prevalence of diarrhoea and its causes.

4. Government statistics, mainly those from the M.O.H. and from other government departments that collaborated with the health services.

The order of collecting these results was roughly that above. The observations were made from the very beginning when the mother walked into the interview room at Roda clinic and were continued throughout the study at the home visits. Appendix (ii) contains short summaries or impressions of the visits and general information on the families made originally in Arabic and later translated into English.

The questionnaire was filled by the worker, after the initial interview at the clinic and in the home set-up during the first home visit. Sometimes it was necessary to update information on the questionnaire for example upon a new birth, mother going back to work or a new maid joining the household. Historical recollections were noted at this stage or during the home visits- as they came up in conversations.

The laboratory analysis went on throughout the study period. The background data was noted during the study period and re-checked at the end of the study period by thoroughly going through the laboratory records.

Statistical analysis was done at the end of the study when all the data was complete.

The Preliminary Study

From the 152 specimens; collected over a 2 month period; that arrived at Central Hospital laboratory the following isolations were made:

Table (11): Organisms isolated according to age-group

Pathogen	No. isolations From various age groups in months				Total
	0 - 5	6 - 11	12- 18	> 19	
Rotavirus	4	7	2	3	16
G.lamblia	--	--	17	22	39
Ent.histolytica	--	--	--	--	--
Yersinia sp.	--	1	1	1	3
Salmonella sp.	1	--	--	--	1
Shigella sp.	2	--	1	4	7
E.coli	15	15	15	15	60
Unknown	4	8	8	6	26
Total	26	31	44	51	152

Giardia lamblia was the most recognised pathogen at 25.6%. It's isolation came mainly from patients whose laboratory forms requested parasite testing (microscopy) only. Only 2 patients with diarrhoeal complaints as indicated on these forms were positive for G.lamblia. They came from the 19 month to 5 year old group, both had E.coli isolated as well. G.lamblia isolation in general occurred from the older children with a 43% in the 12-18 month group and the remaining 56% occurring in the 19 month-5 year old group and no isolations in any of the other groups. There were no E.histolytica (which were specifically requested on two hospital forms) or any other parasite isolates.

E.coli were isolated in 39% of all cases and equally distributed amongst all age groups. No serotyping was done and therefore it is not possible to comment on whether these E.coli fell into any of the known pathogenic serogroups. Their isolation coincided with 95% of all cases stated as diarrhoea or gastroenteritis on the forms. 7% of all the isolates were found to be comprised of Yersinia spp. (3 isolates), Salmonella sp. (1 isolate) and Shigella spp. (7 isolates).

Rotavirus was isolated from all the age-groups and in 10% of all specimens tested. Isolation was greater in the under 2 year old group, peaking at the 6-11 month group. There were 2 isolations from children who were older than 2 years old, one of which was asymptomatic. There was a small number of asymptomatic infections (% not available). Rotavirus testing was not done routinely at Central Hospital at the time of this study so there was no available information on prevalence, carriage or seasonality. A Rotazyme test kit was used and when that became unavailable a Rota-latex kit was used. It is possible that some rotavirus infections were missed using the latex kit, as the latex test can sometimes to be less sensitive than the rotazyme test. Or some infections were missed as a result of the time of specimen collection relative to onset of symptoms, as it has been shown that rotavirus particle shedding was less in the second week of infection than the first (Pai et al; 1985).

17% of all the diarrhoeal specimens were recorded as non-identifiable. It was not possible to designate an organism to the isolates cultured from these specimens using the Roche Enterotube II for the identification of Enterobacteriacae and as such were classified as non-identifiable.

The nationalities or ethnic groups to which these study children belong to are not known in detail as this information is not normally included on the request forms, but all patients seen at this hospital or the clinics it caters for are non-nationals of the United Arab Emirates.

The Main Study

Qualitative Results:

Family Recruitment:

Of the initial 98 mothers approached at Roda clinic during the 2 month recruitment period, only 36 ended up participating in the study (fig 8)

Figure(8): Scheme of Mother Recruitment in the Study.

98 → mothers approached at Roda clinic

↓

78 ← 18 mothers declined to be interviewed

↓

66 ← 12 mothers declined after 1st interview at clinic

↓

58 ← 8 dropouts when calls were made to confirm 1st home visit

↓

53 ← 5 dropouts when homes couldn't be located and no telephones in homes to re-direct

↓

48 ← 5 dropped as homes too far to be included in study

↓

46 ← 2 dropped due to uncomfortable home set-up

↓

36 ← 10 dropouts after 1st home visit.

A total of 52 children from those families were involved in the study. Figure (9) shows the total number of children in each family, broken down into numbers participating and non-participating in the study

The First Visit:

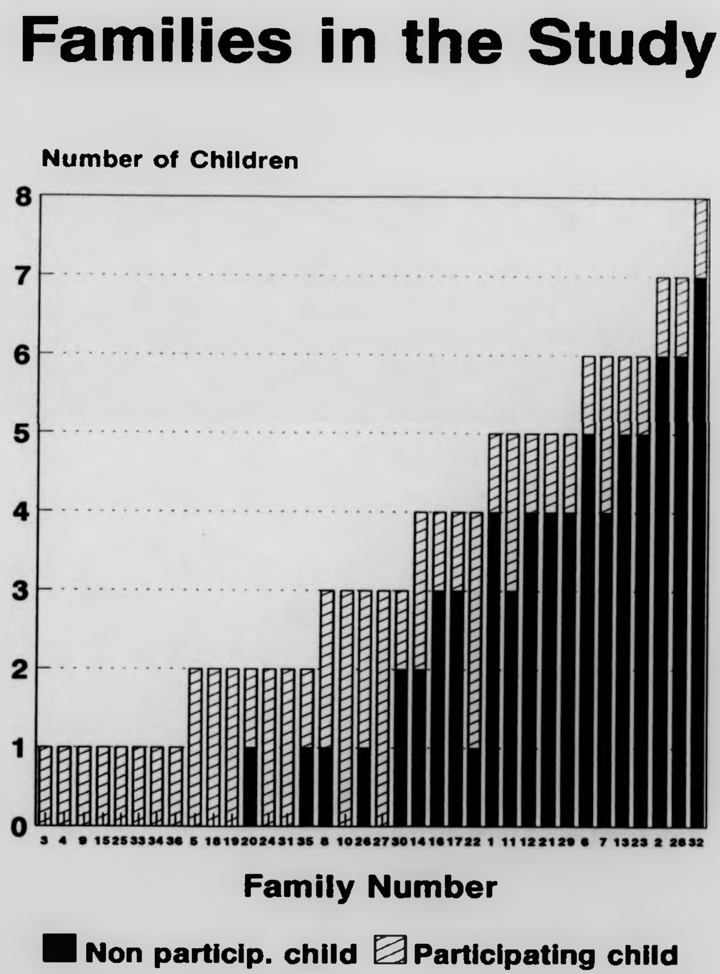
As mentioned in Chapter 3 page 89 much emphasis was laid on the first visit to take full advantage of the fact that the worker came from the same cultural background as the mothers in the sense that they all spoke the language with the same dialect, all were moslems, and all were mothers, most had lived in Abu-Dhabi city for over 10 years. Armed with all this the worker tried to cause as little a disturbance in the homes visited yet get on with her work.

Sample Collection:

Originally it had been hoped to make collections every week but that proved too ambitious given a work force of only one. So the visits were done usually on a fortnightly basis, thus giving time to process the specimens collected. If 20 specimens were collected from each of the 52 children in the study over the duration of the study period, then 1060 would have been expected to be collected. But never were 52 specimens collected during any one week and a total of specimens collected was 500. Figure (10) shows the distribution of the specimens collected and includes a breakdown according to definition of diarrhoea and identifiable pathogen isolated as well as method of feeding.

It was not always possible to get away quickly while making the collections as some mothers insisted the worker came in for Arabic coffee, giving the opportunity for a

Figure (9): Families in the Study.



Key to Figures (10-13) a,b:

F Family Number

C_x y Child$_x$: child number, y: age in months

PB Partially breast-fed

SB Solely breast-fed

B Solely bottle fed

No specimen available yhat week

Secimen available that week

Mother's definition of diarrhoea

Laboratory's definition of diarrhoea

Typable <u>E.coli</u> isolated

<u>Shigella sp.</u> isolated

<u>Salmonella sp.</u> isolated

Rotavirus detected

Figure 10 (a): Distribution of Stool Specimens Collected from 1/10/89 - 30/6/90 from Children in Families F_1-F_{10}

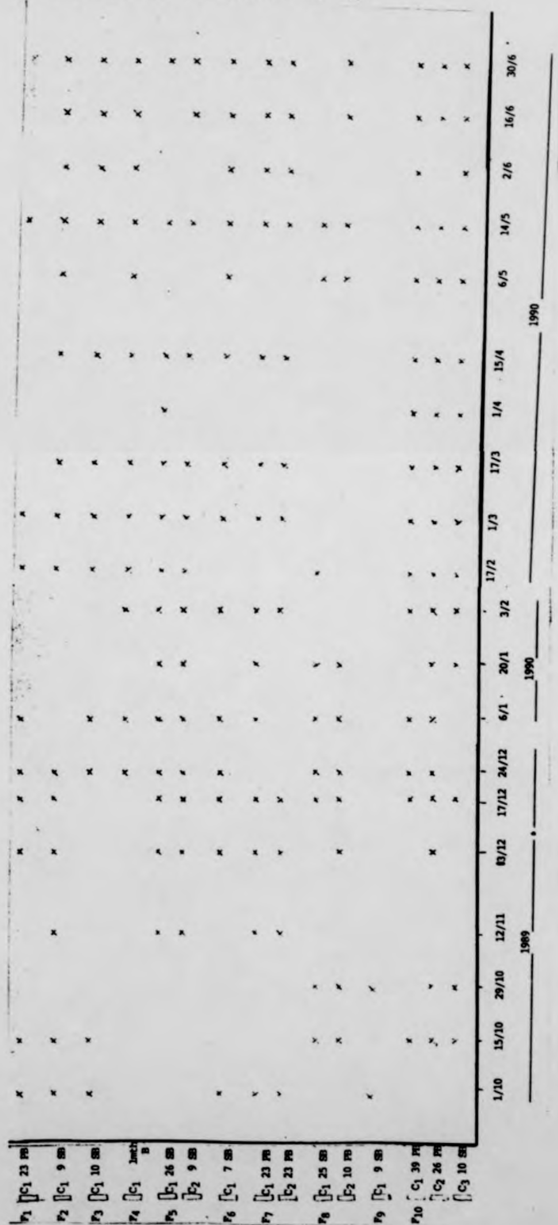


Figure 10 (b): Distribution of Pathogens Isolated from Specimens Collected as per Figure (10 a)

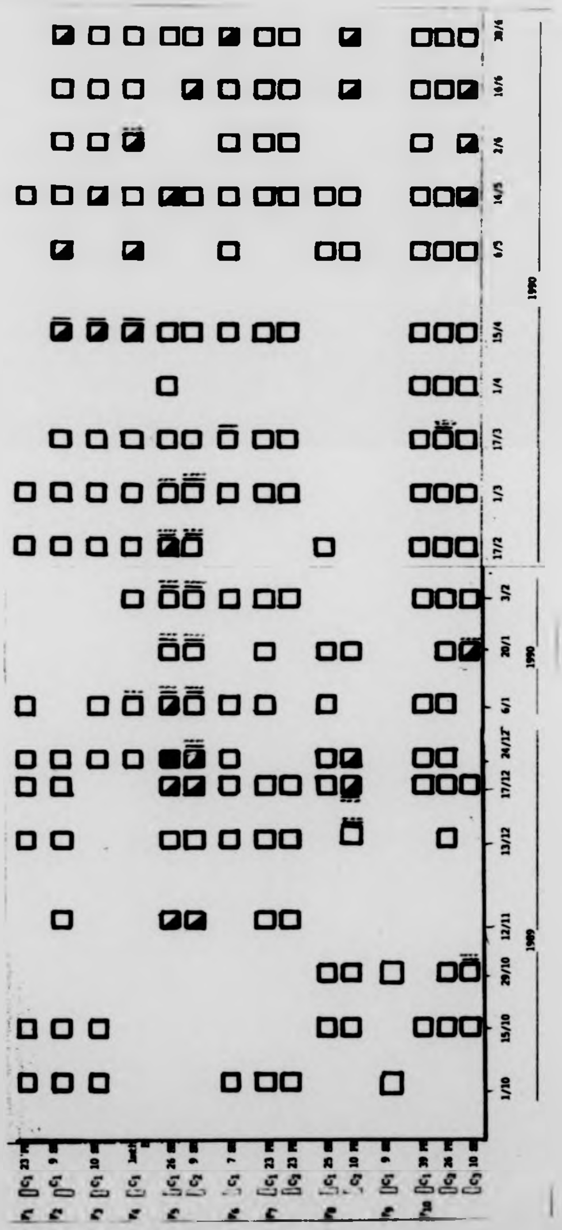


Figure 11 (a) : Distribution of Specimens Collected from 1/10/89 - 30/6/90 from Children in Families F_{11}-F_{21}

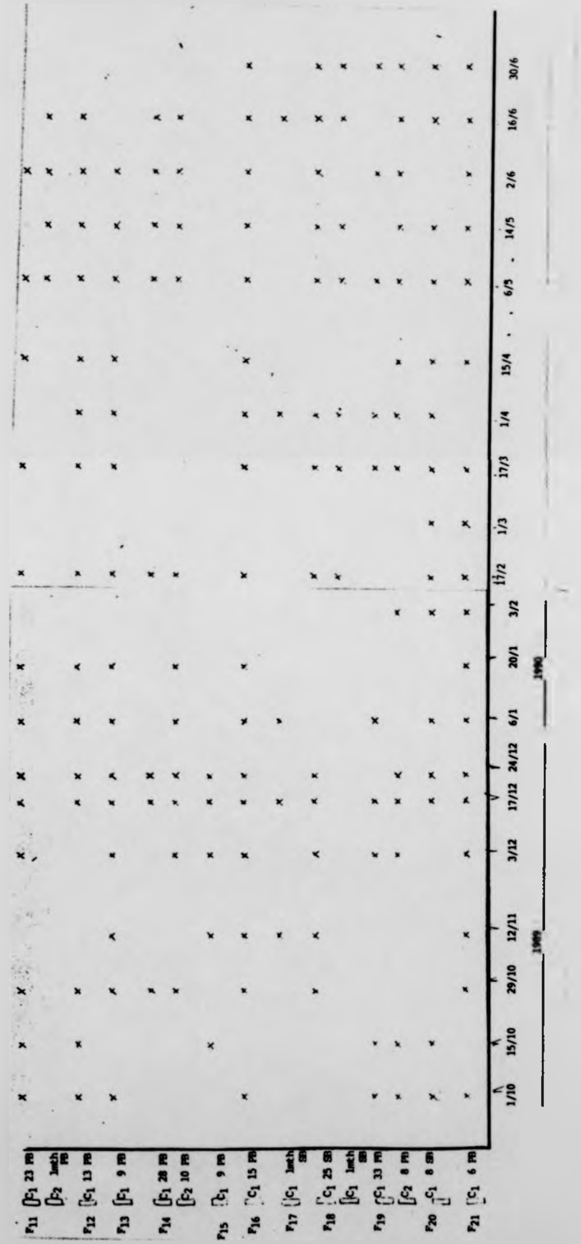


Figure 11 (b): Distribution of Pathogens Isolated from Specimens Collected as per figure 11 (a)

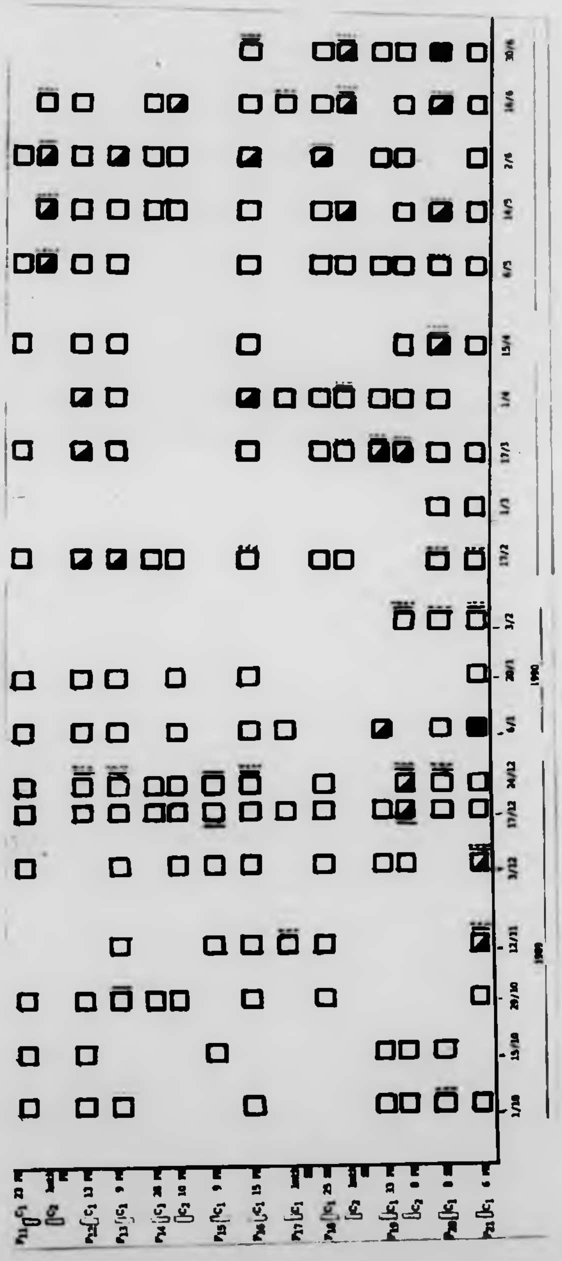


Figure 12 (a): Distribution of Stool Specimens Collected from 1/10/89 - 30/6/90 from Children in Families F_{22}-F_{30}

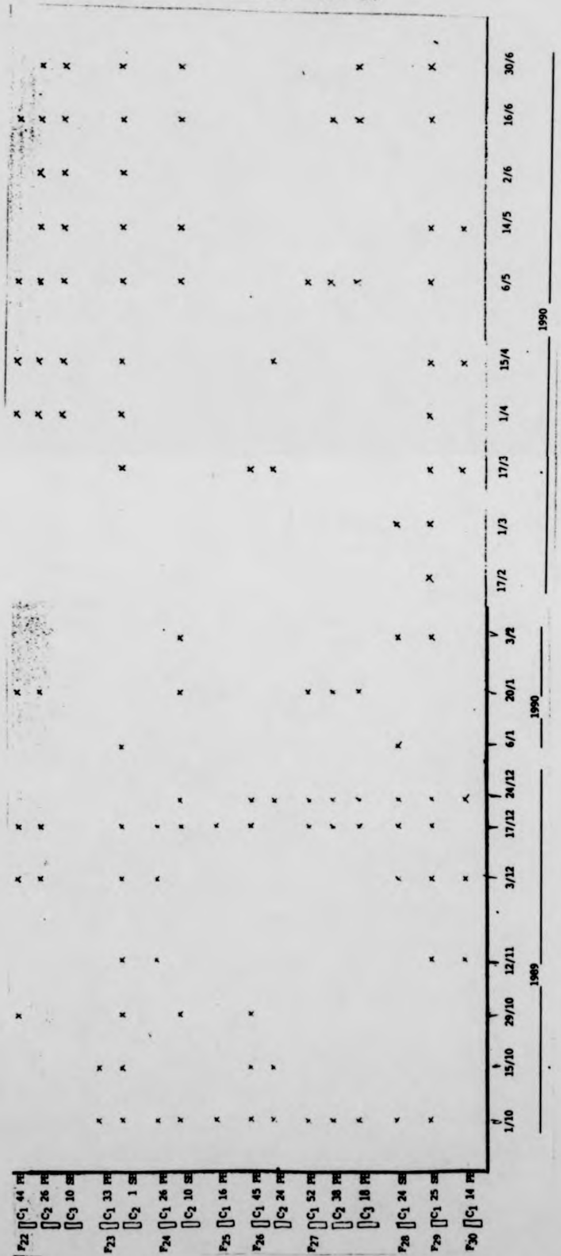


Figure 12 (b): Distribution of Pathogens Isolated from Specimens Collected as per Figure 12 (a)

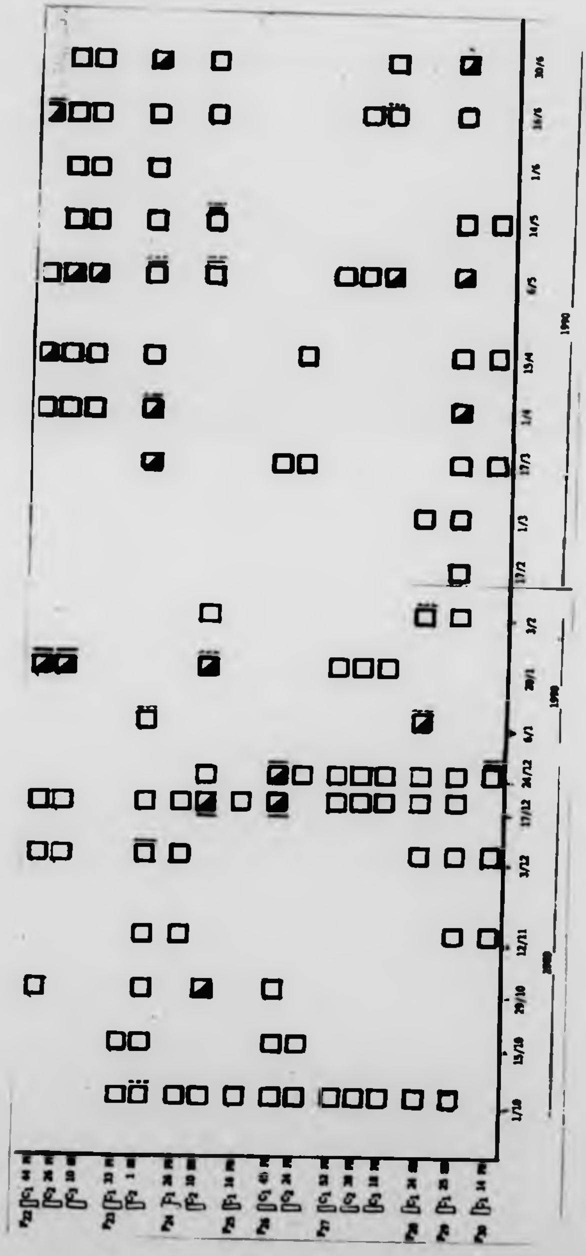


Figure 13 (a): Distribution of Specimens Collected from 1/10/89 - 30/6/90 from Children in Families F_{31}-F_{36}

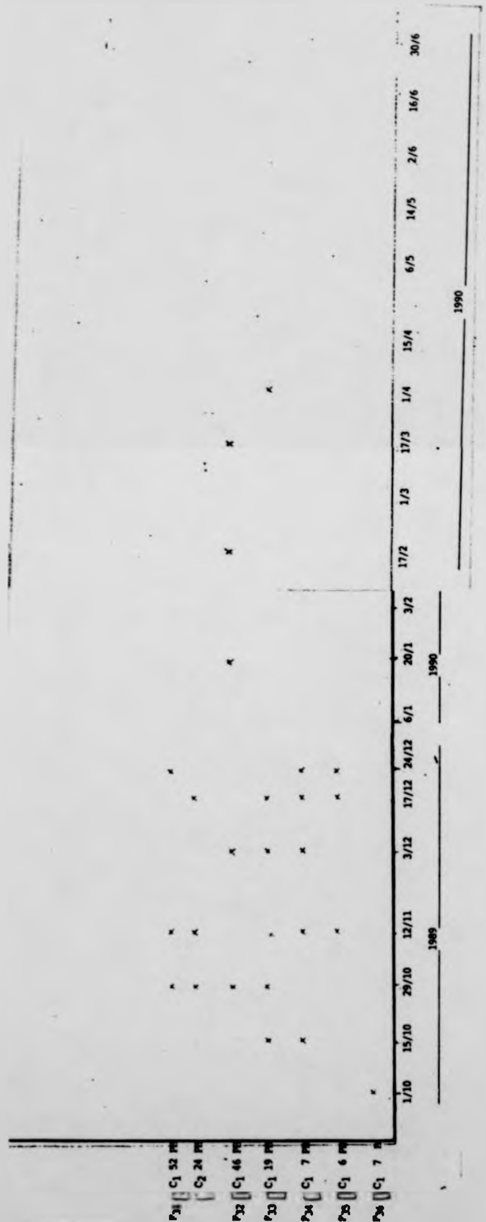
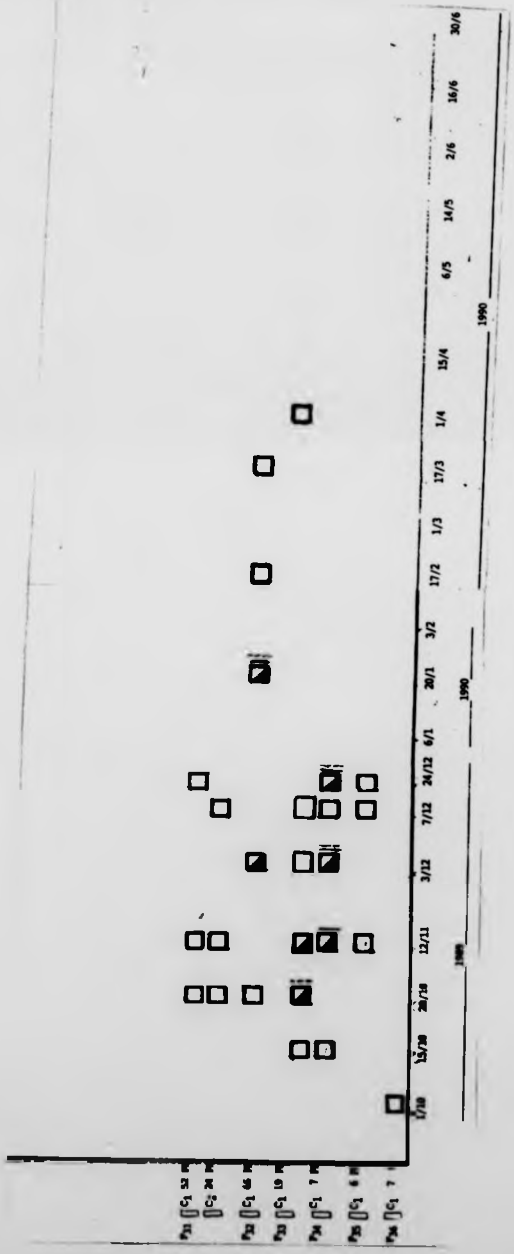


Figure 13 (b): Distribution of Pathogens Isolated from Specimens Collected as per Figure 13 (a)



short rest as well as a chance to ask about the children in the study, their siblings and answering any queries the mother might have concerning the welfare of her children. These visits became links between the medical system and the mother, where the latter would ask questions she didn't ask the doctor or nurse for any number of reasons. Impressions from those first home visits are included in appendix (ii). They were largely made as an assessment of the level of acceptance of the mother of the study. Additional remarks may have been added along the way. Generally the mothers seemed to be happy to join the study group even though there was no incentive for joining as one mother remarked "it's not diarrhoea you should be looking at but constipation". From personal experience acquired by being part of the Abu-Dhabi community, it was observed that the main lunch menu of most local households - especially those where the mother was originally from the U.A.E. - was fish, white rice, dates and sometimes purified butter with very little emphasis on vegetables or salads or any other kind of food that contained roughage, a diet that might very well contribute to the constipation noted by that mother.

Another mother suggested I would have more volunteers if I was working on "coughs and colds". From the worker's own experience as a mother living in the same city and from hospital and from personal observations, it would seem that there was more of a prevalence of respiratory tract diseases than diarrhoea, it may be speculated that the life-style which depends on air-conditioning systems in the summer and the temperature variations during winter, have a lot to do with that kind of complaint especially where little children are concerned as they move about more and are more susceptible to these infections as a result of size and age. What little diarrhoea there was, was not life threatening, and most mothers seemed to know about Oral

Rehydration Treatment (O.R.T.) and Oral Rehydration Solutions (O.R.S.), in fact O.R.S. were given freely at Roda clinic to any mother who complained her child had a bout of diarrhoea and/or vomitus (personal experience and personal observation at Roda clinic).

There was a feeling amongst most of the mothers that after 4 months of age breast milk alone was not sufficient to allow the babies to grow adequately into nice round babies and that was the time to start weaning foods. Most mothers tried to solely or partially breast-feed for that length of time or longer if possible. The notable exceptions to this were the working mothers. Since maternity leave is officially limited to 6 weeks from the date of giving birth, all had to supplement feeds during absences from the home during working hours. The method of choice of bottle sterilisation was boiling with only one mother using sterilisation tablets. 2 mothers (from the same extended family) cleansed the bottle and teats by washing them with Fairy liquid soap solution and tap water. Some mothers gave additional boiled water especially in the hotter months, a few gave a home preparation at 6 weeks, the purpose of this was to clear colic and induce sleep. The preparations contained among other things dates, which have a high content of sugars and calcium, mixed with finely ground cardamon seeds and un-crystallised sugar all ground and strained into a fine puree.

It is advised in the holy Koran that breast-feeding be continued until the age of 24 months, for those able to do so. Breast-feeding and rooming in with the new-born babies is a strongly advocated policy of the local and only maternity hospital, additionally a lot of mothers used breast-feeding as an extra form of contraception and believed it was beneficial otherwise the holy Koran would not have mentioned it. So although the mothers were not able in all cases to explain scientifically why breast-feeding was more desirable, they pointed to the fact that the maternity hospital encouraged it and the Koran mentioned it, so science was merely confirming what their mothers had practised religiously.

Weaning food usually consisted of boiled carrots, courgettes and potatoes to begin with, in addition to powdered baby foods, such as Cerelac readily available in any food outlet, and that are made by reconstituting with boiled water or milk. All mothers said they prepared each meal freshly thereby cutting out the standing time at ambient temperatures that is one of the main causes of the "Weanling's Dilemma". 33/36 of the house-holds had domestic help in the form of maids and/or cooks. The division of labour varied greatly from households where the mother did everything because she had no domestic help, to the mother doing nothing and the domestic help doing all the work. In 11 of the houses especially those where the mother's original nationality was from the U.A.E. all the cooking was done by a cook and preparation of the babies' food was normally the task of the maid. But in most of the other houses the mother and the maid shared the duties of looking after the children.

The level of literacy among the mothers was generally good. Two had no formal education and could read only with difficulty. Fifteen of the remaining mothers having achieved a primary or intermediate level of education and 19 of the mothers achieved a secondary or higher level of education (figure 14 a). In other words they were capable of reading simple literature on child-care and hygiene. The ages of the mothers varied from 19-40 (figure 14 b). 8 of the mothers were full-time working mothers and 14 of the 36 families lived as extended families with one more relatives living with the family in the same house.

Mother Age (in Years)

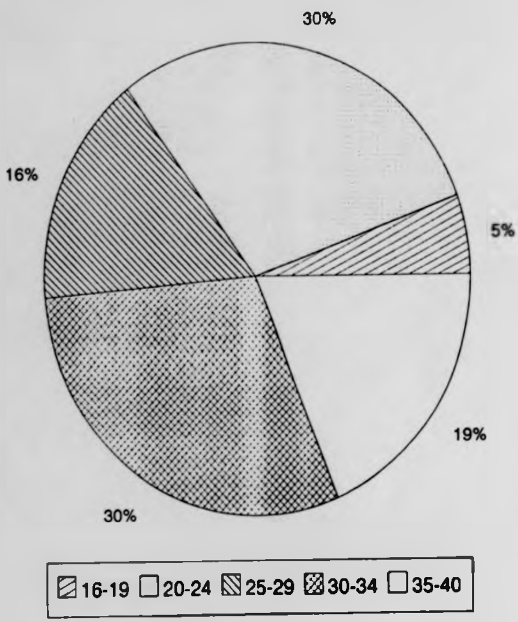


Figure (14 a): Mother Education

Figure (14 b): Mother Age (Years)

Fig 14 (a)

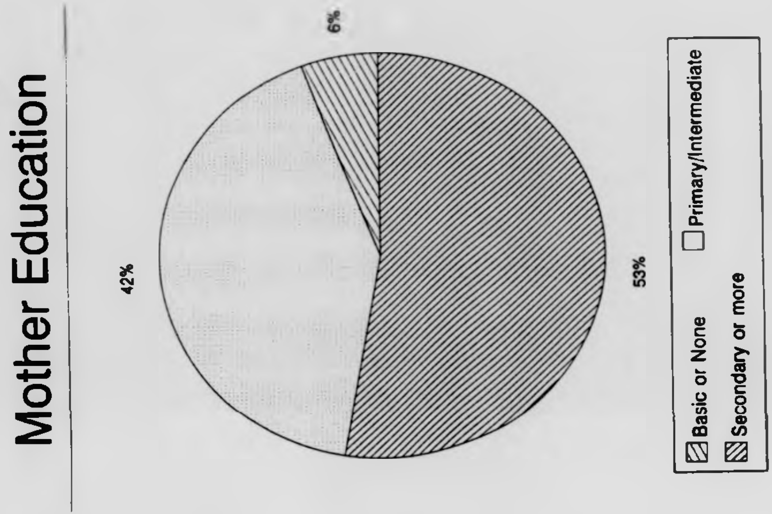


Fig (b)

It is difficult to place these families into socio-economic groups as there is no formal system in the U.A.E. for grouping people according to socio-economic class due to the lack of sufficient demographic data. All the families in this study owned a television set, a fridge, a car, air-conditioned accommodation and most had domestic help in the houses. Income might have been a good indicator, however in a lot of the cases men are the main earners and women are not told precisely what the family income is or if they are told are reluctant to discuss it. The occupation in many cases may not be the sole source of income for a family. Housing type was not a good indicator of status as the government has schemes for housing its employers according to job held and number of dependents in the family. Home owners are made to participate in government schemes that are almost 100% subsidised. These schemes involve the updating of old houses to build more modern ones in an effort to improve and maintain the standard and appearance of housing in the city.

A very rough classification system would be according to social standing:

whether any of the families came from the ruling family,

or whether any of the families were high government officials,

or whether the main bread-winner in the family was government employed, self-employed or not employed,

whether either or both of the parents were educated and to what level.

For the purpose of this study it may be safely stated that none of the families came from the ruling family or high government officials. The main bread-winners of all the families were the men. Only one father was self employed and the remaining 35 were government employed. The level of education of the fathers was not noted in the questionnaire. The level of literacy among the mothers has already been discussed above.

It was felt that all mothers showed a remarkable sense of home-pride in the sense that they wanted to appear in the best possible light and for fear of being exposed as careless or ignorant deliberately failed to report a diarrhoeal episode or refused to acknowledge the fact that their child had a diarrhoeal infection, as was the case with family 3 and 6 respectively, yet the mothers were among the well educated in the group (see appendix ii), this was the main reason that 2 definitions of diarrhoea (chapter 3 page 92) were used in the study, to crosscheck the mother's reports and the appearance of the specimen. In the original interviews 2 mothers who brought their children with diarrhoeal complaints were amongst the ones who could not be convinced into joining the study. Yet 3 mothers whose children suffered diarrhoeal attacks during the study made a point of contacting the worker to let her know of the attack and were interested in the findings of the tests, as was the case with family 5, 20 and 21. The mothers from these 3 families had different levels of education (see appendix ii).

The request to inspect the kitchens and toilets used by the children was abandoned very early on in the study as it was felt to have offended the sensibilities of one family and not wanting to run the same risk, the outright request was abandoned in favour of passive observation of the level of hygiene of the reception areas of the homes visited, the presentation of the children and the method and style of food and drink offered to guests - the worker coming under that category. On the whole the level of hygiene was adequate in all houses, but only 3 of the mothers made a point of washing their hands after handling the specimens. Most of the kitchens seen were clean although some were untidy especially where the cooks were in sole charge of the kitchen or where extended families lived together, as there would be more work going on in the kitchens. All houses had running water reaching them. No rubbish was left uncovered and the municipal services are such that rubbish is collected daily from all rubbish tips. Only 2 families had domestic animals around the house; one kept a cow and the other some goats. Such animals would have been kept previously to supply milk mainly for the children of the household.

Quantitative Results:

The Questionnaire:

Socio-economic Association:

According to the mother's definition of diarrhoea 69/500 (14%) of specimens sent to the laboratory were diarrhoeic, and according to the laboratory's definition 52/500 (10%) of specimens sent to the laboratory were diarrhoeic. Of the 69, 35 (50%) were diarrhoeic by both definitions and similarly of the 52, 35 agreed with the mother's definition of diarrhoea. The mother's definition of diarrhoea was found to have a more significant association (P=0.001) with diarrhoea according to pathogens isolated.

The frequency of isolation and detection of organisms usually associated with diarrhoea and those not usually so, is shown in the table below (table 12). The figures add up to more than 500, (the number of specimens collected) as more than one organism was isolated or detected in many cases. There were a group of bacteria that could not be identified by the Roche Enterotubes. Since there is no reference laboratory in the U.A.E. the non identifiable group remained so without any attempts being made to further identify them. No organism isolated was reported for those specimens where a total failure to grow any organisms from the specimens provided. All such cases were related to the use of antibiotics for a variety of complaints such as respiratory tract infection, ear infections or fever. Many bacteria, such as Citrobacter sp., Enterobacter sp., Serratia sp. that are not normally associated with diarrhoeic diseases were isolated and are grouped under "others". Campylobacter jejuni was not looked for routinely in Jazeira or Central Hospital laboratories. The maternity hospital had a limited success rate in isolating them although the controls strains were successfully grown. For the purpose of this study, and taking into consideration the reports of all 3 local hospitals, attempts to isolate any campylobacter that may have been present were dropped early on, as the success rate was nil even though the controls were working.

Table (12): Number of isolates by organism from 500 specimens collected during the study

Organism isolated/detected	Number (%)
Salmonella sp.	10 (2)
Shigella sp.	31 (6)
Typable *E.coli*	2 (0.5)
Yersinia sp.	8 (2)
Rotavirus	48 (10)
Non identifiable	14 (3)
Others	488 (96)
No organism isolated	17 (3)

Pathogens (organisms usually associated with diarrhoea) were isolated from 85 of the 500 specimens. 26 of those 85 (31%) had a significant (P=0.0001) association with the mothers definition of diarrhoea and 31 of those 85 (37%) had a significant (P=0.0001) association with the laboratory's definition of diarrhoea. When the pathogens were looked at individually, it was found that Shigella sp. showed a significant association to diarrhoea according to the laboratory's definition. It was also found that the 20/48 (42%) of rotavirus detected agreed significantly with the mother's definition and the laboratory's definition of diarrhoea (P=0.00001 and 0.0001 respectively). No such patterns were found for the 9 Salmonellae sp. or 2 typable E.coli isolated.

Figure (13) shows a summary of the significances of the different variables from the questionnaire yet to be discussed.

Fig.(13): Summary of Significances of Variables from the Questionnaire

Variable	Definition	
	Mother	laboratory
mother age	significant	significant
mother education	non significant	non significant
mother occupation	non significant	non significant
mother nationality	significant	non significant
family type	significant	significant
maid nationality	significant	non significant
prep.food mother	non significant	non significant
prep. food maid	non significant	non significant

Mother age was divided into the following age groups:

16-19, 20-24, 25-29, 30-34, 35-40 (fig. 14 b), and was found to have a significant (P=0.011) association with diarrhoea according to the mother's definition. A trend test using the Epi Info. package showed that children belonging to mothers in the younger age group were more likely to be reported to have diarrhoea according to the mother's definition. Although the mother age was found to bear a significant (P=0.01) association to the laboratory's definition of diarrhoea, no trend such as that found with the mother's definition was found when a trend test was done.

The education of the mothers varied from basic to university (fig. 14a), but in this study it was found to bear no significant relationship to diarrhoea according to either definition. The same applies to mother occupation where the mothers group was broken down to 2 groups: working full time and non working or house wives.

The original nationality of the mother was an interesting variable. The mother group was divided into 2 groups for this purpose: those who were ethnically of the U.A.E. (22/36) and those who were not ethnically of the U.A.E. but have U.A.E. status (14/36). It was found that the ethnic U.A.E. mother group was slightly significantly (P=0.04) associated with diarrhoea according to the mother's definition. Presence of a maid in the house was found to have an association with diarrhoea according to the mother's definition only. That association being significant when the nationality of the maid was Sri-Lankan. Of the 133 specimens belonging to children who had Sri-Lankan maids in the house 23 (17%) were significantly associated with the mother's definition of diarrhoea (P=0.07). No such association was made with any of the other maid nationalities. 10 of the study families had Sri-Lankan maids. When the ethnic nationality of the mother and the nationality of the maid were taken in consideration to diarrhoea according to the mother's definition of diarrhoea, it emerged that of 19/105 (18%) of specimens collected from children belonging to U.A.E. ethnic mothers, who had a Sri-Lankan maid (7/10) showed a significant association with this definition of diarrhoea (the mother's). A similar trend was found with the laboratory's definition of diarrhoea, Where 22/105 (21%) of the specimens were associated with the laboratory's definition of diarrhoea. No such associations according to either definition were found in the non ethnic mother group who also had Sri-Lankan maids (3/10).

An association was found between the family type ie. whether the family lived as a single unit on its own or whether relatives lived with it and diarrhoea according to both definitions. The strongest association being between the mother's definition and extended families. 15/36 of the families lived as extended families, 10 of those were also families where the mother was ethnically U.A.E. It was found that 20/37 (70%) of specimens from children whose mothers were ethnically U.A.E. and who lived as extended families showed a significant (P=0.0017) association with diarrhoea according to the mothers definition of diarrhoea, no such association was found with the non-ethnic group of mothers.

Mother education and mother occupation and the involvement of the mother in the preparation of the children's food all had no association with either definition of diarrhoea. Interestingly there was no association with diarrhoea according to either definition when the maids were involved in the preparation of the children's food, even when the nationality of the maid and the ethnic group of the mother was taken into consideration.

Children were grouped into 3 groups: solely breast fed (SB), partially breast fed (PB) and solely bottle fed (B). A significant association was found between the laboratory's definition of diarrhoea and partially breast fed children. An association between the laboratory's definition and the age of complete weaning from the breast was found, the association being significant (P=0.002) with the 4-6 month age group. In this study group a high (95%) rate of breast feeding from birth was found. 2 children of 2 mothers were the only ones to be solely bottle fed from birth. 25/52 were almost solely breast fed up to 4 months of age. Supplementary or mixed feeding was practised on the remaining 25 after 4 months of age. There were differences in the time and type of foods introduced before 4 months. U.A.E. ethnic mothers were more likely to give their babies the home preparation mentioned on page (142), while non-U.A.E. mothers were more likely to offer their babies just cooled boiled water with perhaps a little sugar added to it. Mothers belonging to this group were also more likely to include vegetables and salads in their daily meals than the former group.

From the above analysis it would emerge that there is not much diarrhoea in this study group. When it was found it was found more in association with:

- the mother's definition of diarrhoea
- the younger age group of mothers
- children whose mothers were ethnically from the U.A.E., who lived in extended families and whose houses had maids who came from Sri-Lanka

Pathogen Detection:

Shigella and rotavirus were found to the most associated of the pathogens with diarrhoea in this study as mentioned in page (150) above. These 2 agents of disease were found to have differing associations with the type of feeding of the babies as shown in table (14) below.

Table (14): Diarrhoea Occurrence According to Organism Present and Type of Feeding

Feeding method	Organism present (%)	
	Shigella sp. (n=30)	Rotavirus (n=48)
Sole breast fed	19 (63)	16 (33)
Breast and bottle	10 (33)	30 (62)
Sole bottle fed	1 (3)	2 (4)
Significance	0.007	NS

E.coli are one of the most well documented pathogens causing diarrhoea and were the most organism frequently isolated in this study but only 2 could be further identified by serotyping, one belonged to serotype O124 K27 and the other belonged to polygroup III with no further identification possible. Even then these E.coli that were successfully identified were isolated with a Salmonella paratyphi c and rotavirus respectively raising questions as to which, if not both, pathogen was the cause of the diarrhoea. Serotyping was not always successful in the Jazeira hospital laboratory and for that reason and for time and effort conserving reasons, E.coli were only typed when isolation occurred from a specimen that agreed with the mother, laboratory or both definitions of diarrhoea. None of the isolates were identified by the DNA-DNA hybridisation assay. Therefore: a vast majority of the E.coli strains isolated (227/229) were not enteropathogenic when they were serotyped against the standard serotypes used, and none of them were enterotoxigenic when a controlled DNA-DNA hybridisation assay was done as the "tox" genes did not show up. The absence of red blood cells and white blood cells in the diarrhoeic specimens was taken as an indicator that the strains present were neither EIEC nor EHEC, but specific tests for these groups were not included in the study design.

Tissue Culture:

A tissue culture assay to test for LT presence was done on unrelated samples that were sent from Abu-Dhabi table (10 a,b), 40 of those samples were from children who were hospital patients and who visited the hospital for a variety of complaints and the remaining 15 were sent from the Quality Food Control Laboratory as mentioned in chapter 3 page (112).

There are 5 known toxin effects on mammalian cell lines as observed in tissue culture assays. 3 of those were exhibited to varying degrees in this study:

1. Cytotoxic effects that cause cell rounding and death (plate 14)
2. Cytotonic effects that cause alteration in cell physiology resulting in dendrite formation (plate 15)

3. Protease effects that cause cell movement apart (plate 16)

By convention, toxin effect on 50% of all the cells at a dilution of 1:4 or more is considered as a positive result for the presence LT (Vadivelu; 1985).

The bacterial filtrates showed varying but on the whole weak effects on the vero cell monolayers. The effects when found were seen alone or in combination with each other. 70% of the 55 isolates had no effect at all on these monolayers. Of the 31 isolates that were identified as E.coli. only 4 showed a 1:4 toxin effect on the vero cell monolayer and these came from the hospital patients. Of the 11 isolates belonging to children whose laboratory request forms stated gastroenteritis or diarrhoea, 7 were identified at the laboratory of origin as E.coli. 3 were identified as Klebsiella sp. and 1 isolate was identified as a coliform. The Klebsiella sp. that was isolated from a child whose clinical data specified diarrhoea, showed a cytotoxic effect at a 1:256 dilution. A Pseudomonas sp isolated from a water sample, showed a cytotoxic effect up to a 1:16 dilution and a cytotonic and cytotoxic effect up to a dilution of 1:64.

No serotyping or DNA-DNA hybridisation assay was done on these isolates.

In this study 3 salmonellae isolations were made. A Salmonella paratyphi C was isolated from an infant from whom E.coli O124 K72 was also isolated. This infection was thought, by the mother to have been acquired from food eaten at a picnic a day before the onset of symptoms. An asymptomatic Salmonella paratyphi C was isolated from a child whose brother had contracted food poisoning at a boy's scout camp. The other asymptomatic infection of Salmonella typhmurium was unaccounted for. The mother denied her son had a diarrhoea or any abnormal bowel movements, the stools were formed for the duration of the salmonella isolations.

30 shigellae isolations were made in this study making it the highest after rotavirus. It's isolation was associated to diarrhoea only according to the laboratory's definition of diarrhoea. All isolates were first identified using the Roche Enterotubes (chapter 3 page 101), E.coli from diarrhoeic specimens, shigellae and salmonellae were then confirmed by serology. None of the shigellae isolated were serotypable. They were recorded as Shigellae on the basis of repeated and consistent isolation from the same children and biochemical identification, they may or may not have been shigellae. The shigellae isolated showed a significant (P=0.007) association with the mode of feeding (table 14), in the solely breast fed group.

Plate (14): Cytotoxic Effects of Etec on Vero Cells

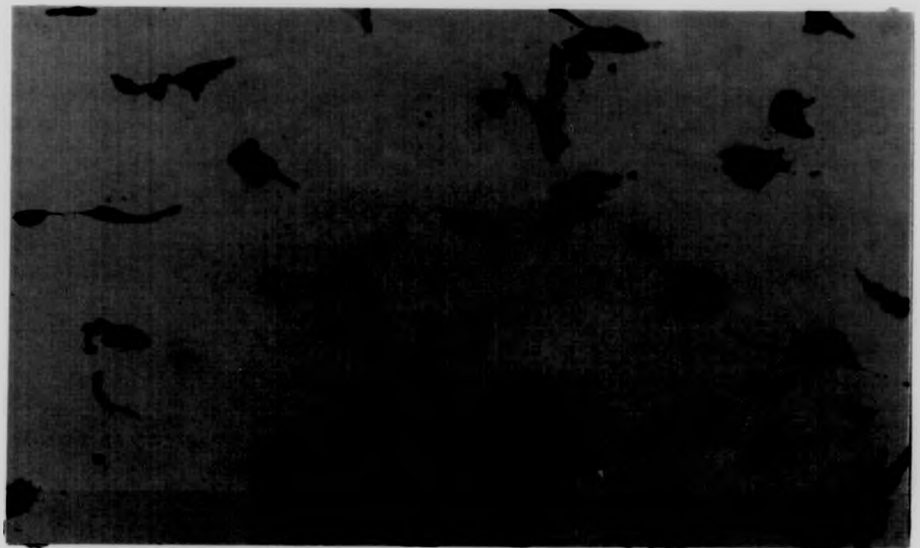


Plate (15): Cytotonic Effects of Etec on Vero Cells

Plate (16): Protease Effects of Etec on Vero Cells

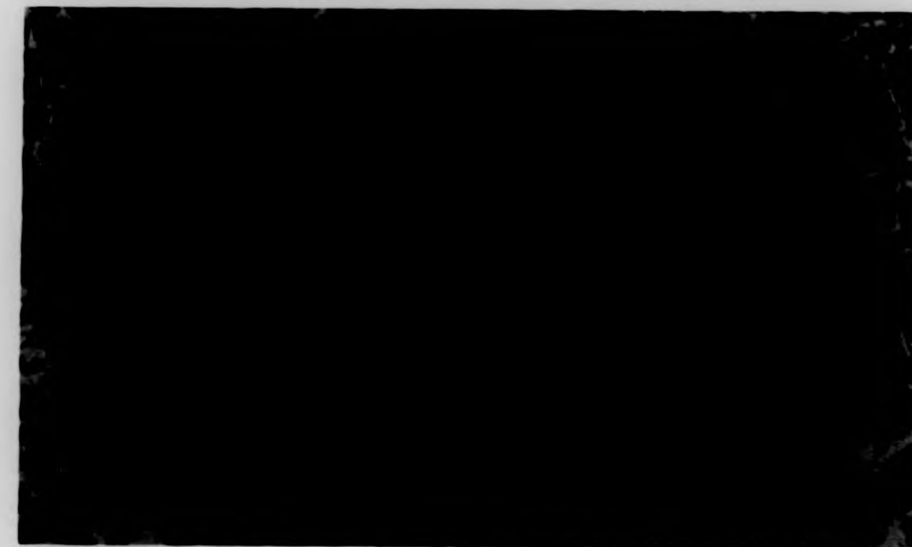


Plate (17): Positive Control Using Cholera Toxin

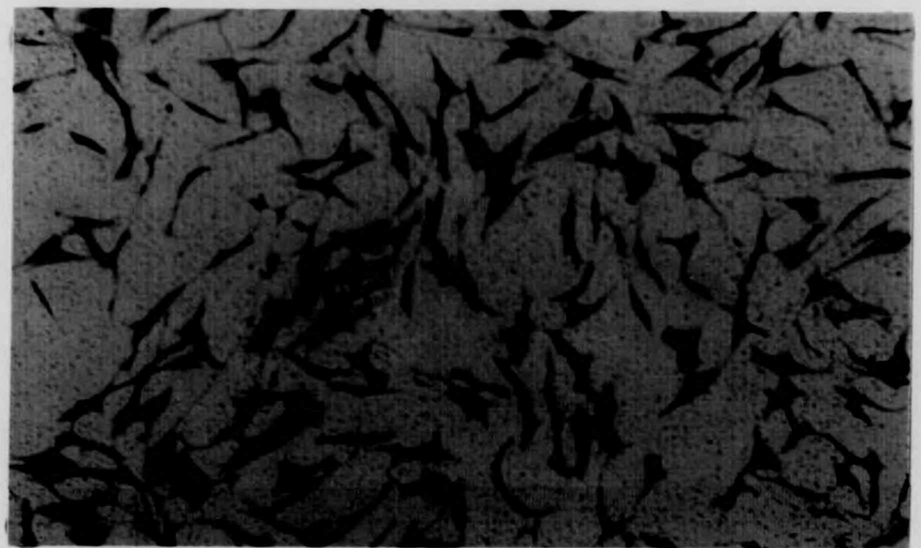


Plate (18): Negative Control

Rotavirus:

The seasonal calender of the U.A.E. maybe divided into a long summer (May to October), a short winter (November to February) and an even shorter spring (March and April). Most rotavirus isolations were made during the winter months, followed by those during the summer and the least being made during the short spring.

Figure (10 a,b-13 a,b) shows the isolations made as per week they were made in. It also shows if either, both or none of the definitions of diarrhoea accompanied that isolation. It emerges that more isolations were made during the winter months. Bearing in mind that the carriage rate of rotavirus infections is thought to be 3 weeks on either side of the appearance of symptoms, it was found in this study that rotaviral infections still prevail during the winter months. However a substantial number of rotavirus isolations came from specimens that agreed with neither definition of diarrhoea. These maybe asymptomatic infections but a carriage rate is not possible to work out since there are no symptoms to go by.

The mean temperatures as supplied in the climatological summary of 1989 (table 1) show that the spring temperatures are the most suitable for outdoor activities, summer and winter temperatures and conditions were considered,by the mothers, as either too hot or too cold to allow their small children to come and go freely. It may be argued that due to children being more frequently indoors and in close association with other children, the respiratory route of rotavirus infection, suggested by many workers, assumes a more active role making these infections more common during the winter and summer seasons than the spring season.

There were more rotaviral isolations during the winter months. This is in keeping with data from neighbouring countries such as Kuwait and Saudi Arabia as well as other parts of the world (table 9). Since spring is a short and considerably "new" season, that has become increasingly evident as a result of the tireless efforts of making Abu-Dhabi verdant, then if the spring and summer isolates are grouped together, rotavirus isolation was found to have occurred throughout the year with a small peak in winter. This is again in keeping with data from neighbouring Kuwait and Saudi Arabia as well as other parts of the world (table 9).

Infections occurred in all age groups, but mostly in the under 24 month old group with a peak in the 12-17 month group. Symptoms ranged from diarrhoea and vomiting to no symptoms at all. Table (15) shows the distribution of rotavirus infection according to age and season.

Table (15): Isolation of Rotavirus According to Age-group and Season

Age groups in months	Season			
	Winter Nov.- Feb.	Spring Mar.- Apr.	Summer May - Oct.	total
0-5		1	1	2
6-11	3	4	5	12
12-17	14		2	16
18-24	9	2	7	18
>25	26	7	15	48

6 specimens of the most strongly positive rotazyme test results were used for electron microscopy. This had to be done at the electron microscope unit at the London School of Hygiene and Tropical Medicine, as a further confirmation of the Elisa and latex tests. It would have been impractical to have this procedure done on all the positive specimens since the electron microscopy as the Elisa test is a diagnostic one and it was required merely to confirm the findings of the Elisa. The long term effect of freezing followed by thawing that were bound to occur during transport were expected to have an adverse effect on the virus particles to be viewed, therefore only strongly positive specimens were used to ensure that some particles remained intact to be seen under the electron microscope.

Sharper images were obtained with the Uranyl Acetate stained particles than the Ammonium Molybdate stained particles. Plate (19)

Plate (19): Electron Micrograph of Rota virus isolates

Electron micrograph of Rotavirus particles using Uranyl Acetate stain

Electron micrographs of Rotavirus using Ammonium Molybdate stain

Microscopy/Parasitology:

Microscopy in this study was done on specimens that confirmed to either or both definitions of diarrhoea, and was used for 2 purposes:

1. To look for red blood cells and/leucocytes in an initial attempt to recognise the presence of bacteria that cause invasions of the colon such as Shigellae EIEC or EHEC. The microscopy was done by the examination of saline smears.
2. To look for parasites such as G.lamblia and Ent. histolytica. This was done by the examination of saline and iodine smears.

For both purposes the results were largely negative. 98% of the specimens examined were negative for leucocytes and red blood cells. The very few that were positive were not at all related to either definition of diarrhoea or pathogen isolated.

No parasites were identified at all. To confirm and compare these results, laboratory records of all paediatric patients visiting Jazeira Hospital at the same time the study was being carried showed very little presence of parasites: of a total of 454 children with a variety of complaints and whose stool specimens were sent to the laboratory for analysis, G.lamblia was isolated from 10 (2%) patients only and Ent. histolytica from only one. This would fortify the finding that parasites were not a problem of major concern in this sector of the population.

Background Results: (Laboratory findings from Jazeira and Central Hospitals):

The results from the preliminary study and the main study showed on the whole very little diarrhoea. Comparative surveys of laboratory findings from Jazeira Hospital and Central Hospital much agreed with this.

The results of the preliminary study showed there was a diarrhoea problem amongst the under 5 year age group. This was not the case with the study group- where diarrhoea was not found to be a problem. The children of the study group would be expected to use the facilities at Jazeira Hospital, so a comparison was made with children attending the hospital at the same time as the study period.

Of 454 children under the age of 5 years old visiting Jazeira Hospital at the same time as the study was carried out and presenting with a variety of conditions, G.lamblia was isolated from 10 children, Shigella spp. from 10 children, Yersinia spp. from 5 children and Ent.histolytica from 1 patient only.

Children under 5 years old visiting Central Hospital during the months of February and March 1990 were 144 in number. The clinical data on the laboratory request forms of 38 (26.3%) of those children stated gastroenteritis or diarrhoea. Only one diarrhoeal case was found to be caused by Shigella boydii; the remaining 37 were of unknown etiology. Campylobacter were not felt to be a major cause of diarrhoea in this area (a finding verbally confirmed at the maternity hospital in Abu-Dhabi), and were hence not looked for routinely. Information from the preliminary study and main study, suggests that some these cases of diarrhoea of unknown etiology might have been due to other agents usually associated with diarrhoea such as rotavirus (which at the time was not routinely looked for), E.coli, Cryptosporidia or perhaps other agents hitherto unassociated with diarrhoea.

Government Statistics:

The results obtained at the end of the main study were discussed at the M.O.H. No data on diarrhoeal diseases in the United Arab Emirates is available, but there is data on the immunisation program in the country. M.O.H. statistics estimate that an 80% success rate has been achieved with the immunisation scheme (M.O.H. annual report; 1989). It was felt that the success of the scheme could be extrapolated to diarrhoeal diseases since the vast majority of mothers would normally bring their children to the immunisation centres to receive their immunisations. These immunisation centres were looked after amongst others by the M.C.H. units in the clinics and by the Public Health authorities. It was felt that these units were target areas for instilling new and educational ideas on hygiene and child care into the mothers, a place where they could ask any questions concerning contraception, their child's welfare; diarrhoea presumably being one of those concerns; and where Oral Rehydration Treatment was easily given with instructions of use and where the mothers could meet other mothers and discuss matters concerning their children, be it mode of feeding, teething, diarrhoea, immunisation or any other problem. All this along with the other facilities provided by the government such as the sewage system, the water system, municipal services, were felt by the Ministry of Health to have attributed to the low incidence of diarrhoea.

The acquisition of immunisation according to medical outlet is provided by the M.O.H. and is broken into figures for U.A.E citizens, expatriates or foreigners and combined for both, Table (16).

Table (16): Acquisition of Immunisation According to Ethnic Origin and Medical Outlet.

Outlet	UAE citizens	Expatriates	Total
MCH clinic	35 %	45 %	80 %
Government hospital	33 %	26 %	29 %
Government clinic	27 %	25 %	26 %
Private	5 %	3 %	4 %
Mobile teams	1 %	0 %	1 %
Abroad	0 %	1 %	1 %

Although there is a choice of health outlets where immunisations may be taken, the M.C.H. clinics and the government hospitals were the most outlets sought for this purpose, arguing that ministry targeting reaches the majority of the people at some stage during the immunisation process.

Coverage rates for BCG (tuberculosis vaccine), diphtheria, polio, pertussis, measles mumps and rubella are shown in table (17) below.

Table (17): Immunisation Coverage Rate

vaccine	UAE citizens	Expatriates	Both
BCG	94 %	98 %	96%
DPT 1*	89 %	96 %	93%
DPT 2*	84 %	93 %	90%
DPT 3*	76 %	90 %	85%
Measles	62 %	82 %	75%
M.M.R	59 %	68 %	65%
All 8 doses < 12 mos	60 %	80 %	73%
All 9 doses < 19 mos	49 %	62 %	57%

* = includes polio vaccine

M.M.R.= measles mumps and rubella

BCG is given at birth, DPT 1 at 2 months of age, DPT 2 at 4 months of age, DPT 3 at 6 months of age, MMR at 8 months of age The rate of coverage amongst the expatriate population is higher than amongst the local population and both show a steady decline with age. Snyder and Merson (1982) have found that the morbidity due to diarrhoea was highest in the 6 - 11 month group (fig.5), so the mothers of this group should be the main group to be targeted for any prevention of diarrhoea plans that in any way involve education.

But from the table above it would seem that the best coverage of vaccination is up to 6 months of age after which the rate of coverage drops below 75%. A higher attendance from the 6 month onward age group is necessary to reach the target group that is most effected by diarrhoea. However there was not much diarrhoea amongst the study group of children or amongst the children surveyed from the laboratory data books in spite of the drops in vaccination attendance. Therefore it would seem that in addition to targeting there was another factor that was responsible for the protection against diarrhoea.

Chapter 5. Discussion

Contents of Chapter 5

Global diarrhoea:

Much work has been done and much literature is available on the prevention, intervention and treatment of diarrhoea. The W.H.O. has issued numerous papers recommending various methods for achieving these goals. Walsh and Warren (1978) have laid out a policy stressing the advantages of, and the difficulties in achieving a primary health care system. Realising that such a health care system would be too expensive for the countries that need it most, they suggested, in the same paper, alternatives to their proposed primary health care system, which would go a long way to controlling many infectious diseases at a lesser cost, thereby making them theoretically more attainable for these countries.

Diarrhoea or gastroenteritis and malnutrition amongst other diseases prevail under conditions of poverty, over-crowdedness and lack of hygiene - all predicaments unfortunately associated with third world or under-developed countries (Snyder and Merson; 1980, Guerrant et al; 1990).

Gastroenteritis in the U.A.E.

Not much is known about the epidemiology and etiology of diarrhoeal diseases in the U.A.E. Laboratory investigations and general observations showed diarrhoea was not, at present, a leading cause of infantile morbidity and mortality as might be expected of a developing country. Despite a high finding of diarrhoea in the preliminary study the MOH's list of important childhood diseases does not include diarrhoeal diseases. In neighbouring Kuwait and Saudi Arabia, as well as other countries the world over, diarrhoeal diseases are considered a major cause of infant mortality and morbidity, The results that came out of this study therefore came as a surprise.

Critique of the Study:

This study was supposed to have been carried out for a period of 24 weeks, from October 1989 - May 1990 with specimens being collected on a weekly basis. In actual fact it was carried out over a period of 28 weeks that spread between October to June 1990. This occurred due to the collection of specimens on a fortnightly basis rather than a weekly basis and also due to the fact that various holidays (a school 2 week mid-term holiday, a one week unforeseen public mourning holiday and 2 other shorter public holidays) that coincided and interfered with specimen collection days. Specimen collection was extended to the month of June in an effort to boost the numbers collected, but it could not be increased more than that as the onset of the summer and the closure of the schools saw the families leaving Abu-Dhabi to the less humid parts of the Emirates or abroad altogether.

With the benefit of hindsight it is useful to look at the study critically and point out it advantages and limitations and how the methods may have been improved.

The greatest advantage of the study was that the researcher shared many common factors, such as being female, moslem, Arabic speaking from the same country, with the mothers which made it easier for the mothers to accept her and allow her access into their homes. These coupled with the curiosity that many of the mothers expressed about a woman who was able to combine between her family life and academic ambitions made the worker more attractive to the mothers, some of whom might have wished to have had the opportunity to fulfil their own ambitions, made the worker more attractive to these mothers and who in turn gave her the access required into their homes.

The study had its share of limitations. The main one upon which everything else ensued was the work force. To get a good representation of the 96 or so nationalities that live in Abu-Dhabi recruitment would have had to have been from more clinics, both government and private. For this a larger study number would have been necessary. To arrive at this number more time would have had to be spent at the recruiting centres (such a method would select against people who did not attend MCH clinics and a method would need to be devised to include such people). If this was to be done by one person then the study group would not be of the same cohort as recruiting would not be at the same time. The collection of the specimens for this study and their delivery to the hospitals was time consuming, tiring, and frustrating when no specimen was available. For one person to have to do this for a larger number of families and then expect to process the collected specimens in the laboratory alone, this would be a very difficult if not impossible task. For this reason the study group had to be limited. The basis on which this was made was by choosing a group whose existence was most relevant to population of Abu-Dhabi. This was felt to be the local population as they are the most permanent one. However, due to the various government protection schemes, this sector of the population was also the most affluent one and its problems and needs need not reflect those of the rest of the population. Therefore they are not really representative of the population as a whole but they may be representative of the locals (who are estimated to make up about 30% of the total population).

Representativeness of the Study Group:

There are no demographic data that are easily available in the UAE, so the representativeness of the study group to other UAE local families in the population is subject to discussion. The sample number in this study was a consecutively selected one of mothers arriving at the MCH unit at Roda clinic. Since it was not practical to calculate a sample size due to the limitation of the workforce and as it turned out later, due to the high drop-out rate, a time limit of 2 months was set for the recruitment of as many mothers who came to the clinic and who agreed to join the study. Of a total of 98 mothers interviewed at the clinic, 36 (34%) only continued with the study. The high drop-out rate as well as the low ratio of collected specimen : ratio of expected specimens, obtained was taken as a reflection of how seriously these mothers thought the problem of diarrhoea was to their children, indeed the laboratory findings of the main study showed no evidence of it being of widespread occurrence.

The mothers in this study group were all of U.A.E. nationality, but some (14/36) were not ethnically from the U.A.E. Maternal ages varied from 19 to 40 years and the family size varied from 1-8 children. The mothers who attended the clinic were all aware of the health facilities and were actively seeking some kind of medical attention. This might be the typical picture as these facilities are free to all locals after a health card had been bought- the price of that was nominal. The health system does not provide a home delivery of babies service and all babies are delivered at the local maternity hospital, where births are recorded. It is also the policy to give mothers their first post-natal appointment 3 weeks after delivery at a clinic closest to their homes or at Roda clinic if the mother has local status. These appointments are "open" in the sense that no given time is allocated to each mother for her appointment, they function on a first come first serve basis. There are no data on the number of women who turn up for this appointment and those who do not, but based on data from the immunisation programs, the MOH assumes that approximately 4 visits / year are made to these clinics during the first year of life in 80% of the children. So in that sense a majority of the mothers would seek the medical facilities.

The cultural race to join the developing nations of the 20th century and the arrival of highly educated expatriates have been greatly responsible for the adoption of ostentatious manifestations of progress and development, some frivolous such as materialistic objects (cars, jewellery etc.) and others more profound such as hygiene, health, cleanliness and education.

The government has extensive programs for the eradication of illiteracy that are looked after by the Women's Federation groups and the Ministry of Education. These programs included paying the students to come to school and providing them with a free meal when the educational system was first set-up. This practice continued for 10 years or so until the concept was accepted by the mothers and children. The government programs initially included non-competitive university entrance requirements (these requirements have since become more competitive), in an effort to encourage students to pursue their studies and with that the promise of better job prospects.

Educationally the group might be said to represent the UAE society with all its gradations in the educational system. Older women are expected to be of lesser education than younger ones as the system was not set then. Any educated UAE women from the older groups either lived abroad with their families, as quite a few families did move to Kuwait or Saudi Arabia or Bahrain before the oil boom, and were therefore educated abroad, or were ethnically not from the UAE and were likely to have received their education elsewhere.

A 53% level of secondary or higher education may not seem that high in comparison to secondary education levels, in England for example, where education is compulsory up to secondary standard and one should therefore expect almost 100% achievement at that stage. In the U.A.E. the educational system, which has been set-up in its modern form only in the last 25 years, is not compulsory at any stage. The government gives the incentives and it is up to the individuals to make use of them. Women turn more to education because they are more limited by social traditions in their freedom to come and go. Education is a good way of filling up time as well as enhancing a social standing or improving career prospects.

Family sizes in the study group are also thought to be representative of those of other families in the UAE, with the older women having the most children and the youngest having the least. Contraception is not the policy of the government, which feels that the local population (which is 30% of the total population) is a small one and that in fact should be encouraged to grow. Such encouragements include social benefits for each child born, free education, nominally charged health fees. So contraception when given, is given by choice and is usually used as a method of spacing out children rather than controlling numbers.

The ages of the mothers and the family sizes may be considered as normal in an arab, moslem society as that of the U.A.E. where family duties and obligations are considered a woman's first priority before education and a career. But it is not unusual to find married women with families who are still pursuing their studies or even more recently having a career as there is usually plenty of back-up in the home. Living in extended families or in close contact with parents or relatives in addition to the affluence that allows most families to be able to afford a maid/nanny has given mothers more room for manoeuvre.

Socioeconomically, it was difficult to define the study group according to any standards are none are available to use. However the families would fairly be said to reflect other families when accommodation is taken into account as the government promises jobs for all locals and with the job comes the advantage of accommodation. For locals this is given according to number of children, number of dependents and level of education more or less in that order. However some families in this study were dropped as a result of not having a telephone to contact the mothers to confirm the initial home visit or get redirections for the house when those were not clear. This could be a covert indication of socioeconomic standing, although it would obviously need confirmation. But since the first post-natal appointment clinics happens on a first come first serve system and since there is no co-ordinated system between the maternity hospital and the various clinics, that would allow the follow up of all mothers, then it becomes difficult to know how many mothers are or are not turning up for their appointments. A family that does not have a telephone in the house would be automatically selected against by the present system, unless it made the effort to attend.

The ability to have a maid in the house would very much be the same as that in this study, with the majority of households having maids from the poorer Far East, which makes them affordable in a society such as that of the UAE. The perils of heavy dependence on this kind of domestic help is the subject of much heated debate amongst educationalists, sociologists and other intellectual groups, never-the-less they do exist and in large numbers as this study shows.

So from a representative point of view, the study group may said to be representative of a UAE families living in big cities. The same may not apply to dwellers in smaller cities, villages or dwellers of oasis and the desert, all who make up the population of the UAE.

A Different Approach to the Study:

With the benefit of hindsight the study might have been done differently to arrive at more conclusive results.

Firstly: it might have paid off more to conduct the study on an aspect of health the mothers considered relevant to their children such as respiratory tract illnesses.

Secondly: more conclusive results would have been obtained if the study number had been larger, but the limitations to that have already been discussed above

Thirdly: the laboratory work could have been done differently to include more tests and arrive at more results.

Two observations were made from the preliminary study and the main study.

1. More diarrhoea was found in the preliminary study than in the main study. It may be argued that the subjects of the preliminary study were "ill" children visiting the hospital because of their condition which was diarrhoea and that the subjects of the main were initially "well" children visiting the MCH unit at Roda clinic, therefore it is to be expected to find more diarrhoea in the former children. For comparative reasons the laboratory records of Jazeira Hospital were surveyed. It was found that stool specimens from 454 children under 5 years old who visited Jazeira Hospital between October 1989-June 1990 (the duration of the study period) only 26 pathogens were identified, with no specification as to which ones were diarrhoea sufferers. Surveys of Central Hospital laboratory records showed that during the months of February and March 1990, 38 out of 144 children who visited the hospital were specifically diarrhoeic according to the laboratory request forms. It should be pointed out

that laboratory records in Jazeira and Central Hospitals are recorded in different ways. In Jazeira Hospital the name of the patient, his/her hospital number and the type of specimen sent are all that are recorded in the laboratory records, whereas in Central Hospital a copy of the whole request form is kept in the laboratory usually for 2 months after which they are disposed of, all the relevant data presumably being in the patients' files. The comparative results may be a confirmation of the finding of the main study or they may not be. MOH records do not list diarrhoea on its list of important childhood diseases in the UAE, and information such as that above would tend to agree with that.

2. Of the 38 diarrhoea specimens from Central Hospital, that stated diarrhoea on their request forms, a pathogen was isolated from only one of those specimens and over the duration of 9 months only 26 pathogens were identified from 454 stool specimens that were sent to the Jazeira Hospital laboratory (these were: 10 isolations of G.lamblia, 10 isolations of Shigella sp., 5 isolations of Yersinia sp. and 1 isolation of Ent. histolytica). In a modern day laboratory performing all the possible tests available for the identification of diarrhoeal pathogens, an 80% isolation rate would be considered reasonable. Therefore a long trial period would be required, where a large number of tests are tried until the combination that produces the optimum identification results is obtained. This would involve testing of different media, identification kits as well as adding new tests to the laboratory routine such as rotavirus testing, campylobacter isolation and identification and cryptosporidium identification all which were absent from the hospital routine and some which were absent

from the study. It should be realised that there are differences in the isolation and identification methods of bacteria, parasites and viruses such as rotavirus. Virus identification (as used in this study) is independent of experience and may require simple training to use kits such as the latex agglutinating kits or Elisa kits. Whereas bacteria isolation and identification is very much dependent on the training and experience of the workers, for the recognition of bacteria and their characteristic reactions under different laboratory conditions. The same would apply to parasites and the relevant one in this study would have been Giardia which was not frequently isolated. When it was isolated, Giardia was not associated with complaints of diarrhoea except in 2 patients in the preliminary study. This could be due to shortcomings in technique, as only microscopy of saline or iodine smears were used. To overcome this cyst and oocyst concentration techniques ought to become part of the laboratory routine. MOH records show that there is a big parasite problem in the UAE that is largely imported from SE Asia. Giardia isolation rates are highest from people of Bangladeshi or Filipino origin. So it is quite likely that the isolation methods used in the laboratories such as Jazeira Hospital and Central Hospital laboratories, are adequate enough to enable the MOH to realise there is a parasite problem in the population. The fact that the same methods were not picking up any parasites in the study or the observed populations is likely due to the type of patient in this study (under 5 year old local children), where there was a low rate of G.lamblia infection.

Family Recruitment:

As mentioned before the preliminary study showed a considerable amount of diarrhoea in the population surveyed. This was not the finding of the main study. In fact the response of the mothers to the study maybe taken as a refection of how they rated the problem of diarrhoea. Of the 98 mothers who were originally interviewed at Roda clinic, only 36 continued with the study. The remainder dropped out for a variety of reasons, perhaps the most obvious one would be is that many of them did not feel that diarrhoea was a problem of great relevance to them. In fact one of the mothers suggested that there may have been a more enthusiastic response from more mothers had the topic been "respiratory diseases in children", of which there are plenty.

The 36 mothers brought 52 children into the study with them. If one specimen was collected weekly from each of those children, then a total of 1040 specimens were expected to be collected at the end of the study period. Instead only 500 specimens were collected. Again this may say something about the level of commitment of the mothers to the study. While they were supportive of the study were not committed enough to ensure the provision of a specimen weekly.

The Mothers :

A rare insight into the role of women in their environment, and the way they perceived themselves, was obtained from the very beginning during the first interviews at Roda clinic.

It was perhaps not surprising that the mothers felt they should consult with their husbands before allowing a stranger into their homes, but what was surprising was the finding that it was the fathers who decided whether there was a need to join this study, or that was the excuse given by the mothers. One mother actually declined to enter the study as she could see no "social benefit" in doing so. It would be unfair on the mothers who did join to describe them as unsupportive or uninterested. All of their children suffered no diarrhoeal complaint at the time of the initial interview, yet they consented to be part of the study out of curiosity or out of the wish to feel helpful in a small way as not much was being asked of them, or they might have done so to keep up a social appearance especially where a mother knew a friend or another relative had joined the study.

The problem of commitment to the study was encountered when collection rounds were made. It was felt that mothers of children who suffered a diarrhoeal episode were more active in seeking the worker to inform her and in preparing specimens for collection

Qualitative results:

It was felt that many of the mothers had a great need to portray their families and homes as ideally healthy to the extent of one mother refusing to acknowledge a diarrhoeal pathogen was isolated from the stool of her child and another mother refusing to admit the child had a bout of diarrhoea. Another mother refused to allow the worker into the kitchen where the children's food was cooked or into the bathrooms that were used by the children, preferring to drop out of the study than to permit this. Curiously her child showed no diarrhoea according to either definition and no pathogens were isolated from that child during the whole study. The reception areas of that house and the presentation of the children were all considered by the worker as quite adequate.

It was found that diarrhoea, when found was more associated with:

- the mothers definition of diarrhoea,

- the younger age group of mothers,

- children whose mothers were ethnically from the UAE, who lived as extended families and who had Sri Lankan housemaids,

- children given partial or supplemented breast feeding.

- children weaned off the breast at 4-6 months of age.

It is to be stressed that due to the small number involved in the study, it is not possible to consider the Sri Lankan maids as a risk factor for diarrhoea in the population at large, even though they were in this study. Interestingly there was no association with either definition of diarrhoea or with any pathogen isolated when these Sri Lankan maids were involved in the preparation of food for the children. This would indicate that if they were indeed a risk factor for diarrhoea, the route of transmission was other than that of food or that the cases where this occurred were in solely breast-fed children who were not yet weaned.

The education of the mother, her occupation if she had one, the extent of her involvement in preparing the children's meals were all not associated with either definition of diarrhoea or pathogen isolated.

The association between the younger age group of mothers and diarrhoea according to the mothers was probably due to the fact that maybe these mothers, due to lack of experience and the alarmism that might accompany the novel experience of having a baby, were over-reporting.

There was an association between diarrhoea according to the mother's definition and the ethnic mothers in the group. These mothers were likely to give their children a home-made preparation which might account for this association.

Quantitative Results:

This study confirms that the mothers' judgement of what were normal or abnormal bowel movements of her child were more associated with pathogen isolation (pathogens in this study being Shigella sp., typable E.coli, rotavirus, Salmonella sp. and G.lamblia)

The known pathogens of diarrhoea that were isolated in the main study were a few typable E.coli, Salmonellae from 3 children, a few "Shigellae" and the greater number of rotaviral isolations. The E.coli that were typed were all negative except on 2 occasions. This immediately raised doubts about the serotyping of these bacteria. Either the anti-sera were not working and this could have been controlled in the laboratory or the E.coli serotypes that were prevalent in this study were other than those of the classical sero-groups and would therefore not sero-type with the anti-sera used, this would be a finding similar to those reported by other workers from all over the world. Or alternatively EPEC E.coli were of no significance in this study. A study made on the troops of Operation Desert Storm (Hyams et al; 1991) in northeastern Saudi Arabia showed that ETEC E.coli along with Shigella soneii were the most common causes of travellers' diarrhoea. There was no mention of EPEC E.coli, either they were not looked for or they were not the cause of travellers' diarrhoea in that region, which would agree with finding of this study. Many workers have questioned the use of serotyping, however it remains a powerful tool of identification.

The inability to serotype these bacteria might have been due to the fact they were not EPEC E.coli. So ETEC E.coli were looked for by the DNA-DNA hybridisation assay and again this was totally negative. The DNA-DNA hybridisation assay was done to discover whether any plasmid bearing ETEC E.coli were present. It was also carried out to see whether any bacteria of different biochemical identification other than E.coli were carrying the plasmid as inter as well as intra species plasmid transfer is said to be theoretically possible. The outcome was negative in all cases except the positive controls. A tissue culture assay was done to determine in a different manner whether there were any ETEC E.coli in different samples sent from Abu-Dhabi. 70% of all the isolates tested produced no effect at all on the monolayer. The strongest toxin reactions were obtained from a Klebsiella and a Pseudomonas both which gave cytotoxic effects on the vero cell monolayer. The Klebsiella was isolated from a diarrhoeic child. Four E.coli from diarrhoeic children also produced low dilution effects on the vero cell monolayer. The dosage number of bacteria necessary to produce diarrhoea in children is not known. Whether or not the strains that produced toxins that exerted an effect only at low dilutions could be causative in producing diarrhoea in children remains a matter of speculation.

The manifestation of diarrhoea in the body is controlled by various factors:

1. The efficiency of reabsorption in the large gut.
2. Secondary disaccharide deficiency.
3. Toxin adhesion sites.
4. Toxin receptor sites.
5. Amount of toxin produced.

So the production of a toxin and the effects that toxin produce in tissue culture assays does not indicate the occurrence of a diarrhoea in the body, the only way to show that would be by the use of animal model to show physiological effects of the toxin produced on segments of small intestines of animal.

The Shigellae isolated in this study were unusual in the sense that they were isolated from almost the same children and they were consistently identified biochemically as Shigellae and were consistently untypable. It is possible that these so called Shigellae were in actual fact E.coli but misreadings of the Roche Enterotubes lead to their mis-classification. In the absence of a reference laboratory, the mystery might have been solved if the isolates had been sent to a reference laboratory abroad for further identification.

Asymptomatic carriage of Salmonella is well documented in literature and the 2 asymptomatic cases in the study would seem to indicate carrier status. Since the samples belonged to children, who in all likelihood acquired these infections from an adult close by, then the origin of the infection could have looked for by screening all the adults in the concerned house. No attempts were made to find the origins of these infections. The third Salmonella isolation was symptomatic but was also accompanied by the isolation of one of the typable E.coli, raising doubts as to which, if not both of the pathogens was causing the diarrhoea.

Rotavirus was the most isolated pathogen of diarrhoea and it's isolation was made from symptomatic as well as asymptomatic children, thereby supporting the finding of many workers that asymptomatic carriage does exist. The group most affected were the less than 2 year old children, with a peak at the 6-11 month old children. If the calendar year were to be divided into 3 apparent seasons (a long summer, a short winter and an even shorter spring), then isolation seemed to occur throughout the year, with a peak in winter; a finding that would agree with some of the studies from Kuwait, Saudi Arabia and other parts of the world (table 9). And if Spring, which is a relatively new season, is combined with summer, then isolation was found to have occurred throughout the year and again this would agree with other studies from Kuwait, Saudi Arabia and other parts of the world (table 9). Another finding that would be in keeping with other workers' findings, would be the suggestion of a respiratory route of rotaviral infection. Most of these infections occurred when the ambient temperatures were considered by the mothers as either too hot or too cold to allow little children to come and go freely. As a result the closer contact these children have with each other may contribute to their infecting each other through the respiratory route. Electron microscopy was used for obtaining pictorial proof of the strongest of the rotaviral isolations.

Campylobacter was completely dropped from the study for reasons discussed previously. It's isolation and identification were not part of the hospital routine due to the lack of isolates from specimens even though the controls made at the maternity hospital and by the worker at Jazeira Hospital did work. It's absence was strange especially in the light of recent studies that have placed it high on the list of diarrhoea causing pathogens. Various studies have shown that the most important risk factor for paediatric campylobacter infections was the exposure to campylobacter infected chickens. No chickens were kept in any of the homes of the study group. Unpasteurised milk which was another source of infection cited in the literature, was not a big problem as most houses used either fresh pasteurised milk, which is readily available or powdered milk (which is made by re-constituting it with water). Unpasteurised cow or camel's milk (which is much liked for its taste) is available to selected few as the municipality laws make it difficult to keep animals in close proximity of residential areas. So although These 2 reasons might explain the absence of campylobacter infection from the study group, but would not be enough to explain it's absence from the population at large and the negative results obtained from Central Hospitai and the Maternity hospital laboratories.

Microscopy in the study was done as a prelude to identifying invasive bacteria; this was negative and non related to either definition of diarrhoea or pathogen isolated. Microscopy was also done to look for parasites, which was negative in the study group. This was not unexpected as a high prevalence of G.lamblia was noted as particularly high in children under 5 years of age who were malnourished (Gilman et al; 1985) and the children in the study group did not come into this category. The water-borne route of G.lamblia transmission is an important route of transmission. Drinking of untreated surface water seems to be a major risk factor for Giardia infection. Since all the homes in Abu-Dhabi receive water from the desalination plant which had already undergone treatment steps, then an important route of transmission was being interrupted. But there is a parasite problem in the UAE, with G.lamblia being one of the most detectable parasites. Presumably it is an imported problem as most of the sufferers were from SE Asia, notably Bangladesh and the Philippines.

There was a considerable level of diarrhoea of unidentified origin. Pathogens may have been missed due to the limitation of the tests carried out, and which perhaps ought to be added to the hospital laboratory routines. Such tests might include those for Cryptospridium sp., which like campylobacter have come to be associated with diarrhoeal diseases. They might include tests for other viruses, some of which have commercial identification kits such as those for rotavirus. Region specific anti-sera should be determined in order to make the serotyping of E.coli more useful. The isolation techniques of parasites especially G.lamblia seem to be adequately done, but this should be qualified by participation in external quality control schemes and the use of operator-blind quality control schemes in the laboratory.

Although the preliminary study was undertaken before the main study, it's results will be discussed in the light of the main study. The fact that the E.coli group were the highest isolated group, is not really informative as no serotyping was carried out for further identification of whether these isolates were EPEC or not. There was a high rate of G.lamblia detection, although only 2 came from patients whose request forms specified diarrhoea. The lack of G.lamblia isolation in the main study was argued due to the fact that treated water from the desalination plant reached all the houses in Abu-Dhabi in a modern water supply system. Along those lines of argument then a great deal of the isolations in the preliminary are probably of imported origin as the MOH claimed.

By law a clean bill of health is required before a residency permit is issued, so when parasitic infections (which are considered as treatable) are detected treatment is given immediately. Rotavirus testing was done when the kits were available and some may have been missed due to unavailability of the kits, even so the isolation rate was a high one and again it was found in the under 2 year group.

The number of unidentifiable isolates are possibly higher than 26, as it is not known what number of the E.coli isolated were pathogenic in addition to the fact that only 2 of the G.lamblia isolated were associated with diarrhoea in the patients. This makes for a high rate of diarrhoea of unknown etiology, the reasons for which have been covered in the discussion of the main study results.

Background:

The United Arab Emirates could be considered a Third World country by virtue of geographical situation and relative age of the country, and until recently this certainly was the case. It is unique in the sense that many changes have occurred in the past 25 years and have accompanied the wealth due to oil-production. Historically this time span is short enough for many to remember the conditions before the oil boom. In the absence of hard data about diseases and their prevalences, recollections from people and comparisons with countries with similar backgrounds and conditions as the UAE are used to postulate what the situation might have been like and what interventions were most effective in changing those conditions.

The literature is full of evidence and information about diarrhoea and the conditions under which it prevails. The Abu-Dhabi city (the chosen study area) of 25 years ago bears little resemblance to the city of today. According to a member of The Evangelical Alliance Mission hospital (TEAM) in Al-Ain, "infant mortality was high, children during those times suffered greatly from diarrhoeas, fevers and protein malnutrition, there was also a great deal of anaemia among the children". The lack of hygienic facilities, be they running water, proper lavatories, or soap and the lack of medication in addition to the difficulty in transport made achieving a reasonable hygienic standard of living and seeking medical attention when needed, difficult. The diet according to the member of Team "was very poorly, depending largely on rice or bread; for those in Al-Ain who grew wheat; fish and dates. When a goat or a camel was slaughtered for food it had to be eaten almost immediately as there were no facilities for food storage. Milk was obtained from the few camels and goats that were kept and from the milk a kind of cottage cheese cum yoghurt called Chammy was made". Limes, mangoes and guavas are grown in places where there was fresh water available such as the oasis of Al-Ain or Dibba and their consumption was subject to availability in places such as Abu-Dhabi.

Children fared badly, Dr. Heard-Bey (personal communication) recalls a visit to Ras-Al-Khaimah in the early 1960s when there was an epidemic of eye diseases especially in children that coincided with an unusually high prevalence of flies that year and which was less the following year with the decrease in fly numbers. The early 1960s also saw an epidemic of polio that afflicted many and became widespread as a result of lack of immunisation.

Mothers usually breast-fed up to 2 years or even more although the holy Koran recommends breast-feeding for those able to do so up to the age of 2 years. Diarrhoeas, according to the TEAM member, became more apparent with weaning, the mothers were the most likely source of infection; through the faeco-oral route; in the absence of soap and running water and proper concepts of hygiene. Weaning food itself was neither hygienic or nutritious. Children were given camel or goat's milk, Chammy cheese "and a bone to suck on". Other liquids included water dropped by the mother's fingers into the child's mouth and when they were older, given it from a cup, or variations of the preparation given to the children at 6 weeks of age. This preparation was a finely sieved mixture of dates, cardamon purified butter and crystallised sugar. As the children grew they were introduced to the normal diet of the adults, mentioned earlier. There are verses in folk poetry that mention the difficulties infants go through when teething, comparing and likening that ordeal to dying. In actual fact what may be deduced is that the infants were suffering from the effects of unhygienic surroundings that were causing diarrhoeas and dehydration and quite likely death, rather than teething being the cause of these grievances.

Similar to other diseases, diarrhoea was treated using herbal medicines brought from India and Iran using natural products such as Halool (senna leaves), Murr (Commiphora spp. possibly Bdellium) and Harmal (leaves of Pegarum harmala). It is interesting to note that Halool is used by the locals to treat constipation as well as diarrhoea. It contains an astringent which causes absorption of water from the gut. It also contains anthracene glycosides which have a purgative effect and so relieve constipation. So depending on the concentration used these two opposite conditions are treated by the same treatment. Rhubarb also contains the same substances and has been known to be used for the same purposes in Western countries.

In contrast the Abu-Dhabi of today is an affluent modern city with all the facilities and conveniences that are available in many of the developed world cities, these include:

- Electricity which reaches the whole city.
- A desalination plant that provides the island with piped fresh water and produces enough even to supplement the neighbouring city of Al-Ain (figure 15).
- A sewage network system that is connected to the entire island. After treatment the effluent and sludge from this plant are made use of as irrigation water and fertilizer for local use rather than poured off into The Gulf
- A health system of an excellent standard that is accessible to all, and that lays ample stress on aspects such as preventive medicine, immunisation, maternal and child health and welfare etc. Health programs involve media targeting to

reach certain groups such as mothers or smokers in order to deliver and stress certain messages relating to health, hygiene, immunisation or breast-feeding to name but a few.

- A transport system that enables the reaching of these medical facilities when needed.

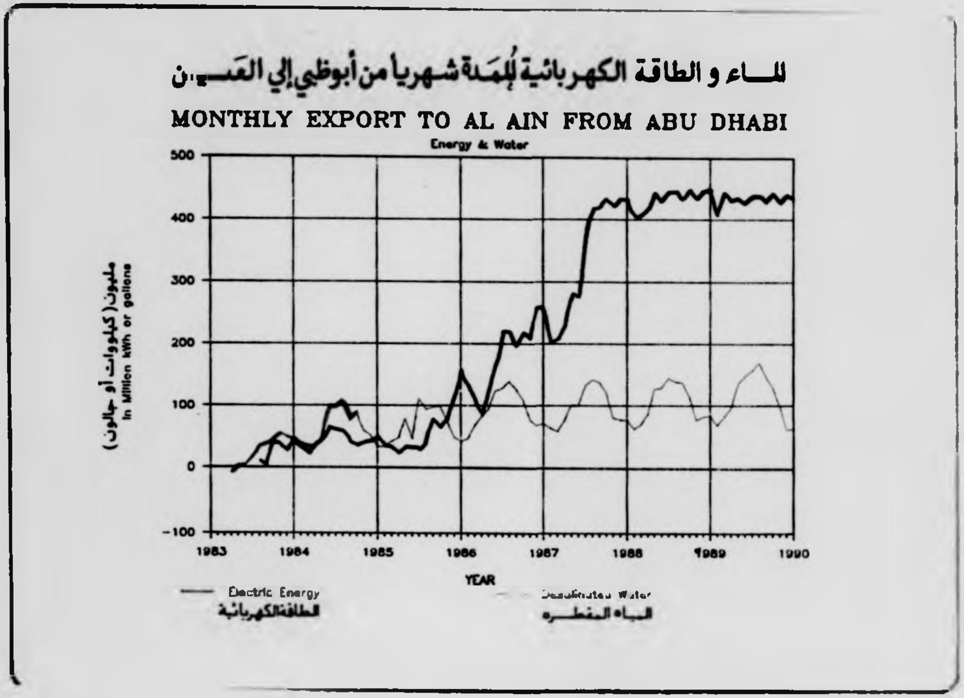


Figure (15): Monthly Export of Water to Al-Ain from Abu-Dhabi

Government Statistics:

Ministry of Health statistics are obtained from the field studies conducted by teams appointed by the ministry, and from statistics and records kept at ministry departments such as The Department of Preventive Medicine, the hospital laboratory records, birth and death records. They are the only source of official data. Birth and death records can be taken as very accurate since every delivery has to be recorded in order for a birth certificate to be issued and the same applies for death records. Statistics on the Immunisation Program have also been much studied and M.O.H. reports show it has been a highly successful program. There are no statistics or studies other than this one on diarrhoeal diseases and there are none on breast-feeding either. The Statistics Department at the ministry has yet to realise its full potential but big and computerised strides are being taken in that direction. However much data is needed to be able to draw significant conclusions. Since the only data available at the present concerns the immunisation system and program, and since the immunisation system in the city of Abu-Dhabi involves bringing the children into the health clinics, some of which have a MCH clinic attached to them, then extrapolations were made regarding diarrhoeal diseases. The results were found to agree with each other and those from the immunisation program went a long way to explaining those from this study concerning diarrhoeal diseases in children.

Apart from those mentioned above there are very few other health statistics available. But there is complementary information available such as data from other government ministries and departments that have been concerned with the setting up of new and modern infra-structural developments which have contributed towards creating a primary health care system. These departments include The Water and Electricity

Department, The Municipality, for the sewage system, the Ministry of Education, the Chamber of Trade and Commerce.

The availability of commodities necessary for maintaining good hygienic standards such as soap, bottle-sterilising kits and medicines in addition to the facilities mentioned above, have made being clean easy. The Abu-Dhabi of today boasts of an affluent and modern society. The change in life-style brought on different problems. It was generally felt by a lot of the mothers participating in this study, friends or even M.O.H. officials, that respiratory tract diseases were more prevalent and problematic in young children. The climatic conditions are such that temperature changes usually occur suddenly without the gradations that occur in temperate climates, making respiratory tract illnesses naturally common in the winter. With the dependence on air-conditioning in modern homes during the summer, little children playing about in the cool in-doors and the hot and humid out-doors are also easily subject to respiratory and tonsillar illnesses.

All this was discussed at the M.O.H., and although there was no data available on diarrhoeal diseases, these diseases were officially not considered a major cause of infant morbidity or mortality. Social attitudes have changed considerably making the task of intervention easier. Communication has become easier with the prevalence of education be that academic education or media targeted at a certain group such as the mothers, for example, who are given their first post-natal appointment, 3 weeks after delivering, at an M.C.H. clinic, usually Roda clinic. This visit is used to check the mother's health after delivery, answer any queries, recommend breast-feeding, advice on bottle-feeding as well as ensure that the mother is aware of the necessity of immunisation and to give a first appointment for that purpose, usually when the baby is 2 months old. The M.O.H. records (Table 17) show a high attendance up to the sixth month or the first measles dose, after which there is a decrease in attendance - probably due to parent complacency about the disease since the children would probably seem healthy and the urgency for immunisation remote. According to Snyder and Merson (1982); and in agreement with others, morbidity due to diarrhoea was highest in the 6 - 11 month age group (fig. 5). And it is precisely at this age when mothers should come into the clinic on an immunisation visit so they be targeted for education about the handling of diarrhoea should it occur. However from this study not much diarrhoea was found and this would explain the lack of pre-occupation of the parents with it, even though in many parts of the world it is a life-threatening problem.

The official response was that this was good proof that the government interventions were highly successful. Providing a sound piped water and sewage system were two of the most important interventions against infectious diseases such as diarrhoea. The ease of living conditions, the availability of electricity as shown by the increase in annual consumption per annum, (Figure 16) meant that meals for the children need not be prepared and left to wait at ambient temperatures (see Chapter 1 page 49) for any length of time before being eaten, again cutting out one of the vital requirements for bacterial multiplication. Adding to all this the medical facilities; available to all at nominal charges; and clinics that have gradually expanded along the years, providing there-by a more than adequate health care system putting the system on par with that of many countries from the developed world. M.O.H. expenditure increased between the years 1980 - 1991. More employees were taken on as indicated in fig (1). Sources of expenditure of the ministry come from the Federal Government of the

U.A.E. as well as the local government of the Emirate of Abu-Dhabi, Dubai Government local health and medical services, the petrol companies' medical services and the Ministry of Defence medical services, there-by allowing more flexibility in spending.

Thus a primary health care system along the lines recommended by Walsh and Warren been set-up, put to the test and proven effective.

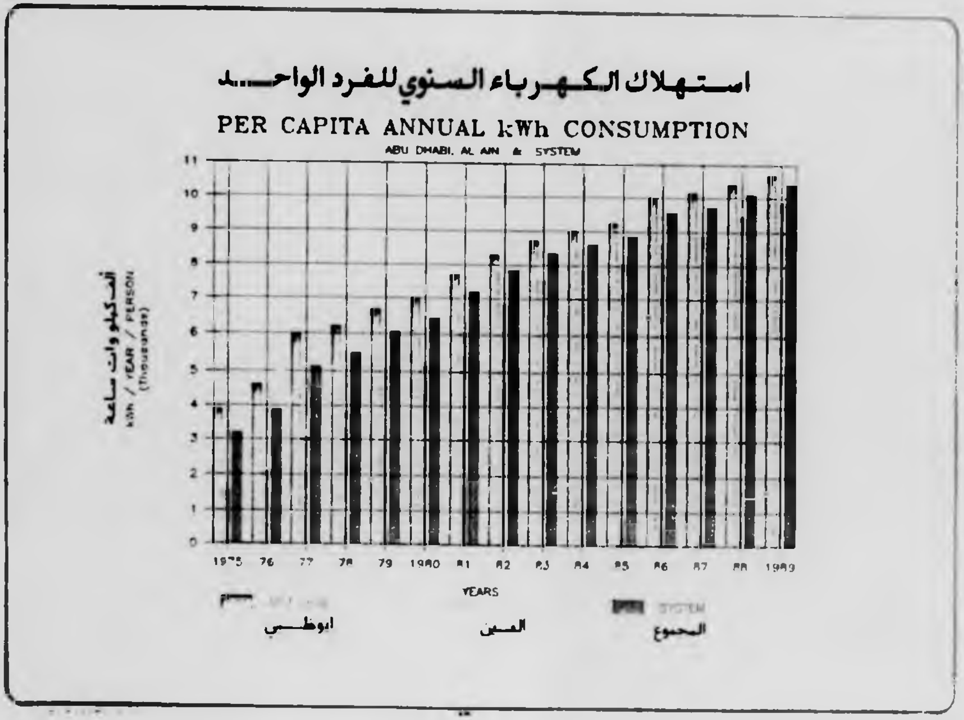


fig (16): Per Capita Annual Electricity ConsumptionIntroduction:

Further suggestions:

This study was a consecutively based clinic study. It posed certain difficulties associated with the recruiting of the families, the level of commitment to the study and the co-ordination between specimen collection and laboratory processing. As well as co-ordination of sending material from the London School of Hygiene and Tropical Medicine (LSHTM) and samples to the LSHTM for further processing.

It would be of interest to do different surveys that are community based or hospital based to investigate whether the same patterns found in this study apply there. Other studies of interest related to this study would involve investigating the E.coli serotypes present in the U.A.E. and studies involving the dietary habits of the area Efforts should be made to co-ordinate with the university and the technical colleges that are beginning to expand in the direction of research, to make use of the facilities that might be available there.

Chapter 6 Conclusion

This study was done to examine the impact of the major infrastructural developments on the health of the children in the U.A.E. A limited laboratory investigation and survey was undertaken and this was embedded in a qualitative study of social attitudes and factors of the mothers. All of this was placed in the context of the historical recollections of the inhabitants. Government statistics as well as health and economic indicators were used to explain aspects of the results. Although found but in small proportions, the absence of diarrhoeal diseases from the list of serious childhood diseases in the city of Abu-Dhabi was a pleasant and un-anticipated surprise. The picture here being quite different to that in neighbouring Kuwait for example, although climatic, social, geographical and economical conditions are very similar. Upon investigation and research it was found that infrastructural interventions and a primary health care system provided by the government that included a modern up-to-date piped water system, a modern sewage system, a good and affordable health care system, had resulted in the reduction of the routes of bacterial infection and multiplication. Added to that good media targeting, a good road system that make seeking medical attention easy when needed, good municipal services such as daily rubbish collection from all parts of the city, a pest controlling system that controls the presence of insects such as flies and a high standard of living that makes the obtaining of hygiene commodities such as soap affordable were all factors that led to efficiently controlling infectious diseases such as the diarrhoea investigated in this study.

Appendix i

The Questionnaire

Family Name:

Family No.:

Date:

Name of child: 1. **DOB:** 1.

2. 2.

3. 3.

Treatment Centre:

1. Jazeira Hospital
2. Roda clinic
3. Private doctor
4. Other (state)

Immunization record:

1. BCG
2. DPT, Polio a) 1st dose
   b) 2nd dose
   c) 3rd dose
3. Measles and Mumps
4. Booster doses
5. Others (state)

Child no.

Name of father: DOB:

Occupation:

1. Government employed
2. Private sector employed
3. Self employed
4. Unemployed
5. Other (specify)

Name of mother: DOB:

Education Level:

1. Primary
2. Intermediate
3. Secondary
4. University
5. Uneducated
6. Other (specify)

Occupation:

1. Housewife
2. Teacher
3. Social Worker
4. Doctor
5. Other (specify)

Original Nationality:

1. U.A.E.
2. Egyptian
3. Lebanese
4. Indian
5. Sudanese
6. British
7. Other (specify)

Type of feeding babies:

1. Solely breast-fed
2. Mixed breast and bottle
3. Bottle only

If not 2 or 3 above, type of milk used:

1. Cow and Gate
2. SM-26
3. Promil
4. Fresh milk
5. Powder milk
6. Other (specify)

Approx. age of weaning:

(if unweaned = 0)

Type of weaning food:

1. Readymade bottled food
2. Powdered food
3. Home-prepared food (strained)
4. Home-prepared food (unstrained)
5. Other (specify)

Any domestic help in the house ? [Y] [N]

How many ?

Nationality of help:

1. Indian
2. Sri-Lankan
3. Egyptian
4. Palestinian
5. Other (specify)

No. of maids: Nationality:

No. of cooks: Nationality:

No. of drivers: Nationality:

Who prepares food ?

1. Mother
2. Maid
3. Cook
4. Nanny
5. Other (specify) [ ]

Who helps children bathe ?

1. Mother
2. Maid
3. Elder sister
4. Child itself
5. Nanny
6. Other (specify)

Who feeds Children ?

1. Mother
2. Maid
3. Elder sister
4. Child itself
5. Nanny
6. Other (specify)

Who helps children use toilet ?

1. Mother
2. Maid
3. Elder sister
4. Child itself
5. Nanny
6. Other (specify)

<u>Total no of Family members living together in one house:</u>

<u>No. of parents:</u>

<u>No. of children including study children:</u>

<u>Ages of children excluding study children:</u>

<u>No. of grandparents if any:</u>

<u>No. of aunts if any:</u>

<u>No. of uncles if any:</u>

<u>No. of cousins if any:</u>

<u>Others:</u>

<u>Hygenic level of the house:</u>

1. Very clean
2. Clean
3. Not clean
4. Dirty

<u>Bathroom floors:</u>

<u>Bathroom sinks:</u>

<u>Kitchen floors:</u>

<u>Kitchen sinks:</u>

<u>Kitchen cupboards:</u>

<u>Kitchen utensils:</u>

<u>Fridge:</u>

<u>Dining room or eating quarters:</u>

Appendix ii

Impressions

Family number:	1
Number of children in family:	5
Number of children in study:	1
Sex of children in study:	F
Extended or single family:	Single
Mother education:	Primary
Original nationality of mother:	UAE (Abu-Dhabi)
Mode of feeding before 4 months:	Mixed

Age which food other than breast-milk introduced: 3 months

Age of complete weaning from breast: 3 months

Reasons: not enough milk

comments: Mother very nice but half-hearted about joining the study group. Gave the impression she agreed to join out of curiosity and after that became satisfied continued so as not to appear ignorant especially in view of her comparative lack of education. House was big with many staff (6 in all). All cooking done by cook and maids generally fed and looked after children. When the mother was asked for permission to see her kitchen and bathrooms, she refused saying that was embarrassing and she would rather withdraw than give this permission. Her children seemed healthy and active. Their intake of fruits and vegetables was little. During the study one of the children developed pneumonia and the little sister developed a milder form.

Family number: 2

Number of children in family: 7

Number of children in study: 1

Sex of children in study: F

Extended or single family: Single

Mother education: teacher training

Original nationality of mother: UAE (Um Al-Quain)

Mode of feeding before 4 months: Breast

Age which food other than breast-milk introduced: not yet

Age of complete weaning from breast: not yet

Reasons: At time of interview child was newborn and had not had anything other than breast-milk

comments: Mother had 7 daughters. Very hospitable and wanting to help. All cooking done by cook. Maid helped mother with children. Main reception areas in house were clean and tidy in spite of the number of children. Mother believed that breast-feeding was the best method for the child and hoped to continue as long as possible.

Family number:	3
Number of children in family:	1
Number of children in study:	1
Sex of children in study:	F
Extended or single family:	Extended
Mother education level:	University
Original nationality of mother:	UAE (Abu-Dhabi)
Mode of feeding before 4 months:	Breast

Age which food other than breast-milk introduced: 4 mos

Age of complete weaning from breast: 12 months

Reasons: Mother believes breast-feeding is best as it is the most natural. It was mentioned in the Koran and it would seem to be a more cleaner way of feeding. Also the bond developed between mother and child with this kind of feeding is of important value.

comments: The mother was willing to partake in the study after she was reassured that the only person making the house visits would be the interviewer herself. Wanted the worker/interviewer to come home and meet her mother-in-law to assure her of the study and its purposes. Visits to this family normally took longer than usual as the ladies usually insisted that the worker come in for a chat with the ladies of the house. These chats would evolve around the children of the house, the progress of the study and the worker's choice of research subject. The grandmother agreed that the subject was interesting while one of the daughters in law; who was pregnant but joined the study group a few weeks later upon delivery; thought it was a curious choice and their must have been more aesthetic choices of study.

For all their interest, later on in the study when the daughter was testing positive for rotavirus and the mother confirmed their was a slight diarrhoea, when the worker tried to explain that this was a viral diarrhoea the mother's attitude changed, changing her mind that their was any irregular bowel movement let alone any viral infection.

Family number: 4

Number of children in family: 1

Number of children in study: 1

Sex of children in study: M

Extended or single family: Extended

Mother education: intermediate

Original nationality of mother: UAE (Dubai)

Mode of feeding before 4 months: Bottle

Age food other than breast-milk introduced: From birth

Age of complete weaning from breast: Bottle fed from birth

Reasons: Didn't have enough milk and was not really keen to breast-feed

comments: This family lived as an extended family with family number 3 and their in-laws. The mother was amused by the choice of research topic, always teasing about it. All cooking done by the cook in this family. This mother and the mother in Family 3 tended their own children. The house was generally clean and the children were always well presented. A cow was kept in the courtyard of the house.

Family number: 5

Number of children in family: 2

Number of children in study: 2

Sex of children in Study: F, M

Extended or single family: Extended

Mother education: Intermediate

Original nationality of mother: UAE (Ras Al-Khaimah)

Mode of feeding before 4 months: Breast for both

Age food other than breast-milk introduced: 4 Mos,not yet

Age of complete weaning from breast: 8 months and not yet

Reasons: Cheaper, easier and cleaner to breast-feed.

comments: The mother was aware that there was some benefit to be gained from this study to all parties and as such was very helpful answering questions, calling when her children had diarrhoeal attacks. Her sisters were less enthusiastic and almost unwilling to help. The visits were pleasant and the mother used them to ask any questions about her children's health. Her children had a long episode of watery diarrhoea for which no agent could be discovered by the worker or the hospital, the children didn't respond quickly to the doctor's medication. Although the diarrhoea persisted for a long time, the children did not become dehydrated. They suffered a lot of respiratory tract infections throughout the study, with the children reinfecting each-other, the son was asthmatic. The cooking was done by a cook and the children's food was prepared by the mother or the grandmother. The children's presentation was not always clean. The family go to the town of origin for weekends and public holidays where there are more domestic animals in close proximity to the children.

Family number: 6

Number of children in family: 6

Number of children in study: 1

Sex of children in family: M

Extended or single family: Single

Education of mother: Secondary

Original nationality of mother: UAE (Dubai)

Mode of feeding before 4 months: Mixed

Age which food other than breast-milk introduced: 2 months

Age of complete weaning from breast: Not yet

Reasons: One of breasts removed and so felt not enough milk for baby.

comments: Mother was helpful even offering to ask among friends if any would like to join the study. Cooking done by cook and maid helped with the babies's food preparation and handling. Child seemed healthy and always well presented. But for all her enthusiasm, after the isolation of rotavirus from one of the weekly specimens, when the worker asked her whether her child had a diarrhoeal episode, she said yes, adding she didn't think it was important to report even though the question was asked at each collection.

Family number: 7

Number of children in family: 6

Number of children in study: 2 (twins)

Sex of children in family: M, F

Extended or single family: Single

Mother education: Primary

Original nationality of mother: Egypt

Mode of feeding before 4 months: Mixed

Age food other than breast-milk was introduced: 3 months

Age of complete weaning from breast: 3 months

Reasons: Didn't have enough milk for the twins.

comments: Mother willing to participate although she didn't think the question of diarrhoea was as relevant to her as constipation which her children suffered. All cooking and handling of children done by mother. Children well presented and house always clean and tidy. Twins were born underweight. They don't like to eat much fruit and vegetables, a factor she felt contributed to their constipation.

Family number: 8

Number of children in family: 3

Number of children in study: 2

Sex of children in study: F, M

Extended or single family: Extended

Mother education: Intermediate

Mode of feeding before 4 months: Breast and mixed

Age food other than breast-milk introduced: 4 mos, 3 mos

Age of complete weaning from breast: 12 months and not yet.

Reasons: Believes breast is better for child and had enough to continue for a long time. With second child, she had resumed studying and was supplementing some of feeds

comments: Mother initially was anxious to know who would be making the home-visits. Once reassured she was happy to oblige. Mother did all cooking and tended children herself-no domestic help in the house.

Her younger son was prescribed antibiotics for diarrhoea and colds and for a long time it was not possible to isolate any bacteria from his stool.

Family number: 9

Number of children in family: 1

Number of children in study: 1

Sex of children in study: F

Extended or single family: Extended

Mother education: Primary

Original nationality of mother: Yemen

Mode of feeding before 3 months: Breast

Age food other than breast-milk was introduced: 4 months

Age of complete weaning from breast: Not Yet

Reasons: Has plenty of milk and believes its healthier for the child, "There must be a reason for its mention in the holy Koran"

comments: Lived with family number 8 (husbands were brothers). Reception areas of house were clean. Mothers shared household chores. This family dropped out as father was transferred to another town.

Family number: 10

Number of children in family: 3

Number of children in study: 3

Sex of children in study: F, M, M

Extended or single family: Extended

Mother education: University

Original nationality of mother: Yemen

Mode of feeding before 4 months: Breast

Age food other than breast-milk introduced: 4 months all.

Age of complete weaning from breast: 6 mos, 4 mos, not yet.

Reasons: Babies were crying a lot and friends advised she supplement as babies seemed to be unsatisfied. She was trying to feed the youngest for longer.

comments: Grandmother lived with this family and used to help with the baby-sitting and occasionally the cooking otherwise mother cooked and tended her children. Very interested in the study although slightly apprehensive of storing the specimen in the fridge. When specimens were ready she used to call to confirm she had a sample to be collected. Reception areas of house were clean as was the kitchen and the children were always well presented. Younger son suffered meningitis during the study but recovered completely.

Mother married before she finished her final year at university. Admired working mothers and wished she had finished her studies.

Family number: 11

Number of children in family: 4

Number of children in study: 1

Sex of children in the study: F

Extended or single family: Single

Mother education: University

Original nationality of mother: Bahrain

Mode of feeding before 4 months: Breast

Age food other than breast-milk was introduced: 4 months

Age of complete weaning from breast: 6 months

Reasons: Tired and didn't think baby needed it any-more

comments: Mother had no domestic help in the house. she was very clean, one of the few who washed her hands after handing the specimen to the worker. Her kitchen was clean and tidy and her children well presented. During the study she gave birth to a girl who was also recruited into the study. A university graduate who was not making use of her degree because of family commitments, although she would have liked to.

Family number: 12

Number of children in family: 5

Number of children in study: 1

Sex of children in study: M

Extended or single family: Single

Mother education: Primary

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Breast

Age food other than breast-milk was introduced: 4 months

Age of complete weaning from breast: Not yet

Reasons: Breast feeding is natural and better and so chose to continue.

comments: Married at the age of 13. Reception areas of house very clean, children always very presentable. Cooking done by cook, while maid looked after child with the mother. Regretted not having much of an education. Child contracted a Salmonella (which was confirmed by biochemical and serological tests) and which mother said did not present as a diarrhoea, no-one else had diarrhoea in the house. Origin of this isolation which was repeated from the next few specimens collected was not found.

Family number: 13

Number of children in family: 6

Number of children in study: 1

Sex of children in study: M

Extended or single family: Extended

Mother education: Primary

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Breast

Age food other than breast-milk was introduced: 4 months

Age of complete weaning from breast: Not yet

Reasons: Age recommended for weaning by the doctor and production of breast-milk was decreasing

comments: Neighbours of family number 12. All cooking done by cook, mother mostly tended her child although maid also helped. Reception areas were not that clean and children were not that well presented. The child in this study seemed prone to colds and fevers that accompanied it. Mother willing to help especially after she knew neighbours were recruited also. Wanted to be reassured who would collect specimens.

Family number: 14

Number of children in family: 4

Number of children in study: 2

Sex of children in study: M, F

Extended or single family: Single

Mother education: University

Original nationality of mother: Yemen

Mode of feeding before 4 months: Mixed occasionally

Age food other than breast-milk introduced: 4 mos, Not yet

Age of complete weaning from breast: 8 months, Not yet

Reasons: Had enough milk to carry on and it was advised by her doctor.

comments: Reception areas of house were adequately clean and children were well presented. All cooking done by mother and maid helped with cooking and the looking after the children. Complained children didn't eat much fruit or vegetables. Specimens always present although not always possible to collect them if mother was out as she locked door and maid didn't have a key. Sister-in-law of family 8 and 9, but each recruited separately.

Family number: 15

Number of children in family: 1

Number of children in study: 1

Sex of children in study: F

Extended or single family: Extended

Mother education: Teacher training

Original nationality of mother: UAE (Ras-Al-Khaimah)

Mode of feeding before 4 months: Mixed

Age food other than breast-milk was introduced: 6 weeks

Age of complete weaning from breast: Not yet

Reasons: Couldn't continue to breast-feed as she went back to work

comments: Lives with the mother's family. Mother's mother and grandmother both very interested in natural or traditional medicine, which they say was used more when there were no proper hospitals and which they believe is safer than modern medicine. They told stories of how much children suffered from diarrhoea in the olden days and how the mortality rate was high due to that. One of the few families that gave its children something other than milk at 6 weeks. This was a preparation made from dates, Fenugreek and sugar to induce sleep in the child thereby giving the mother or tender an easier time. Visits always long-drawn as they were used in comparing the benefits of modern and traditional medicine.

Family number: 16

Number of children in family: 4

Number of children in study: 1

Sex of children in study: F

Extended or single family: Single

Mother education: University

Original nationality of mother: Sudan

Mode of feeding before 4 months: Breast

Age food other than breast-milk was introduced: 4 months

Age of complete weaning from breast: 8 months

Reasons: Had enough milk to continue for that long.

comments: Reception areas of house and kitchen were clean. Cooking and looking after the children were done by the mother. Mother aware of benefits and advantages of this research.

Family number: 17

Number of children in family: 4

Number of children in study: 1

sex of children in study: M

Extended or single family: Extended

Mother education: Primary

Mode of feeding before 4 months: Breast

Age food other than breast-milk was introduced: Not yet

Age of complete weaning from breast: Not yet

Reasons: Believes breast-feeding is good and is easier also as a form of contraception

comments: Lives as extended family with family 16. Reception areas of her part of the house not too clean, children not well presented. Mother could not really see the point of the study but continued anyway since visits were being made to the house.

Family number: 18

Number of children in family: 1

Number of children in study: 1

Sex of children in study: F

Extended or single family: Extended

Mother education: intermediate

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Breast

Age food other than breast-milk was introduced: 4 months

Age of complete weaning from breast: 24 months

Reasons: Mother has plenty of milk and it's better for child

comments: At time of interview child was over 18 months and still being breast-fed. Kitchen and reception areas of house were adequately clean, children not always well presented and daughter was prone to colds. During study mother gave birth to another daughter who was also recruited for the study. Shigella was isolated from this child repeatedly although the isolations couldn't be confirmed by serology. Mother was very helpful and keen to know about the study. Elder daughter didn't didn't eat much fruit or vegetables.

Family number:	19
Number of children in family:	2
Number of children in study:	2
Sex of children in study:	F, F
Extended or single family:	Single
Mother education:	Primary
Original nationality of mother:	Yemen
Mode of feeding before 4 months:	Mixed
Age food other than breast-milk introduced:	3 mos for both
Age of complete weaning from breast:	3 months, not yet
Reasons:	

comments: Reception areas and kitchen very clean, children well presented. Mother washed her hands after handing the sample to the worker. Very helpful, keen to know about the study. Complained daughters didn't eat well and were constipated a lot.

Family number: 20

Number of children in family: 2

Number of children in study: 1

Sex of children in study: M

Extended or single family: Single

Mother education: University

Original nationality of mother: Oman

Mode of feeding before 4 months: Mixed

Age food other than breast-milk was introduced: 3 months

Age of complete weaning from breast: Not yet

Reasons: Knows breast-feeding is beneficial, also easier and cleaner.

comments: Mother well educated. Cooking and tending the children done by mum and maid. Reception areas and kitchen very clean. Very supportive of study and its purposes. The only mother to report precisely the number of stools passed during an episode of diarrhoea which was found to be caused by Salmonella and the origin of that traced back to food eaten at a picnic.

Family number: 21

Number of children in family: 5

Number of children in study: 1

Sex of children in study: M

Extended or single family: Extended

Mother education: Primary

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Mixed

Age food other than breast-milk was introduced: 2 months

Age of complete weaning from breast: Not yet

Reasons: Not enough milk

comments: Reception areas and kitchen very clean. Visits took long time. Mother although young was not well educated-could and barely read, but was very eager to know about the study and how it would be useful to the researcher. Also used up time asking medical or health related questions concerning her baby. All cooking done by cook but mother prepared salads and desserts. Child was tended by mother and maid. When child had an episode of diarrhoea, mother contacted worker and had specimen ready for immediate collection. Her sisters however found the choice of subject amusing and commented on this several times to both worker and mother.

Family number: 22

Number of children in family: 4

Number of children in study: 3

Sex of children in study: M, F, F

Extended or single family: Single

Mother education: Intermediate

Original nationality of mother: UAE (Dubai)

Mode of feeding before 4 months: Mixed, Breast, Mixed

Age food other than breast-milk introduced: 4 mos, 1 mos, not yet

Age complete weaning from breast: 5 mos, 3 mos, not yet

Reasons: Didn't have enough milk

comments: Mother did cooking and tended the children with help of maid. House was not very clean and children not well presented. To begin with mother didn't seem eager to join or even know about the study. This changed at the home visit when she gave a different impression trying very hard to oblige even offering to deliver specimens when worker was very busy, calling worker when specimens were ready.

Family number: 23

Number of children in family: 6

Number of children in study: 1

Sex of children in study: F

Extended or single family: Single

Mother education: Intermediate

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Breast

Age food other than breast-milk was introduced: Not yet

Age of complete weaning from breast: Not yet

Reasons: Breast is natural and mentioned in the Koran, and method of contraception.

comments: Cleanliness of reception areas and kitchen were adequate. All cooking done by cook. Baby taken care of by mother and maid. Mother was recruited into the study at the anti-natal clinic of Roda clinic. Wanted to join study as she knew her friend (family number 6) was recruited. Her baby had severe nappy rash at one stage of the study. Shigella isolated from the stools of baby that was un-confirmable by serology. One of her close friends never failed to make affectionate fun of the choice of study topic, but mother always defended the need for such a study.

Family number:	24
Number of children in family:	2
Number of children in study:	2
Sex of children in study:	F, M
Extended or single family:	Extended
Education of mother:	Intermediate
Original nationality of mother:	UAE (Abu-Dhabi)
Mode of feeding before 4 months:	mixed, Breast
Age food other than breast-milk introduced:	4 months both
Age of complete weaning from breast:	8 months, not yet

Reasons: Discontinued breast-feeding the first child when she discovered she was pregnant. Second child is happy to breast-feed and she has enough milk. Uses this as a method of contraception but occasionally supplements with formula milk.

comments: Reception areas and kitchen all clean. Children always well presented. Mother very interested in the progress of the study and used visits to ask about any questions concerning the health and welfare of her children. Admired independent and educated women. Kept goats in the backyard. All cooking done by cook, mother prepared children's weaning food. Visits to this family were long-drawn.

Family number: 25

Number of children in family: 1

Number of children in study: 1

Sex of children in study: F

Extended or single family: Extended

Mother education: Secondary

Original nationality of mother: Yemen

Mode of feeding before 4 months: Mixed

Age which food other than breast-milk introduced: 3.5 months.

Age of complete weaning from breast: 11 months

Reasons: Milk was decreasing, didn't want to give up breast-feeding so supplemented.

comments: Lived as extended family with family 26 and in-laws. Reception areas and kitchen were not very clean. Mother complained daughter sometimes had constipation. Cooking done by cook. Sterilised feeding bottles by washing with tap water and soap only.

Family number: 26

Number of children in family: 3

Number of children in study: 2

Sex of children in study: F, F

Extended or single family: Single

Mother education: Secondary

Original nationality of mother: Yemen

Mode of feeding before 4 months: Mixed for both

Age food other than breast-milk introduced: 3 mos, 4 mos

Age of complete weaning from breast: 7 mos, 8 mos

Reasons: Considered children had enough.

comments: Mother tended her children alone and did all cooking. Didn't have maid at the beginning. Reception areas were clean, children well presented. Mother needed reassurance that only worker would make home visits as husband worked on oil rig and was away a lot. Willing to help and interested in purpose of study.

Family number: 27

Number of children in family: 3

Number of children in study: 3

Sex of children in study: F, F, M

Extended or single family: Single

Mother education: University

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Mixed

Age food other than breast-milk introduced: 6 weeks for all

Age of complete weaning from breast: 3 mos, 3 mos, 4 mos

Reasons: Mother working and too busy to breast-feed.

comments: Mother was always out, children tended by maids and cooking by cook. Grandmother gave children home preparation to induce sleep. Mother willing to help the worker in any possible way and was glad some woman was furthering her study even though married and with family.

Family number:	28
Number of children in family:	7
Number of children in study:	1
Sex of children in study:	1
Extended or single family:	Single
Mother education:	Teacher training
Original nationality of mother:	UAE (Abu-Dhabi)
Mode of feeding before 4 months:	Breast
Age food other than breast-milk introduced:	4 months
Age of complete weaning from breast:	7 months

Reasons: Continued full breast-feeding up to 4 months old even though she was working

comments: Happy and content mother. All cooking done by cook and maid tended child. House was adequately clean. Child often had constipation and mother said she didn't eat fruit or vegetables. Forgot to take child for immunisations. Amused by choice of study and gave the impression only continued the study to keep up with sister -in-law (family 29).

Family number: 29

Number of children in family: 5

Number of children in study: 1

Sex of children in study: M

Extended or single family: Single

Mother education: Intermediate

Original nationality of mother: UAE (Dubai)

Mode of feeding before 4 months: Mixed

Age food other than breast-milk was introduced: 4 months

Age of complete weaning from breast: 9 months

Reasons: No milk.

comments: House clean and children well presented. Mother very enthusiastic about study and asked what more she could do to help more. Salmonella was isolated from the stools of her son later on in the study which did not present with a diarrhoea. The origin of this was traced back to food poisoning acquired by the elder son while away at camp, that infection did present with a diarrhoea.

Family number:	30
Number of children in family:	3
Number of children in study:	1
Sex of children in the study:	M
Extended or single family:	single
Mother education:	Secondary
Original nationality of mother:	Kuwait
Mode of feeding before 4 months:	Mixed
Age food other than breast-milk was introduced:	3 months
Age of complete weaning from breast:	12 months

Reasons: Wanted to make sure her son was getting enough also added method of contraception.

comments: Level of hygiene of house was adequate. Mother was not much concerned about study but carried on since she didn't have to make much effort .

Family number: 31

Number of children in family: 2

Number of children in study: 2

Sex of children in study: M, M

Extended or single family: Single

Mother education: Teacher training

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Mixed for both

Age food other than breast-milk introduced: 2 mos & 6 weeks

Age of complete weaning from breast: 8 mos & 6 weeks.

Reasons: Mother had to go back to work and couldn't do both.

comments: Mother did cooking and maid helped with cooking and the caring of the children. Mother said she was too busy to personally contribute to this study, but maid would supply specimens. Maid didn't speak much Arabic or English. In general mother gave impression of being disinterested and maybe doing it out of a sense of social duty.

Family number: 32

Number of children in family: 8

Number of children in study: 1

Sex of children in study: F

Extended or single family: Extended

Mother education: Koranic school

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Mixed.

Age food other than breast-milk introduced: 4 months.

Age of complete weaning from breast: 24 months.

Reasons: Had the child much later and wanted to make sure she was doing the utmost for her and so carried on breast-feeding to the maximum period recommended in the Koran as it was bound to be right and she had enough milk.

comments: Level of hygiene adequate. Cooking done by cook and maids helped tend the children. Mother had child much later on than other children, not possible to tell exactly at what age as she didn't know her exact age.

Family number: 33

Number of children in family: 1

Number of children in study: 1

Sex of children in study: F

Extended or single family: Single

Mother education: University

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Mixed

Age food other than breast-milk introduced: 2.5 mons.

Age of complete weaning from breast: 2.5 mons

Reasons: Baby refused breast at 2.5 months so completely weaned her.

comments: Reception areas of house were adequately clean. Child looked after by mother and maid. "Just as well the baby didn't want the breast because I have to go back to work", was mother's response to how she felt about not being able to breast-feed, but added she would make sure she was getting her vaccination on time. Eager to know about weanling food and how to start baby on solids.

Family number: 34

Number of children in family: 1

Number of children in study: 1

Sex of children in study: F

Extended or single family: Single

Mother education: University

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Mixed

Age food other than breast-milk introduced: 2 months

Age of complete weaning from breast: not yet

Reasons: Had to go back to work and supplemented to make sure baby was getting enough.

comments: Reception areas clean. All cooking done by mother upon return from work. Child looked after by mother and maid. Mother very supportive of this and any other study that would benefit mothers and children.

Family number: 35

Number of children in family: 2

Number of children in study: 1

Sex of children in study: F

Extended or single family: Single

Mother education: University

Original nationality of mother: UAE (Abu-Dhabi)

Mode of feeding before 4 months: Mixed

Age food other than breast-milk was introduced: 3 months

Age of complete weaning from breast: Not Yet

Reasons: Had to go back to work and so supplemented feeds.

comments: Mother delivered the specimens to the hospital herself. Didn't think the study was very relevant to the area but still continued participating. Her sister was also recruited but dropped out when she found out one of her children had Leukaemia.

Family number:	36
Number of children in family:	1
Number of children in study:	1
Sex of children in study:	M
Extended or single family:	Extended
Mother education:	Secondary
Original nationality of mother:	Yemen
Mode of feeding before 4 months:	Bottle
Age food other than breast-milk was introduced:	At birth
Age of complete weaning from breast:	Never breast-fed

Reasons: Son was born underweight and put in an incubator and hospital automatically put him on formula feeds which she continued with.

comments: This family dropped out very early on in the study as husband moved out of town. Lived as extended family with family 27.

Chapter 6. References

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