Cifuentes, Enrique (1996) Impact of wastewater irrigation on intestinal infections in a farming population in Mexico. PhD thesis, London School of Hygiene & Tropical Medicine. DOI: https://doi.org/10.17037/PUBS.00682293

Downloaded from: http://researchonline.lshtm.ac.uk/682293/

DOI: 10.17037/PUBS.00682293

Usage Guidelines

Please refer to usage guidelines at http://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by-nc-nd/2.5/

IMPACT OF WASTEWATER IRRIGATION ON INTESTINAL INFECTIONS IN A FARMING POPULATION IN MEXICO/

~~THE MEZQUITAL VALLEY~~

ENRIQUE CIFUENTES

Thesis submitted to the University of London
in Fulfillment of the Degree of
Doctor of Philosophy (Faculty of Medicine)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
UNIVERSITY OF LONDON
1995

ABSTRACT

An opportunistic study was carried out in central Mexico, where one of the world's largest wastewater reuse schemes for agricultural production is located. This scheme provided a unique opportunity to assess the health impact of exposure to wastewater of different qualities on intestinal parasitic infections and diarrhoeal diseases. The central objective of the study was to evaluate the effect of hydraulic retention on reducing the health risks associated with wastewater use. Exposure groups were defined according to eligibility procedures and to the quality of irrigation water. Microbiological quality was measured using nematode eggs and faecal coliforms as indicators. The exposure groups involved households: a) exposed to untreated wastewater; b) exposed to wastewater retained in a single reservoir; c) exposed to wastewater which had passed through two reservoirs in series, and been retained for some time in both; and d) nonwastewater-exposed households (controls). The study outcomes included risk of *Ascaris lumbricoides*, *Entamoeba histolytica* and *Giardia lamblia* infections, as well as the risk of diarrhoeal diseases. The study design was based on two cross-sectional surveys (rainy and dry seasons), and the analyses focused on both comparison of risks between the different exposure groups as well as the identification of at-risk groups. The two surveys involved different intermediate groups - (b) and (c) above - and the main purpose was to assess the effects of single versus double hydraulic retention. They are distinguished mainly for this purpose, rather than the evaluation of possible seasonal fluctuations of the study outcomes. Other variables (i.e. socioeconomic, hygiene and sanitation) were analysed as confounders using a multivariate model.

In young children the prevalence rates of *A. lumbricoides* infection were considerably higher in the raw wastewater group (13.7%), and lower prevalences were observed with decreasing exposure (11.8% in the single reservoir group, 3.3% in the double reservoirs group, and 0.6 - 2.5% in the control group). A high prevalence rate of *G. lamblia* was observed in children (17 - 20.5%), but no association with untreated wastewater was found. The prevalence rates of *E. histolytica* infection in children from the various exposure groups ranged between 4.8 - 7.0%, but were considerably higher in older individuals: 15.7 and 16.5% in the two surveys among the raw wastewater group, compared with 13.2% and 14.7% respectively in the controls. In addition, a high prevalence of diarrhoeal diseases (two-weeks recall period) was found in the rainy season, particularly in young children from the raw wastewater exposure group, and lower prevalences were observed with

decreasing exposure (29.0% in the raw wastewater group, 26.8% in the two reservoirs group, and 23.0 % in the control group, respectively).

The overall prevalences of *Cryptosporidium parvum* and *Trichuris trichiura* infections were unexpectedly low (below 1% and 4%, respectively), and excluded from further consideration. The intensity of *A. lumbricoides* infection was evaluated in a parallel study, and is not reported here.

The main findings of the present study can be summarised as follows:

- Cropland irrigation with raw wastewater was strongly associated with *A. lumbricoides* infection in farmworkers and their families, with a risk of diarrhoeal diseases, and with a small but significant risk of *E. histolytica* infection in individuals aged over 5 years.
- The differences observed in the prevalences of *A. lumbricoides* infection and diarrhoeal diseases were similar in both seasons, but the prevalences in the control group were lower in the dry season; thus, the relative effect of wastewater use was greater in the dry season.
- Retention of wastewater in two reservoirs in series (2-6 months) reduced substantially the risk of *A. lumbricoides* infection, and to a lesser extent the risk of *E. histolytica* infection, and possibly the risk of diarrhoeal diseases in young children.
- Retention of wastewater in a single reservoir (1 - 7 months) did not reduce the risk of *A. lumbricoides* or *E. histolytica* infection, but may reduce the risk of diarrhoeal diseases in children by 20%.
- No association between exposure to raw wastewater and infection with *G. lamblia* was detected in this research.
- Parasitic intestinal infections and diarrhoeal diseases showed significant associations with variables describing personal and domestic hygiene, basic sanitation and socioeconomic characteristics.

These results are discussed in relation to local regulations and health protection measures, as well as in light of the WHO 1989 revised guidelines for restricted crop irrigation.

ACKNOWLEDGMENTS

Dr Ursula Blumenthal and Professor G. Ruiz-Palacios both deserve special acknowledments; they provided substantial contributions throughout the development of this project, which this thesis resulted from. Many thanks for their encouragement and supervision.

I also thank Dr Stephen Bennett for his enthusiastic support. I really enjoyed working with him and appreciate his statistical help.

To Maria Quigley for her support and supervision (and patience) during the analysis and data presentation, and for performing the logistic regression analysis. Thanks again.

To Dr Sandy Cairncross, for his constructive comments during the final version.

To Caroline Smart, for taking care of endless faxes, and the rest.

Here, in Mexico, I would like to thank the field team, laboratory technicians, and data entry staff as well as to Juanita Cortez (IMTA) for all their valuable help. Also, to the staff of the Irrigation Districts and of the National Water Comission (CNA). In addition, I would like to thank the European Economic Community, the Mexican Foundation for Health (FUNSALUD) and CONACYT, for their support.

To Mauricio Hernandez, for his valuable comments and friendship.

Finally, many thanks to Janine for her patience during the final edition, and her tenderness during difficult times... above all; *ni mistasojta miak.*

And to Helena, as always, to start with.... *no kualtzin xochisuapil,*
uan nochi noyollotl ...

Temascaltepec, August 1995.

TABLE OF CONTENTS

	Page No.
Abstract	2
Acknowledgments	4
Table of Contents	5
List of Figures	11
List of Tables	12
List of Appendices	15
Glossary	15
CHAPTER ONE. INTRODUCTION	16
CHAPTER TWO. LITERATURE REVIEW	
2.1 INTRODUCTION	19
2.2 HISTORICAL OVERVIEW	19
2.3 POTENTIAL BENEFITS AND HAZARDS FROM WASTEWATER REUSE	22
2.4 EPIDEMIOLOGICAL EVIDENCE OF ENTERIC INFECTIONS AND DIARRHOEAL DISEASES ASSOCIATED WITH WASTEWATER REUSE	23
2.4.1 Consumption of vegetable crops irrigated with raw wastewater	24
2.4.2 Health effects from occupational risk and risks of living in communities exposed to wastewater irrigation	26
2.4.3 Health effects associated with occupational exposure to partially treated wastewater	29
2.4.4 Epidemiological evidence in Mexico of the impact on health of wastewater reuse in agriculture	32
2.5 EVOLUTION OF HEALTH GUIDELINES AND REGULATIONS FOR THE SAFE USE OF WASTEWATER	33
2.6 WASTEWATER REUSE IN MEXICO	36
2.7 SUMMARY	41
CHAPTER THREE. DESCRIPTION OF THE STUDY AREA	
3.1 INTRODUCTION	42
3.2 GENERAL CHARACTERISTICS OF THE MEZQUITAL VALLEY	42

3.3 INSTITUTIONAL FRAMEWORK OF THE IRRIGATION DISTRICTS (ID) AND MICROBIOLOGICAL QUALITY OF WATER 45

3.4 IRRIGATION SYSTEM 46

3.4.1 Development of the irrigation districts 46
3.4.2 Raw wastewater 47
3.4.3 Stored wastewater 49
3.4.3.1 Endho storage reservoir 49
3.4.3.2 Rojo Gomez storage reservoir 50
3.4.3.3 Vicente Aguirre storage reservoir 50

3.5 MICROBIOLOGICAL QUALITY OF WASTEWATER 50

3.6 AGRICULTURAL PATTERNS 54

3.7 CROP RESTRICTIONS AND OCCUPATIONAL EXPOSURE 56

CHAPTER FOUR. STUDY DESIGN AND METHODS

4.1 INTRODUCTION 58

4.2 OBJECTIVES 58

4.3 STUDY DESIGN 58

4.4 SAMPLE SIZE CALCULATION, ADJUSTMENTS AND CHANGES 60

4.5 METHODS FOR PRELIMINARY FIELD WORK 61

4.5.1 Characterization of the study area 61
4.5.2 Design of questionnaires 62
4.5.2.1 Focus group discussions 62
4.5.2.2 Field trial of questionnaires and interviewers' training 62
4.5.3 Study site office, laboratory and computing facilities 63
4.5.3.1 Laboratory 63
4.5.3.2 Computing facilities 63

4.6 THE CENSUS 63

4.7 ELIGIBILITY PROCEDURES 64

4.8 EXPOSURE CATEGORIES 70

4.9 PILOT STUDY 70

4.10 ASSESSMENT OF WASTEWATER QUALITY 71

4.11 CROSS SECTIONAL SURVEYS 73

4.11.1 Rainy season survey 73
4.11.2 Dry season survey 73

Page No.

4.12 MEASURES OF OUTCOME 74

4.13 OTHER VARIABLES 74

4.14 DATA COLLECTION METHODS 75

4.14.1 Interviews 75
4.14.2 Laboratory tests 75
4.14.3 Wastewater sampling 76
4.14.3.1 Faecal coliforms 76
4.14.3.2 *A. lumbricoides* 76

4.15 DATA ENTRY AND MANAGEMENT 77

4.15.1 Questionnaires 77
4.15.2 Parasitology tests 78
4.15.3 Wastewater quality 78

4.16 QUALITY CONTROL PROCEDURES 79

4.16.1 Field work 79
4.16.2 Data management 80
4.16.3 Laboratory tests 80

4.17 DATA ANALYSIS 80

4.17.1 Descriptive analyses 80
4.17.2 Further analysis 80

4.18 LOGISTICS 81

4.19 JUSTIFICATION OF THE STUDY DESIGN 82

4.20 SOURCES OF BIAS 83

CHAPTER FIVE. CHARACTERISTICS OF THE STUDY POPULATION

5.1 SOCIODEMOGRAPHIC PROFILE 85

5.2 CHARACTERISTICS OF THE EXPOSURE GROUPS 85

5.2.1 Age and gender distribution of the exposure groups 86
5.2.2 Agricultural features 87
5.2.3 Literacy of care-givers 89
5.2.4 Housing conditions 89
5.2.5 Drinking water supplies 91
5.2.6 Basic sanitation 91
5.2.7 Other facilities 92

5.3 SUMMARY 92

Page No.

CHAPTER SIX. RAINY SEASON SURVEY RESULTS

6.1 INTRODUCTION 94

6.2 RAINY SEASON SURVEY RESULTS 95

6.2.1 *A. lumbricoides* infection 95
6.2.1.1 Prevalence of *A. lumbricoides* infection 95
6.2.1.2 Exposure and confounding factors in children 0-4 yrs. 95
6.2.1.3 Effect of exposure and confounding factors in individuals aged 5 years and older 95
6.2.2 *G. lamblia* infection 98
6.2.2.1 Prevalence of *G. lamblia* infection 98
6.2.2.2 Exposure and confounding factors in children 0-4 yrs. 98
6.2.2.3 Effect of exposure and confounding factors in individuals aged 5 years and older 99
6.2.3 *E. histolytica* infection
6.2.3.1 Prevalence of *E. histolytica* infection 100
6.2.3.2 Exposure and confounding factors in children 0-4 yrs. 100
6.2.3.3 Effect of exposure and confounding factors in individuals aged 5 years and older 101
6.2.4 Diarrhoeal diseases 102
6.2.4.1 Prevalence of diarrhoeal diseases 102
6.2.4.2 Exposure and confounding factors in children 0-4 yrs. 102
6.2.4.3 Effect of exposure and confounding factors in individuals aged 5 years and older 104

6.3 DISCUSSION OF THE RAINY SEASON SURVEY RESULTS 104

6.3.1 *A. lumbricoides* infection 104
6.3.1.1 Effect of raw wastewater exposure 104
6.3.1.2 Effect of the reservoirs 105
6.3.1.3 Other factors 105
6.3.2 *G. lamblia* infection 105
6.3.2.1 Effect of raw wastewater exposure 106
6.3.2.2 Effect of the reservoirs 106
6.3.2.3 Other factors 106
6.3.3 *E. histolytica* infection 106
6.3.3.1 Effect of raw wastewater exposure 107
6.3.3.2 Effect of the reservoirs 107
6.3.3.3 Other factors 107
6.3.4 Diarrhoeal diseases 108
6.3.4.1 Effect of raw wastewater exposure 108
6.3.4.2 Effect of the reservoirs 108
6.3.4.3 Other factors 108

6.4 SUMMARY OF THE RAINY SEASON SURVEY 109

CHAPTER SEVEN. DRY SEASON SURVEY RESULTS

7.1 INTRODUCTION 110

7.2 DRY SEASON SURVEY RESULTS 110

7.2.1 *A. lumbricoides* infection 111
7.2.1.1 Prevalence of *A. lumbricoides* infection 111
7.2.1.2 Exposure and confounding factors in children 0-4 years 112
7.2.1.3 Effect of exposure and confounding factors in individuals older than 5 yrs 112
7.2.2 *G. lamblia* infection 114
7.2.2.1 Prevalence of *G. lamblia* infection, 114
7.2.2.2 Exposure and confounding factors in children 0-4 years 114
7.2.2.3 Effect of exposure and confounding factors in individuals older than 5 yrs 114
7.2.3 *E. histolytica* infection 116
7.2.3.1 Prevalence of *E. histolytica* infection 116
7.2.3.2 Exposure and confounding factors in children 0-4 years 116
7.2.3.3 Effect of exposure and confounding factors in individuals older than 5 yrs 117
7.2.4 Diarrhoeal diseases 119
7.2.4.1 Prevalence of diarrhoeal diseases 119
7.2.4.2 Exposure and confounding factors in children 0-4 years 119
7.2.4.3 Effect of exposure and confounding factors in individuals older than 5 yrs 119

7.3 DISCUSSION OF THE DRY SEASON SURVEY RESULTS 121

7.3.1 *A. lumbricoides* infection 121
7.3.1.1 Effect of exposure to raw wastewater 122
7.3.1.2 Effect of the reservoir 122
7.3.1.3 Other factors 122
7.3.2 *G. lamblia* infection 123
7.3.2.1 Effect of exposure to raw wastewater 123
7.3.2.2 Effect of the reservoir 123
7.3.2.3 Other factors 123
7.3.3 *E. histolytica* infection 124
7.3.3.1 Effect of exposure to raw wastewater 124
7.3.3.2 Effect of the reservoir 124
7.3.3.3 Other factors 124
7.3.4 Diarrhoeal diseases 124
7.3.4.1 Effect of exposure to raw wastewater 125
7.3.4.2 Effect of the reservoir 125
7.3.4.3 Other factors 125

7.4 SUMMARY OF THE DRY SEASON SURVEY 126

CHAPTER EIGHT. COMBINED RAINY AND DRY SEASON DISCUSSION

8.1 INTRODUCTION 128

8.2 EXPOSURE TO RAW EXPOSURE 128

8.2.1 *A. lumbricoides* infection 128

Page No.

8.2.2 *G. lamblia* infection 128
8.2.3 *E. histolytica* infection 129
8.2.4 Diarrhoeal diseases 130

8.3 EFFECT OF THE RESERVOIRS 130
8.3.1 *A. lumbricoides* infection 130
8.3.2 *G. lamblia* infection 131
8.3.3 *E. histolytica* infection 131
8.3.4 Diarrhoeal diseases 132

8.4 OTHER FACTORS 132

8.4.1. *A. lumbricoides* infection 132
8.4.2. *G. lamblia* infection 133
8.4.3. *E. histolytica* infection 134
8.4.4 Diarrhoeal diseases 134

8.5 STRENGTHS AND WEAKNESSES OF THE STUDY 135

8.6 SUMMARY OF THE OVERALL DISCUSSION 138

CHAPTER NINE. CONCLUSIONS AND POLICY RECOMMENDATIONS

9.1 EFFECTS OF UNTREATED WASTEWATER 140

9.2 EFFECTS OF A SINGLE STORAGE RESERVOIR 140

9.3 EFFECTS OF TWO STORAGE RESERVOIRS 141

9.4 IMPLICATIONS OF THE PRESENT RESULTS 142

9.4.1. Local implications and policy recommendations 142
9.4.2. Implications of results on WHO guidelines for restricted crop irrigation 144

REFERENCES 147

APPENDIXES 157

1 QUESTIONNAIRE FORMAT AND TRANSLATION

2 EXAMPLE OF COMMUNITY / TOWN MAP

3 ARTICLE PUBLISHED IN REV. SALUD PUBLICA DE MEXICO

<u>LIST OF FIGURES</u>

Figure 2.1	Irrigation Districts, Mexico
Figure 3.1	Map of Mexico, the State of Hidalgo and the Mezquital Valley
Figure 3.2	Retention Time in Endho Reservoir (1989-1991)
Figure 3.3	Retention Time in Rojo Gomez Reservoir (1989-1991)
Figure 3.4	Retention Time in Vicente-Aguirre Reservoir (1989-1991)
Figure 3.5	Mexico City's Major Wastewater Reuse Scheme, the Mezquital Valley
Figure 4.1	Study Design
Figure 4.2A	Eligibility Flow Diagram. Remove ineligible families and classify them for rain-fed areas
Figure 4.2B	Eligibility Flow Diagram. Classify landowners by irrigation canal
Figure 4.2C	Eligibility Flow Diagram. Classify landowners by type of community
Figure 4.2D	Eligibility Flow Diagram. Classification of paid labourers
Figure 4.3	The Irrigation System and Sampling Points
Figure 4.4	Wastewater Reuse Study; Data Management
Figure 5.1	Categories of Farmers in the Exposure Groups, the Mezquital Valley, 1990
Figure 5.2	Land Tenure for Exposure Groups in the Mezquital Valley, 1990.
Figure 5.3a	Agricultural Land Use by Farmers who own their Farm, the Mezquital Valley, 1990
Figure 5.3b	Agricultural Activities of Paid Labourers in the Mezquital Valley, 1990
Figure 5.4	Literacy of the Care-giver, the Mezquital Valley, 1990
Figure 5.5	Roof Materials used by Exposure Groups in the Mezquital Valley, 1990
Figure 5.6	Water supply for houses in different exposure groups, of the Mezquital Valley, 1990
Figure 5.7	Defecation in the Yard's Soil by the Exposure Groups, the Mezquital Valley, 1991
Figure 5.8	Rubbish disposal facilities in Different Exposure Groups, the Mezquital Valley, 1991.

LIST OF TABLES

Table 2.1	Agricultural Application of Wastewater, Selected Examples
Table 2.2	Current Wastewater Reuse Schemes, Selected Examples
Table 2.3	Increase of Crop Yield through Wastewater Irrigation
Table 2.4	Relative Health Risks from the Use of Untreated Excreta and Wastewater in Agriculture and Aquaculture
Table 2.5	California State Department of Health Standards for Safe and Direct Use of Reclaimed Wastewater for Irrigation and Recreational Impoundments
Table 2.6	Recommended Wastewater Quality Guidelines for Use in Agriculture, WHO, 1989
Table 2.7	Major Agricultural Schemes in Mexico which Use Wastewater for Irrigation
Table 2.8	Wastewater Reuse Planned in Mexico
Table 3.1	Age and Gender Distribution for the State of Hidalgo, 1990.
Table 3.2	Social Profile, Population of the Mezquital Valley (1990).
Table 3.3.	Development of the Irrigation Infrastructure in the Mezquital Valley.
Table 3.4.	Crop Restriction Policy, Mexico, 1990.
Table 3.5	Quality of Wastewater and Effluents from Reservoirs in ID 100 -03: Faecal Coliforms MPN/100ml (1990-1991).
Table 3.6	Quality of Wastewater and Effluents (helminth eggs/litre) from the Reservoirs, ID 03, 100 (1990-1991).
Table 3.7	Crops Produced in Irrigation Districts 03 and 100 Hidalgo, 1988.
Table 3.8	Selected Examples of Agricultural Production in the Study Areas, 1986-1987.
Table 3.9	Main Crops Cultivated in the Mezquital Valley, 1990.
Table 3.10	Crops and Irrigation Characteristics in the Mezquital Valley.
Table 3.11	Cultivated Crops and their Economic Value, the Mezquital Valley, 1990.
Table 4.1	Eligible Household Categories.
Table 4.2	Final Definition of Exposure.
Table 5.1	Demographic Profile of the Censused Population, the Mezquital Valley, 1989.

Table 5.2 Exposure Categories of Households in the Mezquital Valley, 1991

Table 5.3. Gender and Age Distribution of Exposure Groups, the Mezquital Valley, 1991

Table 5.4. Distribution of Selected Variables by Exposure Group, the Mezquital Valley, 1991

Table 6.1 Rainy season survey prevalence of intestinal parasitic infections the Mezquital Valley, 1990.

Table 6.2 Rainy season survey children aged 0 - 4 years with *Ascaris lumbricoides* infection according to exposure and other factors; the Mezquital Valley, 1990.

Table 6.3 Rainy season survey individuals aged 5 years and older, with *Ascaris lumbricoides* infection according to exposure and other factors; the Mezquital Valley, 1990.

Table 6.4 Rainy season survey children 0 - 4 years of age with *Giardia lamblia* infection according to exposure and other factors; the Mezquital Valley, 1990.

Table 6.5 Rainy season survey individuals aged 5 years and older with *Giardia lamblia* infection according to exposure and other factors; the Mezquital Valley, 1990.

Table 6.6 Rainy season survey children aged 0-4 years with *Entamoeba histolytica* infection according to exposure and other factors; the Mezquital valley, 1990.

Table 6.7 Rainy season survey individuals aged 5 years and older with *Entamoeba histolytica* infection according to exposure and other factors; the Mezquital Valley, 1990.

Table 6.8 Rainy season survey prevalence of diarrhoeal diseases according to exposure and age categories; the Mezquital Valley, 1990.

Table 6.9 Rainy season survey children aged 0-4 years with diarrhoeal diseases according to exposure and other factors; the Mezquital Valley, 1990.

Table 6.10 Rainy season survey individuals aged 5 years and older with diarroheal diseases according to exposure and other factors; the Mezquital Valley, 1990.

Table 7.1 Dry Season Survey Prevalence of Intestinal Parasitic Infection according to Exposure Group and Age; the Mezquital Valley, 1991.

Table 7.2 Dry Season Survey Children aged 0-4 years with *A. lumbricoides* Infection according to Exposure and Other Factors; the Mezquital Valley, 1991.

Table 7.3 Dry Season Survey Individuals aged 5 years and Older with *A. lumbricoides* Infection according to Exposure and Other Factors; the Mezquital Valley, 1991.

Table 7.4 Dry Season Survey Children aged 0-4 years with *G. lamblia* Infection according to Exposure and Other Factors; the Mezquital Valley, 1991

Table 7.5 Dry Season Survey Individuals aged 5 years and Older with *G. lamblia* Infection according to Exposure and Other Factors; the Mezquital Valley, 1991.

Table 7.6 Dry Season Survey Children aged 0-4 years with *E. histolytica* Infection according to Exposure and Other Factors; the Mezquital Valley, 1991.

Table 7.7 Dry Season Survey Individuals aged 5 years and Older with *E. histolytica* Infection according to Exposure and Other Factors; the Mezquital Valley, 1991.

Table 7.8 Dry Season Survey, Prevalence of Diarrhoeal Diseases according to Exposure and Age Categories, the Mezquital Valley, 1991.

Table 7.9 Dry Season Survey Children aged 0-4 years with Diarrhoeal Diseases according to Exposure and Other Factors; the Mezquital Valley, 1991.

Table 7.10 Dry Season Survey Individuals aged 5 years and Older with Diarrhoeal Diseases according to Exposure and Other Factors; the Mezquital Valley, 1991.

Table 8.1 General Prevalences of Intestinal Infections and Diarrhoeal Diseases in the Mezquital Valley, 1991.

LIST OF APPENDICES

APPENDIX 1 Survey Questionnaire
APPENDIX 2 Example of Community Map
APPENDIX 3 Reprint of article Rev. Sal. Publ. Mex.

GLOSSARY

CNA	Comisión Nacional de Agua (National Water Comission)
CONAPO	Consejo Nacional de Población (National Population Council)
DGE	Dirección General de Epidemiología (General Directorate of Epidemiology)
INEGI	Instituto Nacional de Estadistica, Geografía e Informática (National Institute of Statistics, Geography and Computing)
INI	Instituto Nacional Indigenista (National Institute for Indigenous Populations)
INN-SZ	Instituto Nacional de la Nutrición "Salvador Zubirán" (National Nutrition Institute "Salvador Zubirán)
ID	Distritos de Riego (Irrigation Districts)
IMTA	Instituto Mexicano de Tecnología del Agua (Mexican Institute of Water Technology)
PRONAR	Programa Nacional Reaprovechamiento de Agua (National Programme for Water Reuse)
SARH	Secretaría de Agricultura y Recursos Hidaulicos (Ministry of Agriculture and Water Resources)
SSA	Secretaría de Salud y Asistencia (Ministry of Health)
SEDUE	Secretaría de Recursos Naturales y Pesca (Ministry of Natural Resources and Fisheries)

CHAPTER 1. INTRODUCTION

Wastewater reuse is increasingly recognized as an appropriate solution for cropland irrigation, as long as adverse health effects can be prevented. The rationale for wastewater reuse is economically oriented and takes on higher valuable importance in semiarid regions of developing countries. In the foreseeable future, both the benefits and hazards associated with wastewater reuse will continue to evolve parallel to the growing need for supplementary water sources. Water shortages worldwide have motivated even industrialized countries to consider the advantages of wastewater reuse: water recycling and substantial reduction of environmental pollution.

In agricultural areas of developing countries, major motivations for wastewater reuse are considerable savings from not using chemical fertilizers, multiple planting seasons and increased harvests. Stable jobs and farmers' greater profits (and thus the possibility of better living standards), constitute the practices' central attractions. Unfortunately, in many developing countries, agricultural practices involving exposure to untreated wastewater are frequently tolerated. However, despite widespread cropland irrigation with untreated wastewater, reliable data which assess the health impact resulting from such practices are notoriously scarce. This absence of data is the basis for an almost complete lack of clarity over safety regulations, for official neglect and tolerated illegal agricultural practices, all of which result in considerable environmental and illness burden.

One of the world's largest wastewater reuse schemes is located in central Mexico. Wastewater from the country's major cities represents already a severe disposal problem, which in some cases is currently being alleviated through reuse schemes. Indirect reuse (i.e. rivers receiving raw sewage) is much more common. In the near future, vast areas of agricultural land may be incorporated into these irrigation programmes, in an effort to reduce growing conflicts between urban and rural areas, and thus alleviate increasing water shortages and demographic imperatives. An adequately managed expansion of the reuse programme would release fresh water for the domestic demands of millions in city slums and scattered rural settlements.

Given current financial constraints in Mexico, full treatment of large volumes of wastewater will not be feasible for agricultural schemes. Crop restriction is likely to continue as the primary health protection policy, although it is increasingly recognized that addresses only consumers' risk. Protection for occupational exposed farmers and their families needs to be assessed through evaluation of the impact of additional protection measures on the health of farmers and their families. In conjunction with these evaluations, planners must define low cost wastewater treatment systems capable of reducing the risk associated with occupational exposure. Safe use wastewater programmes consistent with low cost treatment systems will need to set achievable water quality guidelines, assess cost-effectiveness of wastewater treatment and management options, as well as determine appropriate compliance regulations. Parallely, integrated reuse schemes will need to epidemiologically monitor the health effects of each option.

This thesis presents the results of an observational study consisting of two cross-sectional surveys carried out between 1989 and 1991 in the Mezquital Valley, central Mexico. The primary objectives of the research were to provide evidence of measurable risk of intestinal parasitic infections and diarrhoeal diseases in a farming population exposed to untreated wastewater, as well as the potential beneficial effects of hydraulic retention in reducing these health risks. An additional objective was to evaluate the revised WHO guidelines for restricted irrigation. The study focused on "opportunistic situations" i.e. existing schemes, comprised of large agricultural areas, all receiving wastewater of different characteristics. Due to continuous growth of these irrigation districts (ID), one of the areas involved in the study receives untreated wastewater flowinng 65 - 100 km from Mexico city; this raw wastewater is used primarily to irrigate maize and fodder crops through flood farming techniques. Surplus volumes of wastewater and run-off continue further north, and is retained in a series of interconnected reservoirs from which it is released several months later, depending on the farming demands of nearby communities. These reservoirs provide partial treatment of the wastewater through hydraulic retention, thereby improving water quality prior to cropland application. These rapidly expanding areas are surrounded by several dozen rain-fed farming villages.

The study presented herein was designed to address major limitations of previous studies and, hence, focused particularly on sample size, correct classification of exposure, strict definitions of outcomes and the control of major confounding variables in the analysis. In addition, different at-risk groups, including children of agricultural workers were evaluated. Wastewater was monitored through monthly sampling, in order to determine levels of water pollution and to monitor any improvement in wastewater quality attributable to hydraulic retention in the storage reservoirs.

The thesis is organised as follows. Chapter Two is a review of research literature relevant to the present research. Chapter Three provides a detailed description of the study area, the Mezquital Valley. Chapter Four describes the study objectives and methodology, while Chapter Five presents characteristics of the study population including basic hygiene and sanitation variables, socioeconomic features and other descriptive variables. Chapters Six and Seven summarise the results of rainy and dry season surveys and contain season-specific discussions of each. A global discussion of study findings is presented in Chapter Eight. The final chapter summarises conclusions and health policy recommendations in the expanding wastewater reuse programme of Mexico; some of the remarks in the last chapter may, or may not be applicable to other semi-arid regions of the world.

CHAPTER 2. LITERATURE REVIEW

2.1 INTRODUCTION

The earliest documented land application of sewage and wastewater can be traced back to the second half of the nineteen century, when community collection systems developed in urban centres (Metcalf & Eddy 1972, Jewell & Seabrock 1979). Since then, our understanding of both the benefits and the hazards associated with wastewater reuse have evolved. Attitudes towards water and wastewater reuse will continue to evolve along with economic, environmental and health issues which shape public and official perceptions.

This chapter reviews the published evidence of the impact of agricultural wastewater reuse on enteric infections, with special reference to semiarid areas in developing countries. Historical aspects and current trends of wastewater reuse worldwide will be summarised, followed by a review of the impact on health of wastewater reuse. Other aspects of wastewater reuse have been reviewed in other studies (e.g. Feachem *et al.*. 1984; Shuval *et al.*. 1986). The third section is a review of the evolution of health guidelines and related policies, while the prevailing situation in Mexico is summarised at the end of the chapter.

2.2 HISTORICAL OVERVIEW.

Human waste has been used to restore soil nutrients for farming practices since ancient times. The first farming application of wastewater can be traced back to ancient Athens, where it was applied for both irrigation and waste disposal system (Metcalf 1972). In the nineteenth century, the rapid growth of Europe's major cities resulted in widespread sewage soil-application on the outskirts of urban centres. "Sewage farms" were used by authorities advocating resource recycling and prevention of river pollution policies (Feachem *et al.*. 1983). Disposal of untreated sewage and sludge for cropland irrigation was the only feasible alternative treatment for overwhelming river pollution (Hespanhol, 1993).

At the end of the last century, the "germ theory" of diseases was rapidly adopted in most western societies, fostering ideas of aseptic environments achievable through new and promising technologies. Simultaneously, rapid urban growth around sewage farms, increasing land values, public complaints (e.g. odors and flies), as well as growing concerns regarding transmission of communicable diseases all contributed to the decline of sewage farming in many recently industrialised areas (Gunnerson *et al.*. 1984).

There was a resurgence in farming application of wastewater in the western hemisphere during the 1950s and 1960s, as a result of advances in wastewater treatment technology and of the increasing scarcity of fresh water resources for urban populations. Strauss (1988), Mara and Cairncross (1989), and Strauss and Blumenthal (1992) have provided sound reviews of wastewater schemes around the world. Tables 2.1 and 2.2 illustrate the extent of these practices, emphasizing the types of waste used (treated versus untreated), examples of water treatment provided and the range of crops cultivated.

Cropland applications of wastewater are currently practiced on every continent, with the exception of Antarctica (Chang & Page 1993). Perhaps one of the pionneering examples of such policies is that of Israel, where water reuse priority is to recycle 80% of total treated wastewater volume within the next few years. In the United States, more than 3,400 projects using treated wastewater for various reuse purposes were recorded in 1980. Many other examples of wastewater reuse schemes are located in Australia, China, Germany, the former Soviet Union, India, South-Africa, Tunisia, Jordan, Kuwait and Egypt (IRCWD News 1988, Shuval 1986). In Latin America, one example of the use of sewage for irrigation is the use of 80% of Santiago's sewage to irrigate 16,000 hectares of land (Yanez 1980). In Mexico, nearly 250,000 hectares are irrigated with sewage, while in the desert coast of Peru, 33 reuse projects irrigate 2,300 hectares surrounding Lima (Bartone 1985).

Table 2.1 Agricultural Application of Wastewater, Selected Examples.

Country and City	Irrigated Area (ha)
Argentina, Mendoza	3,700
Australia, Melbourne	10,000
Bahrain, Tubli	800
Chile, Santiago	16,000
China, all cities	1,330,000
Germany, Braunschweig	3,000
Other cities	25,000
India, Calcutta	12,500
All cities	73,000
Israel, several cities	8,800
Kuwait, several cities*	12,000
Mexico, Mexico City	90,000
All cities*	250,000
Peru, Lima*	6,800
Saudi Arabia, Riyadh	2,850
South Africa, Johannesburg	1,800
Sudan, Khartoum	2,800
Tunisia, Tunis*	4,450
Other cities*	2,900
United States, Arizona	2,800
Bakersfield, California	2,250
Fresno, California	1,625
Santa Rosa, California	NO DATA
Lubbock, Texas	3,000
Muskegon, Michigan	2,200

*Includes planned expansion of existing reuse
Source: Bartone 1987

Table 2.2 Current Wastewater Reuse Schemes, Selected Examples

COUNTRY	WASTE USED	CROPS PRODUCED	APPROX. AREA HECTARES
SOUTH AMERICA			
Argentina	Primary effluent at times diluted and dried STP - sludge	Lettuce, onions, tomatoes, artichokes	2,000
Chile	City's wastewater untreated or diluted	Lettuce, cabbage, celery, cereals, grapes	6,000
Peru	Wastewater treated & part. treated	Misc. edible and non edible crops	5-6,000
S. Martin	Wastewater untreated	Tomatoes, radish, spinach,	2,000
ICA	Primary (facult) pond effluent	Fodder, cotton, maize, grapes	400
NORTH AMERICA			
Mexico	Untreated wastwater or diluted or impounded	Maize, wheat, oats, green tomatoes, fodder, alfalfa and chillies	250,000
Mexico City Mezquital Valley	Untreated wastewater or diluted or impounded	Maize, wheat, oats, green tomatoes, fodder, alfalfa and chillies	87,250
USA			
California	Wastewater effluent from aerated lagoons	Barley, field corn, cotton, pasture, tomatoes, chillies,	2,000
	- effluent from tertiary treat. plants	asparagus, broccoli, cauliflower, corn and citrus orchards	>5,000
EUROPE			
FR Germany	Wastewater mixed 90% sec. effluent 10% raw	Cereals, sugar beets and potatoes	2,800
UK	Lagooned, digested STP - sludge	Field and horticultural crops	------
NORTHERN AFRICA AND WESTERN ASIA			
Tunisia	Wastewater second. treatt	Citrus trees	600
Jordan	River consisting of treated sewage	Trees, industrial, crops vegetables eaten cooked or raw	500
Kuwait	Wastewater tert. treatment plant effluent	Fruit trees, fodder, maize, wheat, raw or cooked vegetables	2,000
Saudi-Arabia	Wastewater tert. treatment plant effluent	Wheat, fodder, date palms, lemon trees and fodder	2,500 800
ASIA			
China	Wastewater untreated, diluted; partially treated	Paddy rice, maize, wheat, sorghum vegetables, fodder	1,330,000
SOUTH EAST ASIA			
India	Wastewater untreated, diluted; partially treated	Paddy rice, maize, wheat, sorghum vegetables, fodder, aquaculture	>70,000

Source: (Modified) Strauss M. 1988

2.3 POTENTIAL BENEFITS AND HAZARDS FROM WASTEWATER REUSE.

The rationale for most wastewater recycling practices is economic. Even in countries without severe drought problems, governments are continuously searching for supplemental sources of water to cope with increasing demands for this valuable resource. In many arid and semiarid regions of the world, water has become a limiting factor for sustainable development (Hespanhol, 1993). This is particularly relevant in many Latin American countries which face the consequences of population growth and critical water shortages; 20% of land in Latin America is arid or semiarid and receives only 5% of regional water resources, most of which are severely polluted (Bartone 1985). As a result of these limited resources, environmental, water and agricultural planners are increasingly interested in the rational reuse of wastewater. Some priorities oriented to substitution policies (clean water for human consumption in exchange for treated wastewater to be used for cropland irrigation near population centres), involve major environmental benefits.

Wastewater cropland irrigation has been the traditional disposal method for municipal sewage in many countries due to the fact that farmers are aware of multiple advantages involved in such practices (Romero Alvarez, 1995). One of the first attributes is the potential increase of crop yields and, therefore, farmer's profits from local markets. Further justification of wastewater cropland irrigation include considerable savings from not using chemical fertilizers and the opportunity for several planting seasons. Such nutrient input can reduce the need for commercial fertilizers, whereas the mixture of wastewater and nutrients provides organic matter acts as a soil conditioner, thus increasing the capacity of the soil to store water.

Table 2.3 Increase of Crop Yields through Wastewater Irrigation.

Irrigation Water Category	Crop Yields (tonnes/hectare/year)				
	Wheat	Moong beans	Rice	Potato	Cotton
Raw wastewater	3.34	0.90	2.97	23.11	2.56
Settled wastewater	3.45	0.87	2.94	20.78	2.30
Stabilization pond effluent	3.45	0.78	2.98	22.31	2.41
Fresh water + Chemical fertilizer(N,P,K)	2.70	0.72	2.03	17.16	1.70

Source: Mara & Cairncross 1989.

Table 2.3 illustrates that, in fact, an effluent from conventional secondary treatment, with typical concentration of 15 mg/l of total phosphorous at the usual irrigation rate of about 2 metres per year, would supply a substantial load of nitrogen and phosphate for land application (300 and 60 kg/ ha/ yr, respectively).

Well-operated wastewater reuse schemes also provide the opportunity for land reclamation in semiarid settlements. A further positive effect from wastewater reuse is preservation of fresh and clean drinking water, which contributes to a cleaner environment by preventing discharges of sewage into rivers and lakes. If used to irrigate tree belts around urban settlements, wastewater reuse may contribute to the control of dust storms, thereby preventing land erosion and further desertification. In coastal areas, wastewater reuse may reduce or prevent water level depletion and salt intrusion due to over-pumping of ground water (Hespanhol, 1993).

Well-managed wastewater irrigation schemes have the potential to improve the overall socioeconomic conditions and quality of life in many rural communities by increasing the availability of jobs, harvests and the nutritional status and health of the population. This is especially important in semiarid areas of developing countries where conflicts between urban centres and agricultural suburbs are of growing concern. Potential negative effects of reuse programs that should be carefully monitored by environmental and health authorities are ground water pollution and disease vector breeding (e.g. mosquitoes), in addition to the health impact referred below.

2.4 EPIDEMIOLOGICAL EVIDENCE OF ENTERIC INFECTIONS AND DIARRHOEAL DISEASES ASSOCIATED WITH WASTEWATER REUSE.

Despite widespread wastewater reuse, epidemiological studies addressing the impact of this policy on health are scarce and few of those that exist utilize modern investigative methods. Except for half a dozen articles, publications often contain only descriptive anecdotal information. The following review focuses on three main aspects of enteric infections and diarrhoeal syndromes: first, the impact of raw wastewater on health, including risks from crop consumption; second, occupational risk and risk of living in communities exposed to wastewater irrigation; and third, the scarce evidence regarding the reduction of health risks resulting from effective pathogen removal through wastewater treatment. The final section summarises primary features of studies carried out in Mexico and current wastewater reuse related practices.

2.4.1 Consumption of vegetable crops irrigated with raw wastewater.

Several studies have demonstrated that the consumption of uncooked vegetables irrigated with raw wastewater is an important route of infectious disease transmission. One of the first known reports comes from Khalil, Egypt (Shuval *et al.*. 1986). This report describes prisoners eating crops cultivated inside the prison which had been irrigated with sewage. Although these vegetables were normally consumed cooked, infection through contact or consumption of raw vegetables must be considered ("the hands of all inmates working in the kitchen were contaminated") and probably contributed to the spread of *Ascaris lumbricoides* inside the prison.

Further circumstantial evidence of an association between raw wastewater irrigation and the consumption of vegetable crops and infection with *Ascaris lumbricoides* and *Trichuris trichiura* in Jerusalem has been reviewed by Shuval *et al.*. (1986). Information collected between 1932 and 1982 demonstrated an important decrease in these infections after interruption of the use of wastewater irrigation practices which occurred during the partitioning of the Israeli territory in 1948. When wastewater irrigation of vegetables was reintroduced, there was a steep rise in *A. lumbricoides* and *T. trichiura* infections among residents of Jerusalem. However, improvement of the socioeconomic level of the general population should be considered while interpreting the almost total disappearance of these infections. Apart from this, no data on the microbiological quality of the water was provided in the study.

A series of cholera outbreaks in Israel during the 1970s represent additional examples of the risk associated with consumption of vegetable crops contaminated through irrigation with raw wastewater (Fattal *et al.*. 1986). The first outbreak was shown to have been transmitted by consumption of salad crops irrigated with raw wastewater, which had been sold in markets around the city of Jerusalem. Cholera was not endemic in Jerusalem and, therefore, there was either low, transient or non-existent immunity to the pathogen. Originally, the outbreak was suspected of having originated from an imported clinical or subclinical case which had entered the city from a neighbouring epidemic area. Further surveillance, however, showed that during the epidemic, nearly 20% of wastewater samples were positive for the same serotype of *Vibrio cholerae* as that isolated from the majority of clinical cases. Cholera vibrios were also isolated from vegetables grown in wastewater irrigated plots (Shuval 1986). Interestingly, the outbreak subsided almost immediately after the vegetables irrigated with wastewater were confiscated.

In addition, it was observed that primary infections occurred also among farm workers (through occupational exposure), who then transmitted the infections to their families. A confounding factor in this study was that farmers may have consumed locally grown contaminated vegetables, so that both occupational and consumer exposures may have been parallel routes of transmission. Contaminated crops were, however, considered to be the main secondary route of transmission of the cholera outbreak and not occupational exposure. Further outbreaks in Israel have reported *V. cholerae* and phages from wastewater samples and irrigated vegetables, as well as from drinking water supplies. As a result of these outbreaks, authorities have enforced, with some success, a ban on raw wastewater irrigation of vegetables in Israel.

Recent reports of enteric infections in wastewater irrigated areas of Chile describe ecological conditions surrounding sewage farms, but do not provide solid epidemiological evidence of increased risk of enteric infections (e.g. typhoid fever, hepatitis; Monreal 1994). Several outbreaks of enteric infections transmitted mechanically through consumption of fish or clams harvested from faecally polluted waters have been reported. Briefly, unpublished reports from China indicate that individuals consuming raw fish (contaminated with untreated sewage) have a 62.2% prevalence of clonorchiasis (Ling Bo *et al.*. 1990) and that approximately 100% of the population in Guandong province who consumed raw or undercoated fish could have been infected with *Clonorchis sinensis.* Aquaculture practices short-circuit the faecal-oral route in these villages, resulting in a high risk of parasitic infections. The same source reported an outbreak of Hepatitis A virus (HAV) affecting 2 million individuals in Shanghai in 1988, attributed to the consumption of shellfish contaminated with raw sewage. Although these reports provide data regarding the populations concerned, they give no information about the microbiological quality of water in these areas.

In conclusion, consumption of uncooked crops irrigated with raw wastewater may be associated with enteric infections such as ascariasis, trichuriasis, cholera and viral hepatitis (Shuval *et al.*. 1986, Feachem & Blum 1984, Rose & Gerba 1991). Theoretically, other risks are possible (e.g. protozoan infections), but have not been adequately documented. In areas where viral infections are endemic, it is difficult to detect any excess risk of infection due to wastewater exposure since the population develops certain immunity based on early life exposure due to poor hygiene and low sanitation in the immediate environment (Shuval 1986; Table 2.4).

2.4.2 Health effects from occupational risk and risk of living in communities exposed to wastewater irrigation.

One of the pioneering studies on the risk of intestinal parasitic infections in farm workers exposed to raw sewage was carried out in India by Krishnamoorthi and colleagues in 1973. The original report (reviewed by Shuval 1986) illustrated a significant excess of *Ancylostoma duodenale, A. lumbricoides and T. trichiura* infections among farmers exposed to flood irrigation with raw wastewater. The prevalence of all parasitic infections combined was 87% in farm workers exposed to raw sewage (n=466), while it was 50% for controls (n=432). While 70% of the farmers using raw sewage had hookworm infection, only 33% of the controls were infected. The difference in *A. lumbricoides* infection rates between farm workers using sewage and controls was even greater than that for other infections (47% vs. 13%, respectively). In addition to the increase in infection prevalence, this study also reported an increase in intensity of the parasitic infections. When data from this study was reanalysed (Shuval 1986: 89), evidence of secondary health effects (e.g. hookworm infection and high rates of anemia) were found. However, the study does not provide relevant information regarding characteristics of the control population, wastewater quality or any measurable definition of exposure.

Table 2.4 Relative Health Risks from the Use of Untreated Excreta and Wastewater in Agriculture and Aquaculture.

Type of Pathogen/Infection	Excess Frequency of Infection or Disease
Intestinal nematodes *Ascaris lumbricoides* *Trichuris trichiura* Hookworms	High
Bacteria Bacterial diarrhoeas (e.g. cholera, typhoid)	Lower
Viruses Viral diarrhoeas Hepatitis A	Lowest
Trematodes and cestodes Schistosomiasis Clonorchiasis Taeniasis	High to nil, depending upon the method of excreta use and local circumstances

Source: Shuval *et al.*. 1986.

The use of night soil and sewage in rural farms in China has been reported by the Chinese Academy of Preventive Medicine (Ling Bo *et al.*. 1990). The areas described in these reports practice self subsistence farming and thus a considerable share of their harvests are locally consumed. The prevalence of ascariasis, ancylostomiasis and trichuriasis in rural populations practicing night soil farming was 93.8%, 65.0% and 92.5%, respectively. The studies demonstrated the presence of parasite eggs on vegetables and a relationship between the presence of ova and the distance of vegetables from ground level. However, no detailed information was given regarding the study population or on the type of exposure, and no control population was included in the study. The authors highlighted the possible synergism of occupational and consumer risk in rural environments of developing countries.

Despite the report of no health risks for workers exposed to sewage in studies conducted in developed countries (Burge & Marsh 1978), recent outbreaks of enteric infections in sewage plant workers have raised the question of possible occupational risk in these individuals. A report from Norwich (UK) has shown a higher prevalence of protozoan infections (e.g. *Giardia lamblia*) in sewage plant workers as compared with the general population (Jefferson & Betton 1991). However, another study from India found that 25% of sewage workers were infected with *Ancylostoma duodenale*, compared with 7.7% in controls. Contrary to the report from Norwich, the study from India showed no significant differences between the prevalence of *G. lamblia* among workers exposed to treated versus untreated sewage (Sehgal & Mahajan 1991). Other studies from Egypt reported higher prevalence of *Entamoeba histolytica* and helminth infections in sewage workers than in general populations (Hammouda 1992). Low standards of hygiene in India and Egypt may explain the high prevalence of protozoan infection in the general population: those exposed to sewage and wastewater however, may become resistant to the infection, while the general population acquires some immunity from repeated exposure.

Situations like those described above may be different in industrialized countries. Although there is potential risk in these latter populations, there are no studies relating the microbiological quality of sewage with health problems in populations and this risk appears to have limited relevance for the health of farming groups exposed to wastewater of varying qualities. Sewage workers from three cities in the United States were tested for 28 different viruses (Clark *et al.*. 1981). A study of 500 volunteers, including controls, found higher gastrointestinal illness rates among inexperienced workers as compared with experienced or control workers. However, immunoglobulin levels were not significantly higher in wastewater-exposed workers as compared with controls. The inadequacy of clinical diagnostic criteria, the small sample size studied and the lack of appropriate environmental data all shed doubt on the study's conclusions.

In another study carried out in Muskegon, Michigan, workers at a spray irrigation station (n=35) and road workers (n= 41) were compared for prevalence of viral infections (Linnemann *et al.*. 1984). Low-pressure spray irrigation workers from corn farms were classified into high, intermediate and low categories according to levels of exposure to partially treated sludge (lagoon). Study participants (including control individuals chosen from distinct occupational groups) were medically examined monthly and data were maintained in the form of clinical records. Samples from wastewater influent and air (collected during spraying) were cultured for viruses and bacteria, although no data on the quality of effluent was given. Although various viruses were isolated from the wastewater influent to the aeration basin, none were recorded from the influent to the irrigation rigs or from air samples. Monthly viral cultures from the irrigation workers were negative and there was no seroconversion even among seasonal workers. In addition, there were no significant differences in either clinical illness or antibody levels between the exposure groups. After adjustment for age, there were no significant differences between either Hepatitis A or legionnaire's disease antibodies between the study groups. The study's conclusions were that workers in direct contact with partially treated wastewater had a very low risk of infection with the viruses and bacteria studied, although this risk was detected in the group with the highest exposure.

The same research group also studied the risk of infection with Norwalk agent and rotaviruses among wastewater treatment plant workers (Clark *et al.*. 1985). Sera from a group of workers (n=48) and from controls were tested for antibodies to rotavirus, Norwalk agent and *Prototheca sp.* Observations on the work environment and air samples were used to categorize aerosol exposure levels. Exposure was determined by questionnaires and by observation, and workers were categorized according to period of experience in their job. The study was conducted over a period of 42 months and data were analysed according to antibody response, age, sex, geographic location, exposure group and socioeconomic status. Results revealed that inexperienced wastewater-exposed workers had higher levels of antibody to Norwalk agent than did experienced or control workers. Analysis for *Prototheca* antibody was negative. The authors concluded that antibody levels were not related to the length of wastewater exposure and that occupation in modern treatment plants does not pose a major risk of viral infection to wastewater workers.

Parasitic infection rates (protozoan and nematode) between sewage workers (n=56) and controls, in this case highway maintenance workers (n=69), were studied in a prospective survey carried out in Cincinnati (Clark *et al.* 1984). Sewage workers were divided into two main exposure categories: direct and indirect contact with sewage. Surprisingly, highway workers had a higher rate of infection (14.5%) as compared with sewage workers (5.4%). However, they reported that all infections were asymptomatic and that most of the parasites identified were non pathogenic (*Entamoeba coli* and *Endolimax nana*). Only four cases of pathogenic protozoa were detected (*Isospora sp.* and *G. lamblia*). One of the study conclusions was that low infection rates were perhaps due to the low prevalence of infection in the general population.

2.4.3 Health effects from occupational risk and risk of living in communities exposed to partially treated wastewater.

A series of epidemiological studies on the potential transmission of salmonellosis, typhoid fever, shigellosis, infectious hepatitis and "enteric diseases" through partially treated wastewater irrigation were carried out by Katzenelson *et al.*. (1976), Fattal *et al.*. (1986) and Shuval *et al.*. (1989) in populations of agricultural communities in Israel, where partially treated wastewater is used for cropland irrigation. Methodological inconsistencies of the first two studies, led to conflicting results making it impossible to demonstrate significant excess of enteric diseases in effluent-irrigating kibbutzim. Major methodological problems involved misclassification of exposure of the inhabitants of kibbutzim, information bias, misuse of "control" diseases and loose definitions of outcomes.

Further prospective studies evaluating the association between partially treated wastewater sprinkler irrigation and enteric infections were conducted taking into consideration the previous methodological flaws, both through serological and morbidity surveys (Fattal *et al.*. 1986, Shuval *et al.*. 1989). The first of these prospective studies involved 30 kibbutzim, which were divided into three major categories, and six subcategories, according to wastewater utilization practices and distance from residential areas. The first category used sprinkler irrigation with wastewater effluent within 600 meters of residential areas, the second category included kibbutzim at 1,000 m or more from residential areas. This latter group was assumed not to have been exposed to aerosol sewage, but to have been exposed through contact with sewage irrigation workers. The third major category consisted of kibbutzim not using wastewater for any purpose. The wastewater effluent used in all kibbutzim for sprinkler irrigation had undergone partial treatment (5 to 10 days of retention in waste stabilization ponds), which resulted in effluent of poor microbiological quality: 10^6 - 10^8 total coliforms/100 ml. The outcomes (enteric diseases) were defined through clinical record monitoring and from two serum specimens for viral antibodies (infectious hepatitis, coxsackie viruses, ECHO, polio viruses and varicella zoster). Most of the former are transmitted by the fecal-oral and fecal oral/respiratory routes, whereas varicella-zoster virus was included as a control infection. Drinking water, aerosol and wastewater samples were monitored for viruses and bacteria, the results of which suggested, according to the researchers, the possibility of transmission of pathogens through aerosols.

The analysis of all outbreaks of enteric diseases did not provide evidence of excess outbreaks associated with wastewater irrigation. The only significant difference in prevalence between exposed and control populations were for ECHO 4 virus (this antibody increase was observed only in the 0-5 yrs age group) and for antibodies to *Legionella pneumophilia.* Morbidity data, however, did not support an excess of clinical disease or reported illness, although the authors concluded that under non epidemic conditions, exposure to aerosols or indirect contact to the wastewater does not normally lead to an increase in prevalence of viral infections. Although the evidence is not conclusive, the authors concluded that viruses from wastewater may spread to agriculturally adjacent populations resulting in a higher risk of viral infections (not clinical disease) in highly susceptible children. They suggested that in exposed communities, increased transmission of enteric viruses occurs to the highly susceptible young age group during the irrigation season and that this transmission decreases at other periods of the year. In noneffluent-irrigating communities, constant spread by multiple concurrent routes occurs so that the level of transmission is the same in all age groups over the entire year.

In the second prospective study, a total of 20 agricultural communities were evaluated on a matching basis (Shuval *et al.*. 1989). Wastewater used for irrigation was partially treated in stabilization ponds and resulted in an effluent with 10^4 - 10^5 faecal coliforms/ 100 ml. All kibbutzim had similar irrigation periods and were divided into three categories: the first was exposed to sprinkler irrigation within 300-600 m of the residential area; the second consisted of communities using wastewater, but no exposure to aerosols; and the third category was a control which used water from clean sources. A selected list of "enteric conditions" reported to the health posts was chosen as the outcome for the study. Overall rates of these "enteric conditions" for all ages were not significantly different among the three exposure groups. In contrast to the previously described retrospective studies, the authors concluded that wastewater irrigation workers and their families may be protected from a range of enteroviruses by their high levels of immunity.

Most of the previously described studies are methodologically deficient in that they lack a clear definition of outcome, they used inadequately small sample sizes and they lack quantitative data on the microbiological quality of wastewater, all of which result in misclassification of exposure. These deficiencies made it difficult, if not impossible, to interpret the findings from these population studies.

It is possible to summarize: a) Credible epidemiological data regarding risk reduction resulting from pathogen removal by wastewater treatment is notoriously scarce. Few available data overlap studies on populations living near reuse sites, and occupational exposure. Conflicting results arise from lack of adequate wastewater reuse health criteria (i.e. the microbiological standards for wastewater reuse prevailing until the last few years), and ambiguous meaning in the real world. In fact, the Israel experience has forced a more critical approach to existing standards, and the development of new guidelines based on epidemiological, rather than microbiological criteria. b) The actual risk associated with treated wastewater irrigation may be much lower than previously estimated. The consensus today is that early standards for effluents used for unrestricted irrigation (e.g. vegetables and salad crops usually eaten uncooked) have been unjustifiably restrictive, particularly in respect of bacterial pathogens. c) Available evidence indicates that it is unjustified to disregard intestinal parasitic infections (i.e. helminths) which represent a primary risk associated with the use of insufficiently treated wastewater.

Based on current knowledge and gaps, the revised World Health Organization guidelines (WHO, 1989) has relaxed the coliform requirement for unrestricted irrigation, and added an intestinal nematode guideline to protect both consumers of crops as well as farmers occupationally exposed to wastewater. To date, however, there has been no evidence assessing the effects of exposure to wastewater at the new recommended levels, achievable by wastewater treatment (e.g. using waste stabilization ponds). Finally, there is an urgent need for data evaluating the epidemiology of protozoan infections, diarrhoeal syndromes and other enteropathogens (i.e. viruses) in situations where the WHO quality guidelines have and have not been met.

2.4.4 Epidemiological evidence in Mexico of the impact on health of wastewater reuse in agriculture.

Epidemiological evidence of wastewater reuse from Mexico is scarce and those studies that do exist are in the form of unpublished reports and postgraduate theses. One such report describes the potential risks of wastewater irrigation on the health of young school children in the Mezquital Valley, Irrigation District 03, as compared with a control community not practicing wastewater irrigation (Sanchez Leyva, 1976). A total of 405 children were included in that study (207 from the wastewater communities and 98 individuals from the "control" population). Protozoan and helminth infections as well as diarrhoeal diseases were the main outcomes which were assessed through the evaluation of stool samples and individual interviews for diarrhoeal diseases. The recall period for the latter was three months. There was no significant excess in prevalence of intestinal parasites or diarroeal diseases in children from wastewater-irrigating villages, despite "agricultural compounds near irrigated fields which provide plenty of chances for children to come into direct contact with wastewater flowing throughout the canals and plots". No information on irrigation water quality or to treatment through retention in the nearby storage reservoir is provided in these reports. Furthermore, no details were given regarding the type of exposure concerned, the authors used an unduly long diarroeal disease recall period, the study population was insufficiently small and no consideration was given to the farmers' exposure.

Another unpublished report from Rivera (1980) describes the comparison of intestinal infection prevalence (e.g. bacteria, protozoa and helminths) in two agricultural communities, one which used raw wastewater and a control area. Local health service records from a five year period were used to determine the prevalences of these infections. Enteric infections, particularly amoebiasis, were more prevalent in those communities irrigating with raw wastewater as compared with control areas which used fresh water for irrigation. The authors suggested that the increase paralleled the expansion of both the sewage system in the metropolitan area of Mexico city and the wastewater irrigation network in Tula, Hidalgo, in the 1970s. Due to the presence of pathogens in the drinking water supplies (from wells) it was concluded that the reliability of information gathered from the health services was limited for this type of analysis.

In another attempt to study the impact of wastewater use (from waste stabilization ponds) on the health status of agricultural workers, Rivera and Acevedo (1985) compared 50 workers exposed to raw sewage with 50 farmers irrigating with ground-water. Data were collected from questionnaires and stool parasitology and the study found a significant increase in prevalence of *G. lamblia* infection in the exposed group (17% vs. 4%, respectively). The prevalence of *A. lumbricoides* was also considerably higher in the exposed group (50% vs. 16%), although the prevalence of amoebiasis was equally high in both groups (80%). Interestingly, it was discovered during the course of the study that the irrigation influent had been diverted by local farmers, so that raw sewage was used for irrigation, and not treated wastewater.

None of the latter studies provide direct evidence of major health risks involved in raw wastewater reuse. The length of recall periods used in the studies was too long, the quality of information used for analysis was unreliable (i.e. medical records from the local health services), data on actual exposure was not adequately recorded and the irrigation water quality was not adequately assessed. In addition, the last of these studies used insufficient sample sizes to detect significant differences and none of the previous studies appropriately defined exposure.

2.5 EVOLUTION OF HEALTH GUIDELINES AND REGULATIONS FOR THE SAFE USE OF WASTEWATER.

The first health regulations regarding wastewater reuse were implemented in the state of California, United States, in 1918 (Calif. State Dept. Hlth. 1968, Shuval 1991). These guidelines were developed during an adverse period for agriculture and the economy, due to the current drought, and sewage farming became an attractive solution for disposal of urban waste, as well as a means to increase crop yields for greater profits. Microbiological methodology improved over this same period, opening the way for detection and identification techniques for a wide range of pathogens. Concurrently, a growing concern developed regarding the potentially negative impact of wastewater reuse on the health of communities. On the one hand, the state governments needed to identify inexpensive water resources for irrigation, through which they could generate income (e.g. taxes from crop production), and on the other, these authorities did not want to face epidemics resulting from wastewater reuse. Both concerns contributed to the development of pioneering wastewater reuse regulations.

Shortly after their implementation, California's regulations were modified and tightened (Ongerth & Jopling 1977). One of the major components amended was that of crop restriction recommendations (Table 2.5). A quality standard of 2.2 coliforms/100 ml was recommended for wastewater applied to crops eaten raw, a similar threshold for drinking water quality. Such standards, however, could only be achieved by highly sophisticated biological treatment, followed by heavy chemical disinfecting (Shuval 1964). The resulting regulations remain the most restrictive in use to date.

California's regulations were rapidly adopted in many developing countries soon after World War II. By 1973, however, the World Health Organization recognized that recommendations to achieve a standard of 2.2 coliforms/100 ml were impractical in many developing countries. In addition, most rivers in Europe and North America commonly had higher coliform counts than allowed by this guideline. Subsequently, the WHO recommended certain treatment processes and new, less restrictive guidelines, were issued. At that time, the guidelines recommended effluent with no more than 100 coliforms/100 ml in 80% of water samples for unrestricted irrigation (WHO, 1973). Although these standards are less stringent that those implemented in California, they are currently considered unjustifiable (given the absence of epidemiological data). California's regulations and the 1973 WHO guidelines are both examples of "maximum feasible" approaches, but also reflect incongruencies based on a "zero risk" concept. As noted above, natural rivers in developed countries rarely meet these standards, despite the fact that they are a major source for unrestricted irrigation and bathing (Shuval 1991).

Table 2.5 California State Departament o⊡f Health Standards for Safe and Direct Use of Reclaimed Wastewater for Irrigation and Recreational Impoundments.

	Description of Minimum Required Wastewater Characteristics			
Use of Reclaimed Wastewater	Primary[a]	Secondary and Disinfected	Secondary Coagulated Filtered[b] and Disinfected	Coliform MPN/100ml Median (daily sampling)
Irrigation				
Fodder crops	x			N R
Fiber crops	x			N R
Seed crops	x			N R
Produce eaten raw, surface irrigated		x		2.2
Produce eaten raw, spray irrigated			x	2.2
Processed produce, surface irrigated	x			N R
Procedded produce, spray irrigated		x		23
Landscapes, parks, etc.		x		23
Creation of impoundments				
Lakes (aesthetic enjoyment only)		x		23
Restricted recreational lakes		x		2.2
Non-restricted recreation lakes			x	2.2

a Effluent not containing more than 10 ml/L/h settleable solids
b Effluent not containing more than 10 turbidity units
Source: Ongerth & Jopling 1977.

In 1983, the World Bank/UNDP initiated a comprehensive review of research conducted on the quantifiable impact of wastewater irrigation on health and the reevaluation of current standards (Shuval et al. 1986). One of the main contributions of that evaluation was the resolution regarding overly conservative public health policies. Soon after the publication of this report, a group of scientists met in Engelberg, Switzerland, and reviewed available epidemiological evidence gathered by Shuval and colleagues (1985). The group formulated new microbiological water quality guidelines for treated wastewater reuse in agricultural irrigation. These revised guidelines liberalized earlier "zero risk" criteria, suggesting that effluents containing less than or one helminth egg per litre and a geometric mean faecal coliform concentration of 1000/100 ml could be used for crops eaten raw (Engelberg, 1985). The primary purpose of these guidelines was to provide criteria for both effective helminth egg and faecal coliform removal, as well as faecal coliform removal for unrestricted irrigation, mostly through the use of appropriate wastewater treatment systems in less developed areas. The Engelberg report did not refer specifically to all helminths and protozoa of public health importance, but its rationale implied that recommended guidelines would perform well as indicators for most of the settleable pathogens, including some protozoa (Shuval 1988).

The WHO scientific working group on health guidelines for the use of wastewater in agriculture and aquaculture convened in Geneva at the end of the last decade. Based on data available at that time, the group recommended further relaxation of the coliform guideline for unrestricted irrigation and the addition of a nematode guideline to protect both consumers of crops as well as farmers occupational exposed to wastewater (Table 2.6). These new guidelines were developed to assist engineers and health planners in the choice of technical alternatives to achieve required standards. Given appropriate design of treatment systems, wastewater treated by natural processes and long hydraulic retention can remove helminth ova by sedimentation (Saenz-Forero 1994). Recommendations, therefore, evolved to protect both consumers and agricultural workers. However, proposed guidelines are being implemented, despite scant epidemiological evidence and little data of their impact on health (Stott *et al.* 1992).

Table 2.6 Recommended Wastewater Quality Guidelines for use in Agriculture, WHO,1989.

Category	Reuse conditions	Exposed group	Intestinal nematodes[b] (arithmetic mean eggs per litre[c])	Faecal coliforms (geometric mean per 100 ml[c])	Wastewater treatment required to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked sports fields, public parks[d]	Workers, consumers, public	≤1	≤1000[d]	A series of stabilization ponds designed to achieve the water quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops fodder crops, pasture and trees[e]	Workers	≤1	No standard	Retention in stabilization ponds for 8-10 days or equivalent, helminth and faecal coliform removal
C	Localized crop irrigation in category B if no exposure of workers and the public	None	Not applicable	Not applicable	Pre-treatment as required by irrigation technology, but not less than primary sedimentation

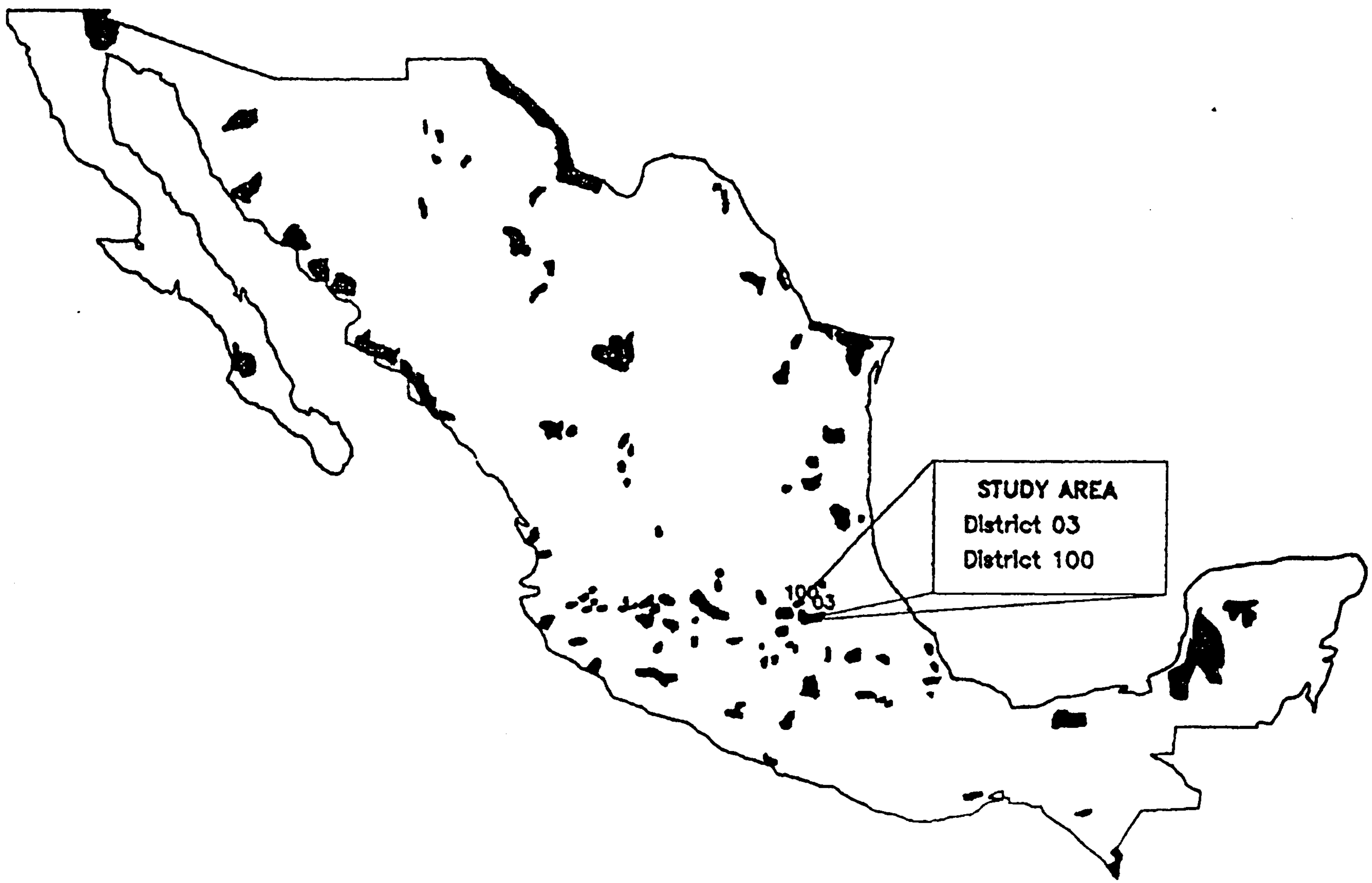
a. In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.
b. *Ascaris* and *Trichuris* species and hookworms.
c. During the irrigation period.
d. A more stringent quideline (< 200 faecal coliforms/ 100 ml) is appropriate for public lawns, such as hotels, with which the public may come into direct contact.
e. In the case of fruit trees, irrigation should cease before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

2.6 WASTEWATER REUSE IN MEXICO.

Approximately 85% of water resources in Mexico are located at an altitude below 500 meters above sea level, whereas 70% of the population lives in settlements above such level. Estimates of overall fresh water deficits are 30%, although the actual figure in slums and remote rural areas is considerably higher. It is estimated that wastewater is produced by major cities at the rate of approximately 160 m^3/sec, creating a severe dilemma for disposal, given potential environmental and public health consequences (Romero Alvarez 1995). Nearly 80% of agricultural land in Mexico is dependent on irrigation schemes, and it is estimated more than 250,000 hectares are currently irrigated with raw wastewater (Table 2.7, from Mejia 1993). Available information indicate that nearly 800,000 hectares need to be incorporated into current irrigation programmes in the next few years, in order to cope with future food demands. A rational approach will require sound consideration of several conflicts between rural and urban areas. In order to relieve increasing fresh water deficits, water conservation programmes have resorted to increasing land application of wastewater in areas with low annual rainfall (PRONAR, 1986).

Water resources, including wastewater, are administered in Mexico by the Sub-Secretaries of Agriculture and Irrigation Resources (SARH and CNA). The existing 77 irrigation districts (ID) have evolved within this institutional framework, and are responsible for 60% of irrigated land (Figure 2.1). Thus, SARH and CNA, along with the Minister of Health and Social Security (SSA), are the responsible authorities regulating water quality guidelines for agricultural production, as well as for other health protection measures. The target population for this guidelines are primarily consumers. Existing national regulations set a microbiological standard of no more than 1,000 total coliforms/100 ml, for water used to irrigate crops which are consumed raw (SSA 1990). Until the early 1990s, no irrigation water quality restrictions existed for crops consumed cooked or for those that do not come into contact with soil. Since the wastewater used in the irrigation districts is mostly untreated, the crops that can be grown are restricted to fodder crops, those grown above ground or those eaten cooked. Current information indicates that 250 000 hectares in the whole country are irrigated with untreated wastewater. Crop restriction represents the primary health protection policy for wastewater use in Mexico, and it is recognized that the target population for these regulations are primarily the consumers of agricultural products. Crop restriction, however, is not totally effective; in the early 1990 it was estimated that about 25 000 hectares (10% of the total irrigated area) were cultivating vegetables and crops eaten raw (Mejia, 1993). Lettuce, cabbage, beetroot, coriander, radish, carrot, spinach and parsley are increasingly prohibited for wastewater irrigation, despite conflicts with farmer's associations, defending their profits. A further issue is the widespread availability of non-cultivated wild-greens, which are part of the local staple diet, and cannot be regulated.

Figure 2.1 Irrigation Districts



The National Wastewater Reuse Programme (PRONAR) is the surveillance and regulatory agency which assesses and upgrades available water resources at the national level (PRONAR 1986). It establishes and plans for future wastewater reuse, which currently targets to expand the irrigation system to include a further 430,000 hectares. In order to complete this expansion, PRONAR is currently developing feasibility proposals for nationwide reuse schemes.

Table 2.7 Major Agricultural Schemes in Mexico which use Wastewater for Irrigation.

CITY	ANNUAL VOLUME ($10^6/m^3$)	IRRIGATED SURFACE (hectares)	PRIMARY CROPS
1. ACAMBARO	1.24	112	wheat, maize,
2. AGUASCALIENTES	41.94	3,813	wheat, maize,
3. CELAYA	10.44	949	wheat, maize,
4. CHIHUAHUA	37.56	3,414	cotton, wheat,
5. CHILPANCINGO	1.72	156	copra, beans,
6. COLIMA	10.86	987	maize, beans,
7. CO.LAGUNERA	44.84	4,076	cotton, alfalfa,
8. DURANGO	23.79	2,157	wheat,sorghum,
9. FRESNILLO	6.25	568	alfalfa, maize,
10. HERMOSILLO	27.88	700	citrus, wheat,
11. LA PAZ	6.90	627	wheat, cotton,
12. LA PIEDAD	2.88	262	maize, beans,
13. LEON	40.55	3,686	maize, beans,
14. MATAMOROS	10.24	931	wheat, maize,
15. MONCLOVA	6.00	504	wheat, sorghum.
16. MONTERREY	158.85	14,440	citrus, wheat,
17. MORELIA	24.44	2,223	maize, beans
18. NAVOJOA	2.32	211	sesame,cotton,
19. OAXACA	2.61	237	maize, beans
20. CD. OBREGON	36.04	3,277	cotton, maize,
21. REYNOSA	7.73	702	cotton, beans,
22. SALTILLO	26.16	2,378	wheat, sorghum
23. SAN LUIS (Son)	14.37	1,036	wheat, saffron,
24. TEPIC	8.61	783	beans, maize,
25. TOLUCA	18.32	1,666	maize, beans,
26. ZACATECAS	4.68	425	barley, maize,
27. MEZQUITAL VALLEY ** IRRIGATION DISTRICT 03 **	980.00	87,200	maize,alfalfa, oats, greens
28. PUEBLA	6.20	17,583	maize, alfalfa,
29. CD. JUAREZ	31.40	3,000	cotton, wheat,
30. TULANCINGO	3.40	300	alfalfa, maize.
TOTALS	1617.76	156,453	

* Source: SARH "Wastewater Irrigation Districts in Mexico".

In the near future, wastewater reuse schemes will seek to incorporate a wider scope of health protection strategies other than crop restriction (cereal, fodder, etc). Crop export requirements will contribute to improved practices, although current financial constraints may hinder certain health and environmental protection measures. SARH is currently expanding to include management of a further eleven (possibly twelve) additional IDs. Recent estimates suggest a potential area of a further 230,000 hectares to be irrigated with wastewater in the 1990s. This expanded programme would release demands on first-use water for domestic use by nearly 30 million inhabitants from major urban areas. Essential characteristics of the SARH expansion programme are outlined in Table 2.8.

PRONAR's primary objectives for this expansion programme are initially to improve actual wastewater reuse. Where wastewater does not meet quality standards, SARH and health planners will seek to enforce crop restrictions and wastewater treatment through decentralised schemes or through private companies. A secondary objective is to promote an extended and safe use of this water supply to areas with restricted water resources, while reducing environmental contamination and restoring degraded areas through a rational wastewater reuse scheme.

Table 2.8. Wastewater Reuse Planned in Mexico.

CITY	Total area which can be irrigated (hectares)	Annual wastewater flow available as % of total irrigation water supplied
SINALOA	223 000	1.3
GUANAJUATO	102 000	5.6
B. CALIFORNIA N.	207 000	1.5
MORELOS	34 600	2.6
COAHUILA & DURANGO	150 000	2.1
MICHOACAN	33 900	7.2
TAMAULIPAS	79 500	1.5
SONORA	93 800	1.3
ZAMORA (Mich)	17 900	2.0
V. DEL FUERTE (SINALOA)	223 000	0.2
VERACRUZ	1 600	2.6

** Source: Strauss & Blumenthal (1992)

Other wastewater reuse projects in Mexico include land reclamation of saline soils for crop production, the halting of further desertification on the edge of Mexico City (Lake Texcoco). This latter project involves 10,000 hectares within the Mexico Valley, which prior the Spanish colonial times was covered by a large lake, whith remarkable agricultural and transportation features. This area currently receives water from sewage, which has been treated in an activated sludge plant and in a waste stabilization pond system. The reestablished lake will be used for irrigation, aquaculture, reforestation and bird sanctuaries. Other wastewater recycling projects in Mexico are targeted for irrigation of main urban green-belts, recreational parks and reuse of industrial wastes. At present, there are a total of 130 sewage treatment plants (STP) (activated sludge) located in major cities. Reuse of chlorinated effluent from these STPs for recreational and industrial purposes is a common practice, although a large proportion of these schemes do not use water resources efficiently (Strauss & Blumenthal 1992).

2.7 SUMMARY

Available evidence indicates that consumption of uncooked crops irrigated with raw or insufficiently treated wastewater is associated with an excess of enteric infections, particularly ascariasis, trichuriasis and cholera. Other potential risks exist, but have not been adequately documented (e.g. protozoan and viral infections).

In endemic areas, farmers exposed to raw or insufficiently treated wastewater may have increased infections with *Ancylostoma duodenale, Ascaris lumbricoides and Trichuris trichiura* . Evidence exists that there is a higher prevalence of protozoan and hookworm infections in sewage plant workers (e.g. *G. lamblia, E. histolytica* and *A. duodenale*).

It has not been demonstrated that sprinkler irrigation with partially treated wastewater facilitates spread viruses and bacteria in exposed communities, since there is a lack of data demonstrating actual risk of disease transmission. In endemic areas, most infections with enteropathogens occur in early life, due to low standards of hygiene and sanitation. Prospective studies have failed to demonstrate an increase of enteric diseases in individuals exposed to partially treated wastewaster.

Epidemiological data demonstrating risk reduction resulting from effective enteropathogen removal through wastewater treatment is notoriously scant. Since there have been no further studies demonstrating the effects of exposure to wastewater at the new recommended levels, the 1989 WHO guidelines and choices of technical alternatives to achieve required water quality standards remain unevaluated.

The safe use of wastewater involves compliance with microbiological quality guidelines and these guidelines require continuous evaluation. Epidemiological monitoring will yield data which can be used by water and health planners not only regarding choices for appropriate technologies required to achieve the recommended guidelines, but also for a range of additional health protection measures.

CHAPTER 3. DESCRIPTION OF THE STUDY AREA

3.1. INTRODUCTION.

Relevant features of the study area and its population will be described in this chapter. The first two sections are general descriptions of its inhabitants and their agricultural activities, while section three provides a brief summary of the institutional framework regulating both social policies and economic factors and irrigation policies. Sections four and five focus on the description of the irrigation system and irrigation network, whereas sections six and seven emphasize the importance of crop cultivation and occupational exposure to wastewater.

3.2. GENERAL CHARACTERISTICS OF THE MEZQUITAL VALLEY.

The study area is commonly known as the Mezquital Valley. It is situated in the country's central plateau, 120 miles north of Mexico City, in the state of Hidalgo. The Mezquital Valley itself covers nearly 30% of the total surface of the state territory (Figure 3.1). The region is approximately 2,000 meters above sea level (6,500 ft.) and the average annual temperature is 18° C. (maximum 34.5° C, minimum 5.5° C). It is a semiarid region, with a mean annual rainfall of 450-500 mm, which falls mostly during the months of July, August and September over 70% of the state. Due to the disparate distribution of rainfall during the course of the growing season, a quarter of the state of Hidalgo is currently receiving irrigation, mostly with urban wastewater.

The total population in 1990 was 485,000, of which nearly 20% were below the age of 10 years (Table 3.1). The annual growth rate is estimated at 2.23% and the population density in the Mezquital Valley is 85 inhabitants/km^2, twice the national average and one of the highest in the country.

According to criteria of the National Population Council, 75% of the population in the Mezquital Valley live in a rural environment. However, important socioeconomic differences exist between rural and urban settlements within the state of Hidalgo (INEGI 1990). Almost 10% of the population is made up of the Nha-nhu indigenous group, most of whom live under the poverty level (INI 1992). In most of the agricultural communities in the Mezquital Valley, up to 30% of adults are illiterate and nearly half of the individuals over 15 years of age do not complete primary education (Table 3.2). Hygiene and sanitary conditions in agricultural villages can be summarised by the fact that three quarters of the households lack human waste disposal facilities and 30% of the dwellings have no piped running water (Censo Nacional de Poblacion 1990). This figure may be lower in urban centers equipped with sewage collection systems.

Figure 3.1 Map of the Mezquital Valley, State of Hidalgo, Mexico

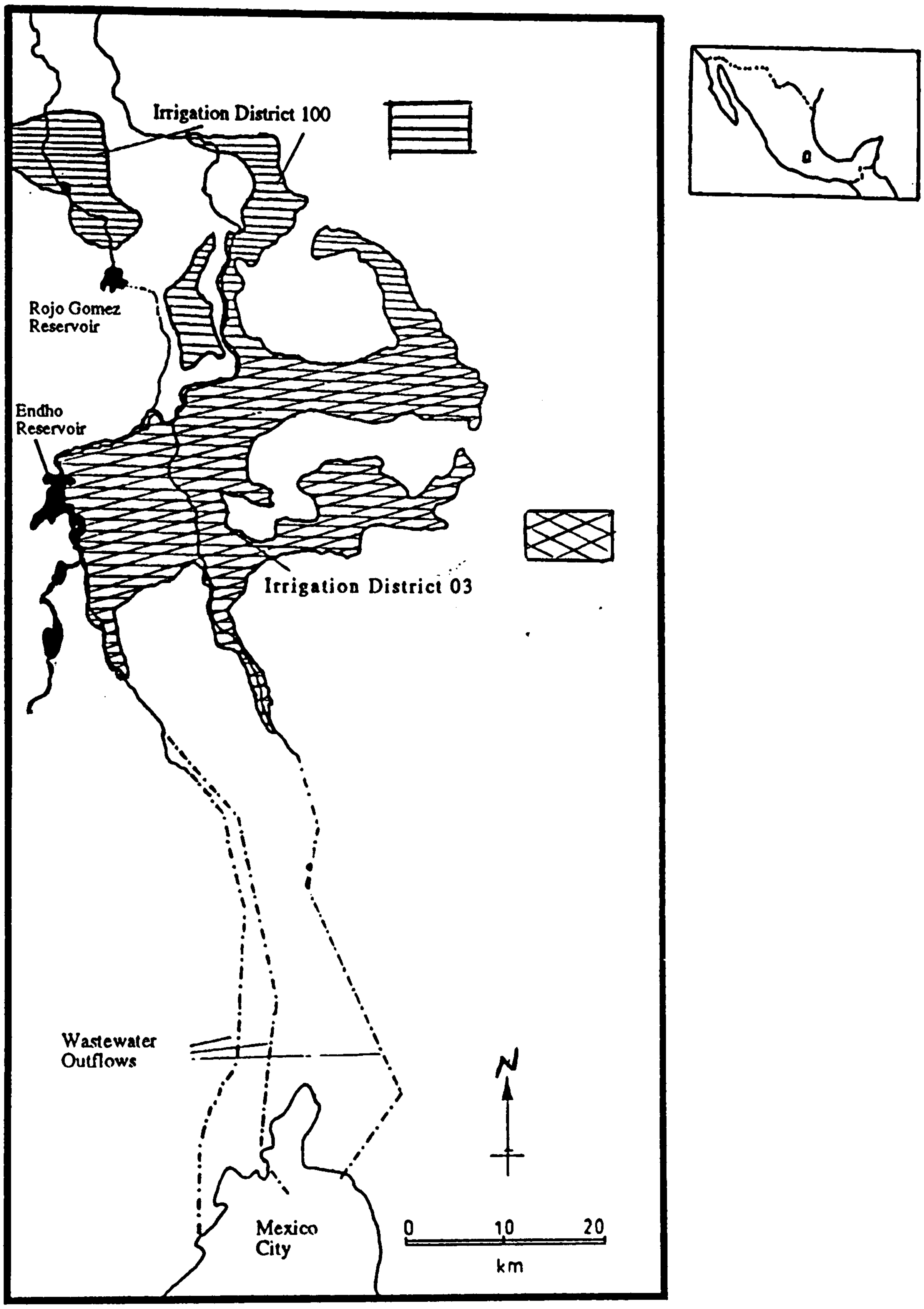


Table 3.1 Age and gender distribution for the state of Hidalgo, 1990.

AGE	GENDER MALE	 FEMALE
	%	%
LESS THAN 1 YEAR	1.3	1.2
1-4 years	5.3	5.3
5-9 years	7.7	7.0
10-14 years	6.6	7.6
15-19 years	5.8	5.7
20-24 years	4.1	4.4
25-29 years	3.5	3.8
30-34 years	2.9	3.2
35-39 years	2.5	2.3
40-44 years	1.8	1.9
45-49 years	1.7	2.0
50-54 years	1.7	1.5
55-59 years	1.0	1.1
60-64 years	0.9	0.9
65 AND OLDER	2.1	2.0
NOT SPECIFIED	0.7	0.5
TOTAL	49.5	50.5

Source: Dirección General de Epidemiología, 1988

Table 3.2 Social Profile, Population of the Mezquital Valley, 1990.

Characteristics	Urban	Rural
Population aged over 15 years,		
- illiterate	7%	30%
- incomplete primary education	30%	47%
Households		
- without sanitary facilities	28%	75%
- without piped water	10%	30%
- dirt floor	7%	51%
- in lowest income categories	57%	81%

Source: Censo Nacional Poblacion 1990

As in most of rural Mexico, the basic foodstuffs in agricultural villages of the Mezquital Valley are maize (almost invariably eaten as tortillas), dried-cooked beans and chilli peppers. Meat, milk, eggs and poultry consumption varies according to the purchasing capacity of each family and to a less extent to self subsistence practices. Supplementary diet includes almost every conceivable edible plant as a source of vegetables which are locally available, and the fermented agave juice (pulque). A large range of cacti, wild greens (quelites) and a variety of insects are prepared in traditional dishes providing the ethnic populations a diet of considerable variety and nutritional content (Anderson *et al.*. 1946).

3.3 INSTITUTIONAL FRAMEWORK OF THE IRRIGATION DISTRICTS (ID) AND MICROBIOLOGICAL QUALITY OF WATER.

Since 1989, the National Water Commission (CNA) has administered all water resources in Mexico. In the study area, wastewater administration is controlled through the "Irrigation Districts" 03 and 100 (ID 03, 100). These IDs have their respective and interrelated organizational boundaries. The main sources of irrigation for 45,000 hectares in what is known as Irrigation District 03 are the Salado river and the Taximay, Requena and Endho reservoirs. The ID 100, Alfajayucan, is fed by the Rojo Gomez and Vicente Aguirre reservoirs (SARH, 1990). As is valid for all ID in the country, ID 03 and ID100 depend on the Ministry of Water Resources (SARH) as well as on the National Water Commission (CNA). Both ID 03 and ID100 are responsible for the farmer's compliance in relation to crop restrictions and technical extension services. ID staff are also responsible for the maintenance of the irrigation system's infrastructure, operation and wastewater administration.

Farmers requiring irrigation must apply and pay a fee to the ID headquarters. When applying, the farmer states the crop(s) to be cultivated, location and size of the plot, as well as the number of times during the agricultural year his crop(s) will require irrigation. Depending on current restrictions, ID authority issues a printed receipt with written details on crop(s), location of the plot etc... By entering into this contract, the farmer commits himself to the declared conditions and the ID authority "guarantees" that the farmer will receive the water needed throughout the cycle and to the harvest concerned. Given the irrigation techniques utilised, every crop has specific water requirements, so that farmers do not get irrigation more frequently or in larger volumes than those for which they pay. Every time a farmer needs irrigation, he must show his written consent to the gate staff (canaleros), who operate the flow of water through the channels. In order to comply with crop restriction policies, sanctions involving economic loss may range from interrupting irrigation supply to confiscation of crops are imposed, in cases of noncompliance with conditions declared in the original "contract". Illegal practices, e.g the backyard's cultivation of vegetables, may occur in this farming population.

Wastewater used in irrigation throughout the Mezquital Valley receives no treatment *per-se*. Existing health protection policies (for consumer protection) are based on crop restriction. At the end of the 1980s and in the beginning of the present decade, national regulations set a microbiological standard of less than 1,000 coliforms/100 ml for water used to irrigate crops consumed uncooked. No guidelines for the microbiological quality of water used to irrigate crops eaten cooked existed, nor for those crops that do not have contact with the farming soil. In 1991, however, a cholera outbreak was reported in Mexico, and new regulations and standards were issued and in the last five years have experienced a series of modifications (see Table 3.4).

3.4. IRRIGATION SYSTEM.

3.4.1 Development of the irrigation districts.

Irrigation in the Mezquital Valley involves 70% of the state territory. The agricultural use of wastewater for irrigation began at the turn of the present century and has rapidly expanded throughout previously rain-fed areas (Table 3.3). The present size and complexity of the wastewater irrigation system of the districts 03 and 100 (Tula and Alfajayucan, respectively) make this area the largest wastewater reuse scheme in the world. The scheme is a by-product of the explosive growth of its donor, Mexico city.

Table 3.3. Development of the Irrigation Infrastructure in the Mezquital Valley.

Year	Irrigated area (Hectares)		Irrigation works
1900	000	--	Construction of the Drainage Canal discharging Mexico City wastewater
1926	14 000	--	Initiation of irrigation network in the Mezquital Valley
1950	28 000	--	Tunnel of Tequisquiac
1960	38 512	--	Endho canal
1972	39 442	--	Bojay irrigation zone
1978	66 367	--	The central Chilcuahutla and Xochitlan canals, New Alfajayucan zone
1979	66 900	--	Network of "El Tigre", Alto Tepetitlan, Chicavasco and Demacu canals
1984	71 360	--	El Xotho canal
1986	75 384	--	Salto Tlamaco and Upper Alfajayucan
1987	85 095	--	Total surface under SARH 03 and 100 Irrigation district
1993	100000	--	Planned expansion

Source: SARH (1989)

In the last 10 years, the volume of wastewater flowing through the irrigation system has increased from 1,020 to 1,350 million m^3/year, equivalent to 43 m^3/second. A total volume of 2,000 Mm^3/year is expected for the year 2000 (Romero Alvarez 1993). Urban sewage and wastewater constitutes approximately 80% of this volume and the remaining water is mostly storm-runoff, the availability of which is seasonal. The continuous expansion of the irrigation network forecasts that in 1995 a further 15,000 hectares (previously semiarid and dependent on scarce rainfall) will receive wastewater irrigation (PRONAR, CNA 1990). Wastewater and river runoff are flowing throughout 1,050 km of irrigation canals, regulated by means of a complex system of intake dams, spillways, gates and large storage reservoirs. The following six types of water are currently used for irrigation in the Mezquital Valley (see Figure 4.3): (pp 72)

A. - Raw wastewater from Mexico City, flowing through the main collector canals and urban outfalls (65 - 100 km of transfer);

B. - River water and storm runoff stored in Taximay and Requena reservoirs; the latter is fed by the effluent of the Taximay reservoir. These reservoirs receive no wastewater;

C. - Effluents from Taximay and Requena reservoirs, mixed downstream with river water conveying raw wastewater, flowing towards the Endho storage reservoir;

D. - Effluents from the Requena and Endho storage reservoirs, which mix downstream with raw wastewater from the metropolitan area and local towns, and are transported by the Salado river;

E. - Stored wastewater from the Rojo Gomez and Vicente Aguirre reservoirs which receive wastewater via Endho reservoir;

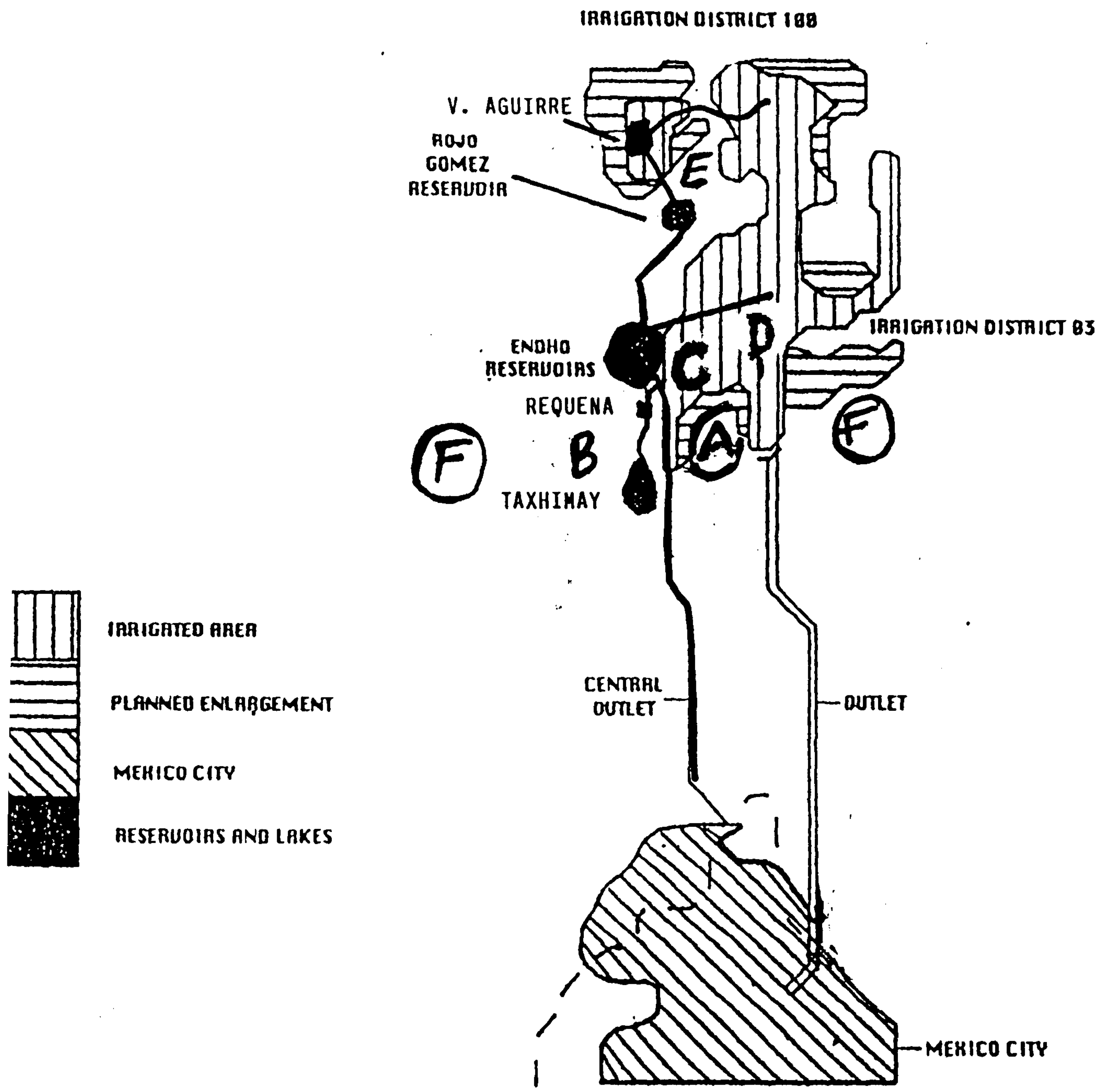
F. - Water from springs and wells.

A series of reservoirs (B, D and E above) serve to store storm runoff and wastewater when the supply is in excess of demand. These reservoirs fill during the rainy season (June - September) with urban wastewater, river water and storm runoff . Wastewater is retained in these reservoirs, during several months, but retention times are variable in each reservoir due to the fact that water is released according to local farming requirements and available volumes (see Figures 3.2, 3.3, 3.4). Since irrigation demands are not completely satisfied by the local rainfall (even during the wet season), available water is used throughout the agricultural year. A simplified, basic description of the function of the irrigation system is given as follows.

3.4.2. Raw wastewater and mixed wastewater.

The Western outfall, the Central outfall and the Great Drainage outfall (Interceptor Poniente, Emisor Central and Gran Canal de Desague) link the metropolitan area (Mexico City) with the Mezquital Valley. The Central and Western outfalls discharge urban sewage from the metropolitan area and storm runoff through the El Salto river, a tributary of the Tula river,

Figure 3.5 Mexico's Major Wastewater Scheme, the Mezquital Valley

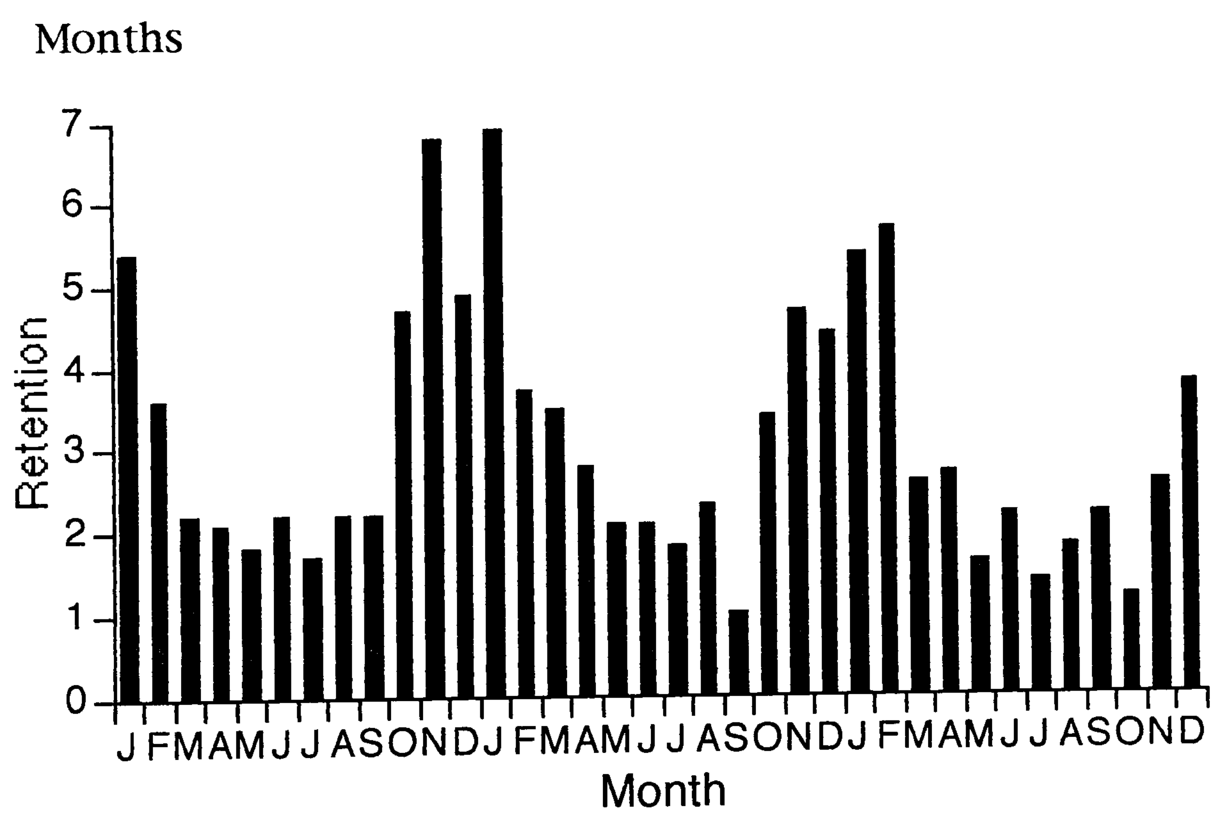


Towards the East of the study area, the Salado river also receives large volumes of raw wastewater and storm runoff from Mexico City, which come through the Great Drainage Canal near Tequisquiac. Further downstream, the Salado river flows south-north crossing the irrigated area and discharges into the Tula river, near Tezontepec, or directly into the Endho canal. The discharge takes place mostly during the rainy season. Therefore, at the junction with the Salado river, both the Endho canal and the Tula river contain a mixture of stored wastewater coming out of Endho reservoir, in addition to diluted raw wastewater flowing through Salado river. Water coming from the Salado river, along with that coming out of the Taximay, Requena and Endho reservoirs, are the main source of water of irrigation for 45,000 hectares, in Irrigation District 03 (SARH,1990).

3.4.3. Stored wastewater.

3.4.3.1.Endho storage reservoir. Raw wastewater flowing through Tula river is the largest component entering the Endho storage reservoir. Smaller volumes of water from Requena reservoir also enter and are stored in the Endho reservoir. Endho effluent flowing downstream dilutes the raw wastewater from the Gran Canal, Tequisquiac tunnel and Salado river (Figure 3.2).

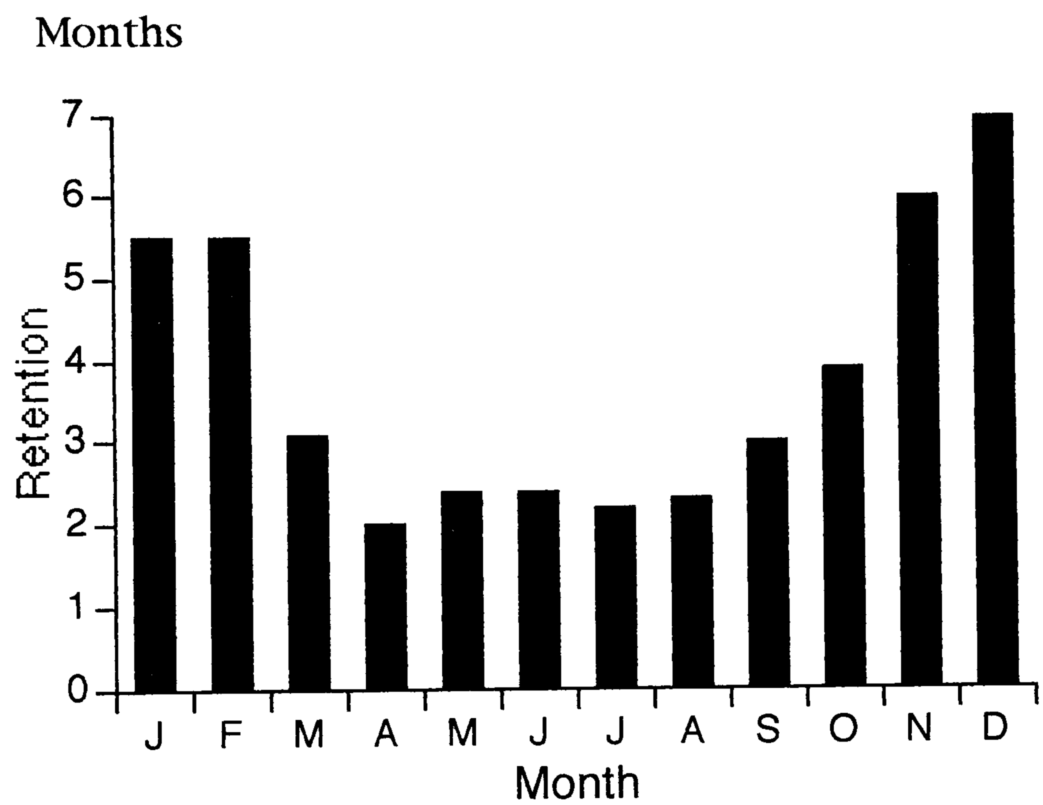
Figure 3.2. Retention time in Endho reservoir (1989-1991).



Source: A. Peasey, Personal communication

3.4.3.2.Rojo Gomez storage reservoir. Wastewater entering Rojo Gomez reservoir through the Central canal initiates in the Endho reservoir. A small proportion of wastewater entering Rojo Gomez reservoir may also come from the Tula river, flowing from Endho's lateral canal. Thus, wastewater coming out of Rojo Gomez reservoir has passed through two retention reservoirs and flows through the principal canal and Alfajayucan river. The principal canal bifurcates, although most of the water deviates through Alfajayucan river towards Vicente Aguirre reservoir (Figure 3.3).

Figure 3.3. Retention time in Rojo-Gomez reservoir (1989-1991).



Source: Cifuentes & Vargas: Unpublished data.

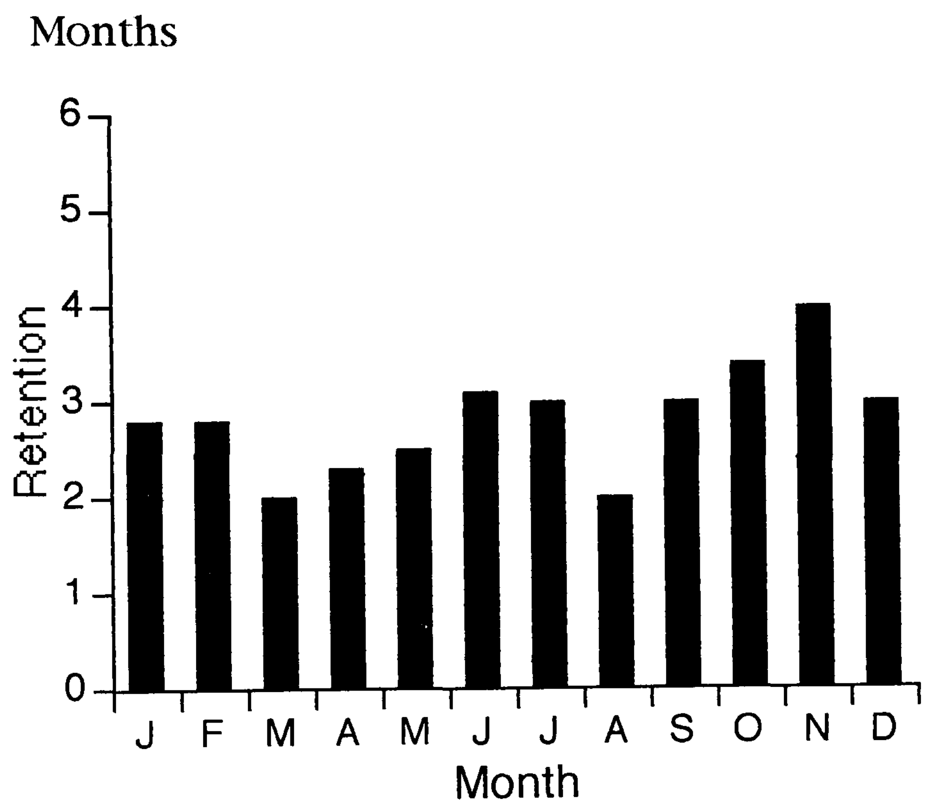
3.4.3.3.Vicente Aguirre storage reservoir. The source of wastewater entering Vicente Aguirre reservoir is the Rojo Gomez reservoir (see 3.4.3.2., above). Wastewater flowing from Vicente Aguirre reservoir has hence been retained in three reservoirs (Figure 3.4).

3.5 MICROBIOLOGICAL QUALITY OF WASTEWATER

Wastewater used in irrigation throughout the Mezquital Valley receives no treatment *per-se*. National revised legislation since 1991-92 (Table 3.4) has faced conflicting situations since farmers often demand irrigation for vegetables which generate more profits. Compliance with these crop restriction policies requires a well organized institutional framework.

A "Technical Norm" (1991) establishes the maximal concentration of faecal coliforms (FC) in wastewater irrigation according to the type of crop, irrigation technique and time interval between the last application and harvest (CNA, 1991). Crop restrictions involve vegetables and those crops eaten uncooked, whenever wastewater irrigation contains more than 1000 FC per 100 ml, or a maximum of 1 helminth egg per litre, adopting the 1989 WHO guidelines.

Figure 3.4. Retention time in Vicente-Aguirre reservoir (1989-1991).



Source: Cifuentes & Vargas: Unpublished data.

Table 3.4 Water Quality Guidelines and Crop Restriction Policy, Mexico, 1990.

WATER USE	MICROBIOLOGIC QUALITY	CROPS PERMITTED	TIME INTERVAL BETWEEN LAST IRRIGATION AND HARVEST (DAYS)
UNRESTRICTED	$\leq 10^3$ FC ≤ 1 helminth egg	All, except vegetables in contact with soil	15
RESTRICTED	$10^3 - 10^5$ FC - no egg guidelines	- Rice; - vegetables eaten cooked; - vegetables eaten raw; but with no contact with soil; - Garlic and onions	20
RESTRICTED	$>10^5$ FC	- Fodder - grains - seeds - industrial crops - flowers - fruit trees	20

Source: Comisión Nacional del Agua, Manual Técnico (1990).

In addition, the current technical norm establishes the minimum interval between the last application and the harvest: 20 days if flooding techniques have been used, and 15 days if furrow techniques are being used (PRONAR/SARH 1992). Since pasture constitutes a major crop, the main justification for this intervals is related to the risk of *Taenia saginata* , as docummented by Feachem *et al* (1983).

Samples of wastewater were collected from selected points of the irrigation network (Fig. 4.3). Raw wastewater samples were gathered from the metropolitan outlets, whereas samples were also collected immediately upstream of the junction , where most of wastewater is discharged to the river Tula. (Tables 3.5 and 3.6). The results then include any 'transport effect", by which the quality of wastewater may have changed and account for the huge difference between the helminth counts in raw wastewater and the influent of the Endo reservoir. These data also indicated a considerable improvement in the microbiological quality of wastewater in the effluent of the second reservoir, possibly as a result of several months of double hydraulic retention.

Table 3.5 Quality of Raw Wastewater and Effluents from Reservoirs ID 100 -03: Faecal Coliforms MPN/100ml (1990-1991).

	April[2]	May[1]	June[1]	Aug.[1]	Sept.[1]	Oct.[1]	Nov.[2]	Dec.[2]
Raw waste/ water	$2x10^8$	$1x10^8$	-	-	$2x10^8$	$2x10^8$	$1x10^7$	$6x10^6$
Raw waste/ water + dilution	$2x10^8$	$2x10^8$	-	-	$5x10^7$	$9x10^5$	$3x10^6$	$3x10^6$
1st (Endho) reservoir								
Influent	$7x10^7$	$5x10^7$	-	-	$7x10^6$	$1x10^7$	$3x10^6$	$6x10^8$
Effluent	$4x10^4$	$9x10^4$	-	-	$2x10^6$	$3x10^6$	$2x10^5$	$1x10^5$
2nd (R. Gomez) reservoir								
Influent	-	-	$1x10^6$	$3x10^6$	-	$8x10^3$	$5x10^3$	$2x10^4$
Effluent	-	$1x10^4$	$6x10^3$	$2x10^5$	$8x10^3$	$2x10^3$	$1x10^1$	$5x10^2$

[1] Rainy season, [2] Dry season

Table 3.6 Quality of Wastewater and Effluents (helminth eggs/litre) from the Reservoirs, ID 03, 100 (1990-1991).

	April	August	December
Raw wastewater	135	105	90
1st (Endho) reservoir			
Influent	3	0	0
Effluent	1	0	0
2nd (R. Gomez) reservoir			
Effluent	0	0	0

3.6 AGRICULTURAL PATTERN.

Census information indicates that 35-60% of economically active individuals in the Mezquital Valley are engaged in agricultural production. These data may vary among rain-fed regions and irrigated areas, and according to the agricultural cycle. Seasonal migrations are common and up to 25% of adults in some villages migrate temporarily to nearby cities or to the United States in search of employment (Censo Nacional de Poblacion 1990).

As in other rural areas of Mexico, many aspects of agricultural production in the Mezquital Valley are deeply rooted in the country's history. Land reforms initiated at the beginning of the present century and the explosive growth of urban populations during the last four decades are two of the most recent events of importance. Prior to social reform and land tenure changes in 1910, the Mezquital Valley was a region of large ranches (haciendas) where the chief industry was the production of pulque (a fermented juice of the "maguey", a succulent cultivated in the area, mostly in the semiarid, nonirrigated areas). When the large estates were subdivided, most families acquired a small plot on which they attempted to plant primarily maize or beans, despite the lack of irrigation. At present, more than 40% of the peasantry own no land. Among farmers who own a piece of land, the average size is between 1 and 3 hectares. Only 20% of farmers make their living from larger holdings. According to official records, about half of the agricultural land is held in a communal form of land tenure, known as "ejidos", and the other half is privately held. One common practice in the Mezquital Valley is to rent the land in exchange for harvest, regardless of whether it is communal or belonging to an individual ("mediero" agreement). In the latter case, the "tenant" provides the labour and farming inputs (e.g. seeds, fertilizers) and at harvest, the yield is shared in halves between the land owner and the tenant (INEGI 1990).

Farming is less mechanised and more oriented to subsistence agriculture in the rain-fed area. Major crops are maize, beans, agave (maguey), barley and some vegetables. Irrigated areas with more technological input for farming have a more diversified agricultural production (Table 3.7). Many families produce their own maize but cash crops are also increasingly cultivated: alfalfa (fodder), oats, barley, beans, chilies, cucumber, wheat, sorghum and green tomatoes and chilies.

Recent calculations estimate that the irrigated area generates up to 75% of the agricultural production of the entire state (SARH 1990, INEGI 1990). When yields per hectare are compared, maize production is four times higher in the irrigated area than in the rain-fed zones (Table 3.8).

Table 3.7. Crops Produced in Irrigation Districts 03 and 100, Hidalgo, 1988.

Crop	Area Cultivated (ha)	Percentage of total (%)	Water requirement (cm)	Net profit per hectare (X 1000 pesos)
Maize	19,668	41.0	100	41.4
Alfalfa	17,972	37.5	158	22.4
Barley	1,852	3.9	72	4.0
Oats	1,706	3.6	72	4.0
Wheat	458	1.0	113	11.6
Chilies	999	2.1	108	154.9
Green tomatoes	587	1.2	141	192.5
Haricot beans	865	1.8	31	20.1
Broad beans	301	0.6	88	18.3
Others	2,574	7.3	97	58.6

Source: Mara - Cairncross, 1992

Agricultural land in the irrigated area of the Mezquital Valley is both extensively and intensively cultivated. During the agricultural season of 1988-89, nearly 85 thousand hectares produced 2,625,000 tons of different crops, with a monetary value of approximately $ 70 million USD (Velazquez 1991, Anuario Estadistico del Inst. Nal. Indigenista 1992). Despite this income, most farming families must rely on additional sources of income due to their small plot sizes or low prices of products in the market. A fair harvest is rarely obtained and in most cases, farmers have to work as hired labourers (including women and children) on other farms with the heaviest duties e.g. ploughing, seeding, weeding and crop picking. In addition, the family's income is often supplemented with money provided by relatives working outside the village. Where the size of the plot and the crop yield is sufficient to guarantee their living, families rely on labour provided by several of its members during intensive farming cycles. A small number of large landowners and 'medieros' who manage to rent several plots, may obtain considerable profits from large harvests.

Table 3.8. Examples of Agricultural Production in the Study Areas, 1986 - 1987.

Crops	Irrigated area Hectares	Yield (tons)	Tons/ha
Maize (*Zea mays*)	39.5	156.9	3.9
Beans (*Phaseolus vulgaris*)	2.9	4.6	1.5
Barley (*Hordeum vulgare*)	2.4	7.0	2.9
**Rain-fed communities			
Maize	31.5	32.0	1.0
Beans	3.8	2.8	0.7
Barley	1.6	4.0	2.5

Source: SARH (1989); INEGI (1990)

3.7. CROP RESTRICTIONS AND OCCUPATIONAL EXPOSURE.

Crops grown with different types of water in the irrigation districts 03 and 100 and those crops cultivated in rain-fed areas are summarised in Table 3.9. By 1989-90 agricultural cycle the main crops produced from raw wastewater irrigated areas were maize, alfalfa, barley, oats, courgettes and chilies. In addition to those crops mentioned above, beans and green tomatoes were also cultivated in the reservoirs areas. In rain-fed and spring water irrigated areas, the main crops recorded were maize, beans and barley. Wild-greens (natural greens) have a high cultural value within the traditional diet. Some of these wild greens may be eaten raw.

Occupational exposure varies according to the farmer's role in agricultural production. The source of irrigation, the techniques used, the crop planted and the frequency of irrigation are all factors which determine an individual's exposure (Table 3.10). Irrigation is carried out by the local farmers mostly through flooding and furrow techniques and often the irrigation-related duties are carried out walking barefoot in the fields. Most farming duties are manual, using spades and hoes for directing the flow of irrigation water through the furrows, involving greater exposure. As a result, farmers flooding their plots have the greatest direct exposure, while crop pickers may have less exposure, since irrigation ceases some days before harvesting.

Table 3.9. Main Crops Cultivated in the Mezquital Valley, 1990.

	TYPE OF IRRIGATION WATER			
CROP	Raw Wastewater (1)	Stored wastewater (2)	Stored wastewater (3)	Spring/Rain (4)
Alfalfa	++	++	++	--
Maize	++	++	++	++
Barley	++	++	++	++
Oats	++	++	++	--
Beans	--	++	--	++
Courgettes	++	--	++	--
Tomatoes	--	--	++	--
Green Tomatoes	--	++	--	--
Chilies	++	--	++	--
Wheat	--	--	++	--
Cactus	+	+	+	++

1). mostly urban sewage and storm runoff
2). stored wastewater with no mixture
3). stored wastewater mixed with some raw wastewater
4). includes villages with rivers, some which receive wastewater
++ Priority crop; + Secondary crop

Source: SARH (1990)

Contact with water may also occur when a member of the household is asked to graze cattle near the canals. It is also common to see men and women with their young children eating their lunch in the crop fields. Women and other individuals in the household have contact when they carry out other activities (e.g. cattle grazing) involving wastewater flowing through the canals. Recreational exposure in children occurs if they play in these canals, around their homes, or while assisting their parents in the field. Consumer's risk (wild-greens) may also have a relative impact on the health of this farming population.

Table 3.10. Crops and Irrigation Characteristics in the Mezquital Valley.

CROPS	PLANTING PERIOD	IRRIGATION PER YEAR (n)	INTERVAL BETWEEN IRRIGATION	IRRIGATION TECHNIQUE
ALFALFA	OCT+JAN	9	40 DAYS	FLOODING
MAIZE	FEB+JUN	5	48 DAYS	FURROW
BARLEY	JUL+JAN MAY+JAN	4	60 DAYS	FLOODING
TOMATO	FEB+APR	5	48 DAYS	FURROW
OATS	OCT+DEC	4	60 DAYS	FLOODING
COURGETTE	FEB+AUG	4	60 DAYS	FURROW
CHILLI	FEB+JUN	6	40 DAYS	FURROW
WHEAT	NOV+JAN	5	48 DAYS	FLOODING
BEANS	FEB+AUG	4	60 DAYS	FURROW
GREENTOMATO	ALL YEAR	5	48 DAYS	FURROW

Source: Adapted, SARH (1991)

Some of the crops not only generate more working days per hectare, but they also represent considerable profits for farmers who grow them (Table 3.11). This profit from cultivation of vegetables may be six times higher than that for alfalfa and maize. Available data indicate that in the 1990 agricultural cycle, every hectare cultivated with vegetables (tomato, green tomato, chillies and courgettes) generated approximately 125 working days, while every hectare cultivated with maize and alfalfa generated only 11 days of labour during the year (Velazquez 1991).

Table 3.11. Cultivated Crops and their Economic Value, the Mezquital Valley, 1990.

CROP	HECTARES	PRODUCTION VALUE*	LABOUR**
Vegetables	5,700	13.6 per hectare	125
Maize and Alfalfa	59,500	2.3 per hectare	11

* MILLIONS OF N. $ PESOS

** Days/Year Source: SARH, (1991)

CHAPTER 4. STUDY DESIGN AND METHODS.

4.1. INTRODUCTION.

The evaluation of the health impact of wastewater reuse is essential, providing they are appropriately designed, in order to determine whether wastewater reuse results in measurable excess of disease or infection. This study was designed after careful consideration of conclusions from previously reported research.

4.2. OBJECTIVES.

The specific objectives of this study were to:

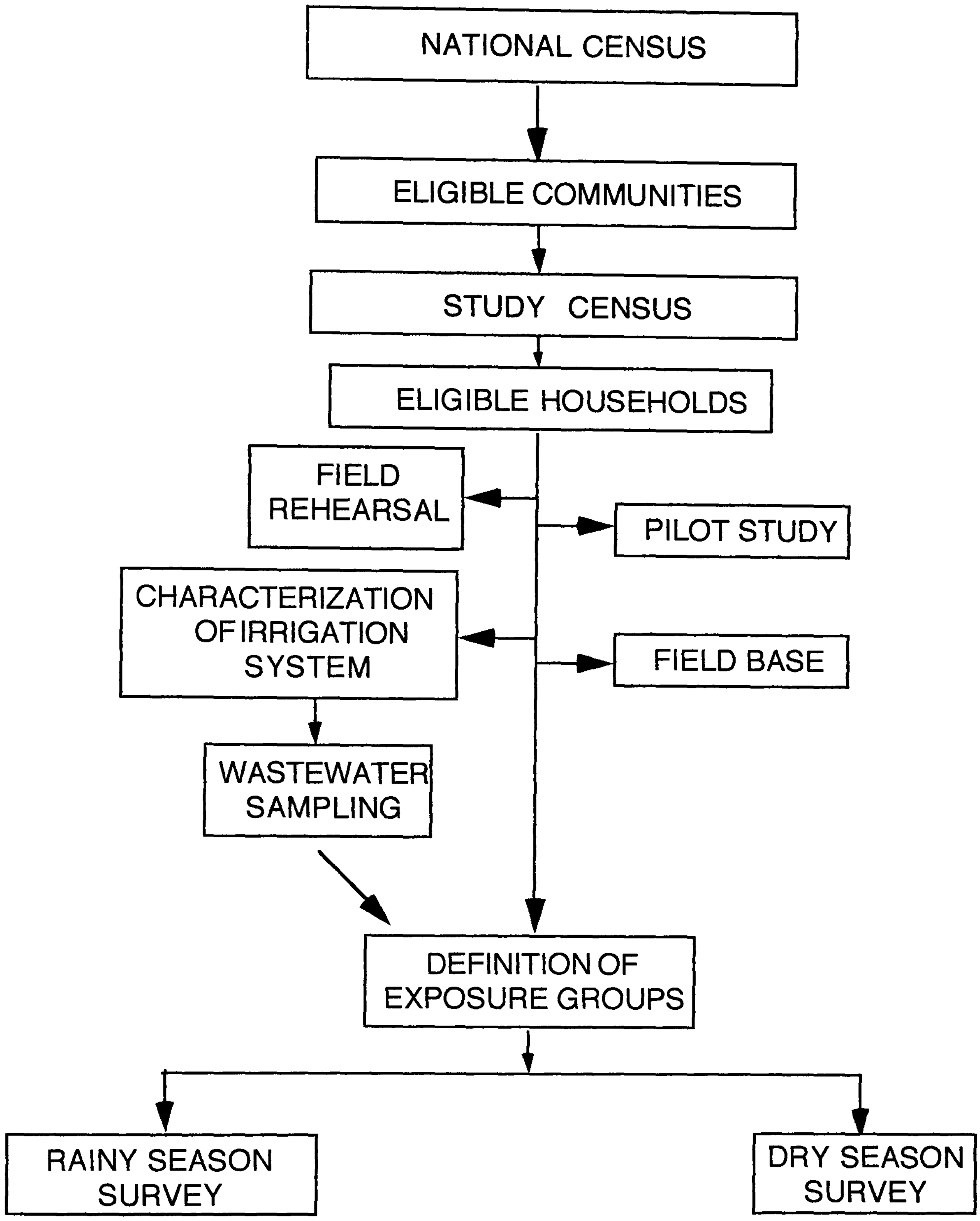
1) evaluate the risk of parasitic infections and diarrhoeal diseases in agricultural families exposed to raw wastewater;
2) identify subgroups at high risk of parasitic infections and diarrhoeal diseases associated with wastewater reuse;
3) assess the effect of hydraulic retention in the storage reservoirs, both on the water quality improvement and on the risk of intestinal infections and diarrhoeal diseases in a farming population;
4) assess the WHO revised guidelines for wastewater reuse in restricted irrigation schemes;
5) identify important risk factors for diarrhoeal diseases and intestinal infections in the study area, in addition to exposure to wastewater;
6) propose appropriate health protection measures for the populations under study.

4.3. STUDY DESIGN.

This project consisted of an observational, opportunistic study of an existing situation (see Chapter 3, Study Area). The prevalence of diarrhoeal diseases and parasitic intestinal infections were assessed in farmers and their families exposed to wastewater of different qualities; farmers using only rainfall were considered as the control population.

Assessment of wastewater quality complemented the study. The purpose of these tests was two-fold: to assess microbiological water quality throughout the irrigation system and to provide a basis for the definition of exposure categories. Primary procedures of the study design are illustrated in Figure 4.1. These procedures resulted in two cross-sectional surveys, one during the rainy season and the other during the dry season. It is important to underline that the whole design took into consideration the possible differences beween single and double hydraulic retention, rather than seasonal fluctuations in the study outcomes.

Fig. 4.1 STUDY DESIGN
the Mezquital Valley, 1989-1992



Exposure Groups involved throughout the study (1990-1992).

EXPOSURE GROUP	RAINY SEASON SURVEY	DRY SEASON SURVEY
Raw wastewater	X	X
One reservoir		X
Double reservoirs	X	
No wastewater (controls)	X	X

4.4. SAMPLE SIZE CALCULATION, ADJUSTMENTS AND CHANGES.

The sampling unit in this study was the household, which was defined as the sum of all members sharing food. Economically active members of the household were individuals who contributed with work, income, or both. Therefore, exposure was defined according to agricultural activities, since occupational exposure was the primary exposure of interest.

The following formula was used to calculate minimum sample size to determine a difference between prevalences in the exposure groups (Fleiss, 1981):

$$N = \frac{2\,p\,(1 - p)\,F}{D^2}$$

where $p = (p_1 + p_2) / 2$ and $F = \frac{(Z\alpha + Z\beta)^2}{2}$ and $D = p_1 - p_2$

α = probability of type I error and β = 1- power
and Z = variable of interest having the standard normal distribution.

Sample size was calculated by using the prevalence results from the pilot study (see section 4.9). According to the prevalence of *A. lumbricoides* infection found in the villages involved in the pilot study, the sample size was calculated to detect a 0.05 difference in prevalence for individuals aged 5 years and older, and 0.10 difference for individuals aged under 5 years. The final sample size was calculated in order to detect significant differences in other study outcomes (e.g. diarrhoeal diseases), of which was found in the pilot study to be more prevalent than *A. lumbricoides* infection. Other infections, however, were dropped from the study because low prevalences detected. This was the case for *Cryptosporidium parvum* and *Trichuris trichiura* infections (below 1% and 4%, respectively).

In addition, the following considerations were taken into account: power of the study of 0.90 and a significance level of 0.05 (two-tailed). The sample size was also adjusted for nonresponse by 0.15, and also for possible confounders by further 0.15.

The sampling unit was the household, but the unit of analysis was the individual. Thus, the initial sample size was calculated assuming the presence of only one agricultural worker per household. However, results from the census (see section 4.6) showed that most households included more than one member performing farming duties, which reduced the number of households needed per water-zone.

Final adjustments indicated that 580 households per group would achieve 0.90 power, to detect 5% differences in the prevalence of *A. lumbricoides* infection, and also covered the sample sizes needed for other study outcomes. Following the inclusion criteria, the total number of eligible units available in the reservoirs groups was slightly greater than the required sample size, and therefore all eligible households were included.

4.5. METHODS FOR PRELIMINARY FIELD WORK.

4.5.1. Characterization of the study area.

Identification of agricultural villages suitable for the study was one of the most important initial steps of the preparatory field work. The basic tasks used in their identification can be summarised as follows: a) the identification of different types of irrigation, their origins and direction of flow; b) the geographic delineation of each zone receiving irrigation; c) identification of crops grown in each water zone, agricultural cycle periods and frequency of irrigation; d) identification of a suitable comparison zone; and e) selection of villages, both from the irrigated and rain-fed zones, which would fulfill the research objectives.

Preliminary sketches of the operational characteristics of the irrigation network were obtained from local authorities (SARH). This information was rechecked through field visits, focusing on the definition of major water zones (e.g. raw wastewater, diluted wastewater stored in the different reservoirs, natural springs, rivers and rainfall). Irrigation canals were identified, mapped and coded. The most important canals were grouped into categories, according to the type of water flowing through them. In the rain-fed zone all rivers, wells and natural springs were also coded.

A list of villages eligible for inclusion in the study was obtained from available National Census files. The primary census eligibility criteria for villages was that at least 30% of its adult population was engaged in agricultural activities, both in the rain-fed and in the irrigated area; further criteria included village population (between 1000 and 10,000 inhabitants). Baseline sociodemographic information of the target population, as well as maps for locating the villages, roads and the irrigation system network were assembled from all possible sources (e.g. census data, local files) to produce a useful reference framework for those communities to be censused. Communities were excluded either because a) they did not receive irrigation year round, b) their irrigation source changed during the agricultural cycle from raw wastewater to stored wastewater, or vice versa, or c) they had outstanding urban features (e.g. public services).

4.5.2. Design of questionnaires.

Questionnaires were designed with input from focus group discussions, field rehearsals and interviewer's training sessions (Annex 1).

4.5.2.1. Focus group discussions. The use of focus groups as a qualitative research technique involves structured, in-depth discussions between a "facilitator" and small groups of individuals from the target population (Scrimshaw & Hurtado 1988). This method provides an understanding about local beliefs, attitudes and practices on the subject of enquiry (Khan & Manderson 1992, Manderson & Aaby 1992). From March to April, 1989, a preliminary list of open questions was checked through a series of discussions with groups of "canaleros" and "regadores", who are the workers in charge of the irrigation systems' operations, and with experienced farmers. Their practical knowledge of agricultural duties improved our checklist of variables to be included in future questionnaires. Focus groups were organized based on discussion of general and agricultural-specific issues: a) everyday activities involving contact with water; b) perceived benefits of different irrigation water types; c) when, how and by whom irrigation is carried out; d) perceived health hazards in relation to irrigation; and e) socioeconomic features of farmers in the area. Information gathered through these interviews was recorded on a blackboard and summarised in a notebook, and was used to design questionnaires.

4.5.2.2. Field trial of questionnaires and interviewers' training. A group of students specializing in rural sociology (Universidad Metropolitan) was trained to conduct the field trial of questionnaires; the same students tested these questionnaires for three weeks in the field. The objectives of this preliminary field trial were a) to test procedures and create a mapping system of villages and homesteads, to be used during the census, b) to define potential logistics problems, and c) to select field supervisors, recruit computing staff and laboratory technicians. A total of 250 households in three small villages which had different types of irrigation for agricultural production (wastewater, stored wastewater and rainfall) were selected for this field rehearsal. Data provided by residents included general sociodemographic information (e.g. extended families, land tenure and major crops), as well as details of farming activities which involved exposure in a combination of open and closed questions. During the final week of the field rehearsal, selected questions were refined and adapted as closed questions in the definitive format.

4.5.3. Study site office, laboratory and computing facilities.

4.5.3.1. Laboratory. A project laboratory was set up within the facilities of Irrigation District 03, where SARH maintains basic equipment. Three technicians were recruited and trained in parasitology techniques by members of the Department of Infectious Diseases, INN.

4.5.3.2. Computing facilities. Two computers (IBM compatible 286 and 386 MHz) were set up at the study site office. One data manager and two local data entry clerks were recruited and trained for data input.

4.6. THE CENSUS.

A census of the study area was conducted due to the lack of suitable sociodemographic information for agricultural workers. The objectives of the census were to gather data about farmers' households, their farming plots and irrigation practices. This information provided a sampling framework of farm workers, as well as a classification system by which households could be allocated into basic categories of exposure. Based on eligibility criteria, each household was allocated to a water use category. The census also provided data necessary for initial comparison of the study groups.

The census was conducted by house to house visits of 11,350 dwellings scattered throughout more than 125,000 hectares of agricultural land. It was carried out between August and October, 1989 (see Figure 4.1). Only households fulfilling the inclusion criterion (a minimum of one member older than 15 years actively involved in farming duties) were fully censused. Selected villages were mapped, boundaries delineated and homesteads located on the maps. The mapping team was also in charge of establishing contact with local authorities, requesting their support and cooperation, while checking the identification of canals and the geographic limits of each village. Detailed maps with all components were photocopied, discussed and delivered to field trial workers every morning prior to the census visits (Annex 2).

Every dwelling visited was assigned with a unique identification number and marked on the maps. The numbers were checked by the supervisors on a daily basis. Data collected from each dwelling during the census were:

a) household structure,

b) occupation of both the head of the household and his spouse,

c) location of the farming plot,

d) identification of irrigation sources,

e) land tenure,

f) crops cultivated,

g) housing characteristics,

h) education of the head of the household and spouse/female head of household.

The census's progress was closely monitored and practical difficulties discussed with the team supervisors, who were responsible for groups of five to seven interviewers. Absent or nonrespondent families were identified and villages with compliance rates lower than 70% were revisited to reduce information bias during the census.

4.7. ELIGIBILITY PROCEDURES.

All households were fitted into eligibility categories by joining sets of data from the census. These categories were then structured into an algorithm to create more refined definitions of household exposure, which were based on adults agricultural activities (Figures 4.2A, 4.2B, 4.2C, 4.2D). Inclusion criteria were developed from these categories for all households:

1) location in agricultural community: wastewater-irrigated or rain-fed villages;
2) land holding of wastewater irrigated or rain-fed plots;
3) occupational contact with wastewater;
4) farming in rain-fed plot (s).

Figure 4.2A. ELIGIBILITY FLOW DIAGRAM
Remove ineligible families and classify them for rainfed area.

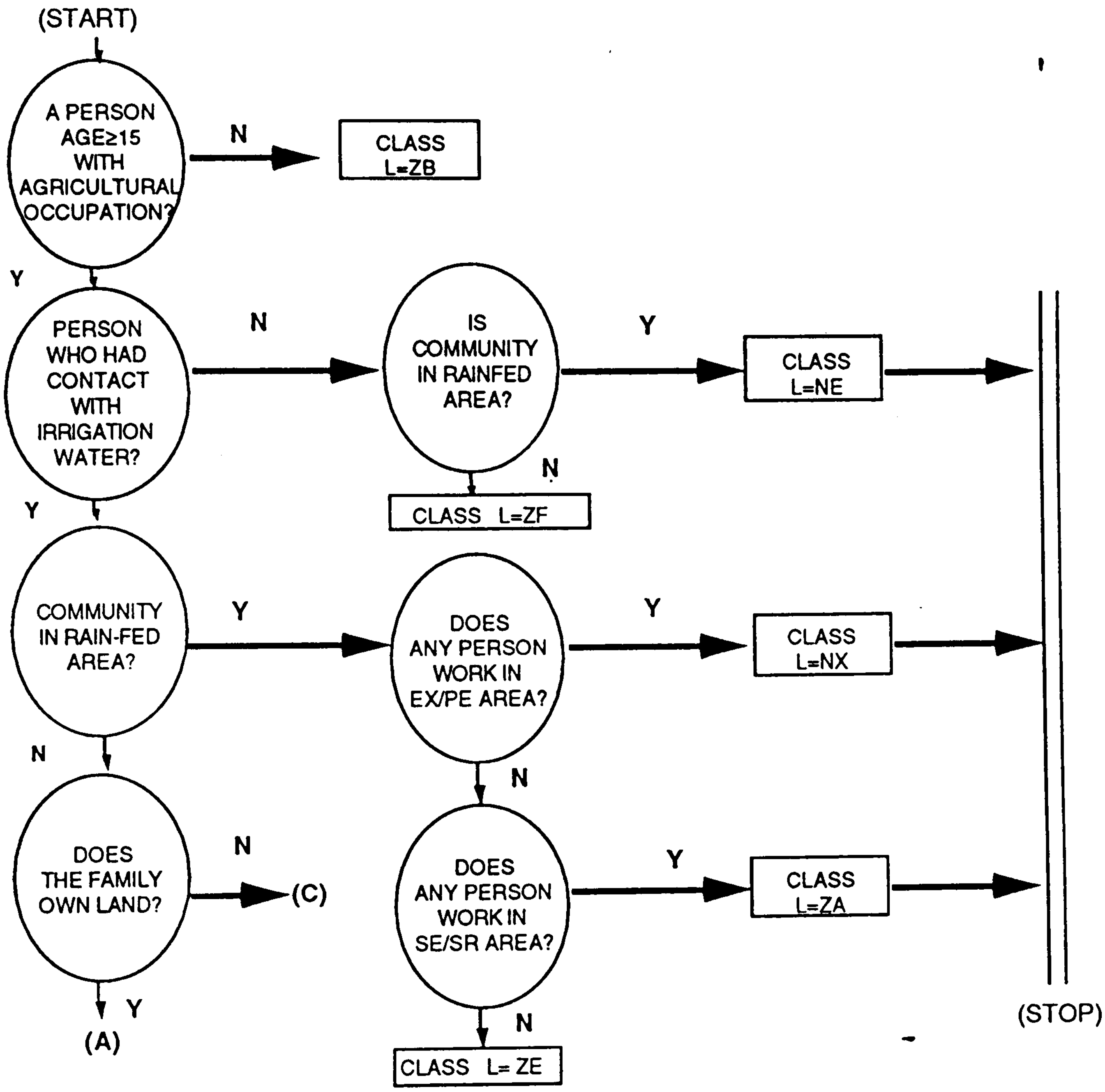


Figure 4.2B. ELIGIBILITY FLOW DIAGRAM
Classify landowners by irrigation canal.

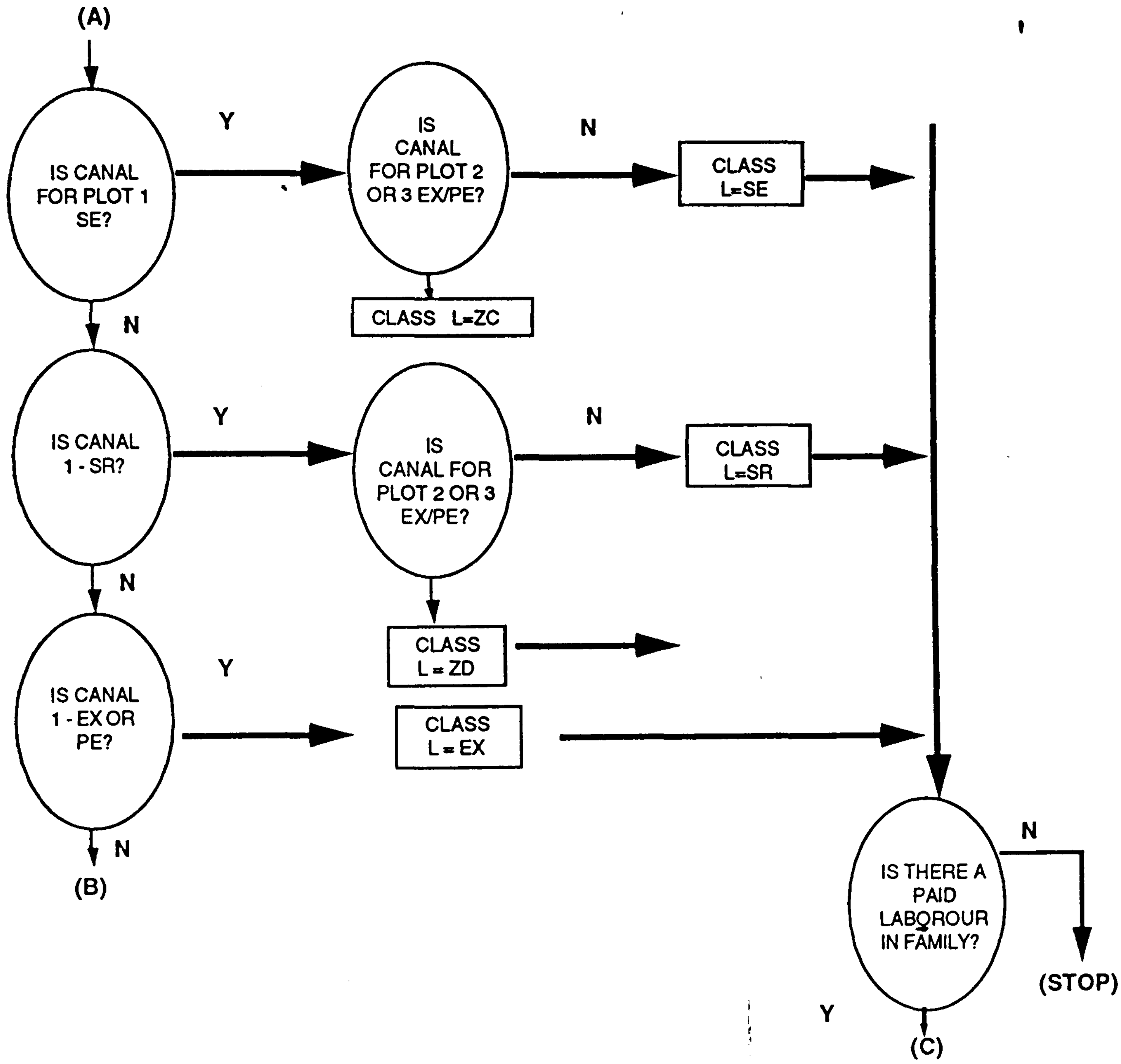


Figure 4.2C. ELIGIBILITY FLOW DIAGRAM
Classify landowners by type of community.

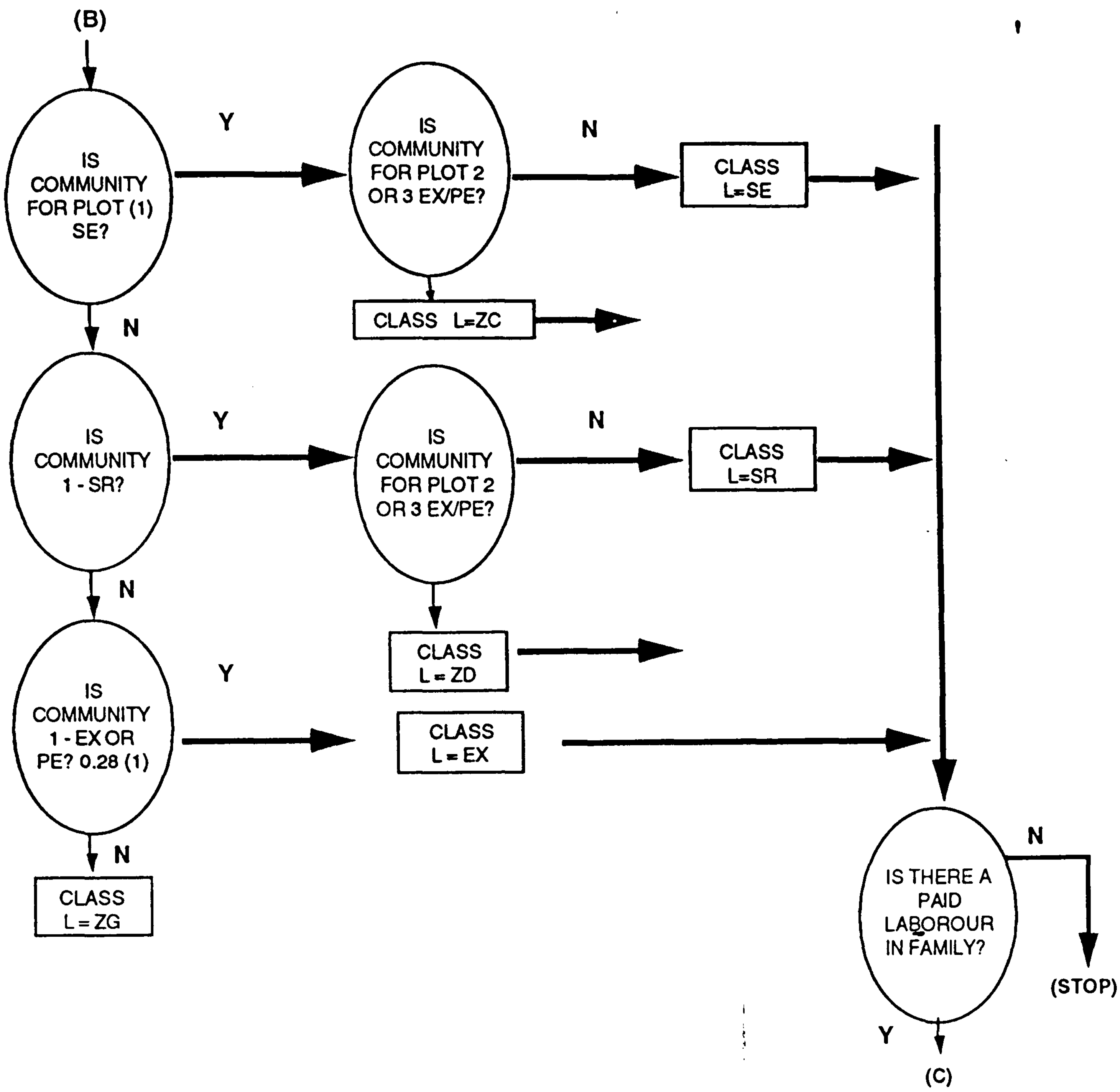
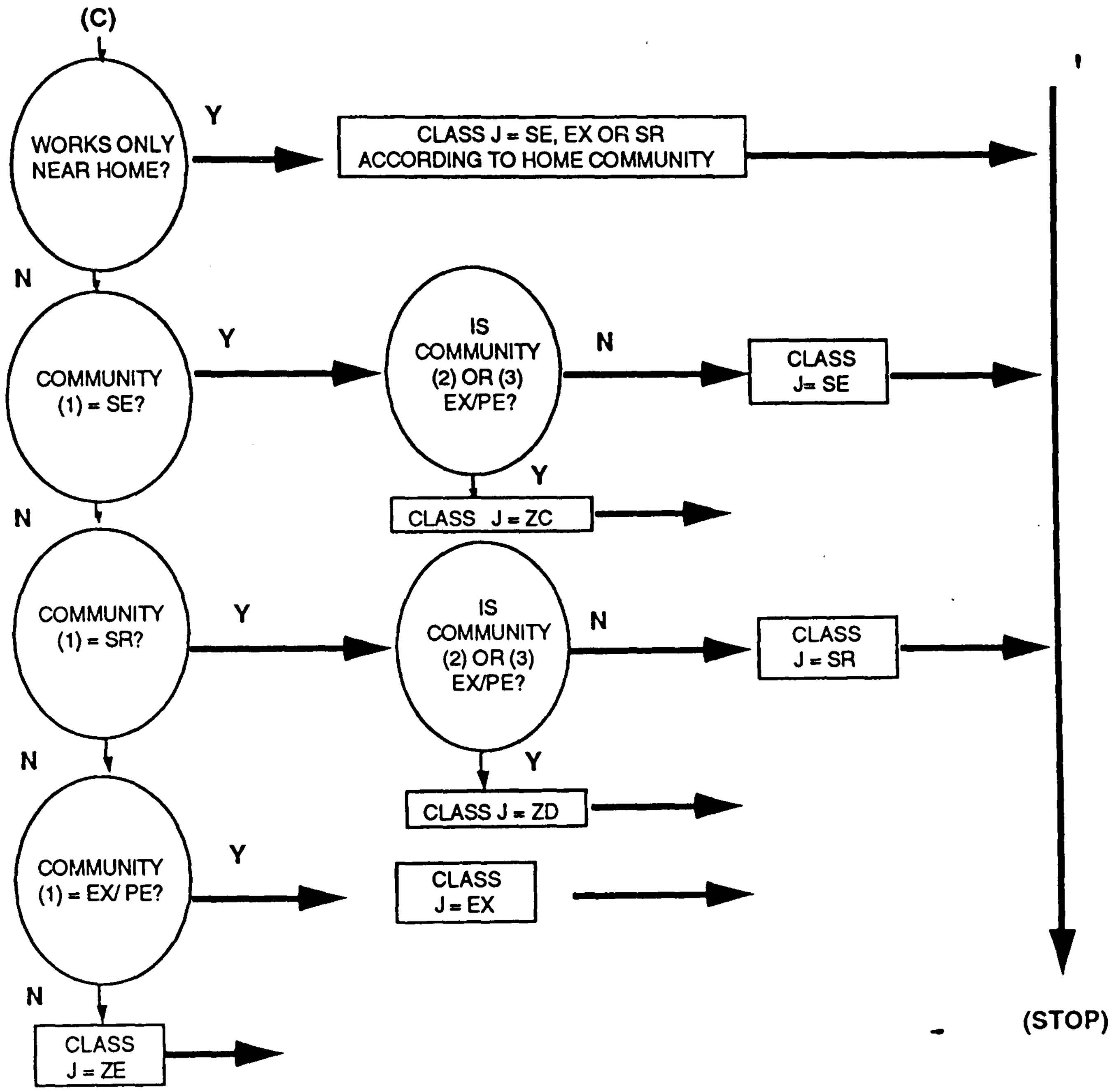


Figure 4.2D. ELIGIBILITY FLOW DIAGRAM
Classification of paid labourers.



All eligibility procedures were dependent on occupational exposure, which was defined as having direct contact with irrigation while farming. Exposure was also measured according to the source of water used in irrigation (e.g. identification of canal(s), natural springs or rainfall). Eligibility procedures were developed to detect nonexposed households in the rain-fed area and contact with other plots (e.g. for paid labourers). The entire household (e.g. women and children younger than 15 years) was classified in the same category as the farmer('s) occupational exposure. In addition, further procedures involved the timing of individuals contact; the primary eligibility categories are presented in Table 4.1 and 4.2:

Table 4.1 Eligible Household Categories.

Category	Description
NE	Live and work in rain-fed village
SE	Live and work in Endho reservoir zone
SR	Live and work in the two reservoirs zone
EX	Live and work in the raw wastewater zone
*NX	Live in rain-fed village, but have contact with raw wastewater
*ZA	Live in rain-fed village, but have contact with reservoirs' water
*ZB	Have no members in agricultural work
*ZC	Have plot in Endho zone and another plot in raw wastewater zone
*ZD	Have plot in the two reservoirs zone and another in raw wastewater zone
*ZE	Plot in unclassified category
*ZF - ZG	Live in rain fed village, but have contact with wastewater although the exposure point is unknown.

* Excluded from survey, and from analysis if found in this category

Exclusion criteria for households were: a) having no members working on the land; b) classification as plot owners (who hire paid labourers), although none in the dwelling had exposure; c) having members working in different locations, and therefore having contact with water from more than one source, each with different characteristics; d) having members irrigating with an unknown type of water or an unclassified canal.

4.8. EXPOSURE CATEGORIES.

Data obtained throughout both cross-sectional surveys defined exposure status more precisely. This definition characterised both the gradient of exposure and the time scale required for analysis (e.g. the prepatent periods). Final exposure categories are presented in Table 4.2.

Table 4.2 Final Definition of Exposure.

Variable	Description	Values
ANYEXP	Level of exposure at <u>any</u> time in past	EX = Exposed to raw wastewater SE = Exposed to Endho reservoir wastewater SR = Exposed to two reservoirs wastewater MEZ = Exposed to mixed waters NE = Not exposed (rain-fed) 99 = Not known or excluded for some reason.
EXR1	If above exposure occurred in previous month	Yes ; No
EXR2	If above exposure occurred within last 2 months	Yes ; No

4.9. PILOT STUDY.

A total of 250 families were involved in a small-scale survey conducted between November and December, 1989. The main objectives of the pilot survey were to:

a) assess the prevalence of intestinal parasites and diarrhoeal diseases in selected households of selected villages to more accurately estimate sample size for the cross-sectional surveys;

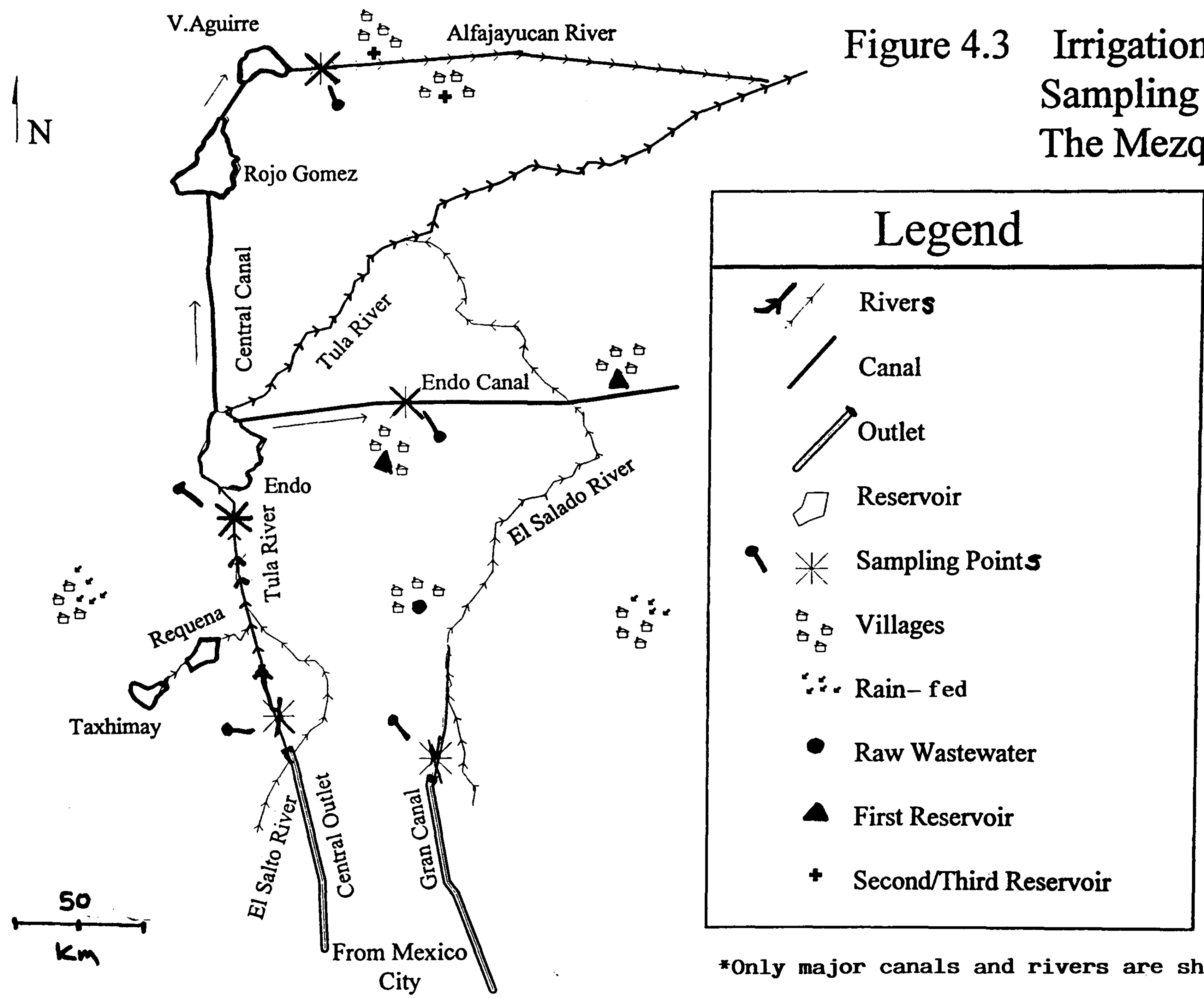
b) assess the acceptability of stool sample collection and estimate expected compliance rates;

c) provide the necessary training for laboratory technicians and field workers (all local villagers) for all procedures which would be incorporated into the large-scale surveys.

4.10. ASSESSMENT OF WASTEWATER QUALITY.

Wastewater samples were collected from previously selected points of the irrigation network of cannals (Figure 4.3). The primary purpose of this activity was to: a) assess the microbiological quality of water used in irrigation; b) determine the effects of wastewater retention in storage reservoirs; and c) provide a basis for the definition of exposure groups. The principal indicators of wastewater quality were faecal coliforms (FC) and *A. lumbricoides* eggs.

Figure 4.3 Irrigation scheme and Sampling Points The Mezquital Valley



*Only major canals and rivers are shown here

4.11. CROSS SECTIONAL SURVEYS

4.11.1. Rainy season survey.

Irrigation requirements in the Mezquital Valley are primarily satisfied by raw wastewater, since rainfall is scarce and erratic in the Valley itself. However, during the rainy season (June to October, 1990) and due to the high rainfall rate in the City's basin, large volumes of storm-runoff and wastewater from Mexico City are transported to and available in the Mezquital Valley. This untreated wastewater enters a series of interconnected reservoirs where it is retained for several months, and then released when farming activities require it for irrigation.

The exposure groups were defined as:

-1. <u>Raw wastewater exposure group</u>: consisting of households in which there is at least one farmer who had contact with raw wastewater;

-2. <u>Two reservoirs group</u>: households in which there was one farmer who had recent contact with the effluent of the <u>second</u> reservoir;

-3. <u>Rain-fed or control group</u>: these were households in which adult farmers used only rainwater for agriculture.

4.11.2. Dry season survey.

The dry season survey was initiated in February, at the beginning of the irrigation period of the agricultural cycle, and conducted through May, 1991. Winter weather conditions do not permit farming activities involving irrigation prior to this period. During the winter months, Mexico City's wastewater is stored in the first reservoir, namely the "Endho" reservoir.

The exposure groups for the dry season survey were:

-1. <u>Raw wastewater exposure group</u>: consisting of households in which there was at least one farmer who had contact with raw wastewater;

-2. <u>Endho reservoir group</u>: households in which there was at least one farmer who had contact with the effluent of the <u>first</u> storage reservoir;

-3. <u>Rain-fed or control group</u>: households in which adult farmers used only rainfall for agriculture.

It is important to underline that both the raw wastewater and the control groups were identical in the two cross-sectional surveys and that only the two reservoirs group or the Endho (single) reservoir group were included alternatively, depending on rainy or dry season. The choice of one or two retention reservoirs was determined by the number of "interventions" required to reduce the health risks associated with raw wastewater reuse. Retention in only one reservoir is a more achievable intervention, although the wastewater quality does not always achieve the 1989 WHO guidelines. Farmers exposed only to wastewater from the Endho reservoir were therefore involved in the dry season survey. Farmers exposed to wastewater from both the second and third reservoirs were included in the rainy season, since the effluent from these reservoirs achieved WHO recommended microbiological quality for restricted irrigation (1989).

4.12. MEASURES OF OUTCOME.

The outcomes of this study are intestinal parasitic infections and symptomatic diarrhoeal diseases. Intestinal parasitic infections were evaluated for the helminth eggs: *Ascaris lumbricoides* and *Trichuris trichiura*,; *Entamoeba histolytica* and *Giardia lamblia* cysts were also tested. No attempt was made to distinguish between *E. histolytica* and *E. Hartmanni.* The prevalence of intestinal parasitic infections was assessed by means of microscopic identification of cysts and for eggs in stool samples. These were recorded as either positive or negative for every individual sampled. Intensity of infection was not recorded as this was subject of a parallel study.

Symptomatic diarrhoeal diseases were defined as the occurrence of three or more loose stools passed in a single day. The recall period for episodes used in the interview was two weeks (WHO 1984). For those individuals with positive answers for diarrhoeal disease, additional data on duration of the episode in days, number of stools passed per day and consistency of faeces during the episodes were recorded.

4.13. OTHER VARIABLES.

Additional variables recorded in the present study included the following hygiene and sanitation related variables:

a) source (s) of drinking water

b) other water related characteristics (i.e. access, storage, boiling, bathing, washing hands and use of soap),

c) excreta disposal facilities (adults and children),

d) washing hands after defecation,

e) rubbish disposal practices.

In addition, socioeconomic status was evaluated using the following criteria:

a) educational status (head of the household and his spouse),

b) dietary patterns, e.g. weekly poultry and meat consumption,

c) house tenure, housing conditions and commodities,

d) land tenure and farming commodities (e.g. tractor, oxen),

e) number of wage-earners,

f) livestock and domestic animals.

Other variables recorded were the source of vegetables consumed (purchased and cultivated), types of crops cultivated and source of irrigation.

4.14. DATA COLLECTION METHODS

4.14.1 Interviews.

Structured and coded questionnaires were used for both household and individual interviews. At the household level, data were collected from the caregiver, usually the spouse of the head of household for household questionnaires. These interviews focused on socioeconomic variables, hygiene and sanitation, as well as potential confounders (see above 4.13). Individual interviews provided information on health status (diarrhoeal diseases) and exposure (occupational, domestic and recreational) and consisted of face to face interviews using structured questionnaires. For individuals absent at the time of the visit or for children under 5 years of age, information was obtained from the closest relative present; mothers provided information regarding their children.

Exposure questionnaires were applied to all individuals in the household over the age of three years, since recreational or domestic contact, and not only farming, are potential sources of exposure, particularly if canals are in proximity of dwellings. Emphasis for questions was placed on the following variables:

1. Recent contact with irrigation water;
2. Activity during exposure; (farming, bathing, playing or domestic duties);
3. If occupational contact was detected, additional data included:
    3.1. exposure point (s) (e.g. canal, river, natural spring etc.....);
    3.2. exposure frequency;
    3.3. date of last exposure;
    3.4. irrigation techniques and farming tools,
    3.5. crops cultivated.

After the interview, field workers made necessary arrangements for a second visit the following day to collect stool samples.

4.14.2. Laboratory tests.

Lists of individuals from eligible households were given to field workers in advance in order to include the complete number of individuals for stool sample collection. Plastic containers were pre-labeled with identification numbers and preservative was added to each container. Stool samples were delivered to the laboratory, where data entry clerks registered only the identification numbers and the lab technicians processed samples on a daily basis. Results were recorded and entered into local computers at the end of each day; double printed lists of samples were re-checked by both field and laboratory supervisors. Check lists of individuals who migrated or those who refused to continue in the study were maintained by field workers and supervisors. Specimen compliance rate was monitored throughout the study and if noncompliance in a given village was higher than 25%, the village was revisited.

The procedure used for parasitological examination was the merthiolate-iodine-formalin-concentration technique (Young *et al.* 1979) Specimens were centrifuged at 500 g for two min. and the pellet was resuspended in Lugol's iodine in order to improve contrast for microscopic observation (Blagg 1955, WHO 1985). The technique dissolves fatty particles thereby obtaining a concentrated, relatively clear stool precipitate. Excess samples were stored for quality control procedures.

4.14.3. Wastewater sampling.

Wastewater samples were collected and processed by a laboratory technician from the Institute of Water Technology (J. Cortez). The sampling points in the irrigation network included the metropolitan sewage outlets, but within the irrigation sites, as well as the influent and effluents of the main reservoirs (Figure 4.3). The shedule was determined by the exposure groups to be surveyed. The techniques used are described below:

4.14.3.1. Faecal coliforms. Faecal coliforms were quantified according to a multiple tube technique using aseptic procedures (APHA, 1989). Wastewater samples were collected from selected points throughout the main canals, as well as from influents and effluents of the three storage reservoirs. Samples were transported on ice (<10°C) to the laboratory and the dilution was prepared by adding 1 ml. of wastewater sample to 9 ml. of phosphate buffer. 1 ml of diluted sample was added to each of a series of 5 tubes. Tubes were incubated at 35°C over 24-48 hours in lactose broth. Confirmatory cultures were carried out in FC medium and tubes were incubated at 44.5°C for 24 hours.

4.14.3.2. *A. lumbricoides*. *A. lumbricoides* eggs from raw wastewater samples were quantified and identified using the Leeds I method (Ayres 1992; more efficient for high debris content), while the Leeds II method was used for samples from reservoir effluents (Ayres 1992). The techniques used are described briefly below.

Leeds I:

1. Grab-samples of 4 l of wastewater were preserved in 10 ml. formaldehyde and transported to the study laboratory;
2. 1 litre of this sample was divided into 100 ml bottles and centrifuged at 700 g for 10 min.;

3. The sediments were washed with 0.01% Triton X100 three times (700 g, 10 min.), transferred to 15 ml tubes and the sediments resuspended in 3-4 ml of a saturated solution of $MgSO_4$;

4. The tubes were then filled with the same solution and centrifuged at 300 g for 1 min.;

5. Cover slips were placed on top of tubes (touching liquid interface) for 30 min. to allow flotation of helminth eggs and then examined under the light microscope.

Leeds II:

1. Allow fixed effluent sample (4 l sample + 10 ml formaldehyde) to sediment at least 1 hr;

2. Supernatant was removed leaving the sediment in 60 ml solution, transferred to a centrifuge tube and centrifuged at 700 g for 10 min.;

3. The supernatant was discarded leaving the sediment suspended in 2-3 ml.; add NaCl (sp. gravity 1.04) to fill 5 cm. depth and allow to settle 1 hr;

4. Siphon off the supernatant leaving 2-3 ml with sediment. A sample of this preparation was then placed in a Doncaster or a Sedgewick-Rafter chamber and checked in a light microscope at 200X, 400X and 1000X magnification.

4.15. DATA ENTRY AND MANAGEMENT

4.15.1. Questionnaires.

Data from each questionnaire were entered into a dBase III database on local computers and statistical analysis conducted with the aid of SAS programming. Data were double entered by two independent data entry clerks; discrepancies and omissions were reported first to the data manager and then to field supervisors. Errors were corrected after checking original and processed data. Data management procedures were designed within the overall data management system (Figure 4.4).

Questionnaires were field-coded and checked by supervisors at the study site headquarters. Each field worker had an individual ID code, which was registered on completed questionnaires. While checking questionnaires (20% of these were checked for quality control), the supervisor was able to identify mistakes and missing data, as well as the identity of the interviewer. Whenever possible, omissions or incomplete information were corrected by a follow-up visit to the household. The author of this report was responsible for the supervisionOf the overall process.

4.15.2. Parasitology tests.

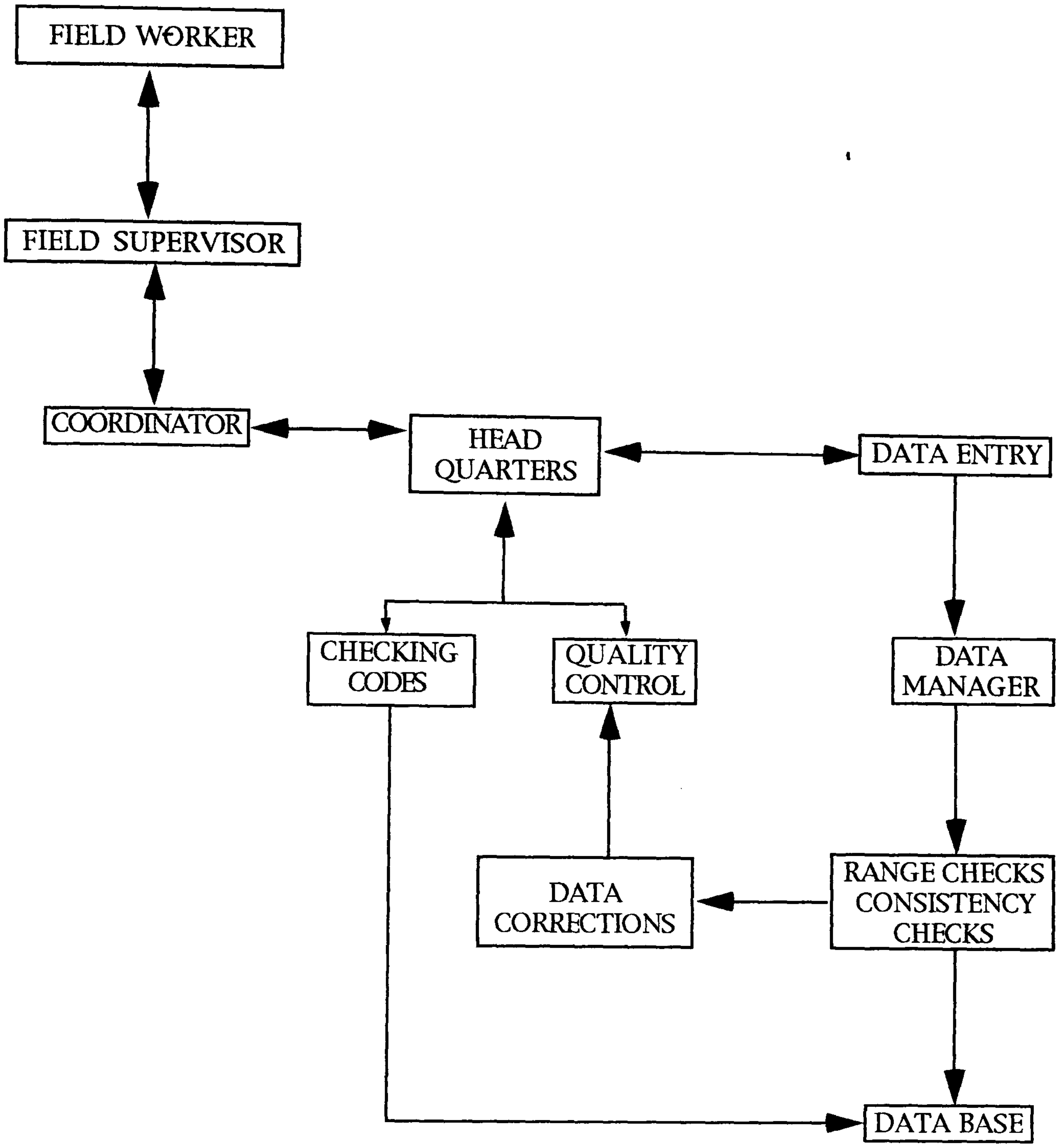
All stool samples were identified and processed at the headquarters laboratory by two trained technicians and one supervisor. Samples were checked for parasite ova (*A. lumbricoides*) as well as for protozoan cysts (*G. lamblia* and *E. histolytica*) and their presence or absence in the stool preparation recorded. Parasitology results were merged with data from the individual questionnaires by using Epi-Info.

Household data record files were used to define eligible units, classify them into exposure categories and classify them for descriptive analysis of socioeconomic, hygiene and sanitation variables. Individual's data were used to create records on exposure, diarrhoeal episodes and parasitological test results.

4.15.3. Wastewater quality.

A sample was positive for faecal coliforms when tubes showed gas production; the most probable number (MPN) of faecal coliforms/100 ml was read from standard tables. Results for faecal coliform counts and *A. lumbricoides* eggs were recorded in the sample data sheets, including the source of the sample, date and other relevant information.

Figure 4.4
Wastewater Reuse Study
Data management
The Mezquital Valley 1989 - 1992



4.16. QUALITY CONTROL PROCEDURES.

4.16.1. Field work.

Field supervisors were trained to manage records, map areas and supervise quality control procedures related to the interviews (e.g. revisiting and checking data consistency). In order to monitor quality control, 1 out of 10 interviews were duplicated by selecting a different interviewer each day. The quality of the data gathered was evaluated by comparing household respondent's answers (field worker vs. supervisor's). Errors were corrected during the second visit (stool sample collection) and interviewers where re-trained when necessary (Fig.4.4). The author of this report was the team's overall supervisor.

4.16.2. Data management.

Frequency distributions and contingency tables were periodically evaluated as were range and consistency checks. Verification of these values was performed using SAS programming. The main procedures were monitored by the principal investigator.

4.16.3. Laboratory tests.

Local lab technicians (diploma 1 year of trainning) were retrained by skilled technicians from the Department of Infectology, INN, during six months and through the pilot study. During the main study, 1 out of 20 preparations was checked by a different microscopist as an internal quality control measure. 1 in 5 of these were examined by the Author of this report. External control was conducted in Mexico City (Laboratory of Infectious Diseases, INN) where remaining sample aliquots were stored. Original results and quality control evaluations were compared by the principal investigator. Technicians making repeated mistakes were retrained; half way of the survey one of the lab microscopists was dismissed because of systematic error.

4.17. DATA ANALYSIS.

4.17.1. Descriptive analyses.

Each household was classified on the basis of the quality of water with which it had contact. Only households with exposure to water of known origin were included in the analysis. The date of exposure was defined for each particular outcome. For individuals having had diarrhoeal diseases and *G. lamblia* infection, recent exposure was defined as having occurred within the previous month, while a definition of past exposure over the previous year was used for infection with *A. lumbricoides* and *E. histolytica*.

The prevalence of each infection was calculated for each exposure group and age category. Initial age categories were defined as 0-4, 5-14 and over 15 years. During final analysis (see below), all individuals over 5 years were grouped into a single age category. Dummy s new variables based on socioeconomic, hygiene and sanitation factors were created (dummies) and were later used for comparative analysis between different exposure groups.

4.17.2. Further analysis.

A list of potential confounders was produced and Mantel-Haentzel tests (Schlesseman, 1982) were performed to ascertain whether these variables were associated with exposure or with the disease (see list below). Those factors which were associated with the outcome, after adjusting for the effect of exposure were analysed in a multiple logistic regression model using (EGRET).

List of confounders:

Drinking water supply	Distance(s) from supplies (drinking water)
Boiling water practices	Storage water practices
Adults' defecation sites	Children' defecation usual sites
Washing hands	Basic sanitation
Literacy	Occupation
Dwelling's material	Commodities
Dietary patterns	Income
Source of vegetables	Cultivation of vegetable crops
Irrigation sources	Hygiene appearance

Potential confounders were fitted into preliminary models as dummy variables, which were then reduced so that they contained only those factors which were significantly associated with the exposure status and the risk of infection or disease. The main analysis presented in this thesis focussed on:

a) comparison between the exposed and control group, and

b) comparison between the reservoir(s) group and control.

4.18 LOGISTICS.

Members of the team were retrained before the start of each stage of the study. Major responsibilities were defined in a flow chart and a manual of procedures was developed, specifying the timing of major activities (Fig. 4.1). All of these procedures were defined and supervised by the principal investigator and coordinated by field supervisors, the data manager and laboratory technicians. A timetable was developed to schedule the sequence of villages to be visited, as well as dates and number of days in each village. This timetable was visible on the wall of the study site headquarters for reference during discussion and surveillance of progress.

This logistical organization made it possible to involve a large number of villages throughout a scattered area; communities of every exposure category (water zones) were visited at the same rate and field workers were rotated when visiting different villages. In order to increase compliance, the objectives of interviews and stool collection were illustrated in printed pamphlets, which were delivered to every family visited. Compliance rates were assessed before moving on to the next village. If non-compliance was higher than 25%, revisiting was included in the following weekly plan.

Stool samples were transported daily from the community to the study site headquarters; identification numbers were recorded and parasitology results entered into the corresponding files. Wastewater samples were gathered only over weekends on a monthly basis due to logistical availability of vehicle and driver. Data gathering for each survey was completed over a period of up to 5 months.

4.19. JUSTIFICATION OF THE STUDY DESIGN.

Various study methods have been used for the assessment of the health impact of wastewater reuse. Although epidemiologists have long recognized that observational studies do not prove cause and effect, this type of research is frequently used in the assessment of causality (Flanders *et al.*. 1992). In studies concerned with acute diarrhoeal diseases, the epidemiologist must pay special attention to the exposure immediately prior to the onset of the diarrhoeal episode. For infections with long prepatent periods (e.g. *A. lumbricoides*), exposure over several months may be a relevant factor.

The present study was based on the simultaneous assessment of both exposure and outcomes, and the central objective was to assess the beneficial effects fro hydraulic retention. As far as possible, the study was designed to allow for the assessment of potential confounders. Both the level and the timing of exposure were carefully characterized from individual questionnaires. The former was based on the type of water used in irrigation, whereas the latter was based on a detailed description of farming activities. Data on diarrhoeal diseases were also obtained from individuals' health questionnaire, while intestinal parasitic infections were defined by stool sample results. Thus, it was considered that the procedures used in this study provided a method to investigate the direct "cause-and-effect" relationship between exposure and disease.

The time-window for each survey was defined by three basic considerations:

a) the timing of the agricultural cycle and farming activities;

b) the seasonal availability of wastewater coming out of the storage reservoirs, (such a situation provided a unique opportunity to test the effect of one versus more reservoirs on the health risks under study);

c) the seasonality of the study outcomes (e.g. diarrhoeal syndromes).

Parasitology techniques were selected on the basis of the following considerations: the MIF-C (a modified technique derived from the original formalin-ether) more effectively separates cysts and eggs, regardless of their size, shape or density. The preparations obtained were "cleaner" (free of debris), since ether removes fatty substances as well as other "artifacts". As a result, helminth eggs and protozoan cysts are more readily identified and counted during microscopic examination. MIF assays are more expedient than conventional techniques (i.e. the stool is centrifuged only once, instead of three times as in other techniques) and in terms of safety, formalin inactivates cysts and eggs, thus the risk of infection among field staff and lab technicians is reduced.

The overall prevalences of *Trichuris trichiura* and *Cryptosporidium parvum* infections were unexpectedly low (below 1% and 4%, respectively), and excluded from further analysis. A parallel study, whose study population was obtained from the sampling frame generated by this research, focussed on the intensity of *A. lumbricoides* infection. Since assessing intensity of infection is labour-intensive, it was decided not to repeat the same procedures in this study, whose sample size was considerably larger.

The microbiological quality of wastewater was assessed by monthly monitoring regimes, using the best experimental techniques available for both raw wastewater and crop irrigation effluents. While a more intensive sampling regime would have been desirable, available information indicates that there are no significant differences between the mean eggs per litre in raw or effluent samples taken on a weekly or monthly basis (Stott *et al.,* 1995). The evaluation of diurnal variation in the number of nematode eggs and faecal coliforms in wastewater was severely restricted by logistical and financial considerations.

4.20 SOURCES OF BIAS.

The target population in this study were farmers and their families, since the core objective was to assess the health effects of occupational exposure to wastewater. The main source of bias in this research, however, is the fact that each study group consisted of only one geographic area, and there may be socioeconomic differences between the areas.

Further, a shorter recall period for assessing the prevalence of diarrhoeal diseases would have been more appropriate, but implied logistical problems extremely difficult to cope with in the field. Additional bias may have resulted regarding data from absent individuals, whose health status and exposure-related data were provided by surrogates (e.g. spouses). These problems were impossible to overcome, since farmers leave home often before 4 AM, or if they have to irrigate, spend most of the night in the plot.

In order to reduce bias in the information collection methods several strategies were implemented, including rotating the field workers through the different areas and the use of uniform quality control procedures (i.e. repeated interviews by supervisors) to corroborate reliability of the data. Selected questions were used to avoid repeating the compete interview. Thus, we assume that by using standard procedures, the same problems affected all exposure groups in the same way.

False negative parasite diagnosis may result from erratic patterns of excretion (i.e. immature or male worms, no production of ova, distribution of cysts or ova in the stool specimen). In order to minimise this problem, more than one stool specimen would have to have been checked. Logistical difficulties did not permit collection or processing of more samples. Correlatively, there is no clear evidence of the significance between in intestinal parasite prevalence estimates after examination of either one or two stool specimens (Gyorkos *et al.* 1989). As above, we assume that the procedures used in this study affected all exposure groups in the same way.

Misclassification and false positive parasite diagnosis may result from confusion of *E. histolytica* with *E. hartmanni*, if cysts are not measured or nuclei not adequately identified. In moderate infections, misclassification of infected individuals to false positives was likely to occur, while differential misclassification was more probable if prevalence of infection associated with one of the exposure groups was lower than in the other exposure groups.

A further source of bias in this study may result from low sensibility of wastewater sampling techniques. In addition, a more rigorous sampling regime would have been highly desirable, in order to have a more thorough evaluation of microbiological water quality in the irrigation network. The evaluation of diurnal variation would have required collection of composite samples over 24 hour periods or a more strict determination of potential diurnal variation in samples.

CHAPTER 5. CHARACTERISTICS OF THE STUDY POPULATION.

5.1. SOCIODEMOGRAPHIC PROFILE.

This study involved a total of 57 villages in the Mezquital Valley. These villages were initially subdivided according to type of water used in agricultural production. A census was conducted on a total of 4,399 households, out of 11,246 dwellings initially numbered. Households with members not engaged in agricultural production were excluded from further consideration. The numbers of villages and households in each study group are presented in Table 5.1. The highest population densities were observed in the raw wastewater and the Endho reservoir area, whereas the lowest density was observed in villages form the two reservoirs water zone.

Table 5.1 Demographic Profile of the Censused Population, the Mezquital Valley, 1989.

WATER ZONES	No. VILLAGES	No. HOUSEHOLDS	PROPORTION (%)
Raw wastewater	9	1311	29.8
Single reservoir	25	1281	29.1
Two reservoirs	11	589	13.4
Rain-fed (control)	12	1218	27.7
TOTALS	57	4,399	100.0

5.2. CHARACTERISTICS OF THE EXPOSURE GROUPS.

All households included in the study were classified according to the farmer's individual characteristics (see eligibility procedures explained in section 4.6). These procedures produced "cleaner" categories of exposure than those defined by the initial "water zone" criteria used during the census. These final exposure categories were further adapted in the analysis presented in the next chapters.

In the raw wastewater zone 163 households were detected irrigating with the effluent of the Endho reservoir and were excluded from further consideration. In communities initially classified within the rain-fed water zone, a total of 66 households irrigated with wastewater (34 raw and 32 from the Endho reservoir) and these were excluded from further analysis (Table 5.2). Households with unclassified or unknown canals were additionally excluded from the final categories. Thus, in the raw wastewater zone a total of 55 households did not know the source or canal of their irrigation, whereas in the Endho reservoir zone, the number of households irrigating with wastewater from unknown sources was considerably higher (574); these were also eliminated from further consideration. In the two reservoirs' zone, 39 households with unclassified canals were identified and excluded. In the rain-fed area, 223 households had unclassified canals and were excluded.

Table 5.2 Exposure Categories of Households, the Mezquital Valley, 1991.

Source of canal	WATER ZONE CATEGORIES Raw Wastewater	Endho Reservoir	Two Reservoirs	Rain-Fed	Totals
Raw wastewater	848	5	0	34	887
Endho reservoir	163	951	3	32	1149
Two reservoirs	0	0	545	0	545
Rain - fed	0	0	0	929	929
Unknown or unclass. canal	55	574	39	223	891

5.2.1 Age and gender distribution of the exposure groups.

A total of 24,983 individuals were included in the main study. Age and gender distributions of the population are shown in Table 5.3. These data indicate that the population profile was young, with more than 40% of the total population under the age of 15 years. The lowest proportion of older individuals was observed in the two reservoirs' group. Slightly fewer females were found in all exposure groups as compared with males, although gender distribution within each age category was fairly balanced in the different exposure groups.

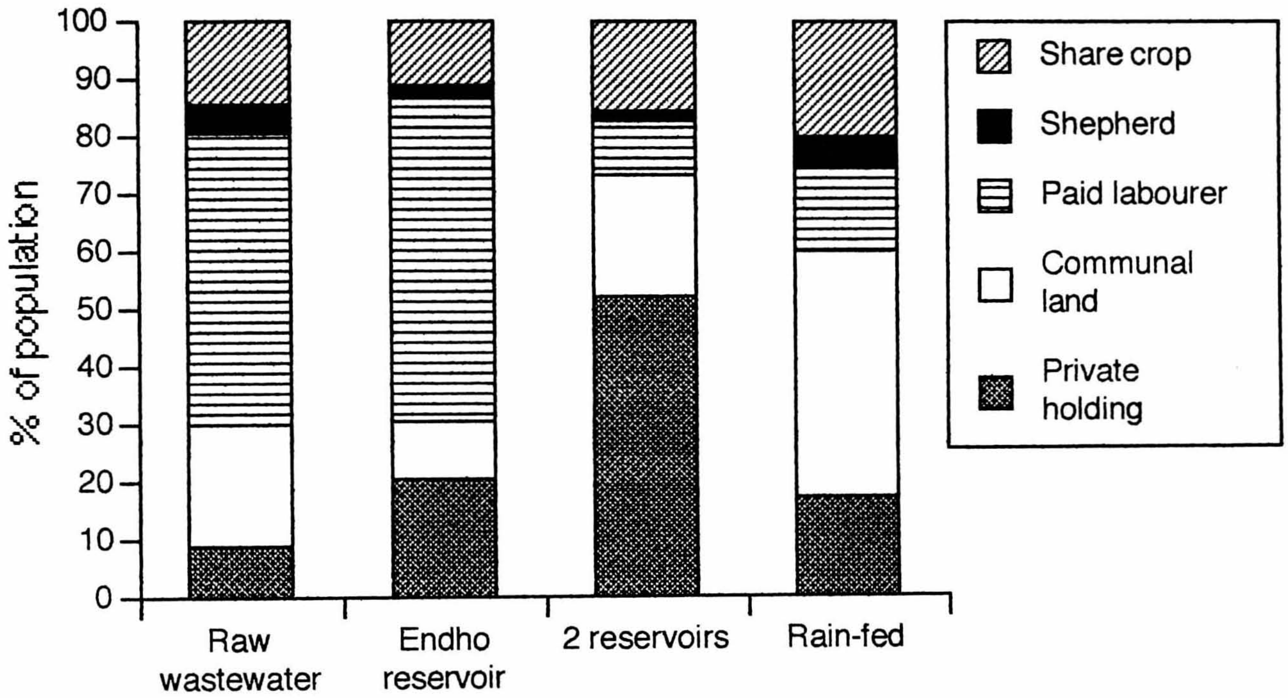
Table 5.3 Gender and Age Distribution of Individuals from the Exposure Groups, the Mezquital Valley, 1991.

	EXPOSURE GROUP							
	UNTREATED		1 RESERVOIR		2 RESERVOIRS		CONTROL	
Age group	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
0 - 11 mo	54 (1.5)	54 (1.5)	49 (1.3)	51 (1.5)	47 (2.5)	43 (2.5)	70 (2.0)	64 (2.0)
1 -4 yrs	359 (9.0)	350 (9.5)	365 (10.0)	332 (9.5)	207 (11.0)	207 (12.0)	302 (8.5)	292 (9.5)
5 - 14 yrs	1126 (28.0)	996 (27.0)	1009 (27.7)	949 (27.0)	582 (31.5)	498 (29.5)	1007 (28.0)	848 (27.5)
15 + yrs	2469 (61.5)	2248 (62.0)	2208 (61.0)	2152 (62.0)	1030 (55.0)	946 (56.0)	2186 (61.5)	1882 (61.0)
TOTALS	4008	3648	3631	3485	1876	1684	3565	3086

5.2.2. Agricultural features.

More than 60% of the households owned their agricultural plot, either as a private asset or as communal land property ("ejido"). The highest proportion of private land holders (pequeno propietario) was found in the two reservoirs group. Landless families who depended on paid labour were more common in the single reservoir and raw wastewater groups (Figures 5.1 and 5.2).

Figure 5.1 Categories of farmers in the exposure groups, the Mezquital Valley, 1990.



Data indicate that there were different land tenure patterns in the Mezquital Valley, as well as differences in the farmer's socioeconomic characteristics. Ejidos (communal farms) were most frequent in the rain-fed group, followed by the two reservoirs and the raw wastewater group. On the other hand, paid labourers were most present in the raw wastewater and the single reservoir exposure groups. Traditional agricultural patterns were more prevalent in the control and the two reservoirs groups. Another form of agricultural activity was shepherding, which occurred most frequently in the rain-fed area and less frequently in the two reservoirs group.

Maize was the most common crop cultivated by agricultural workers from the four exposure groups (Figures 5.3.a. and 5.3.b). Fodder (alfalfa) was the second most common crop and was cultivated in similar proportions throughout the irrigated areas. This was not the case in the rain-fed area, where land owners rarely cultivated this crop. A small proportion of families in all of the exposure groups cultivated some type of vegetable crop.

Figure 5.2. Land tenure by exposure group in the Mezquital valley, 1990; owns land (dark hatch), landless (line hatch).

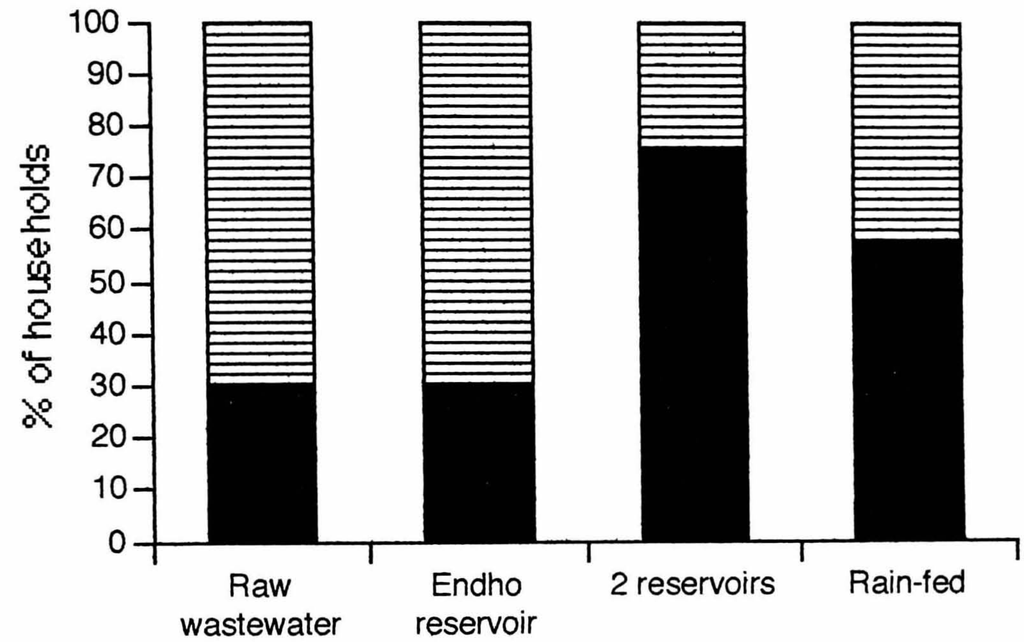
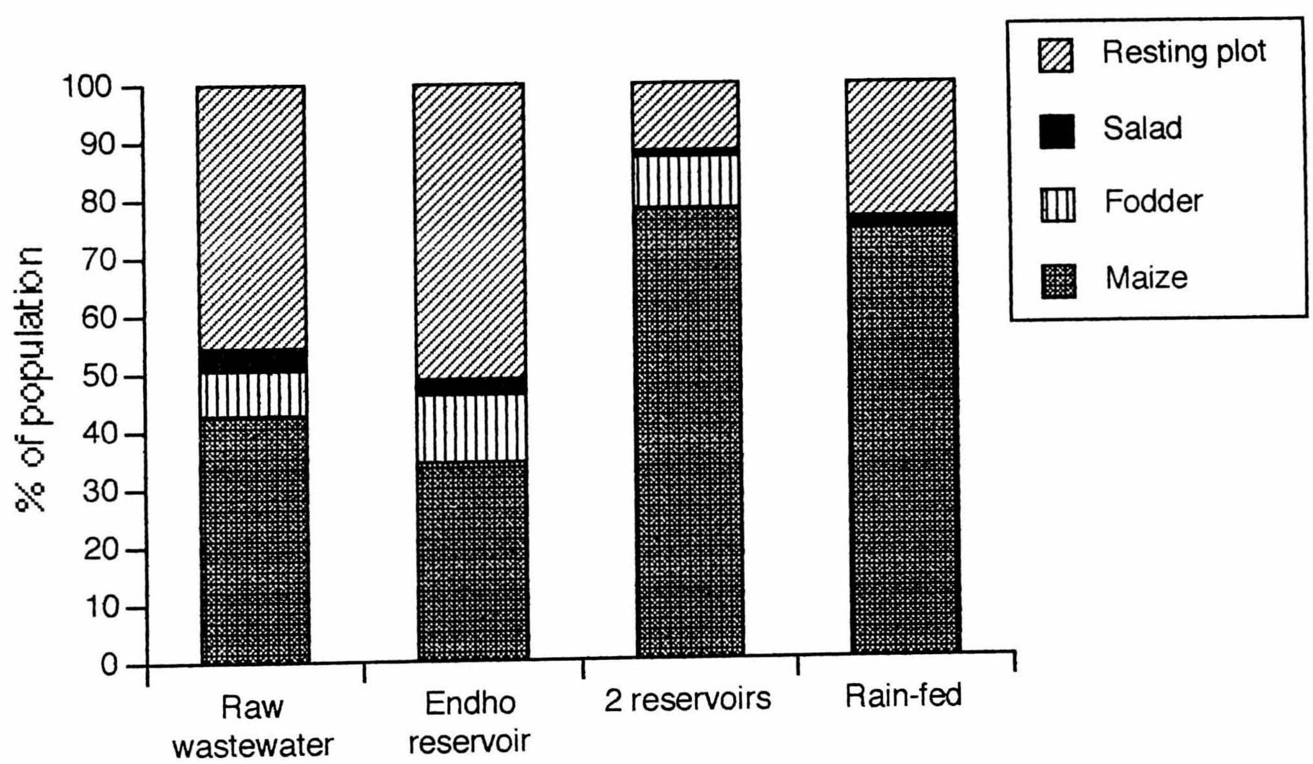
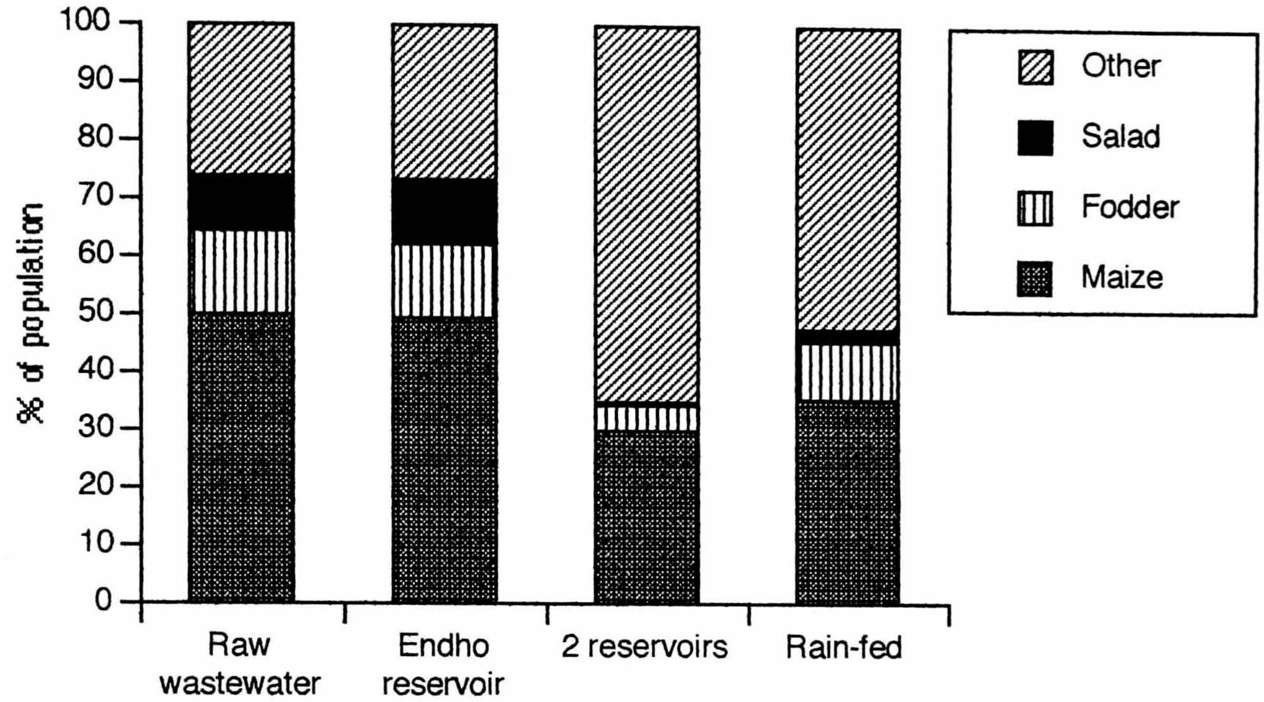


Figure 5.3a Agricultural land use by farmers who own their farm, the Mezquital Valley, 1990.



A considerable proportion of farmers worked on "resting plots" which involved preparation of the soil (e.g. ploughing, clearing) or repairing irrigation canals, all of which was often combined with shepherding.

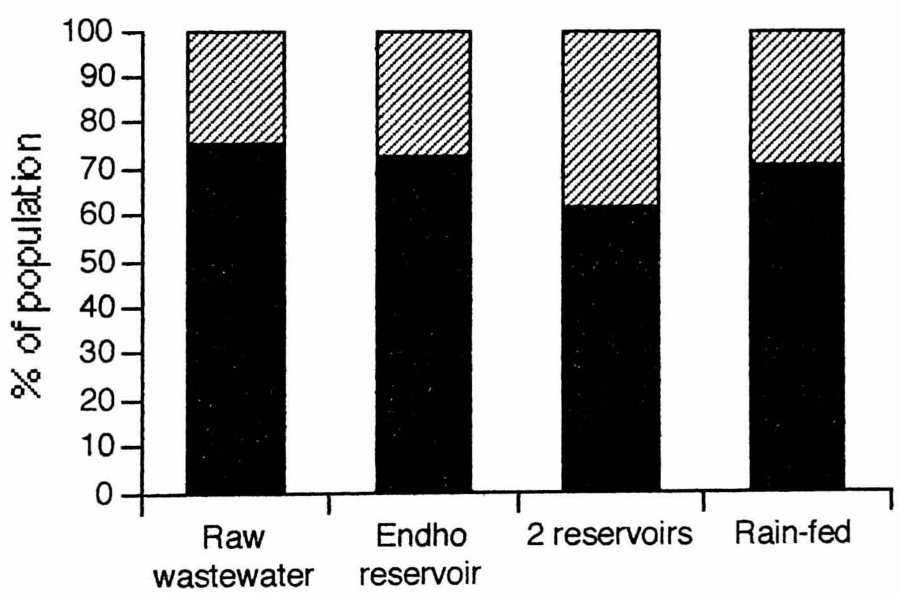
Figure 5.3b Agricultural activities of paid labourers in the Mezquital Valley, 1990.



5.2.3. Literacy of care-givers.

Literacy rates for household care-givers were highest in the raw wastewater and the single reservoir groups suggesting lower sociocultural conditions in the other two groups (Fig. 5.4).

Figure 5.4. Literacy of the care-giver, the Mezquital Valley, 1990: literate (dark hatch), illiterate (diagonal hatch).



5.2.4. Housing conditions.

Roofing materials and drinking water supply were used as basic dwelling socioeconomic indicators (Figures 5.5 and 5.6). The highest proportion of dwellings with a cement roof (considered as an indicator of better housing conditions) was found in the raw wastewater groups. Corrugated roofs were mostly observed in the raw wastewater group, whereas the highest proportion of asbestos roofing was found in the single and two reservoirs groups.

Figure 5.5. Roof materials by exposure groups in the Mezquital Valley, 1990.

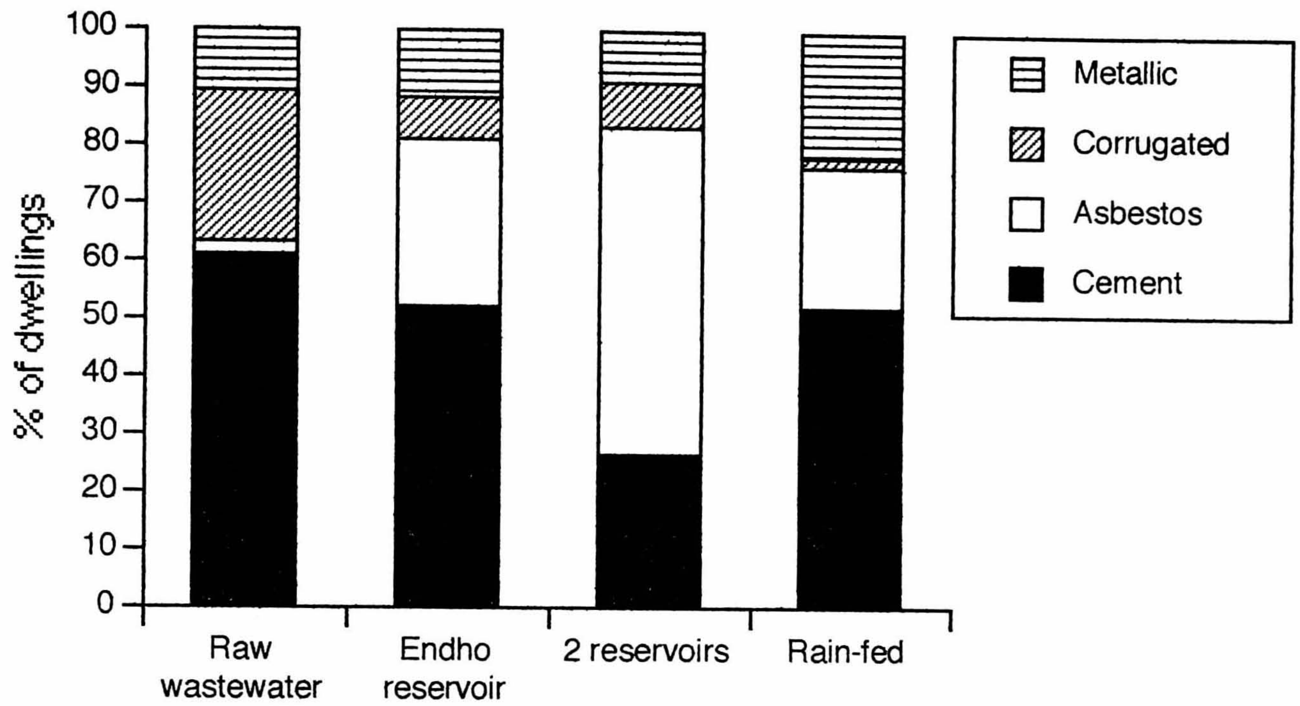
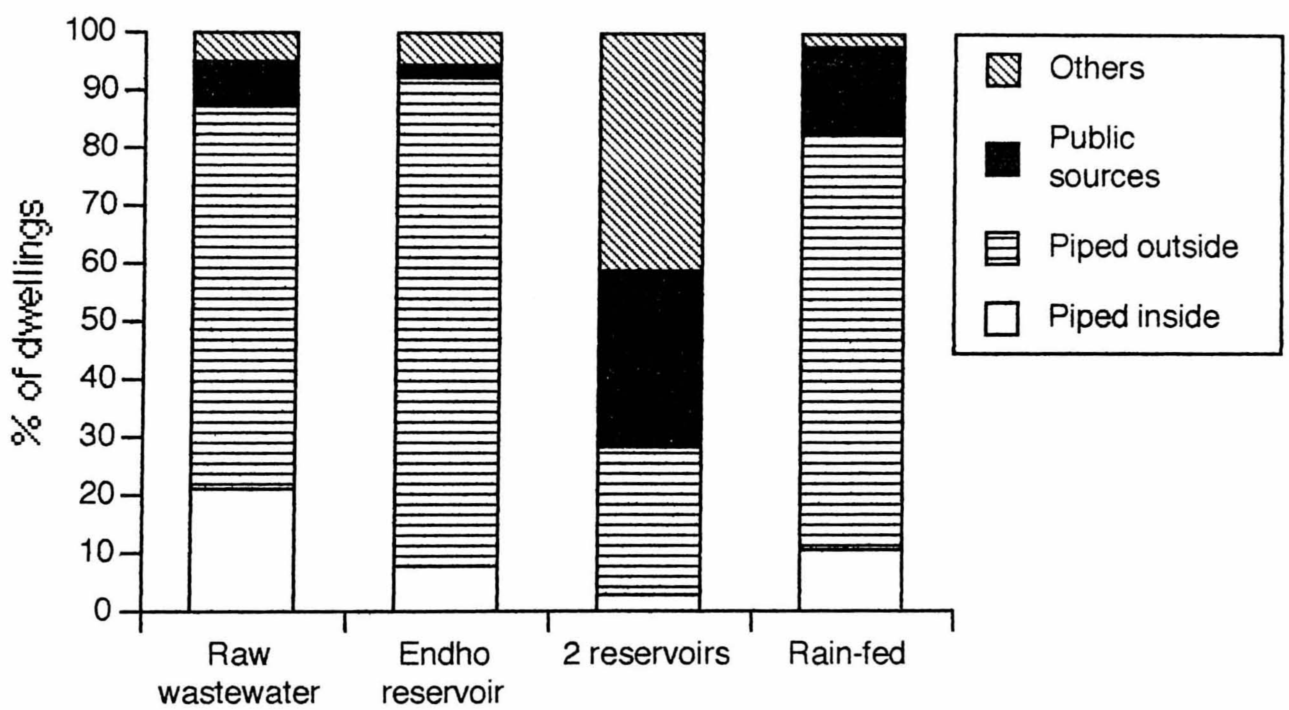


Figure 5.6 Water supply for houses in different exposure groups of the Mezquital Valley, 1990.



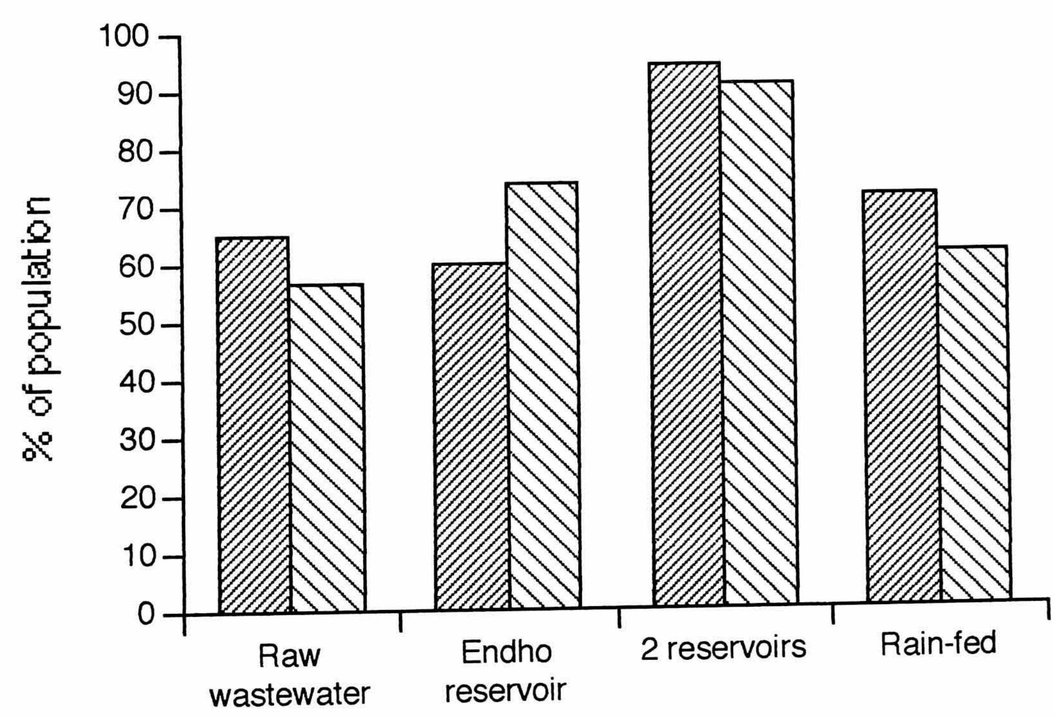
5.2.5. Drinking water supplies.

Nearly 80% of households had piped water supplies (Figure 5.6). However, taps located outside the dwelling were more prevalent than those located inside the dwelling. The Endho reservoir group had the highest proportion of dwellings with water piped outside the dwelling. The highest proportion of intra-dwelling supply was found in raw wastewater households. Households obtaining drinking water from communal sources (e.g. tank trucks, wells) were more common in the two reservoirs and rain-fed groups. The latter two exposure groups also had the highest proportion of alternate sources of drinking water (e.g. commercial bottled water, collected rain water and natural springs).

5.2.6 . Basic sanitation.

Only one variable was recorded as an indicator of dwelling sanitation: defecation practices. Defecation in the yard's soil, both by adults and children is a common practice (Figure 5.7). Nearly all households from the two reservoirs group were found to practice such a habit. Only in the Endho reservoir exposure group was the proportion of adults defecating in the soil higher than that for children. However, greater than 50 % of all groups defecated in the yard's soil.

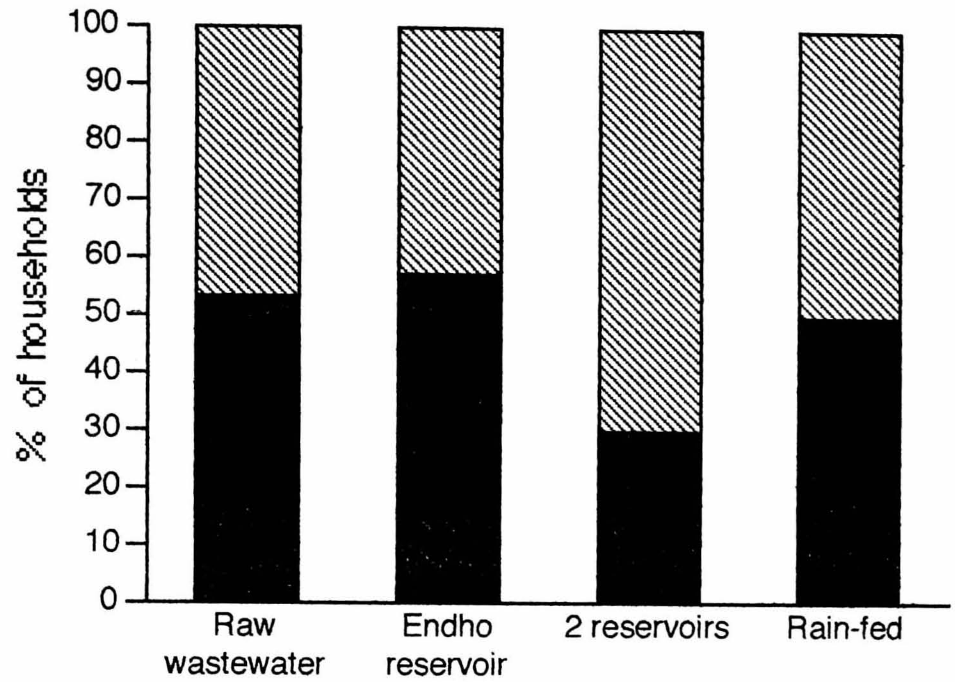
Figure 5.7. Defecation in the yard's soil by the exposure groups, the Mezquital Valley, 1991; children (close diagonal hatch), adults (broad diagonal hatch).



5.2.7. Other facilities.

More than half of the households from the Endho reservoir, raw wastewater and control groups had some form of rubbish disposal (Figure 5.8).

Figure 5.8 Rubbish disposal facilities in different exposure groups, the Mezquital Valley, 1991; with facilities (dark hatch) and without (diagonal).



5.3. SUMMARY

Households from the two reservoirs group had the lowest socioeconomic status, as defined by the mother's literacy rates, numbers of bedrooms in the dwelling and the quality of housing materials (e.g. asbestos roof). This group was also characterised by a lower level of hygiene and sanitation status, by drinking water supplies and through the availability of toilet and rubbish disposal facilities. Overall, the raw wastewater and single reservoir groups were quite similar (Table 5.4). A description of these associations are included in the following analysis (chapters 6 and 7).

Table 5.4 Distribution of Selected Variables (expressed as percentage for individual group) by Exposure Group, the Mezquital Valley, 1990.

VARIABLE	PROPORTION OF EXPOSURE GROUP WITH CHARACTERISTIC			
	UNTREATED WASTEWATER (%)	ONE RESERVOIR (%)	TWO RESERVOIRS (%)	CONTROLS (%)
Households (n=)	848	950	545	929
Occupation of head of household				
Land holder farmer	32	31	86	81
Landless labourer	55	58	10	18
Other (e.g. shepherd)	13	11	4	11
Mother's literacy	79	77	61	86
Completed primary sch.	25	26	24	24
Housing roof				
Cement	61	52	27	51
Corrugated	26	5	7	2
Asbestos	2	31	58	25
Others	11	12	8	22
Number of bedrooms				
1 - 2	78	81	86	79
3 +	22	19	14	21
Source of drinking water				
Outside and public sources	75	84	54	86
Piped inside dwelling	21	7	22	10
Other (wells, bottled)	4	9	24	4
Hygienic appearance				
Clean	6	3	4	5
Unclean	94	97	96	95
Wash hands				
Usually	95	97	81	95
Store drinking water				
In uncovered recipient	31	34	23	33
Toilet facility				
No	58	71	89	65
Animal excreta in backyard	64	57	42	55
Local source of vegetables	59	42	16	64
Diet: chicken/week				
1	74	73	43	56
2 +	26	27	57	44

CHAPTER 6. RAINY SEASON SURVEY RESULTS.

6.1. INTRODUCTION.

The primary concern of the present study was the variable 'exposure to wastewater', followed by the relative importance of potential confounders. The study outcomes were:

1. *A. lumbricoides* infection,
2. *G. lamblia* infection,
3. *E. histolytica* infection,
4. Acute diarrhoeal diseases.

The exposure groups in the rainy season were:

1. Raw wastewater,
2. Wastewater from two reservoirs,
3. Nonexposed (controls).

Age categories analysed were 0-4, 5-14, and over 15 years of age. During final analyses, however, the 5-14 and over 15 years were grouped into one single age category (over 5 years), since the effect of exposure was not significantly different between the two categories. Analyses presented in the present chapter are focused on the following:

1. Comparison between exposed and control groups, and
2. Comparison between the two reservoirs group and controls.

These comparisons are focused on the assessment of parasitic intestinal infections and diarrhoeal diseases associated with exposure to raw wastewater, and on the evaluation of the effect of these storage reservoirs on such risks.

Initially, potential confounders were screened and were fitted into multivariate models as dummy variables. During final analyses the effect of exposure was retained in the model, and only those factors associated with the outcome, after adjusting for the effect of wastewater exposure, were used in the multiple regression analysis. Outcomes were coded as binary variables ("Yes" or "No"); odds ratios presented throughout the results section refer to the crude values for exposure (OR_1 first column) and to adjustment for exposure and confounders in the final model (OR_2 column on the right hand side), respectively. P values refer to OR_2. Unless stated otherwise, the term "risk" is used here to refer to the excess of infection or excess of disease in those exposed to raw or treated wastewater, as compared with the control group.

6.2. RAINY SEASON SURVEY RESULTS

A total of 1,900 households and 9,433 individuals were analysed from the rainy season. A total of 7,665 stool samples were collected throughout the survey, representing an 81% compliance rate. It should be noted, however, that denominators were different in the analyses of intestinal infections and diarrhoeal diseases, since compliance rates for interviews (diarrhoea) and stool samples (intestinal infections) were not the same.

The prevalence of *A. lumbricoides*, *E. histolytica* and *G. lamblia* infections by age and exposure group are summarised in Table 6.1. Tables 6.2 to 6.7 are the results of logistic regression analyses of all parasitic infections. Table 6.8 summarises the prevalence of diarrhoeal diseases and Tables 6.9 and 6.10 are the results of logistic regression analyses of diarrhoeal diseases.

6.2.1. *A. lumbricoides* infection.

6.2.1.1. Prevalence of *A. lumbricoides* infection. The raw wastewater group had the highest prevalence rates of *A. lumbricoides* infection and lower prevalences were observed with decreasing levels of exposure (Table 6.1). In relation to age, individuals under 14 years from the raw wastewater group had a significantly higher prevalence of infection than older individuals. Prevalences for the two reservoirs group was similar to that of the nonexposed group (95% CI = 0.54-3.22).

6.2.1.2. Effect of exposure and confounding factors in children 0 to 4 years old. Logistic regression analysis was conducted to calculate the magnitude of the risk of infection with *A. lumbricoides* associated with wastewater exposure, while allowing for the confounding effects of other factors (Table 6.2). Children aged 0 - 4 years from the raw wastewater group had a higher prevalence of infection than controls (95% CI= 2.95 - 12.75), whereas the prevalence in the two reservoirs group (no eggs in wastewater) was not significantly different to that found in the control group (95% CI= 0.54 - 3.22). After adjusting for other confounding factors, the risk from raw wastewater exposure remained high (OR= 5.71). Adjustment for confounders did not alter the lack of significant difference between the two reservoirs and control groups (OR= 1.29). Young children from landless households and those living in the poorest dwelling's roof category had a higher prevalence of infection with *A. lumbricoides* than those families having a plot and better quality of roof.

6.2.1.3. Effect of exposure and confounding factors in individuals aged 5 years and older. In children aged 5-14 years and individuals over 15 years, the effect of exposure was originally assessed separately.

Table 6.1 Rainy Season Survey
Prevalence of Intestinal Parasitic Infections
the Mezquital Valley, 1990.

	EXPOSURE GROUP		
	RAW WASTEWATER	NONEXPOSED	2 RESERVOIRS
Ascaris lumbricoides			
0 - 4 yrs.	13.7 (46/335)	2.5 (9/356)	3.3 (11/333)
5 - 14 yrs.	16.5 (115/698)	1.2 (10/855)	2.0 (15/729)
15 + yrs.	5.6 (82/1457)	0.5 (71/440)	1.2 (13/1091)
Giardia lamblia			
0 - 4 yrs.	17.3 (43/249)	17.1 (61/356)	18.8 (59/314)
5 - 14 yrs.	12.0 (63/525)	11.2 (96/855)	15.2 (103/676)
15 + yrs.	3.7 (18/490)	3.7 (53/1440)	6.2 (43/696)
Entamoeba histolytica			
0 - 4 yrs.	6.5 (22/335)	7.0 (25/356)	4.8 (16/333)
5 - 14 yrs.	15.9 (111/698)	12.0 (103/855)	16.0 (117/729)
15 + yrs.	15.6 (227/1457)	13.9 (200/1440)	14.5 (158/1091)

Table 6.2 Rainy Season Survey
Children aged 0 - 4 years with *A.lumbricoides* Infection
According to Exposure and Other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	9	(2.5)	356	1	1	
Two reservoirs	11	(3.3)	333	1.32 (0.54-3.22)	1.29 (0.49-3.39)	NS
Raw wastewater	46	(13.7)	335	6.14 (2.95-12.75)	5.71 (2.44-13.36)	<0.001
Land holding						
Yes	26	(3.8)	683	1	1	
No	40	(11.7)	341	2.11 (1.22-3.67)	2.20 (1.25-3.84)	0.006
Dwelling´s roof						
Cement	30	(6.5)	461	1	1	
Tiles	11	(3.5)	316	1.67 (0.62-4.48)	1.65 (0.61-4.41)	NS
Corrugated	15	(13.0)	115	1.47 (0.75-2.88)	1.45 (0.74-2.85)	0.06
Others (metal)	9	(7.0)	120	2.28 (0.98-5.28)	2.23 (0.96-5.22)	NS

OR_1 = Odds ratio adjusted for exposure.

OR_2 = Odds ratio adjusted for exposure, land holding and dwelling´s roof.

P value refers to OR_2

Note: Factors significantly associated in the univariate analysis : time in getting water, storage practices, house tenure, commodities farming goods, crowding and literacy.

However, due to the lack of significant differences between the two age groups, they were combined into one age category (5 yrs and older). As shown in Table 6.3,the highest prevalence of infection was found in the raw wastewater group (95% CI= 8.18 - 22.21), although individuals from the two reservoirs group had a two-fold increase in the risk of *A. lumbricoides* infection as compared with controls (95% CI= 1.14 - 3.84). After allowing for confounding factors, the prevalence in those exposed to raw wastewaterremained 13 times higher than that in controls (95% CI= 7.51-23.12), while the risk in the two reservoirs group, although low, remained higher than controls (95% CI = 1.01 -3.71).

Table 6.3 Rainy Season Survey
Individuals Aged 5 years and Older, with *A. lumbricoides* Infection
According to Exposure and Other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	17	(0.7)	2295	1	1	
Two reservoirs	28	(1.5)	1820	2.09 (1.14-3.84)	1.94 (1.01-3.71)	0.04
Raw wastewater	197	(9.1)	2155	13.48 (8.18-22.21)	13.18 (7.51-23.12)	<0.001
Disposal of rubbish						
Yes	90	(3.1)	2892	1	1	
No	152	(4.5)	3378	1.96 (1.49-2.58)	1.72 (1.29-2.29)	<0.001
Source of vegetables						
Outside village	100	(2.9)	3395	1	1	
Local	142	(4.9)	2875	1.57 (1.19-2.07)	1.43 (1.07-1.92)	0.01
Land holding						
Yes	114	(2.4)	4660	1	1	
No	128	(8.0)	1610	1.94 (1.47-2.54)	1.53 (1.14-2.04)	0.004
Dwelling roof						
Cement	105	(3.6)	2912	1	1	
Tiles	29	(1.7)	1741	1.59 (0.92-2.74)	1.59 (0.90-2.80)	NS
Corrugated	83	(10.9)	758	1.94 (1.43-2.64)	1.76 (1.28-2.41)	<0.001
Other (metal)	19	(2.4)	791	1.21 (0.72-2.01)	1.07 (0.63-1.80)	NS
Age						
5 - 14 years	140	(6.1)	2282	1	1	
15 + years	102	(2.6)	3988	0.33 (0.26-0.44)	0.38 (0.29-0.50)	<0.001

OR_1 = Odds ratio adjusted for exposure

OR_2 = Odds ratio adjusted for exposure, disposal of rubbish, source of vegetables, land tenure, dwelling roof and age group.

P value refers to OR_2

Note: Factors significantly associated in the univariate analysis included drinking water house tenure, commodities, crops and crowding.

In the final analysis, while allowing for potential confounding factors, the risk of *A. lumbricoides* infection among individuals aged 5 years and older was also associated with other variables, such as lack of rubbish disposal facilities (95% CI =1.29 - 2.29), acquisition of vegetables from a local supply (95% CI= 1.07 - 1.92), not owning a plot of farming land (95% CI = 1.14 - 2.04) and living in a dwelling with a corrugated roof (95% CI = 1.28 - 2.41). In addition, age was an important confounder with a decrease in prevalence of infection with increasing age.

6.2.2. *Giardia lamblia* infection.

6.2.2.1. Prevalence of *G. lamblia* infection. In children aged 0-4 years, the prevalence of infection with *G. lamblia* was similar in all three exposure groups (17.3 and 17.1% in the raw wastewater and control groups and 18.8% in the two reservoirs group; Table 6.1). In individuals aged 5 to 14 years and in the oldest age category, the prevalence of infection was significantly higher in the two reservoirs group than in controls (P < 0.007).

*

Table 6.4 Rainy Season Survey
Children 0 - 4 years of Age with *G. lamblia* Infection
According to Exposure and Other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	61	(17.1)	356	1	1	
Two reservoirs	59	(18.8)	314	1.12 (0.75-1.66)	0.93 (0.50-1.72)	NS
Raw wastewater	43	(17.3)	249	1.01 (0.66-1.55)	0.73 (0.39-1.39)	NS
Age						
0 - 11 months	3	(2.2)	135	1	1	
1 - 4 years	160	(20.4)	784	11.27 (3.54-35.85)	11.73 (2.84-48.40)	<0.001
Distance from canal						
> 20 min.	43	(24.9)	173	1	1	
5 - 20 min.	50	(17.1)	292	0.63 (0.40-0.99)	0.64 (0.40-1.01)	0.05
<5 min.	2 5	(14.2)	176	0.47 (0.27-0.83)	0.50 (0.28-0.89)	0.01

OR_1 = Odds ratio adjusted for exposure.

OR_1 = Odds ratio adjusted for exposure, walking distance from canal and age.

P value refers to OR_2

Note: Factors significantly associated in the univariate analysis included animal excreta in the yard dwelling´s roof, rubbish disposal facilities, animal excreta yard, crowding home, literacy of the mother.

6.2.2.2. Effect of exposure and confounding factors in children 0-4 years old. There was no risk associated with exposure in children aged 0 to 4 years, either in the raw wastewater or in the two reservoirs groups, even after the effect of other factors was taken into account (Table 6.4). In the final model, children aged 1 to 4 years had a much higher prevalence of infection with *G. lamblia* than those aged 0 to 11 months (95% CI= 2.84 - 48.40). Those children from this category living in households located closer to the irrigation canals had a lower prevalence of infection (OR= 0.50, with 95% CI = 0.28 - 0.89).

6.2.2.3. Effect of exposure and confounding factors in individuals older than 5 years. No association was found between raw wastewater exposure and infection, even after allowing for potential confounders (OR= 1.25). The prevalence of *G. lamblia* infection in the two reservoirs group was significantly higher than controls (10.6%, and 6.5%,; Table 6.5) and was greater following logistic regression analysis (95% CI= 1.35 - 2.18). In the final analysis, the risk of infection in the two reservoirs was reduced, although it remained significantly higher than controls (95% CI= 1.10 - 1.86). Individuals aged over 15 years, those from families not renting land and those living in a dwelling with a corrugated roof had a lower risk of infection (95% CI = 0.26 - 0.41; 0.64 - 1.02 and 0.34 - 0.87).

Table 6.5 Rainy Season Survey
Individuals aged 5 years and Older with *G. lamblia* Infection
According to Exposure and Other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	149	(6.5)	2295	1	1	
Two reservoirs	146	(10.6)	1372	1.72 (1.35-2.18)	1.43 (1.10-1.86)	0.007
Raw wastewater	81	(8.0)	1015	1.25 (0.94-1.66)	1.23 (0.90-1.69)	NS
Age						
5-14 years	262	(12.7)	2056	1	1	
15 and over	114	(4.3)	2626	0.32 (0.25-0.40)	0.32 (0.26-0.41)	<0.001
Renting land						
Yes	115	(9.4)	1217	1	1	
No	261	(7.5)	3465	0.80 (0.63-1.00)	0.81 (0.64-1.02)	0.07
Dwelling's roof						
Cement	165	(7.9)	2092	1	1	
Tiles	146	(10.0)	1454	1.20 (0.92-1.56)	1.15 (0.88-1.51)	NS
Corrugated	22	(5.1)	433	0.54 (0.34-0.87)	0.54 (0.34-0.87)	0.02
Others (metal)	35	(5.3)	663	0.71 (0.48-1.04)	0.76 (0.51-1.11)	NS

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, age, land and dwelling's roof.
P value refers to OR_2
Note: Factors significantly associated in the univariate analysis included drinking water supply, time to get water, adult defecation practices, disposal of rubbish, source of vegetables, irrigation source, crops, number of wage-earners, illiteracy.

6.2.3.1. Prevalence of *E. histolytica* infection. As Table 6.1 illustrates, the prevalence of infection with *E. histolytica* in children aged 0-4 years was similar in the raw wastewater and control groups (6.5% and 7.0%), whereas the lowest prevalence was found in the two reservoirs group. In the category aged 5-14 years, the prevalence in both the raw wastewater and the two reservoirs groups was higher than the control population (16.0% and 12.0%, respectively). In the oldest age category, a higher prevalence was found in the raw wastewater group, followed by the two reservoirs and the controls (15.6%, 14.5% and 13.9%, respectively).

6.2.3. *E. histolytica* infection.

6.2.3.2. Effect of exposure and confounding factors in children 0-4 years. Exposure to the raw wastewater group in children under five years had no association with *E. histolytica* infection (Table 6.6). The raw wastewater and two reservoirs groups overlapped at the 95% CI (0.51 - 1.68 and 0.35 - 1.28, respectively). In the final analysis, the prevalences of infection, both in the raw and two reservoirs groups, were lower than that prior to adjusting for confounders (0.66 and 0.42, respectively). In this age group, the only other variable significantly associated with an increased prevalence of infection was that of not taking amoebicidal medication within 6 months prior to the study (OR= 4.70 and 95% CI= 1.16 - 19.10).

Table 6.6 Rainy Season Survey
Children aged 0-4 years with *E. histolytica* Infection
according to Exposure and Other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	25	(7.0)	356	1	1	
Two reservoirs	16	(4.8)	333	0.67 (0.35-1.28)	0.42 (0.04-3.85)	NS
Raw wastewater	22	(6.5)	335	0.93 (0.51-1.68)	0.66 (0.14-3.04)	NS
Last medication (months)						
Within previous 6 mo.	4	(2.6)	155	1	1	
More than 6 mo.	5	(12.2)	41	4.70 (1.16-19.10)	4.70 (1.16-19.10)	0.03

OR_1 = Odds ratio adjusted for exposure.

OR_2 = Odds ratio adjusted for exposure and medication.

P value refers to OR_2

Note: Factors significantly associated in the univariate analysis included drinking water supply, defecation practices, rubbish disposal, crowding, literacy of mother and housing materials.

6.2.3.3. Effect of exposure and confounding factors in individuals aged 5 years and over. As Table 6.7 shows, a low but significant risk of *E. histolytica* infection was observed in individuals exposed to raw wastewater (95% CI = 1.03 - 1.45), while there was no significant difference between the two reservoirs and control groups (95% CI = 0.98 - 1.40). After adjusting for other factors, the risk of infection associated with exposure to raw wastewater increased slightly (95% CI =1.08 - 1.53), whereas the two reservoirs group remained unchanged (95% CI = 0.88 - 1.29).

Table 6.7 Rainy Season Survey
Individuals aged 5 years and Older with *E. histolytica* Infection according to Exposure and other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	303	(13.2)	2295	1	1	
Two reservoirs	275	(15.1)	1820	1.17 (0.98-1.40)	1.07 (0.88-1.29)	NS
Raw wastewater	338	(15.7)	2155	1.22 (1.03-1.45)	1.29 (1.08-1.53)	0.004
Adult defecation habit						
Toilet	262	(12.5)	2093	1	1	
Yard - soil	654	(15.7)	4177	1.32 (1.12-1.55)	1.18 (1.00-1.41)	0.05
Disposal of rubbish						
Dustbin	375	(13.0)	2892	1	1	
None	541	(16.0)	3378	1.29 (1.12-1.49)	1.19 (1.02-1.39)	0.02
Appearance of respondent						
Very clean	26	(8.9)	292	1	1	
Regular	628	(14.2)	4429	1.71 (1.13-2.58)	1.47 (0.96-2.24)	0.07
Unclean	215	(17.0)	1266	2.14 (1.39-3.30)	1.69 (1.07-2.67)	0.02
Appearance of the yard						
Clean	362	(13.0)	2781	1	1	
Unclean	547	(15.9)	3435	1.28 (1.10-1.47)	1.15 (0.98-1.36)	0.07

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, adult defecation, disposal of rubbish, respondent's appearance and appearance of the yard.
P value refers to OR_2
Note: Factors significantly associated in the univariate analysis included age, drinking water supply, children's defecation habits, last medication, excreta in the yard, crowding, mother's literacy, rubbish disposal, housing materials and toilet appearance.

In the final model, individuals defecating in the yard's soil had a higher prevalence of infection than those with sanitary facilities at home (95% CI = 1.00 - 1.41) and those living in dwellings without rubbish disposal facilities had a higher prevalence of infection as compared with those having a dustbin (95% CI= 1.02 - 1.39). Other variables statistically associated with *E.*

histolytica infection included the unhygienic appearance of the respondent (95% CI= 1.07- 2.67) and unclean dwelling surrounding (95% = 0.98 - 1.36).

6.2.4. Diarrhoeal diseases

6.2.4.1. Prevalence of diarrhoeal diseases. The highest prevalence of diarrhoeal diseases in the rainy season was detected in children aged 0-4 years (Table 6.8). In the 0 to 4 years age category, the prevalence of diarrhoeal diseases was highest in the raw wastewater group (29.0%), with decreasing prevalences according to exposure. The prevalence of bloody diarrhoea was less than 1%.

Table 6.8 Rainy Season Survey
Prevalence of Diarrhoeal Diseases According
to Exposure and Age Categories;
the Mezquital Valley, 1990.

Age Category	EXPOSURE GROUP RAW WASTEWATER	NONEXPOSED	2 RESERVOIRS
0 - 4 yrs.	29.0 (99/341)	23.0 (100/436)	26.8 (111/415)
5 - 14 yrs.	11.8 (94/793)	10.7 (120/1125)	10.3 (97/944)
15 + yrs.	11.7 (83/709)	9.2 (179/1940)	10.8 (99/920)

6.2.4.2. Effect of exposure and confounding factors in children 0-4 years old. Children under 5 years from households exposed to raw wastewater (10^8 FC/100 ml) had a higher prevalence of diarrhoeal diseases than the control group (95% CI = 0.99 - 1.90; Table 6.9). There was no significant difference between the children from the two reservoirs group and controls (95% CI = 0.89 - 1.68). After adjusting for confounding factors, children from households exposed to raw wastewater had a marginally significant risk, as compared with controls (95% CI= 0.96 - 1.85). Children from the two reservoirs group were not statistically different from controls (95% CI = 0.85 - 1.60). In the final analysis, children from households whose drinking water was seldom boiled had a higher prevalence of diarrhoeal diseases than those where the water was usually boiled (95% CI = 0.96 - 2.18). After adjusting for the effect of exposure, the cultivation of salad crops was significantly associated with diarrhoeal diseases (95% CI = 1.37 - 2.93). Other hygiene-related variables such as living in a dwelling with a dirty yard and the unclean appearance of the respondent (95% CI = 0.68 - 0.98), and 0.38 - 0.88, respectively) both had a "protective" effect.

Table 6.9 Rainy Season Survey
Children aged 0-4 years with Diarrhoeal Diseases according to Exposure and other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	100	(23.0)	436	1	1	
Two reservoirs	111	(26.8)	415	1.24 (0.89-1.68)	1.17 (0.85-1.60)	NS
Raw wastewater	99	(29.0)	341	1.38 (0.99-1.90)	1.33 (0.96-1.85)	0.08
Drinking water						
Usually boiled	33	(19.8)	167	1	1	
Seldom boiled	277	(27.0)	1025	1.45 (0.96-2.18)	1.45 (0.96-2.18)	0.07

OR_1 = Odds ratio adjusted for exposure.

OR_2 = Odds ratio adjusted for exposure, age and whether drinking water was boiled.

P value refers to OR_2

Note: Factors significantly associated in the univariate analysis included age, farming goods, crowding, literacy of the mother and housing materials.

Table 6.10 Rainy Season Survey
Individuals aged 5 years and older with Diarroheal Diseases according to Exposure and other Factors;
the Mezquital Valley, 1990.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	299	(9.8)	3065	1	1	
Two reservoirs	196	(10.5)	1864	1.09 (0.90-1.32)	1.06 (0.86-1.29)	NS
Raw wastewater	177	(11.8)	1502	1.24 (1.02-1.50)	1.10 (0.88-1.38)	NS
Primary Crop						
Cereal	581	(10.1)	5727	1	1	
Alfalfa	26	(11.0)	237	1.03 (0.67-1.58)	1.04 (0.67-1.61)	NS
Salad	40	(19.0)	211	1.96 (1.35-2.83)	2.00 (1.37-2.93)	<0.001
Appearance of the yard						
Clean	311	(11.8)	2641	1	1	
Unclean	353	(9.4)	3744	0.78 (0.66-0.92)	0.82 (0.68-0.98)	0.03
Appearance of the respondent						
Very clean	41	(17.5)	234	1	1	
Regular	459	(10.2)	4508	0.53 (0.37-0.76)	0.55 (0.38-0.79)	0.001
Unclean	139	(9.9)	1399	0.52 (0.35-0.76)	0.58 (0.38-0.88)	0.009

OR_1 = Odds ratio adjusted for exposure.

OR_2 = Odds ratio adjusted for exposure, primary crop, hygiene in yard and respondent's appearance.

P value refers to OR_2

Note: Factors significantly associated in the univariate analysis included age, crowding cultivation of vegetables, crowding, housing materials and literacy of the mother.

6.2.4.3. Effect of exposure and confounding factors in individuals aged over 5 years of age. Individuals exposed to raw wastewater initially showed a significantly higher prevalence of diarrhoeal disease than controls (95% CI = 1.02 - 1.50; Table 6.10); there was no significant difference between individuals from the two reservoirs group and controls (95% CI = 0.90 - 1.32). The prevalence of diarrhoeal diseases from raw wastewater exposure, after allowing for other factors, was not significantly different from controls (95% CI = 0.88 - 1.38). Similar results were obtained while comparing the two reservoirs with controls (OR= 1.06).

6.3. DISCUSSION OF THE RAINY SEASON SURVEY RESULTS.

6.3.1. *A. lumbricoides* infection.

The overall prevalence of *A. lumbricoides* infection in the rainy season was lower than initially expected. The prevalence of infection as a whole was lower than those reported by Stoopen & Beltran (1964), Carrada-Bravo (1984), Lara-Aguilera (1984) and by Forrester *et al.*. (1988) in tropical endemic areas of Mexico (Gonzalez *et al*, 1985). The prevalence observed was also lower than those reported in rural Guatemala (Mata *et al*, 1977) and Venezuela (Pierce *et al*, 1962). Different climatic conditions such as the increased humidity in the latter may contribute to the different prevalences. The age-prevalence observed in the present study, however, suggests that the infection is more common in children, as well as in school age individuals, an age pattern which has been reported by Biagi & Rodriguez (1960), Anderson & May, (1985) Anderson (1986) and Bundy *et al* (1987). Such age- prevalence of ascariasis reflects sociocultural characteristics (WHO, 1987; Feachem *et al*, 1983).

6.3.1.1. Effect of raw wastewater exposure. Exposure to raw wastewater (90-120 nematode eggs/litre) was the factor most significantly associated with increased risk of infection with *A. lumbricoides* in this farming communities practicing flood irrigation. Similar findings were reported by Krishnamoorthi *et al. (1973)* in India, and reviewed by Shuval *et al.* (1986 b). The present study, however, constitutes the first case-study integrating data from wastewater quality, a careful definition of exposure and the epidemiological assessment of exposure and confounding factors, focusing on various sub-groups, including children at risk.

Although the prevalence of infection was higher in young children than in older individuals from households exposed to raw wastewater, the association with exposure was greater in the latter category. This may be associated with the magnitude of exposure, which is closely related to agricultural activities (irrigation) generally carried out by adults. Children under 5 years, however, may become infected through household transmission, while accompanying older relatives during farming activities, or while playing with water and soil near the dwelling and in the family's plot.

6.3.1.2. Effect of the reservoirs. The risk of *A. lumbricoides* infection was substantially reduced in individuals exposed to wastewater effluent from the second reservoir. Prevalence of infection in the two reservoirs group decreased to levels similar to that found in the control group. The risk reduction was clearest in young children, whereas the apparently significant difference observed in those over 5 years of age may lack public health importance, due to the low prevalences observed both in the reservoirs and the control groups. It is worth emphasizing that wastewater is stored in these two reservoirs for up to 7 months, while there is a surplus of run-off from the metropolitan area. Retention time in the second reservoir appears to be long enough to remove *A. lumbricoides* eggs since the effluent of the second reservoir contained no detectable eggs.

6.3.1.3. Other factors. Individuals with risk of infection were more likely to come from landless families. Farming land is a valuable asset because it contributes to living standards in a farming population. In addition, individuals living in poor quality dwellings (measured by use of a corrugated roof) and those without basic sanitation facilities (rubbish disposal) were significantly associated with *A. lumbricoides* infection. The role of these factors has been widely documented by Chandler (1954), Kighlinger *et al*, (1995) and Chan (1991), and reviewed by WHO (1987). Finally, vegetables purchased from local shops had a small but significant association with infection, suggesting that some crops were cultivated in faecal contaminated soil, or irrigated or washed with polluted water after harvest. Similar observations have been made by De Leon *et al*.. in the Philippines (1992).

6.3.2. *G. lamblia* infection.

Results obtained in the rainy season demonstrated a prevalence of *G. lamblia* infection close to that expected. This prevalence is similarly high to that reported by Cruz-Lopez *et al* (1989) in other studies carried out in Mexico and Guatemala and in Brazil by Pierce *et al* (1962) and Sawaya & Amigo (1990). As expected, the highest prevalence of infection was observed in the children of pre-school and school ages. Analogous age-prevalence patterns have been reported by Chandler (1954), Tomkins (1981) and Flanagan (1992), suggesting that behavioural, environmental and immunological factors contribute to such prevalences. There was, however, an unexpectedly higher prevalence of infection in individuals from the two reservoirs group than in the other two study groups. It is likely that person to person transmission is mostly involved.

6.3.2.1. Effect of raw wastewater exposure. Children under five years from households exposed to raw wastewater and controls had a similar prevalence of infection with *G. lamblia* , suggesting that raw wastewater was not a significant risk factor in this population. Similarly, individuals aged 5 years and older exposed to raw wastewater showed no differences when compared with controls. These results differ from that reported in India by Sehgal & Mahagan (1991) and Jefferson & Betton (1991), and may be a result of sociocultural and hygiene contexts.

6.3.2.2. Effect of the reservoirs. Young children from the two reservoirs were not significantly different from controls, which reinforces the hypothesis that wastewater is not a major risk factor in this area. It is worth noting, however, that individuals older than 5 years from the two reservoirs group had a higher prevalence of *G. lamblia.* infection than controls. Some confounders not measured in this study may be involved in such an unexpected result (see below).

6.3.2.3. Other factors. The prevalence of *G. lamblia* infection was much higher between one and four years than during the first months of life, indicating that these former children may become infected during the period when they begin to explore and play around the dwelling, and that they are less exposed while they are breast-fed. Similar observations have been reported by Esrey *et al* (1989), Flanagan (1992) and Porter *et al* (1990). Furthermore, school age children from households in the two reservoirs group had a significantly higher prevalence of infection with *G. lamblia* than older individuals.

The "protective" effect of increasing age may simply reflect behavioural and immunological status, or may be related to certain factors not measured in this study (e.g. behaviour, food-hygiene, facilitated the person to person transmission). Similar observations have been reported by Flanagan (1992) and reviewed in WHO (1991). Finally, the association between infection and living at shorter distances from the canal, not renting land and the low quality of the dwelling's roof probably suggest possible socioeconomic confounders, not measured in this study.

6.3.3. *E. histolytica* infection.

There was a high prevalence of *E. histolytica* infection in this farming population, particularly in the population aged over 5 years (15%). Such prevalence, however, is lower than that reported in other studies carried out in Mexico by Cruz-Lopez *et al* (1989), Crevenna (1977) and Gonzalez Galnares (1986). The prevalence obseived in this study was, however, lower than recorded in Colombia, Costa Rica or the Gambia (WHO 1987; Bray & Harris 1977). In addition to regional differences, dissimilar results may be related, to methodological and technical procedures (WHO 1991, PAHO, 1991).

6.3.3.1. Effect of raw wastewater exposure. Older individuals exposed to raw wastewater had a low but significantly higher risk of infection with *E. histolytica.* than controls, while children 0 to 4 years from households exposed to untreated wastewater and controls had similar prevalences of infection. This is the first report of this association and this study provides evidence on the risk of *E. histolytica* infection associated with exposure to untreated wastewater.

6.3.3.2. Effect of the reservoirs. The youngest children from the reservoirs group had a slightly lower prevalence of infection with *E. histolytica* than controls. However, there was no difference between the two reservoirs and control groups in individuals aged 5 years and older, suggesting that there was a positive effect from double hydraulic retention. This hydraulic retention time may be sufficient to remove cysts from wastewater and therefore reduce the risk of *E. histolytica* infection associated with exposure to wastewater.

6.3.3.3. Other factors. According to available literature, it is likely that more than 15% of paediatric population suffering any kind of gastrointestinal disorder receive some type of medication, either as self prescription practices or at health centres in Mexico (Biagi *et al*, 1960). In this study it was observed that nearly 15% of the study population had recently received medication, and children under 5 years of age not having recent medication (i.e. treatment for cyst passing or clinically defined cases of amoebiasis), had an increased prevalence of infection as compared to those who did. These data may support the quality of the parasitological results. No other associations were detected for *E. histolytica* infections in young children, possibly because factors commonly associated with transmission (i.e. person to person transmission and feeding practices) were not adequately measured or were not considered at all in this study. In individuals aged over 5 years, however, nonwastewater factors associated with risk of infection included lack of basic sanitation (defecation around the dwelling and lack of rubbish disposal) and the unhygienic appearance of the respondent (suggesting person to person transmission). These variables may all highlight faecal-oral transmission within the dwelling (reviewed by Spencer 1976, Sole & Croll 1980 and Engaeck & Larsen 1979).

6.3.4. Diarrhoeal diseases.

During the rainy season there was a considerably higher prevalence of diarrhoeal diseases (two weeks recall) than expected. The highest prevalence occurred in children under 5 years of age. This prevalence was much higher than that reported in the Gambia (Pickering *et al.*. 1987), Nigeria (Huttly *et al.*. 1987) and other areas in Mexico, including from the same state of Hidalgo (DGE 1984). The high prevalence of diarrhoeal diseases in this farming population indicated low socioeconomic and hygiene status, as well as precarious health conditions.

<u>6.3.4.1. Effect of raw wastewater exposure</u>. Children under 5 years from households exposed to raw wastewater showed an increasing trend of diarrhoeal diseases, indicating a health risk from untreated wastewater reuse. Such a trend however, was not observed in individuals aged over 5 years. Raw wastewater samples data (10^8 faecal coliforms/ 100 ml) pointed out serious faecal water pollution.

<u>6.3.4.2. Effect of the reservoirs.</u> There was no significant difference between children from the two reservoirs and control group. Overlapping values of CI with the raw wastewater group suggested an intermediate risk which may have resulted in a significant difference had the sample size been larger. Microbiological water quality indicated a substantial improvement of the second reservoir effluent, which nonetheless may have been insufficient to protect children from exposure to partially treated wastewater.

<u>6.3.4.3. Other factors</u>. Drinking unboiled water was marginally associated with diarrhoeal diseases in young children. Health risks from bacterial contamination of drinking water have been reported by Huttly in Nigeria (1987) and Martinez-Garcia in Mexico (1989). The prevalence of diarrhoeal diseases was significantly higher in individuals over 5 years cultivating salad crops. Despite crop restrictions in the irrigation districts prohibiting the cultivation of crops eaten raw, some farmers conducted illegal farming of vegetables (irrigated with faecal contaminated water). Analogous observations regarding outbreaks of cholera in Israel have been reviewed by Shuval *et al* (1986 b). It is important to state here that at the time when the present research was conducted, no cholera outbreak was detected in the study area. After the study was concluded, a first outbreak was reported in the country, but not in the study area.

Finally, there was an apparent protective association between a fouled dwelling yard and lack of hygiene by respondents in the oldest age category. Individuals with low hygiene status have probably been constantly exposed to enteropathogens since early life and therefore have acquired certain immunity, leaving them less susceptible to enteric infections.

6.4. SUMMARY OF THE RAINY SEASON SURVEY

We have described the epidemiology of intestinal parasitic infections and diarrhoeal diseases during the rainy season. The primary focus of analysis has been the effect of exposure to raw wastewater and the possible benefits from double hydraulic retention. Other factors were also assessed.

Exposure to raw wastewater was significantly associated with *A. lumbricoides* infection and decreasing exposure was associated with lower prevalences. The prevalence observed in the population exposed to wastewater from the two reservoirs strongly suggests a positive eggs-removal effect, presumably related to increased hydraulic retention time. Other risk factors associated with infection included low socioeconomic status, poor sanitation and consumption of vegetables from local shops. Age showed strong confounding effects.

An unexpectedly high prevalence of *G. lamblia* infection was found in this farming population, in age categories. Children between one and four years had a higher prevalence of infection than infants under 11 months, suggesting hygiene and behavioural factors (e.g. feeding practices and multiple alternate transmission routes). Individuals aged 5 to 14 years had a significantly higher prevalence of infection, as compared with those over the age of 15 years. Other factors showing a "protective" effects (corrugated roof or not renting land) were possibly suggesting socioeconomic confounders.

Exposure to raw wastewater was significantly associated with *E. histolytica* infection only in individuals aged 5 years and over. The prevalence of infection in those exposed to the reservoirs effluent was not different from that in the controls, suggesting a beneficial effect from double retention. Other factors associated with increased infection included lack of rubbish disposal facilities, adult defecation on yard's soil and lack of respondent's hygiene.

Children under five years of age from the untreated wastewater group showed an increasing trend of diarrhoeal diseases, when compared with controls. The trend detected while comparing children from the reservoirs group with controls, although non significant, suggested an intermediate risk. Not boiling drinking water was associated with diarrhoeal diseases in young children only and in older individuals, the cultivation of salad crops was significantly associated with diarrhoeal diseases. Other confounders included low hygiene status.

CHAPTER 7. DRY SEASON SURVEY RESULTS.

7.1. INTRODUCTION.

The outcomes of the dry season survey were intestinal infections with *A. lumbricoides* , *G. lamblia* and *E. histolytica* and diarrhoeal diseases. As explained in Figure 4.1, the exposure groups in the dry season were:

1. Raw wastewater,
2. Wastewater from a single reservoir,
3. Nonexposed or controls.

Age categories analysed were 0 - 4, 5 - 14 and over 15 years. Since the effect of exposure was not significantly different between the 5-14 years and the one found in those older than 15 years, individuals older than 5 years were grouped into a single group. Analysis of the dry season data presented in this chapter focuses on:

1. The comparison between exposed and control groups,
2. The comparison between the single reservoir group and controls.

These comparisons have addressed assess parasitic infections and diarrhoeal diseases associated with exposure to raw wastewater, as well as the evaluation of wastewater retention though only one reservoir.

Potential confounders were fitted into the multivariate model as dummy variables, while the effect of exposure (raw wastewater and effluent) was retained in the final model. Only factors (non-wastewater) statistically associated with the outcome, after adjusting for the effect of exposure, were kept in the final analysis. The study outcomes were coded as binary variables ("Yes" or "No"). Odds ratios presented in the first column (OR_1) refers to exposure crude values; OR_2 on the right hand side refers to the adjusted values, for exposure with confounders. P values refer to the OR_2. Unless otherwise stated, the term "risk" will be used here to refer to the excess of infection or disease in those exposed to raw or treated wastewater, as compared with the control group.

7.2. DRY SEASON SURVEY RESULTS.

A total of 2, 049 households, involving 10,489 individuals were analysed from the dry season survey and a total of 8,487 stool samples were collected throughout the survey (83% compliance rate). It should be noted, that denominators were different in the analysis of intestinal infections and diarrhoeal diseases, since compliance rates for interviews (diarrhoea) and stool samples (intestinal infections) were not the same.

The prevalence of *A. lumbricoides*, *G. lamblia* and *E. histolytica* infections are summarised by age and exposure groups in Table 7.1, whereas

Tables 7.2 to 7.7 display the results of logistic regression analysis of the study. Tables 7.8, 7.9 and 7.10 display both the general prevalence of diarrhoeal diseases, as well as the results from the logistic regression analysis.

7.2.1. *A. lumbricoides* infection.

7.2.1.1. Prevalence of *A. lumbricoides* infection. The highest prevalences of *A. lumbricoides* infection were found in individuals aged 5 - 14 years and younger, whereas the lowest prevalence was detected in the oldest age category. Children under 5 years from the raw wastewater and the reservoir groups had similarly high prevalences of infection (Table 6.1). In the 5 to 14 years age category, however, individuals from the raw wastewater group had a higher prevalence of infection than the reservoir group (12.5%, 8.5% and 1.0%, respectively). A similar pattern was observed in the oldest age category, although prevalences were considerably lower (4.5%, 2.5% and 0%, respectively). The lowest prevalences were detected in the control populations (1% or less) .

Table 7.1 Dry Season Survey
Prevalence of Intestinal Parasitic Infections
according to Exposure Group and Age;
the Mezquital Valley, 1991.

	EXPOSURE GROUP		
	RAW WASTEWATER	NONEXPOSED	ONE RESERVOIR
Ascaris lumbricoides			
0 - 4 yrs.	10.0 (34/341)	0.6 (2/327)	11.8 (42/357)
5 - 14 yrs.	12.5 (94/759)	1.0 (8/809)	8.5 (67/795)
15 + yrs.	4.5 (60/1394)	0.0 (0/1243)	2.5 (39/1515)
Giardia lamblia			
0 - 4 yrs.	19.8 (43/217)	20.5 (67/327)	16.5 (38/230)
5 - 14 yrs.	13.5 (60/442)	12.5 (101/809)	14.0 (66/480)
15 + yrs.	4.5 (16/347)	4.0 (48/1243)	6.0 (28/472)
Entamoeba histolytica			
0 - 4 yrs.	6.5 (22/341)	6.7 (22/327)	6.4 (23/357)
5 - 14 yrs.	16.7 (127/759)	14.0 (113/809)	20.3 (161/795)
15 + yrs.	16.4 (229/1394)	15.1 (188/1243)	17.3 (262/1515)

7.2.1.2. Effect of exposure and confounding factors in children 0-4 years old. Children under 5 years from households exposed to raw wastewater, as well as those from households exposed to the reservoir wastewater both had a much higher prevalence of *A. lumbricoides* infection, when compared with controls (95% CI= 4.29 - 75.55, and 5.20 - 90.27, respectively; Table 7.2). When allowing for confounders, the risk of infection in these former groups remained considerably high (95% CI= 4.10 - 79.16 and 5.06 - 88.93, respectively). In the final model, children living in dwellings constructed with low quality materials (e.g. tiles or corrugated roofs), had a higher prevalence of infection than those with better roof (i.e. cement; 95% CI= 1.28 - 4.60). Children aged between 1 and 4 years have a higher prevalence than those in the first year of life (95% CI= 1.29 - 69.65).

Table 7.2 Dry Season Survey
Children aged 0-4 years with *A.lumbricoides* Infection
according to Exposure and other Factors;
the Mezquital Valley, 1991.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	2	(0.6)	327	1	1	
One reservoir	42	(11.8)	357	21.67 (5.20-90.27)	21.22 (5.06-88.93)	<0.001
Raw wastewater	34	(10.0)	341	18.00 (4.29-75.55)	18.01 (4.10-79.16)	<0.001
Housing roof						
Cement	34	(6.1)	561	1	1	
Tiles	20	(8.9)	225	1.78 (0.92-3.44)	2.06 (1.04-4.07)	0.04
Corrugated	19	(15.2)	125	2.20 (1.18-4.12)	2.43 (1.28-4.60)	0.006
Other (metallic)	5	(4.7)	107	1.00 (0.37-2.68)	1.08 (0.40-2.93)	NS
Age group						
0 - 11 months	1	(0.8)	118	1	1	
1 - 4 yrs.	77	(8.5)	907	11.46 (1.57-83.54)	9.49 (1.29-69.65)	0.02

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, washing hands, housing roof and age.
P value relates to OR_2
Note: Factors significantly associated in the univariate analysis included, drinking water supply, time to get water, boil water, crops, dietary patterns, pigs in the yard, animal excreta, water in the plot, crowding and literacy of the mother.

7.2.1.3. Effect of exposure and confounding factors in individuals older than 5 years. The effect of exposure on the 5 - 14 years and on the over 15 years groups was initially assessed separately, with no significant differences encountered on the risk of infection. These two age categories were, therefore, grouped into one age category (Table 7.3). Individuals aged 5 years and older exposed to raw wastewater had a much higher prevalence of infection with *A. lumbricoides* than controls (95% CI=

9.64 - 40.17) and individuals exposed to wastewater from the single reservoir also had a higher prevalence of infections when compared with controls (95% CI= 5.97 - 25.27). After allowing for potential confounders, the risk of infection in both raw wastewater and the single reservoir groups remained significantly higher than in controls (95% CI= 6.35 - 28.63, and 4..45 - 19.94 accordingly).

Table 7.3 Dry Season Survey
Individuals aged 5 years and older with *Ascaris lumbricoides* Infection according to Exposure and other Factors; the Mezquital Valley, 1991.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	8	(0.4)	2052	1	1	
One reservoir	106	(4.6)	2310	12.29 (5.97-25.27)	9.42 (4.45-19.94)	<0.001
Raw wastewater	154	(7.2)	2153	19.68 (9.64-40.17)	13.49 (6.35-28.63)	<0.001
Water for family plot						
No irrigation	78	(2.3)	3458	1	1	
Some	190	(6.2)	3057	1.69 (1.28-2.24)	1.59 (1.18-2.13)	0.002
Drinking water						
Usually boiled	81	(2.9)	2759	1	1	
Seldom boiled	187	(5.0)	3756	1.43 (1.09-1.88)	1.31 (0.99-1.73)	0.06
Animal excreta around yard						
None	137	(3.5)	3967	1	1	
Some	131	(5.2)	2536	1.64 (1.28-2.11)	1.39 (1.07-1.81)	0.01
Housing roof						
Cement	116	(3.3)	3550	1	1	
Tiles	43	(3.5)	1235	1.53 (1.03-2.27)	1.16 (0.77-1.74)	NS
Corrugated	76	(9.8)	775	2.19 (1.60-3.00)	1.93 (1.39-2.68)	<0.001
Other (metallic)	27	(3.0)	890	1.33 (0.86-2.06)	1.20 (0.77-1.88)	NS
Number of bedrooms						
1	120	(5.6)	2130	1	1	
2	110	(3.9)	2793	0.72 (0.55-0.94)	0.82 (0.62-1.04)	NS
3	31	(2.5)	1257	0.45 (0.30-0.67)	0.61 (0.40-0.94)	0.02
4 +	7	(2.1)	334	0.32 (0.15-0.70)	0.47 (0.21-1.05)	0.06
Age group						
5 - 14 years	169	(7.2)	2363	1	1	
15 + years	99	(2.4)	4152	0.29 (0.23-0.38)	0.30 (0.23-0.40)	<0.001

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, water for family plot, drinking water animal excreta around yard, housing roof, number of bedrooms and age group.

P value relates to OR_2

Note: Factors significantly associated in the univariate analysis included, time to get water, cultivated crops, boil drinking water, diet, pigs in the yard, hand washing and literacy of the mother.

In the final analysis, individuals over 5 years from households in which irrigation for the family plot was available, as well as those who said they seldom boiled drinking water, and the group in which animal excreta was observed around the dwelling had higher prevalence of infection with *A. lumbricoides* (95% CI= 1.18 - 2.13; 0.99 - 1.73, and 1.07 - 1.81). Living in a dwelling with a corrugated roof was also significantly associated with infection, whereas an increasing number of bedrooms and aging (+ 15 years) were both variables showing "protective" association (95% CI= 1.39 - 2.68; 0.40 - 0.94, and 0.23 - 0.40, respectively).

7.2.2. *G. lamblia* infection.

7.2.2.1. Prevalence of *G. lamblia* infection. The highest prevalence of *G. lamblia* infection was found in children under five, followed by the 5 to 14 years and then the oldest groups (Table 7.1). Children 0 to 4 years from households in the raw wastewater and control groups had similar prevalence of infection which was higher than children from the single reservoir group (19.8%, 20.5% and 16.5%, respectively). Prevalences in the 5 - 14 years and in the youngest categories were similar among the three exposure groups (13.5%, 14.0% and 12.5%, respectively). In the oldest age category, the prevalence of *G. lamblia* infection was also similar in all exposure groups (6.0%, 4.5% and 4.0% respectively).

7.2.2.2. Effect of exposure and confounding factors in children 0-4 years. Children from the raw wastewater and the single reservoir groups were not significantly different to controls (Table 7.4). This lack of association between exposure and *G. lamblia* infection was observed even after allowing for confounders (95% CI= 0.69- 1.77, and 0.56 - 1.42). In the final analysis, living in dwellings without piped water supplies and a lower number of bedrooms were significantly associated with infection (95% CI= 1.02 - 2.85, and 1.14 - 2.61). Children aged 1 - 4 years had an 8-fold increase in risk of infection with *G. lamblia* as compared with children under one year of age (95% CI= 2.45 - 25. 44).

7.2.2.3. Effect of exposure and confounding factors in individuals older than 5 years. Older individuals from the raw wastewater and the single reservoir groups had similar prevalences of *G. lamblia* infection than controls (Table 7.5). The difference between these and controls was not significant, even after other factors were taken into account (95% CI= 0.72 - 1.44, and 0.84 - 1.56). In the final analysis the only factor significantly associated with infection in this age category was storing drinking water unrefrigerated (95% CI= 1.04 - 1.77). On the other hand, purchasing vegetables from local shops, having pigs in the household (95%

CI= 0.48 - 0.83, and 0.59 - 1.00) and aging (95% CI= 0.23 - 0.40) appeared to have a "protective" association with infection. In the final analysis, other factors significantly associated with infection were a longer time required to get drinking water and being a child of an illiterate mother (95% CI= 1.09 - 4.63, and 1.16 - 3.68, respectively).

Table 7.4 Dry Season Survey
Children aged 0-4 years with *G. lamblia* Infection
according to Exposure and other Factors;
the Mezquital Valley, 1991

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	67	(20.5)	327	1	1	
One reservoir	38	(16.5)	230	0.77 (0.49-1.19)	0.89 (0.56-1.42)	NS
Raw wastewater	43	(19.8)	217	0.96 (0.62-1.47)	1.11 (0.69-1.77)	NS
Water supply						
Piped	118	(18.0)	657	1	1	
Not piped	30	(25.6)	117	1.57 (0.95-2.59)	1.70 (1.02-2.85)	0.05
Number of bedrooms						
1	48	(16.1)	298	1	1	
2	75	(23.7)	316	1.63 (1.09-2.44)	1.73 (1.14-2.61)	0.05
3	20	(16.3)	123	1.01 (0.57-1.78)	1.08 (0.61-1.93)	NS
4 +	5	(14.3)	35	0.89 (0.33-2.42)	0.95 (0.35-2.62)	NS
Age group						
0 - 11 months	3	(3.4)	87	1	1	
1 - 4 years	145	(21.1)	687	7.45 (2.32-23.92)	7.90 (2.45-25.44)	<0.001

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, age, water supply and number of bedrooms.

P value relates to OR_2.

Note: Factors significantly associated in the univariate analysis: time to get water and storage, cultivation of crop, source of vegetables and excreta in the yard and literacy.

Table 7.5 Dry Season Survey
Individuals aged 5 years and older with *G. lamblia* Infection according to Exposure and other Factors; the Mezquital Valley, 1991.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	149	(7.3)	2052	1	1	
One reservoir	94	(9.9)	952	1.40 (1.07-1.83)	1.15 (0.84-1.56)	NS
Raw wastewater	76	(9.6)	789	1.36 (1.02-1.82)	1.02 (0.72-1.44)	NS
Storage of drinking water						
Refrigerator	150	(7.3)	2048	1	1	
Other, outside	106	(9.8)	1078	1.37 (1.06-1.78)	1.36 (1.04-1.77)	0.02
Pigs in the yard						
Yes	122	(10.2)	1198	1	1	
No	197	(7.6)	2595	0.73 (0.57-0.92)	0.63 (0.48-0.83)	<0.001
Source of vegetables						
Market outside village	160	(9.6)	1674	1	1	
Local shops	159	(7.5)	2119	0.80 (0.63-1.01)	0.77 (0.59-1.00)	0.05
Age group						
5 - 14 years	227	(13.1)	1731	1	1	
15 + years	92	(4.5)	2062	0.32 (0.24-0.41)	0.30 (0.23-0.40)	<0.001

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, storage of drinking water, pigs in the yard, source of vegetables and age group.
P value relates to OR_2
Note: Factors significantly associated in the univariate analysis included drinking water supply, time getting water, cultivated crops, irrigation, animal excreta, sanitation, crowding, respondent appearance, mother literacy and dwelling roof.

7.2.3. *E. histolytica* infection.

7.2.3.1. Prevalence of *E. histolytica* infection. The highest prevalence of *E. histolytica* infection was detected in the 5 to 14 age category and in those aged over 15 years groups, from all exposure groups (Table 7.1). In the 5 - 14 years category the highest prevalence was detected in the reservoir group (20.3%), whereas in the oldest category the prevalence of infection was equally high in the three exposure groups (16.4%, 17.3% and 15.1%, respectively). Children under five years had lower prevalences, but these were similar in all three exposure groups (6.5%).

7.2.3.2. Effect of exposure and confounding factors in children under 5 years. Children from the raw wastewater and the single reservoir groups had a similar prevalence of infection to that observed in the controls (Table 7.6). When other factors were considered, the prevalences of infection in these groups were not significantly different from controls (95%

CI= 0.59 - 2.09, and 0.54 - 1.92 respectively).

Table 7.6 Dry Season Survey
Children aged 0-4 years with *E. histolytica* Infection according to Exposure and other Factors; the Mezquital Valley, 1991.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	22	(6.7)	327	1	1	
One reservoir	23	(6.4)	357	0.95 (0.52-1.75)	1.02 (0.54-1.92)	NS
Raw wastewater	22	(6.5)	341	0.96 (0.52-1.76)	1.11 (0.59-2.09)	NS
Time to get drinking water						
Less than 1 min.	10	(3.6)	280	1	1	
More than 1 min.	57	(7.7)	745	2.24 (1.13-4.46)	2.24 (1.09-4.63)	0.03
Literacy of the mother						
Yes	45	(5.6)	806	1	1	
No	18	(11.1)	162	2.11 (1.18-3.75)	2.06 (1.16-3.68)	0.01

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, drinking water and mother's literacy.
P value relates to OR_2
Note: Factors significantly associated in the univariate analysis included age

The final analysis indicated that drinking water which is seldom boiled, bathing in the river, an unclean hygienic appearance and living in a dwelling with tiled roof were all additional variables associated with infection with *E. histolytica* (95% CI= 0.99 - 1.31; 1.02 - 1.37; 1.53 - 3.30, and 1.10 - 1.57, respectively).

<u>7.2.3.3. A Effect of exposure and confounding factors in individuals aged 5 years and older</u>. Individuals aged over 5 years from the raw wastewater and the single reservoir had a higher prevalence of infection with *E. histolytica* than controls (Table 7.7). This difference was statistically significant, even after allowing for the confounding effect of other factors (95% CI = 1.03 - 1.51, and 1.11 - 1.54, respectively).

Table 7.7 Dry Season Survey
Individuals aged 5 years and older with *E. histolytica* Infection according to Exposure and other Factors; the Mezquital Valley, 1991.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	301	(14.7)	2052	1	1	
One reservoir	423	(18.3)	2310	1.30 (1.11-1.53)	1.30 (1.11-1.54)	0.002
Raw wastewater	356	(16.5)	2153	1.15 (0.98-1.36)	1.25 (1.03-1.51)	0.02
Drinking water						
Usually boiled	427	(15.5)	2759	1	1	
Seldom boiled	653	(17.4)	3756	1.16 (1.01-1.33)	1.14 (0.99-1.31)	0.06
Place for bathing						
Shower - tap	766	(15.9)	4823	1	1	
River	314	(18.6)	1692	1.23 (1.06-1.42)	1.18 (1.02-1.37)	0.02
Respondent's appearance						
Very clean	34	(9.9)	344	1	1	
Regular	757	(15.6)	4840	1.69 (1.18-2.43)	1.60 (1.11-2.30)	0.01
Unclean	284	(21.6)	1313	2.51 (1.72-3.67)	2.25 (1.53-3.30)	<0.001
Dwelling's roof						
Cement	537	(15.1)	3550	1	1	
Tiles	247	(20.0)	1235	1.41 (1.18--1.69)	1.31 (1.10-1.57)	0.003
Corrugated	140	(18.1)	775	1.20 (0.97-1.49)	1.11 (0.89-1.38)	NS
Others (metal)	144	(16.2)	890	1.13 (0.92-1.39)	1.08 (0.88-1.33)	NS

OR_1 = Odds ratio adjusted for exposure.
OR_2 = Odds ratio adjusted for exposure, drinking water, place for bathing, respondent's appearance and dwelling roof.
P value relates to OR_2
Note: Factors significantly associated in the univariate analysis included age, time to get water, source of irrigation, diet, yard´s hygiene, sanitation, crowding and literacy of the mother.

7.2.4. Diarrhoeal diseases.

7.2.4.1. Prevalence of diarrhoeal diseases. The highest prevalences of diarrhoeal diseases in the dry season were observed in children under five years of age (Table 7.8). Children 0 to 4 years from households exposed to raw wastewater had the highest prevalence rate (19.4%), with lower prevalences related to decreasing exposure (15.5% in the single reservoir group and 13.6% in controls). Children aged 5 to 14 years had an intermediate position, in relation to older individuals .

Table 7.8 Dry Season Survey
Prevalence of Diarrhoeal Diseases according to Exposure and Age Categories, the Mezquital Valley, 1991.

AGE	EXPOSURE GROUPS RAW WASTEWATER	NONEXPOSED	ONE RESERVOIR
0 - 4 years	19.4 (56/289)	13.6 (55/404)	15.5 (47/302)
5 - 14 years	6.5 (42/656)	4.5 (45/1028)	8.0 (51/651)
15 + years	8.0 (43/546)	7.0 (119/1749)	8.5 (53/631)

7.2.4.2. Effect of exposure and confounding factors in children under 5 years. Children aged 0 - 4 years from households exposed to raw wastewater had a significantly higher prevalence of diarrhoeal diseases, as compared to controls (95% CI= 1.02 - 2.29; Table 7.9). The prevalence of diarrhoeal diseases in children from the single reservoir was not significantly different from controls (95% CI= 0.77 - 1.78). After adjusting for other confounding factors, the prevalence of diarrhoeal diseases in the raw wastewater group remained significantly higher than controls (95% CI= 1.10 - 2.78) and that of the single reservoir remained similar to controls (95% CI= 0.70 - 1.83). In the final analysis, other factors significantly associated with diarrhoeal diseases included drinking water from public taps, occasional hand washing, animal excreta observed around the yard and cultivation of crops in the family plot (95% CI= 1.05 - 2.96; 1.00 - 2.10; 1.08 - 2.21, and 1.11 - 2.52, respectively).

7.2.4.3. Effect of exposure and confounding factors in individuals over 5 years of age. The prevalence of diarrhoeal diseases in the over 5 years group is shown in Table 7.10. Individuals exposed to raw wastewater, as well as those exposed to the effluent of the single reservoir group both had a higher prevalence of diarrhoeal diseases than controls.

Initial analysis showed a marginal risk of diarrhoeal diseases associated with raw wastewater, while the intermediate group (i.e. the single reservoir) had a stronger association with disease (95% CI= 0.92 - 1.59, and 1.09 - 1.82). After allowing for potential confounders, however, these two exposure groups had a significantly higher prevalence of diarrhoeal diseases than controls (95% CI= 1.00 - 1.78, and 1.15 - 1.96, respectively). In the final analysis, other factors statistically associated with diarrhoeal diseases included drinking water from unpiped sources and not washing hands before meals (95% CI= 1.01 - 1.90, and 1.07 - 1.74, respectively). In this age category, it was also observed that the oldest subgroup had a higher prevalence of diarrhoea than younger individuals (95% CI = 1.15 - 1.84), whereas increasing number of bedrooms in the dwelling had a "protective" association (95% CI = 0.46 - 0.90).

Table 7.9 Dry Season Survey
Children aged 0-4 years with Diarrhoeal Diseases according to Exposure and other Factors, the Mezquital Valley, 1991.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	55	(13.6)	404	1	1	
One-reservoir	47	(15.5)	302	1.17 (0.77-1.78)	1.13 (0.70-1.83)	NS
Raw-wastewater	56	(19.4)	289	1.52 (1.02-2.29)	1.75 (1.10-2.78)	0.01
Drinking water supply						
Piped dwelling	131	(15.3)	858	1	1	
Not piped	27	(19.7)	137	1.70 (1.03-2.81)	1.77 (1.05-2.96)	0.03
Hand washing						
Usually	49	(12.9)	381	1	1	
Seldom	103	(17.7)	583	1.48 (1.02-2.14)	1.44 (1.00-2.10)	0.05
Animal excreta around the yard						
None	71	(13.1)	542	1	1	
Some	86	(19.1)	451	1.60 (1.32-2.26)	1.55 (1.08-2.21)	0.01
Crops in family plot						
No	44	(12.2)	362	1	1	
Yes	114	(18.0)	633	1.55 (1.05-2.29)	1.67 (1.11-2.52)	0.01

OR_1 = Odds ratio adjusted for exposure.

OR_2 = Odds ratio adjusted for exposure, drinking water supply, hand washing, animal excreta in the yard and crops.

P value relates to OR_2

Note: Factors significantly associated in the univariate analysis included age, boiling water, time to get drinking water, diet, sanitation, pigs in the back yard, crowding of dwelling and literacy of the mother.

Table 7.10 Dry Season Survey
Individuals aged 5 years and older with Diarrhoeal Diseases according to Exposure and other Factors; the Mezquital Valley, 1991.

FACTOR	N=	(%)	TOTAL	OR_1 (95% CI)	OR_2 (95% CI)	P value
Exposure						
Nonexposed	164	(5.9)	2777	1	1	
One reservoir	104	(8.1)	1282	1.41 (1.09-1.82)	1.50 (1.15-1.96)	0.003
Raw wastewater	85	(7.1)	1202	1.21 (0.92-1.59)	1.34 (1.00-1.78)	0.04
Drinking water supply						
Piped	299	(6.5)	4594	1	1	
Not piped	54	(8.1)	677	1.42 (1.04-1.95)	1.39 (1.01-1.90)	0.04
Hand washing						
Usually	237	(6.2)	3800	1	1	
Seldom	115	(7.9)	1460	1.27 (1.00-1.60)	1.36 (1.07-1.74)	0.01
Age group						
5-14 years	138	(5.9)	2335	1	1	
15 and over	215	(7.3)	2926	1.32 (1.06-1.66)	1.45 (1.15-1.84)	0.002
Number of bedrooms						
1	147	(8.6)	1707	1	1	
2	143	(6.1)	2335	0.70 (0.55-0.89)	0.72 (0.57-0.92)	0.008
3	51	(5.4)	939	0.63 (0.45-0.87)	0.64 (0.46-0.90)	0.009
4 +	12	(4.3)	279	0.48 (0.26-0.87)	0.52 (0.28-0.95)	0.03

OR_1 = Odds ratio adjusted for exposure
OR_2 = Odds ratio adjusted for exposure, drinking water supply, hand washing and age.
P value relates to OR_2
Note: Factors significantly associated in the univariate analysis included time getting water, boiling water, crops, diet, pigs in the yard, animal excreta in the yard, irrigation of the family plot, house roof and literacy.

7.3. DISCUSSION OF DRY SEASON SURVEY RESULTS.

7.3.1. *A. lumbricoides* infection.

The prevalence of *A. lumbricoides* in the dry season in this farming population was lower than expected. Climate, and thus more intensive irrigation, would have predicted higher exposure and, therefore, higher prevalences of infection in the dry season (November to May). It is important to point out that during the winter farming activities decline, not to resume until February, when irrigation begins. This agricultural pattern, along with environmental factors (e.g. solar radiation and pronounced dryness), to which *A. lumbricoides* are very susceptible, may both have contributed to these results.

General characteristics of the infection and comparison of results from other studies have been discussed in chapter 6 (section 6.3.1) and will not be repeated here. Available data indicating the importance of sociocultural and age-related characteristics in the epidemiology of ascariasis have been amply reviewed (WHO 1987).

7.3.1.1. Effect of exposure to raw wastewater. Results from the dry season survey provide clear evidence that *A. lumbricoides* infection was significantly associated with exposure to raw wastewater (120-135 eggs/litre). Despite the lower prevalences of infection as compared with the rainy season, the association between raw wastewater and infection was considerably stronger in the dry season. A possible explanation involves climatic and farming activities; during the winter (overlapping with the start of the dry season) agricultural activities decline, not to resume until February, when irrigation begins.

In childen under five years, the prevalence of infection may reflect the magnitude of exposure at times of the year when the weather is drier and hotter. In this age category, exposure is probably related to recreational activities, which implies more frequent contact with water and mud. In older individuals, occupational activities and other variables which imply less direct contact, may explain the similar prevalences observed in both seasons.

7.3.1.2. Effect of the reservoir. Exposure to wastewater from the single reservoir was significantly associated with *A. lumbricoides* infection. This association was strongest for children, but individuals over 5 years also had a significant risk. In fact, the 95% CI of the single reservoir group overlapped with those obtained from the raw wastewater group.

These results contrast with water quality data from wastewater samples which indicated that effluents had low counts of *A. lumbricoides* eggs (1 egg/litre or even less). In order to explain this apparent contradiction, it must be pointed out that the wastewater sampling schedule used in this study and the detection techniques' threshold may both have had low sensitivity. Secondly, maintenance of the run-off and wastewater outlets involved recontamination of the effluent with minor but considerable volumes of untreated sewage from Mexico City (Peasey 1995). This sewage may contain viable eggs in large numbers which mature in the flooded soils, and contaminate the environment, providing exposure while farming, playing or both.

7.3.1.3. Other factors. A significant association between *A. lumbricoides* infection and quality of the dwelling was observed, confirming socioeconomic factors already detected in the rainy season survey. In individuals over 5 years, infection was also associated with water and sanitation-related variables, some of which were not associated with infection in the earlier rainy season survey. Drinking unboiled water and having irrigation for the family plot were both associated with infection, and both suggest low quality of water as a proxy for low hygiene standards. Certain locally grown crops undoubtedly receive polluted water or are washed with faecal polluted water and then eaten uncooked or possibly unwashed (chillies, wild greens), resulting in intestinal parasitic infection.

Other variables associated with *A. lumbricoides* infection such as lack of sanitation (animal excreta around the yard), poor quality roof, or increasing number of bedrooms, all reflect the influence of domestic environment as risk or protective factors in rural populations. The confounding effect of age on the risk of infection was discussed before (rainy season: section 6.3.1).

7.3.2. *G. lamblia* infection.

The prevalence of *G. lamblia* infection in the dry season in young children was slightly higher than in the rainy season. A possible seasonal variation has been suggested by Feachem *et al* (1983). Otherwise, the age-specific prevalence pattern of infection was similar to that reported in other regions of Mexico, while different from other Latin American countries. These differences may reflect different sociocultural contexts (see 6.3.2).

<u>7.3.2.1. Effect of exposure to raw wastewater</u>. In this farming population there was no excess of *G. lamblia* infection associated with exposure to raw wastewater in any of the age categories. As pointed out previously, these results differ from those reported elsewhere (Sehgal & Mahagan, 1991; Jefferson & Betton, 1991).

<u>7.3.2.2. Effect of the reservoir</u>. Contrary to that observed in the rainy season, there was no association between infection with *G. lamblia* and exposure to wastewater from the single reservoir. These results reinforce the possibility of specific regional differences affecting specific sub-groups in the second reservoir population.

<u>7.3.2.3. Other factors</u>. A slight but significant association between *G. lamblia* infection and storage/drinking water practices was observed, possibly indicating lower water quality in dwellings, not detected in the rainy season. An association was also observed with having a lower number of bedrooms, suggesting additional socioeconomic confounders as suggested in other studies Esrey *et al.*. (1989) Chute (1987) and Flanagan (1992). The association with having no pigs in the yard may simply reflect the importance of avoiding proximity with animal reservoirs, as documented by Chute (1987) and Porter (1990). Age was significantly associated with *G. lamblia* infection,

as previously documented in the rainy season survey. Such age-risk associations could be explained by hygiene-behaviour-feeding variables, and/or by immunological-related factors (Pickering & Ruiz-Palacios,1991, WHO 1987, WHO 1991). The association with other variables (e.g. buying vegetables from local shops) is probably reflecting residual confounding.

7.3.3. *E. histolytica* infection.

The prevalence of *E. histolytica* infection in this farming population was lower than that observed previously in other regions of Mexico (Cruz-Lopez *et al* 1989, Gonzalez Galnares 1986) or in other developing countries (WHO 1987, Bray & Harris 1977). These differences may have resulted from differing methodological and technical procedures or as previously discussed (see rainy season: 6.3.3).

<u>7.3.3.1. Effect of exposure to raw wastewater</u>. In young children, there was no association between raw wastewater exposure and *E. histolytica* infection. Older individuals from the raw wastewater group had a moderate but significant risk of infection, similar to that observed in the rainy season. This is the first report of an association between exposure to raw wastewater and infection with *E. histolytica*, although an Egyptian study reported a high prevalence of infection in sewage workers (which was not significantly greater than the general population (Hammouda *et al*, 1992).

<u>7.3.3.2. Effect of hydraulic retention in a single reservoir</u>. .

Individuals aged 5 years and older from the reservoir group had a significantly higher prevalence of *E. histolytica* infection than controls. These results indicate that despite wastewater sampling results showing one nematode egg or less per litre, transmission may have occurred.

<u>7.3.3.3. Other factors</u>. *E. histolytica* infection in young children was statistically associated with the time required to fetch drinking water and with the mother's illiteracy. In older individuals infection was significantly associated with drinking unboiled water and the respondent's unhygienic appearance, reflecting low hygiene status, while river bathing may suggest cyst ingestion from faecal polluted water. Mother's illiteracy and poor quality of the roof indicate the importance of socioeconomic related factors in this farming population.

7.3.4. Diarrhoeal diseases.

The prevalence of diarrhoeal diseases in this farming population was higher than those reported on a national level, although considerably lower than that observed in the rainy season, particularly in young children. The comparison of age-prevalence rates, differing aetiologies and similarities with other studies have been discussed (section 6.3.4).

7.3.4.1. Effect of exposure to raw wastewater. Exposure to raw wastewater was significantly associated with diarrhoeal diseases in both age groups. Despite lower prevalence rates (compared with the rainy season's), the effect of exposure was greatest in the dry season. Young children may become exposed not only while playing, but also when accompanying older relatives in farming duties, who also showed a significantly higher prevalence of diarrhoea than controls. These epidemiological results are consistent with a high degree of faecal contamination of untreated wastewater (up to 10^8 FC /100 ml).

This finding also differ from prospective reports from Israel (Shuval *et al.*. 1986 a, and Fattal *et al.* 1986). These studies reported a seasonal excess risk of "enteric" diseases in children aged from newborns to four years from kibbutzim using partially treated wastewater to irrigate cotton and fodder crops (10^6 and 10^8 FC/ 100 ml, respectively). Further prospective research, however, did not confirm such reported risk (Shuval *et al*, 1989). The present study, however, was designed to test not only the risk from untreated wastewater by the use of a strict definition of diarrhoeal diseases, and seasonal variations, but also the contribution of other variables, some of which are discussed below.

7.3.4.2. Effect of the reservoir. There was no significant difference in prevalences of diarroeal diseases between children under five from the single reservoir and controls, which suggested a beneficial effect from hydraulic retention. However, older individuals from the single reservoir had a significantly higher prevalence of diarrhoeal diseases than controls, possibly indicating a greater exposure to insufficiently treated wastewater than children. In fact, wastewater samples of the reservoir effluent had counts up to 10^4 - 10^5 FC / 100 ml at this time of the year, indicating that transmission of enteropathogens may occur despite a three log reduction of the FC counts due to hydraulic retention.

7.3.4.3. Other factors. The use of drinking water from public sources and failure to wash hands after defecation were two of the factors significantly associated with diarrhoeal diseases in both age groups. Since these associations were not observed in the wet season, both may be reflecting seasonal problems related to the quantity of water available, as well as a poorer quality of that water (Henry & Zeaur 1990, Rahman *et al.*, 1985, Burgers *et al.*, 1988, VanDerslice & Briscoe 1991., Aziz *et al.*, 1981, Han 1986). Diarrhoeal diseases were also associated in the dry season with the presence of and contact with animal excreta (e.g. chickens, pigs, cows, commonly found around the yard in rural villages). Black *et al.* (1989) has proposed that one source of childhood infection with *Campylobacter jejuni* may be animal faces, although other studies have failed to confirm such observations (Clemens & Stanton 1987). Diarrhoeal diseases were also associated with cultivation of crops in the family's plot, suggesting that some of these crops may have been vegetables irrigated with faecal-contaminated water. Wastewater irrigated vegetables have been similarly implicated in a cholera epidemic in Israel (Shuval *et al.* 1986). In addition, the number of bedrooms in dwellings correlated positively with a "protective" effect, reflecting a better standard of living.

7.4 SUMMARY OF THE DRY SEASON SURVEY

The epidemiology of intestinal parasitic infections and of diarrhoeal diseases during the dry season has been discussed in relation to exposure to raw wastewater. Potential protective effects derived from a single storage reservoir, seasonal-related variables and other potential confounding factors have also been analysed. Exposure to raw wastewater was significantly associated with *A. lumbricoides* infection, *E. histolytica* infection and with diarrhoeal diseases. The risk of *A. lumbricoides* and *E. histolytica* infections remained high in individuals exposed to the reservoir's wastewater, even when the effluent complied with the WHO nematode egg guideline. The prevalence of diarrhoeal diseases remained equally high in individuals aged over 5 years from the reservoir group, although the prevalence for younger children from the reservoir group was equivalent to that of controls. Non-wastewater factors associated with ascariasis during the dry season were the poor quality of dwelling roofs, failure to boil drinking water, aging, access to irrigation for the family's plot and the presence of animal excreta in the backyard. While children aged one to four years had a higher prevalence of infection than infants, aging had a "protective" effect in individuals older than 15 years. There was also a "protective" effect associated with increased number of bedrooms.

There was no association between *G. lamblia.* infection and exposure to wastewater. However, these infections were significantly associated with unpiped water supply, storage of water outside the refrigerator and a small number of bedrooms. Children between one and four years of age had a much higher prevalence of *G. lamblia* infection, whereas individuals aged 15 years were "protected". Purchasing vegetables in local shops and not having pigs in the yard also provided a "protective" effect.

Obtaining water at a distance from the dwelling and having an illiterate mother were both factors significantly associated with *E. histolytica* infection. Drinking water seldom boiled, bathing in the river, having a poor quality roof, and the unhygienic appearance of the respondent all had a significant association with infection in individuals older than 5 years.

Diarrhoeal diseases were associated with drinking water from unpiped supplies and not washing hands in both age groups. Children from households where animal excreta was observed, or those cultivating crops in their family's plot, had a higher prevalence of diarrhoeal diseases. Increasing age was associated with a higher prevalence of diarrhoeal disease in individuals over 5 years.

CHAPTER 8 COMBINED RAINY AND DRY SEASON DISCUSSION.

8.1. INTRODUCTION.

This chapter is an integrated discussion of both rainy and dry season results. Comments focus on the health risks associated with exposure to wastewater of different qualities and the positive effects of hydraulic retention in reducing some of these risks. Finally, the contribution of other risk factors is discussed in relation to previously reported literature. Elements highlighted in this chapter form the basis for conclusions and policy recommendations presented in the final chapter.

8.2. EXPOSURE TO RAW WASTEWATER.

The use of raw wastewater schemes in this semiarid region introduces a health risk for those communities practicing flood irrigation. Flood-farming irrigation with raw wastewater affects not only agricultural workers who are occupational exposed, but also their families and in particular the youngest members.

8.2.1. *A. lumbricoides* infection.

More than 85% of the excess prevalence of *A. lumbricoides* infection in the exposed group was attributable to exposure to untreated wastewater (containing 90 - 120 nematode eggs per litre). Prevalence was highest in the rainy season (Table 8.1), and may reflect transmission throughout the agricultural cycle. In older individuals, the attributable risk from exposure to raw wastewater was similarly high in both seasons (93%), while in children younger than 5 yrs, the greatest effect of raw wastewater exposure was observed in the dry season. Higher temperatures over this period may have stimulated recreational exposure in young children. Evidence concerning the risk of helminth infections in agricultural workers and consumers has been rigorously reviewed by Shuval *et al.* (1986) and provides the basis for revised WHO guidelines for the use of wastewater in agriculture and aquaculture (WHO 1989). These previous studies demonstrated a qualitative risk for farm-workers due to occupational exposure, although none of them have quantified the same in children, or allowed for confounders.

8.2.2. *G. lamblia* infection.

Overall prevalence of *G. lamblia* indicated endemicity of this infection in the Mezquital Valley, regardless of the season (Table 8.1). However, no excess prevalence could be attributable to raw wastewater exposure in either age categories or seasons. These results were unexpected, since faecally contaminated water may play an important role in the transmission of this protozoan enteropathogen, as documented following outbreaks attributed to contaminated drinking water supply (Feachem *et al.* 1983). The present results is different from those previously published reports following outbreaks of *G. lamblia* infections in sewage workers (Sehgal & Mahagan 1991, Jefferson & Betton 1991). Baseline prevalence were equally high as those reported herein, although the degree of exposure was distinct. Exposure to sewage implies a highly concentrated source of contamination, while agricultural exposure to raw wastewater at a certain distance from the wastewater source (around 90 kilometers), the dilution effect from storm run-off and the potentially short survival of *G. lamblia* cysts in a semiarid environment may all have contributed to the lack of association in the present study. Despite the lack of quantitative evidence for occupational exposure, alternative routes of transmission (person to person, peridomestic drinking water contamination) may have been the source of *G. lamblia* in this farming population. Proxy factors important in these alternative routes of transmission are discussed below.

Table 8.1 General Prevalences of Intestinal Infections and Diarrhoeal Diseases in the Mezquital Valley, 1991.

		Rainy Season			Dry Season		
	Age Category	Raw Wastewater (%)	Control (%)	Two Reservoirs (%)	Raw Wastewater (%)	Control (%)	One Reservoir (%)
A. lumbricoides	< 5 yrs.	13.7	2.5	3.3	10.0	0.6	11.8
	≥ 5 yrs.	9.1	0.7	1.5	7.2	0.4	4.6
G. lamblia	< 5 yrs.	17.3	17.1	18.8	19.8	20.5	16.5
	≥ 5 yrs.	8.0	6.5	10.6	9.6	7.3	9.9
E. histolytica	< 5 yrs.	6.5	7.0	4.8	6.5	6.7	6.4
	≥ 5 yrs.	15.7	13.2	15.1	16.5	14.7	18.3
Diarrhoeal Diseases	< 5 yrs.	29.0	23.0	26.8	19.4	13.6	15.5
	≥ 5 yrs.	11.8	9.8	10.5	7.1	5.9	8.1

8.2.3. *E. histolytica* infection.

A high prevalence of *E. histolytica* infection was found in these farming populations and these prevalences were similar in both seasons (Table 8.1). Infection in the over 5 years group was significantly associated with exposure to raw wastewater. In this group, however, excess infection attributable to untreated wastewater exposure was relatively small (AR = 15% in the rainy season). There have been no previous studies on the risk of amoebiasis associated with raw wastewater exposure. Av^ilable information from Eygypt and Santiago de Chile contain anecdotal information regarding sewage workers and the general population (Hammouda *et al.*. 1992, Monreal 1994). The sample size in the present study was large enough to detect the effect of occupational exposure in both seasons. However, since this study detected cysts in stools and did not focus on diagnosis or clinical evaluation of *E. histolytica* infection, the results provide more hypotheses for future studies rather than conclusive evidence of association. More sophisticated techniques would have to be used to define the transmission routes, and other variables involved in the infection or disease process.

8.2.4. Diarrhoeal diseases.

The overall prevalence of diarrhoeal diseases was considerably higher in these populations than expected, and children under five years of age had the highest prevalence in both seasons. Even though the prevalence of diarrhoeal diseases was highest in the rainy season, the effect of exposure was greatest during the dry season both in children and older individuals (Table 8.1). The prevalences of diarrhoeal disease in children attributable to raw wastewater exposure ranged from 20 to 30% depending on the season, while they were similar for older individuals in both seasons (17%). Wastewater sampling data were consistent with these results, revealing that untreated wastewater contained between 10^6 to 10^8 faecal coliforms/100 ml throughout the year. The lack of significant risk in the rainy season may be explained by a "dilution mechanism" from concurrent transmission routes which would function mostly during the rainy season (Black *et al.*. 1989).

This study provides the first documented evidence of significant association between diarrhoeal syndromes and occupational exposure to untreated wastewater in agricultural communities. Circumstancial evidence for cholera implicate consumer risk but not occupational risk and unpublished reports from Santiago de Chile provide ecological data "suggesting" risk (Monreal, 1994). Furthermore, a series of studies conducted in Israel have yielded conflicting results regarding this association (Shuval *et al.*. 1986) and prospective studies carried out by the same group reported no excess of "enteric" diseases in kibbutzim exposed to partially treated wastewater as compared to controls (Shuval *et al.*. 1989).

8.3. EFFECT OF THE RESERVOIRS.

8.3.1. *A. lumbricoides* infection.

Retention of wastewater sequentially in two reservoirs led to a substantial decrease in prevalence of *A. lumbricoides* infection both in farmers and in children. Prevalences of *A. lumbricoides* infection in the two reservoirs group were reduced to levels similar to that of controls. These results were substantiated by the lack of detectable nematode eggs in wastewater samples. Although the sampling technique may have lacked sufficient sensitivity, data concerning hydraulic retention in these two reservoirs (> 3 months) suggests efficient egg removal. This is the first evidence for a relationship beween wastewater retention and reduced helminth infection in agricultural populations. In contrast to results from double retention of wastewater, the risk of *A. lumbricoides* infection in individuals exposed to wastewater from a single reservoir were significantly higher than for controls. This unexpectedly high risk did not correlate with data from wastewater samples from the effluent, which showed few detectable helminth eggs per litre (one or less). Although the sampling schedule may have lacked sufficient sensitivity, data on hydraulic retention time (1 to 7 months) indicated that some beneficial effect from the single reservoir would have been expected. Therefore, transmission either occurs below this detection level or an alternative explanation must be found. Short-circuiting and increased velocity of the flow through the reservoir, or temporary discharges of raw sewage may have recontaminated the effluent, and therefore contributed to a high risk of infection in the single reservoir group. In fact, the risk of infection attributable to exposure to this wastewater was above 90%, quite similar to the risk documented from exposure to raw wastewater (see 8.2.1).

8.3.2. *G. lamblia* infection.

Since *G. lamblia* infection was not associated with exposure to raw wastewater, no positive health effect from hydraulic retention would have been expected. Paradoxically, individuals aged over 5 years exposed to wastewater from the two reservoirs had a small but significant risk of infection over controls, while individuals from the single reservoir had similar prevalence to that of controls (Table 8.1). This "area" effect observed in the double retention group may have been the result of sociocultural counfounders, not adequatly measured in this study. Such factors may have included drinking water contamination (i.e. public taps or water at schools), person to person transmission (e.g. pre-school children) and animal reservoirs, among others (Ruiz-Palacios *et al.* 1990, Feachem *et al.* 1983). Other variables associated with infection are discussed below.

8.3.3. *E. histolytica* infection.

Double hydraulic retention had a beneficial effect on *E. histolytica* infection in individuals over 5 years although in individuals exposed to wastewater from the single reservoir the risk of *E. histolytica* infection remained significantly higher than controls. It may be argued that the potential beneficial effect from double retention may be related to longer periods of hydraulic storage, which allows the *E. histolytica* cysts to die-off naturally (Feachem *et al.*. 1983). It was initially expected that retention time in a single reservoir (1 to 7 months) would have also been sufficient to remove amoeba cysts; however, since *A. lumbricoides* ova were not affected, then amoebic cysts were unlikely to be affected either. In addition, transmission may occur below thresholds established as quality indicators (see 8.3.1). In fact, the risk of infection attributable to exposure to the single reservoir effluent was nearly 20%.

8.3.4. Diarrhoeal diseases.

Prevalences of diarrhoeal diseases in children under 5 yrs of age in the two reservoirs group in the rainy season were double that for older individuals. In this latter group, and since there was no risk associated with exposure to raw wastewater, there could be no improvement due to treatment through double retention. However, in children younger than 5 yrs, there was a marginally significant risk associated with exposure. Higher overall prevalences of diarrhoeal disease in these villages probably diluted the expected improvement from double retention despite the improved wastewater quality. It is possible that a clearer effect could have been found in the dry season. Wastewater quality of the second reservoir's effluent revealed faecal coliform counts ranging from 10^3 - 10^5 FC/ 100 ml. This was a 3 - 5 log reduction when compared with raw watewater levels (10^8 FC/100 ml). The excess disease in children attributable to exposure to the second reservoir's effluent was nearly 15%. There was a moderate but significant reduction in risk from exposure to wastewater from a single reservoir in children, despite a lower prevalence of diarrhoeal disease observed in the dry season. Results from wastewater samples demonstrated a 3 - 4 log reduction (10^4 - 10^5 FC/100 ml) in faecal coliforms. The risk attributable to exposure to wastewater from the single reservoir was 12 and 27% both in children and older individuals, respectively.

8.4 OTHER FACTORS.

8.4.1. *A. lumbricoides* infection

A series of non wastewater variables were analysed as confounders in this study. *A. lumbricoides* infection was significantly associated with low socioeconomic status (as measured by 'corrugated roofs' in the dwelling). The type of roof was a good marker of socioeconomic status, as preliminarily suggested by local interviews (see focus groups: chapter 4). Interestingly, a "protective" association between an increased number of bedrooms and prevalence of *A. lumbricoides* infection was observed, although only in the dry season. Landless households were also more vulnerable, but this association was only detected in the rainy season. Although these socioeconomic variables do not demonstrate a direct cause (Hennekens 1987), similar associations (i.e. with bamboo houses, father's job) have been documented in certain studies in Nigeria and Panama (Adenkule, 1986 and Holland *et al.*, 1988), while not validated in other (Killewo *et al.* 1991).

The 'absence of rubbish disposal facilities' and 'presence of animal excreta in the yard' and 'drinking unboiled water' were significantly associated with infection in the dry season. These associations support the potential importance of safe water supplies and sanitation related variables, as previously reported from Ghana and India (Esrey *et al.*, 1990., Annan, 1985., Bidinger *et al.*, 1981). Special attention must be paid to 'purchase of vegetables from local shops', 'local supply of crops', and 'irrigation of the family plot', because these may indicate consumer risk. Similar, information regarding food hygiene has been demonstrated in studies on parasitic contamination of vegetables sold in metropolitan markets of the Philippines (De Leon *et al.*. 1992).

Children aged 1 to 4 years had a higher risk of infection with *A. lumbricoides* than those before their first birthday, whereas in the oldest category, aging provided a "protective" effect. These trends may reflect behaviour-related factors (Feachem *et al.*, 1983, Bundy *et al.*. 1987, Peasey 1995), which were not accounted for in this study.

8.4.2. *G. lamblia* infection

Age was an important risk factor for *G. lamblia* infection. Children aged 1 to 4 years were much more likely to become infected than those in their first year of life, whereas the opposite was observed in individuals over the age of 15 years. The latter had lower infection rates than those in schooling years. This age-specific prevalence may involve a series of hygiene, behaviour and immunological characteristics (e.g. breast milk, weaning contaminated foods, person to person transmission and deficient hygiene at school), as has been summarized in WHO (1991). Some of these characteristics may have specific sociocultural expressions (e.g. beliefs, values and attitudes), which may be "decoded" through qualitative methods not included in this study. Such characteristics may have contributed to the "area" effect observed in the two reservoirs exposure group, where the highest prevalence of *G. lamblia* infection was detected. Presence of animal excreta was also significantly associated with *G. lamblia* infection, suggesting the role of possible reservoirs. Unpiped drinking water supplies and storing water outside the refrigerator were both variables associated with *G. lamblia* infection as previously reported for waterborne transmission by Esrey (1989). However, the latter route is probably only one of many related to faecal-oral transmission in endemic communities (WHO 1991). Other factors having a "protective" association probably arose through residual confounding (i.e. corrugated roof, not renting land, short distances from the canal, and not having pigs).

8.4.3 *E. histolytica* infection

Factors such as belonging to households spending longer time in obtaining water for basic needs, drinking unboiled water, bathing in the river and unhygienic appearence were significantly associated with *E. histolytica* infection in children under 5, particularly during the driest months. Some of these associations may indicate restricted access to sufficient quantities of water for basic hygiene as previously suggested by Cairncross *et al.*, (1987), although waterborne transmission of amoebiasis is also theoretically possible. Despite scant evidence of waterborne trasnsmission (WHO 1991), transmission of *E. histolytica* occurs easily from person to person where there are low standards of personal and domestic hygiene. The lack of rubbish disposal facilities and the unhygienic appearence of the backyard were simply reinforcing the importance of poor sanitation and hygiene practices. It is likely that a combination of approaches, including water and sanitation improvements, as well as hygiene education may have longer lasting benefits for prevention than isolated measures (WHO 1991). In addition, children from illiterate mothers (a key marker of poor socioeconomic background) were also more likely to become infected with *E. histolytica*, Finally, infection showed association with 'no recent medication' (amoebicidal treatment), which possibly reflects the quality of parasitological data reporting.

8.4.4 Diarrhoeal diseases

Diarrhoeal diseases represent a public health problem in these farming populations, particularly in the youngest age group. Widespread faecal pollution, via multiple routes (e.g. in drinking water supplies, by animal faeces, dirty hands and contaminated food) all pose serious challenges. These populations have long been known to be of the poorest and the regions of the most contaminated in the country. In this study, diarrhoeal diseases were significantly associated with a range of water, hygiene-related factors and socioeconomic variables. Children from households in which drinking water was seldom boiled had a high risk of diarrhoeal diseases, regardless of the season, whereas drinking water from public sources, failure to wash hands, presence of animal excreta around the yard and cultivation of salad crops in domestic orchards were significantly associated in the dry season. Similar observations have been documented by Henry *et al.* (1990) and Cairncross & Cliff (1987). There was substantial evidence that the lack of handwashing after defecation or before meals is involved in bacterial diarrhoea (Khan 1982, Alam *et al.*. 1989, Clemens *et al.*. 1987), although the mechanisms of transmission from contaminated public drinking water have not been elucidated (Victora *et al.*, 1988). The role of animals and their excreta in the transmission of diarrhoeal disease remains unclear, although there is some evidence that chickens, cats and dogs may be infected with *Campylobacter jejuni* , and that children may become infected via animal's faeces (Grados *et al.*, 1988., Black *et al.*, 1989). Finally, cultivation of salad crops was an additional vehicle in the transmission of diarrhoeal diseases, possibly as a result of contaminated water being used for cropland irrigation in the family orchard. These vegetables, if eaten insufficiently cooked, may pose considerable risk to consumers, as documented from several outbreaks of cholera (Fattal *et al.*, 1986).

Other confounding factors showing "protective" association in the oldest group were unhygienic appearence of the respondent and of the dwelling surroundings, suggesting a vestige of other confounders. Despite the desire for cleaniness in these farming communities, the lack of financial resources and time are major constraints. References to poverty and "moral deterioration" in rural communities has been discussed by Boot & Cairncross (1993).

8.5. STRENGTHS AND WEAKNESSES OF THE STUDY.

The present study has provided significant contributions by overcoming some methodological deficiencies and by measuring not only the health effects attributable to exposure to untreated wastewater, but more importantly, potential improvements through adequate and low cost wastewater treatment.

The strength of the present research lies in the reduction of misclassification by the definition used for both exposure and study outcomes. These definitions reduced sources of bias, affecting other studies. Potential confounders (i.e. hygiene, sanitation and socioeconomic factors) were analysed by using sophisticated analytical techniques which permitted measurement of risk of diarrhoeal diseases associated with exposure to wastewater, while allowing for the effect of other factors present in this farming population. This assessment was focussed not only in the farmers themselves, but also in other members of their households.

Special attention was paid to the definition of exposure, which utilized data on each individual's water contact with irrigation canals carrying water of known quality, along with specific activities related to this exposure (as obtained from interviews). More reliable information may had been obtained through observational data, however, this would have been beyond logistical and financial possibilities of the project. Special efforts were made to obtain a sound characterization of the irrigation system in order to substantiate farmers statements and to collect data on the microbiological quality of wastewater with which to supplement this definition. Further refinement of the definition of exposure resulted in a composite variable, which integrated the source of irrigation (e.g. reservoir, canal, spring) and the timing of wastewater contact. Only individuals with defined exposure were included in the analysis.

An adequately defined "nonexposed" population was essential for meaningful interpretation of these results. It may be argued, that diurnal activities in rain-fed farming populations are different from those in wastewater farming populations and that baseline differences (i.e. social or environmental) may affect comparability. At the time of the start of this study, there were no known farming communities in the area using ground water for irrigation. Nevertheless, this " natural experiment" or "opportunistic study" of an existing situation is not inconsistent with the concept of random allocation in field epidemiology (Blum & Feachem 1983).

In addition, special attention was given to identifying potential confounders at the start of the study and this task involved a characterization of major variables within the local context. These confounding variables were systematically monitored throughout the analysis, while assessing the chances of inter-village variation. Inevitably, some residual confounding remained, and it is likely that some of these confounders are resulting from ethnographic expressions (Boot & Cairncross 1993). Hence, an "area effect" may have been the result of culture and hygiene in some of those villages (Nha-nhu ethnic group); this "area effect" may have influenced mostly the two reservoirs group. Interestingly, however, such an effect had no significant influence on the central outcomes of the study (*A. lumbricoides* infection and diarrhoeal disease), although it did on secondary outcomes (*G. lamblia* infection).

The sample size and the number of villages involved in the cross sectional surveys reduced potential problems of one-to-one comparisons. Synchronized visits to different water areas reduced "external" changes between exposed populations (section 4.2). The two surveys involved in this study addressed the possible seasonality of the outcomes, exposure fluctuations and different degrees of exposure. By alternating the intermediate exposure group in each season it was possible to compare the impact from a single versus double hydraulic retention. However, due to logistical constraints it was not possible to measure the effect of each reservoir simultaneously in both seasons; this is a weakness of the study, particularly regarding diarrhoeal diseases.

Potential weaknesses in selection and information bias of many studies were overcome in the present study (Henneckens 1987). Selection bias is unlikely to have been a major problem in the present study since only households fulfilling eligibility criteria were included. The interpretation of direction of 'cause and effect' is always difficult in observational studies (Flanders *et al.*, 1992) and may lead to certain information bias. However, special effort was made to reduce bias from misclassification of either exposure or outcome (by using precise definitions). Exposure was defined by a composite variable summarising not only data on water quality in the canals, but also the individual's specific activities, including frequency and timing of "water contact". These data were used to create new variables, which defined households having contact with multiple wastewater sources or those about which there was insufficient data, so that they could be excluded from the analysis. Potential sources of bias may have been introduced by surrogates (e.g. spouse or mother) providing information (i.e. for the diarrhoeal episodes), whereas it was not a problem for parasitology results. Information bias may be particularly important if the disease event was not prolongued or self-limiting. A special effort was made to obtain face to face interviews, and clear-cut standard definitions of 'diarrhoeal episode' were used. Although a shorter recall period for diarrhoeal diseases may have been beneficial, the sample size and logistics limited such possibilities. Compliance rates over 80% were achieved.

Although sampling bias may have occurred due to erratic egg shedding patterns, it remains unclear whether there is a significant difference in results after the examination of either one or two stool specimens (Gyorkos *et al.*, 1989). Certainly one source of bias may have arisen from the omission of size classification of *E. histolytica* cysts (to distinguish *E. hartmanni* from *E. histolytica*) (Gonzalez-Ruiz, 1991).

Regarding the wastewater quality monitoring for *A. lumbricoides* eggs, potential problems regarding wastewater sampling sensitivity, schedules and detection threshold need to be considered, although indicators used to assess the quality of wastewater were clearly defined. The sensitivity of the detection threshold reflects the recovery efficiency and, hence, the potential for presence of eggs in samples despite failure to detect them. Furthermore, data showing 1 egg or less per litre of wastewater does not answer the question of acquisition of eggs from the soil (Ayres *et al.*, 1992), and lack of measuremment of soil contamination as an intermediate variable may had been a major weakness. Optimal evaluation of diurnal egg load on wastewater should be measured by conducting a 24-hour profile prior to initiation of sampling. This profile was not conducted and only monthly samples were collected for this study (due to financial, logistical and staff limitations). Point-source pollution of the effluent, even though in small volumes, may have resulted in contamination of effluent downstream of sampling sites in the dry season. This uncontrolable problem may have affected interpretation of results from the population exposed to the single reservoir effluent.

8.6. SUMMARY OF THE OVERALL DISCUSSION

The main results may be summarised as follows:

a) Cropland irrigation with raw wastewater was stongly associated with *A. lumbricoides* infection in farmworkers and in their families, with a risk of diarrhoeal diseases both in children and older individuals, and also with a small but significant risk of *E. histolytica* infection in individuals over 5 years.

b) The difference observed in the prevalences of *A. lumbricoides* infection and diarrhoeal diseases were similar in both seasons, but the prevalences in the control group was lower in the dry season; thus, the relative effect of wastewater use was greater in the dry season;

c) Double Hydraulic Retention of wastewater in reservoirs in series (2 - 6 months) reduced subtantially the risk of *A. lumricoides* infection, and to a lesser extent the risk of *E. histolytica* infection, and possibly the risk of diarrhoeal diseases in young children (0-5 yrs);

d) Retention of wastewater in a single reservoir (1 - 7 months) did not reduce the risk of *A. lumbricoides* or *E. histolytica* infections; it did reduce the risk of diarrhoeal diseases in children under 5 years by 20%; this beneficial effect, however, was not observed in older individuals;

e) No association between exposure to wastewater and infection with *G. lamblia* was detected in this research;

f) other variables related to personal, domestic hygiene, and sanitation were also involved in the epidemiology of intestinal infections and diarrhoeal diseases in this farming population .

CHAPTER 9. CONCLUSIONS AND POLICY RECOMMENDATIONS

9.1 EFFECTS OF UNTREATED WASTEWATER.

Untreated wastewater introduces a health risk to the Mezquital Valley through cropland flood irrigation. Existing regulations (e.g. crop restriction) fail to protect the health of families of agricultural workers practicing flood irrigation. The main conclusions of this study are presented below:

Exposure to raw wastewater is associated with *A. lumbricoides* infection in both rainy and dry seasons. Wastewater quality results support this association. Farmers and other members of their households may become exposed to *A. lumbricoides* while labouring on the land, although other activities are associated with transmission in younger individuals (e.g. playing). By using regression analysis, the effect of exposure to raw wastewater on infection was greatest in the dry season, particularly in children under five years of age. Older individuals had a high risk in both seasons, suggesting that the burden of transmission in this latter group was influenced by contact that was not seasonally dependendent (i.e. farming). As data indicated, nearly 85% of excess infection was attributable to exposure to untreated wastewater.

There was a high level of both helminth eggs and faecal coliforms in raw wastewater samples, the latter suggesting the presence of bacterial and viral enteropathogens. Thus, exposure to raw wastewater was associated with the diarrhoeal syndrome, particularly in the dry season. The effect of exposure to raw wastewater was greatest in children under five years. Although there was a higher prevalence of diarrhoeal diseases in the rainy season, the association with exposure was marginally significant in this season and this population group. Overwhelming concurrent routes of transmission may have diluted the effect of exposure to raw wastewater in the rainy season. Approximately 30% of excess diarrhoeal diseases in this farming population was attributable to exposure to untreated wastewaster.

Exposure to raw wastewater was also associated with risk of *E. histolytica* infection in individuals over 5 years of age. This effect was minor and had no seasonality. There was no association between exposure to raw wastewater and *G. lamblia* infection. No risk was attributable to exposure to raw wastewater.

9.2 EFFECTS OF A SINGLE RESERVOIR.

An unexpectedly high prevalence of *A. lumbricoides* infection was associated with exposure to wastewater from the single reservoir, particularly in children under five years. The excess of infection attributable to exposure to that effluent was approximately 95%. The near absence of helminth ova in the effluent indicates that transmission occured below the detection threshold or that wastewater sampling techniques had low sensitivity. However, recontamination of effluent downstream from sampling sites may have contributed to this high risk. The risk of infection attributable to exposure to this effluent was similar to that of raw wastewater (90%). Consistent with results for *A. lumbricoides*, individuals over five years exposed to the effluent of the single reservoir had a minor but significant risk of *E. histolytica* infection. Given the potential effects of hydraulic retention (1 - 7 months in the single reservoir) this was an unexpected observation. However, amoebic cysts are considerably lighter than helminth ova and may not be as settleable as the former. Alternatively, low rate "on site" recontamination of the effluent may have contributed to such an unexpected finding.

There was considerable improvement of faecal coliform water quality following retention in the single reservoir (3 - 4 log reduction of faecal coliforms). Nevertheless, the risk of diarrhoeal diseases only decreased in young children in the dry season, but not in individuals over five years exposed to the effluent from the single reservoir. Transmission of diarrhoeal diseases, therefore, probably occurs despite such FC levels.

9.3 EFFECTS OF THE TWO RESERVOIRS.

Wastewater in the second reservoir had been retained up to 6 months, in addition to that time in the single reservoir; no helminth eggs were detected in the effluent during the sampling period. Interestingly, children from the two reservoirs group had a similar risk of *A. lumbricoides* infection to that found for controls. Prevalences in individuals aged five years and older were relatively low in both control and reservoirs groups, thus the apparently higher risk measured for the latter probably lacks public health relevance.

The effluent of the second reservoir had low FC counts (10^3-10^4 FC /100 ml) indicating substantial improvement of quality over untreated wastewater. However, exposure to raw wastewater was not associated with an increased prevalence or risk of diarrhoeal diseases in older individuals. In children under five, the association was marginally significant for exposure to raw wastewater and was improved for those exposed to the effluent of the second reservoir. However, in all groups, the overlap of 95%CIs from raw wastewater and two reservoirs groups indicate that no real difference may exist. The results indicate that diarrhoeal diseases in this farming populations have a clear seasonal pattern, and that may be transmitted principally by other routes different from wastewater irrigation.

There was a positive effect from double hydraulic retention on *E. histolytica* infection in individuals older than five years. Interestingly, both the single reservoir and raw wastewater exposure groups had overlapping CI, indicating no effect on risk from single hydraulic retention.

There was no association between *G. lamblia* infection and exposure to wastewater from the second reservoir in children under five years. Curiously, older individuals showed a higher risk of infection when compared with their respective controls. However, this finding cannot be related to the use of settled wastewater, as no excess prevalence was found in the group exposed to raw wastewater. Pre-school and school-aged children had the highest prevalence of *G. lamblia* infection, indicating the presence of a strong confounder. Differences in sociocultural characteristics ("area effect") may have facilitated person to person transmission.

9.4. IMPLICATIONS OF STUDY RESULTS.

9.4.1. Local implications and policy recommendations

Raw wastewater in the study area contained high numbers of *A. lumbricoides* eggs and high counts of faecal coliforms (90 to 135 eggs per litre and 10^8/100 ml, respectively). These levels are far above those acceptable for the safe use of wastewater in agricultural production, even for restricted irrigation (WHO 1989). As data showed, raw or insufficiently treated wastewater represents a considerable health risk for agricultural workers practising cropland flood irrigation and their families. Crop restriction policy does not protect these families. There was an excess risk of *A. lumbricoides* infection, diarrhoeal diseases, and amoebic infection associated with exposure to raw or insufficiently treated wastewater.

If the rationale for wastewater reuse is economic, schemes should take into consideration other health protection measures (apart from restricted crop irrigation) and protection of specific at-risk groups (i.e. agricultural workers of the scheme). Transport of wastewater in pipes rather than channels would have negligible effect, as exposure takes place mostly in the fields, while irrigating with wastewater. Data presented herein support the recommendation that there is an urgent need for appropriate wastewater treatment, to begin with upgrading the efficiency of existing storage reservoirs in the Mezquital Valley. As data showed, the risk of *A. lumbricoides* and *E. histolytica* infections was not lower for single-stage wastewater retention, but it was following double hydraulic retention. Despite certain hydraulic retention (1 to 7 months, depending on the time of the year) and considerable egg removal, enteropathogens may be transmitted below detection thresholds. As data showed, this level seems to be insufficient to protect those families exposed to the effluent of the first reservoir. Although wastewater sampling techniques used in this study suffered from low sensitivity, occasional unstable contamination of the effluent may have had a "short-circuit" effect. Unfortunately, no wastewater samples were collected from "on-site" areas. There is some evidence from a simultaneous study, that the quality of wastewater downstream (beyond the by-pass) met the nematode egg guideline for restricted irrigation (Peasey A. personnal communication).

In order to prevent such unstable effluent conditions, irrigation districts should amend wastewater management practices and focus on on-site monitoring of effluents for such irregularities. Performance of the single reservoir could be upgraded by the use of low-cost hydraulic techniques designed to achieve greater egg removal (e.g. waste stabilization ponds). These engineering modifications need to take into consideration not only the location of "on-site" discharges, but also seasonal and daily peak flows, heavy storms and maintenance contingencies (Mara, 1983).

Treatment of wastewater through double retention improved its quality sufficiently for it to qualify for restricted irrigation (i.e. helminth egg levels were negative) and the prevalence of *A. lumbricoides* infection in the reservoir group was similar to controls. On the other hand, it must be remembered that WHO did not set a guideline for faecal coliform levels for restricted irrigation. As data showed, however, there was an overlapping risk of diarrhoeal diseases both in the two-reservoirs and the raw wastewater groups. These data suggest that despite nematode egg level compliance, aetiologic agents of diarrhoeal diseases (bacterial or viral infections) may not be represented by this nematode egg guideline. Existing regulations set a microbial standard of no more than 1 000 FC per 100 ml for crops which are consumed raw, but no FC guideline for restricted crop irrigation. Therefore, a standard for faecal coliforms (as indicator organisms) should be included in the national regulations, and the recommended threshold should not exceed 10^4 FC /100 ml for restricted irrigation. This recommendation is based on the assumptions that treatment technology (i.e. WSP followed by maturation ponds) designed to remove the nematode eggs will automatically achieve an effluent with 10^4 FC /100 ml (Stott *et al*, 1994). Therefore, the above recommended FC standard would serve not only as an additional quality indicator (to monitor other infectious agents not explicitly considered in the WHO 1989 guidelines), but would also serve the purpose of encouraging wastewater treatment. WSP provide a considerable greater opportunity for removal than other treatment processes, and have also other advantages (i.e.

cost and maintenance), relevant for developing countries.

It is important to underline, however, that wastewater treatment on its own may not be enough. As the data presented herein demonstrate, the local environment is overwhelmed with other risk factors. This was particularly the case for diarrhoeal diseases among young children, but also for amoebic infection. Community health care strategies could address the promotion of education programs for personal and domestic hygiene (including food and drinking water) and oral rehydration therapy for diarrhoeal episodes (Feachem 1984). Other strategies may be needed i.e. chronic or dysenteric diarrhoeas, in which the effect of ORT may be modest or even nill. In addition, regular and systematic antihelminthic treatment with wide-spectrum drugs may reduce the health burden in this farming population, particularly in children, since *A. lumbricoides* infection may be contributing substantially to their overall morbidity via malnutrition, pneumonia, diarrhoea, and vitamin A deficiency. Finally, appropriate policies should also emphasize the socioeconomic improvement of the community, in order to address multiple risk factors associated with infection and peri-domestic or person to person transmission especially for protozoan infections.

9.4.2. Implications of results on WHO guidelines for restricted crop irrigation.

The purpose of the WHO guideline for restricted irrigation (Category B, see Table 2.6) was to prevent occupational risk involved in wastewater reuse, by compliance with a level of ≤ 1 nematode egg per litre. This level, however, may be inadequate under unstable treatment conditions, in which the effluent receives additional loads of untreated sewage (even if relatively minor). This contamination results in an excess of parasitic infections among individuals exposed to this wastewater. Similar situations may exist in other wastewaster reuse schemes in which the velocity of the flow increases through the WSP. Therefore, appropriate engineering measures (i.e. wastewater management, WSP) are insufficient alone and a sound knowledge of the irrigation network, as well as of potential or occasional discharges of sewage are essential.

If feasible, water quality monitoring should be carried out more frequently in unstable conditions as for stable systems, possibly on a weekly basis. Wastewater sampling techniques should be improved to measure effluent quality arriving at irrigation sites and not only that immediately adjacent to the treatment site. Special attention should be paid to the low sensitivity of wastewater microbiological techniques and improvement of these. Current methods for the enumeration of helminth eggs in treated wastewater should be adapted for higher recovery rates or a larger sample volume should be used to allow for more precise detection levels (Ayres *et al*, 1991).

WHO guidelines use nematode eggs as indicator organisms for all large settleable pathogens (including protozoan cysts). This rationale suggests that most pathogens of interest become non-viable in well-designed wastewater treatment systems and that treatment to the level of ≤ 1 nematode egg per litre would be adequate to protect farmers from other health risks. In addition, when the present study was initiated, no faecal coliform guideline for restricted irrigation existed, due to the lack of evidence concerning risk from bacterial or viral infections to farmworkers. The present results indicate that there is in fact a risk associated with pathogens present in treated wastewater despite compliance with the nematode egg guideline. Therefore, a faecal coliform guideline should be recommended even for restricted irrigation and a mean level of $\leq 10^4$ FC per 100 ml should be considered. The rationale for this recommendation was briefly discussed above.

This study demonstrated that following single or double hydraulic retention, effluent complied with guidelines for restricted irrigation. Data also demonstrated, however, that there was a moderate risk of diarrhoeal disease and amoebic infection, even when the effluent complied with the nematode guideline. These results indicate the need to assess other health risks for farming communities in the developing world (i.e. bacterial, viral and protozoan infections) not specifically referred to in the 1989 WHO guidelines. The above recommendation for inclusion of a faecal coliform guideline would also assist evaluation of these other pathogens.

In summary, wastewater constitutes a valuable resource for agricultural production in vast semiarid regions, since water availability is a limiting factor for crop production and sustainable development. Appropriate definition of wastewater reuse schemes and corresponding treatment requirements are increasingly urgent tasks. Setting standards which are too strict, without measurable public health considerations, will simply contribute to clandestine or unregulated practices. Similarly, guidelines not adapted to local economic and sociocultural conditions will be extremely difficult to enforce. In contrast, unduly liberal guidelines and codes of practice may result in considerable health risks. It must be born in mind that as policy tools, guidelines are intended to provide guidance for decisions related to the protection of public health and the preservation of the environment, as defined by global tendencies and national interests (WHO 1987). Although the results of epidemiological studies should not be confused with legislative concerns, planners and policy-makers need to foster discussion for risk management decisions in conjunction with at-risk communities. In the meantime, the cost and benefit of adapting or modifying the WHO guidelines should be based on sound scientific research. *

REFERENCES

Adenkunle (1986) Family influences on the incidence of intestinal parasites among Nigerian children. JRSH, 2:66-68.

Adin, A., Bachmat, Y., Fattal, B. and Feigin, A. (1990) Water reuse in agriculture: the Israeli experence. Report for the Seminar on Water Reuse in Agriculture: The Mexican and Israeli Experence, 9p.

Alam, N. *et al.* (1989) Mother's personal and domestic hugiene and diarrhoea incidence in young children in rural Bangladesh. Int. J. Epidem. 18(1): 242-247.

Anderson, R.K., Calvo, J., Serrano, G. and Payne, G. (1946) A study of the nutritional status and food habits of Otomi Indians in the Mezquital Valley of Mexico. Am. J. Publ. Health, 36: 883-903.

Anderson, R.M. (1986) The population dynamics and epidemiology of intestinal nematode infection. Trans. Roy. Soc. Hyg. Trop. Med., 80:686-696.

Anderson, R.M. & May, R.M. (1985) Helminth infections of humans: mathematical models, population dynamics and control. Adv. Parasitol., 24:1-101.

Annan, A. (1985) The socioeconomic determinants of malnutrition among preschool children in Ghana. PhD Dissertation, Cornell University, New York.

Ayres, R.M., Stott, R., Lee, D.L., Mara D.D. and Silva, S.A. (1991) Comparison of techniques for the enumeration of human parasitic helminth eggs in treated wastewater. Environ. Technol. 12: 617-623.

Ayres, R.M., Stott, R., Mara D.D. and Lee, D.L. (1992) Wastewater Reuse in agriculture and the risk of intestinal nematode infection. Parasitol. Today 8(1):32-35.

APHA (1981) Standard method for the examination of water and wastewater. APHA, Washington D.C.

Aziz, K.M.A. et al. (1981) A study of interpersonal spread of human faeces in rural Teknaf of Bangladesh. In: Proceedings of an international Conference Cox's Bazaar, June pp. 227-237.

Bartone, C. (1985) Reuse of wastewater at the San Juan de Miraflores stabilization ponds: Public health, environmental and socioeconomic implications. PAHO Bull. 19 (2):147-152.

Bartone & Arlosoroff, S. (1987) Reuse of pond effluents in developing countries. IAWPRC, International conference on waste stabilization ponds, Lisbon, Portugal.

Biagi, F. & Rodríguez, O. (1960) Study of Ascariasis eradication by repeated mass treatment. Am. J. Trop. Med. Hyg. 9: 274-276.

Bidinger, P.D., Crompton, D.W.T. & Arnold S. (1981) Aspects of intestinal parasitism in villages from rural penninsular India. Parasitol., 83:373-380.

Black, R.E., Lopez de Romaña, G., Brown, K.H., Bravo, N., Grados Baltazar, O. and Creed, H. (1989) Incidence and etiology of infantile diarrhea and major routes of transmission in Huascar, Peru. Am. J. Epidemiol., 129(4):785-799.

Blagg, W., Shloegel, E.L., Manson, N.S. et al. (1955) A new concentration technique for the demonstration of protozoa and helminth eggs in faeces. Am. J. Trop. Med. Hyg. 4:23-28.

Blum, D. & Feachem, R.V. (1983) Measuring the impact of water supply and sanitation investments on diarrhoeal diseases: problems of methodology. Int. J. Epidemiol., 12(3):357-365.

Blumenthal, U.J., Strauss, M., Mara, D.D. and Cairncross, S. (1989) Generalised model of the effect of different control measures in reducing health risks from waste reuse. Wat. Sci. Tech., 21:567-577

Boot, M.T. & Cairncross, S. (1993) Actions speak. The study of hygiene behaviour in water and sanitation projects. (IRC) International Water and Sanitation Centre/LSHTM.

Bray, R.S. & Harris, W.G. (1977) The epidemiology of infection with Entamoeba histolytica in The Gambia, West Africa. Trans. Roy. Soc. Trop. Med. Hyg., 71:401-407.

Bundy, D.A., Cooper, E.S., Thompson, D.E., Anderson, R.M. and Didier, J.M. (1987) Age-related prevalence and intensity of *Trichuris trichiura* infection in a Sta. Lucia community. Trans. Roy. Soc. Trop. Med. Hyg., 81:85-94.

Burge, W. & Marsh, P.B. (1978) Infectious disease hazards of landspreading sewage wastes. J. Environ. Quality. 7(1):1-9.

Burgers, L., Boot, M. and Wijk-Sijbesma, Ch. (1988) Hygiene education in water supply and sanitation programmes: Literature review with selected and annotated bibliography. Technical paper series; N° 27, The Hague, Netherlands, (IRC) International Water & Sanitation Centre.

Cairncross, S. & Cliff, J.L. (1987) Water use and health in Mueda, Mozambique. Trans. Roy. Soc. Trop. Med. Hyg. 81:51-54.

California State Department of Health. (1968) Statewide standards for the safe direct use of reclaimed wastewater for irrigation and recreational inpoundments. California Administrative Code. Title 17-Public Health, Berkeley, CA.

Carrada Bravo, T, (1984) Ascariasis infantil como problema de Salud Pública. Bol Méd. Hosp. Inf. Méx., 41(11): 636-640.

Chan, C.T. (1991) Modes of transmission for *Ascaris lumbricoides* and *Trichuris trichiura* among Chinese children in Macao City. Int. J. Parasitol. 21(4):477-478.

Chang A.C., Page, A.L. & Asano, T. (1993) Background document for chemical guidelines for land application of wastes. Report for WHO, Div. Environ. Hlth., Geneva.

Chandler, A.C. (1954) A comparison of helminthic and protozoan infections in two Egyptian villages two years after the installation of Sanitary improvements in one of them. Am. J. Trop. Med. Hyg., 3:59-73.

Chute, C.G., Smith, R.P., and Barow, J.A. (1987) Risk factors for endemic giardiasis. Am. J. Publ. Health., 77:585-587.

Clark, S.C., Linnemann, C.C., Clark, J.G. and Gartside S.P. (1984) Enteric parasites in workers occupationally exposed to sewage. Journal Occupational Medicine. 26(4):273-275.

Clark, S.C., Linnemann, C.C. and Gartside, S.P., Phair, J.P., Blacklow, N., and Zeiss, C.R. (1985) Serologic survey of rotavirus, Norwalk agent and *Prototheca wickerhamii* in wastewater workers. Am. J. Publ. Health., 75(1):83-87.

Clemens, J.D. & Stanton, B.F. (1987) An educational intervention for altering water sanitation behaviors to reduce childhood diarrhea in urban Bangladesh. I. Application of the case-control method for development of an intervention. Am. J. Epidemiol., 125:284-291.

Crevenna, P.B. (1977) Epidemiologia de la amibiasis. Sal. Publ. Mex., 19:411-413.

Cruz López, A., Cortez Riveroll, R., Valerdi, M.G. and Gonzalez, M. (1989) Uso masivo de la coproparasitoscopía con FAF. Sal. Públ. Méx. 31(4):536-540.

De Leon, W.U., Monzon, R.B., Aganon, A.A., Arceo, R.E., Igancio, E.J., and Santos, G. (1992) Parasitic contamination of selected vegetables sold in metropolitan Manila, Philippines. So. East Asian J. Trop. Med. Publ. Hlth., 23(1):162-164.

Direccion General de Epidemiología (1984) Vital Statistics., SSA.Mexico

Direcion General de Epidemiologia (1988) National Health Survey., SSA, México

Duron, M.C. (1985) Mexican experience with wastewater irrigation. In: Treatment and Use of Sewage Effluent for Irrigation. M.B. Pescod and Arar, A. (Eds.) Butterworths, London.

Engaeck, K. & Larsen, S.O. (1979) A comparative socioepidemiological study of families with *Giardia lamblia, Entamoeba coli* and *Trichuris trichiura*. Ugeskrift fur Laeger., 141:1128-1131.

Esrey, S.A., Feachem, R.G. and Hughes, J.M. (1985) Interventions for the control of diarrhoeal diseases among young children: inproving water supplies and excreta disposal facilities. Bull. WHO, 63(4):757-772.

Esrey, S.A., Collett, J., Miliotis, M.D., Koornhof, H.J. and Makhale P. (1989) The risk of infection from *Giardia lamblia* due to drinking water supply, use of water and latrines among preschool children in rural Lesotho. Int. J. Epidemiol., 18(1):248-253.

Fattal, B., Yekutiel, P. and Shuval, H.I. (1986) Cholera outbreak in Jerusalem 1970, revisited: the evidence for transmission by wastewater irrigated vegetables. In: Environmental Epidemiology: Epidemiological Investigation of Community Environmental Health Problems. ed. J.R. Goldsmith, CRC Press.

Fattal, B., Wax, Y., Agursky, T. and Shuval, H.I. (1986b) Comparison of three studies performed in Israel on health risk associated with wastewater irrigation. In: Environmental Quality and Ecosystem Stability. Vol III:783-793, Bar-Ilan University Press.

Fattal , B., Wax, Y. Davies, M. and Shuval. H.I. (1986c). Health risk associated with wastewater irrigation: an Epidemiological Study. Am. J. Publ. Health. 76(8): 977-980.

Feachem, R.G. (1984) Interventions for the control of diarrhoeal diseases among young children: promotion of personal and domestic hygiene. Bull WHO,62(3):467-476.

Feachem, R.G. & Blum, D. (1984) Health aspects of wastewater reuse. In: Proceedings International Symposium on Reuse of Sewage Effluents. Institute of Civil Engineers, T. Thelford (eds), London. 237-247.

Feachem, R., Bradley, D., Garelick, H. and Mara, D. (1983) Sanitation and Disease: Health aspects of excreta and wastewater management. Wiley, Washington, D.C.

Flanagan, P.A. (1992) Giardia diagnosis, clinical course and epidemiology. A review. Epidemiol. Infect., 109:1-22.

Flanders, D.W, Lin, L., Pirlle, L.J. and Caudill, P.S. (1992) Assessing the direction of causality in cross-sectional studies. Am. J. Epidemiol. 135(8):926-934.

Fleiss, J.L. (1981) Statistical methods for rates and proportions . John Wiley & Sons. New York

Forrester, J.E., Scott, M.E., Bundy, D.A.P. and Golden, M.H.N. (1988) Clustering of *Ascaris lumbricoides* and *Trichuris trichiura* infections within households. Trans. Roy. Soc. Trop. Med. Hyg., 82:282-288.

Garcia, L.S. and Shimizu, R. (1981) Comparison of Clinical Results for the Use of Ethyl Acetate and Diethyl Ether in the Formalin-Ether Sedimentation Technique Performed on Polyvinyl Alcohol-Preserved Specimens. J. Clin. Microbiol., 13 (4):709-713.

Gonzalez Galnares, M., Garcia Perez, M., Guicafre Gallindo, H., and Munoz, O. (1986) Comunicacion de un brote de amibiasis invasora en niños de un campamiento de damnificados por el volcan Chichonal. Arch. Invest. Med. (Mex).), 17:365-368.

Gonzalez Richmond, J.A., Madrigal Fritsch, H., Naranjo Banda, A. and Moreno Terrazas, O. (1985) Consumo de alimentos, estado nutricional y parasitosis intestinal en una comunidad indigena. Sal. Públ. Méx., 27(4):336-345.

Gonzalez Ruiz, A. & Ruiz Palacios G. (1991) Entamoeba hartmanni: missing or misidentified? J. Inf. Dis., 164:612-613.

Grados O., Bravo, N., Black, R.E. and Butzler, J.P. (1988) Paediatric *Campylobacter* diarrhea from household exposure to live chickens in Lima Peru. Bull WHO, 66(3): 369-374.

Gunnerson, C.G., Shuval, H. I. and Arlosoroff, S. (1985) Health Effects of wastewater irrigation and their control in developing countries. Proceedings of the Water Reuse Symposium III, San Diego, California. AWWA Research Foundation, Denver,1576-1602

Gyorkos, T.W., MacLean, J.D. and Law, C.G. (1989) Absence of significant differences in intestinal parasite prevalence estimates after examination of either one or two stool specimens. Am. J. Epidemiol. 30(5):976-980.

Hammouda, N.A., El-Gebali, W. and Razek, M.F. (1992) Intestinal parasitic infection among sewage workers in Alexandria, Egypt. J. Egypt. Soc. Parasitol. 22(2):299-303.

Han, A.M. (1986) Personal toilet after defecation and the degree of hand contamination according to different methods used. J. Trop. Med. Hyg. 89 (5): 237-241.

Hennekens, Ch. (1987) Epidemiology in Medicine, Little & Brown, New York.

Henry, F.J. and Zeaur, R. (1990) Transmission of diarrhoea in two crowded areas with different sanitary facilities in Dhaka, Bangladesh. J. Trop. Med. Hyg., 93: 121-126.

Hespanhol, I. (1993a) Reuse of community wastewater: Health and evironmental protection-research needs. Discussion paper. Technical Consultation on Integrated Rural Water Management, WHO & FAO, Rome, 23p.

Hespanhol, I. (1993b) Who guidelines and national standards for reuse and water quality. Water. Res.

Holland, C.V., Taren, D.L., Crompton, D.W.T., Nesheim, M.C., Sanjur, D., Barbeau, I., Tucker, K., Tiffany, J. and Rivera G. (1988) Intestinal helminthiasis in relation to the socioeconomic environment of panamanian children. Soc. Sci. Med., 26(2):209-213.

Huttly, R., Blum, D., Kirkwood, B., Eneh, R.N., Feachem, R.G. (1987) Thew epidemiology of acute diarrhoea in a rural community in Imo State, Nigeria. Trans. Roy. Soc. Trop. Med. Hyg., 81:865-870.

INEGI (1990) Censo Nacional de Poblacion de Mexico.

INI (1992) Anuario Estadistico: Regiones de Habla Indigena en el estado de Hidalgo. Valle del Mezquital, 2:27-57.

IRCWD (1985b) Health Aspects of Wastewater and Excreta Use in Agriculture and Aquaculture: the Engelberg Report. Report of a Review Meeting of Environmental Specialists and Epidemiologists, Engelberg, Switzerland, July 1-4 International Reference Centre for Wastes Disposal., Dubendorf, Switzerland:

IRCWD (1988) Human wastes: Health aspects of their use in agriculture and aquaculture. IRCWD NEWS., 24/25:01-31.

Jefferson, B.A. and Betton, M.L. (1991). Gardiasis and occupational risk in sewage workers. The Lancet., 338(2):1152.

Jewell, W. & Seabrock (1979) A history of land application as treatment alternative. Technical Report 430/9-79-012, EPA; Office of water program operation. Washington, D.C.

Kabler, P.W. & Clark, H. (1960) Coliform group and fecal coliform organisms as indicators of pollution in drinking water. J. Am. Water Works Assoc., 52: 15177.

Katzenelson, E., Buium, I. and Shuval, H.I. (1976) Risk of Communicable Disease Infection Associated with Wastewater Irrigation in Agricultural Settlements. Science., 194:944-946.

Khalil, M. (1931) The pail closet as an efficient means of controlling human helminth infections as observed in Tara prison, Egypt. Ann. Trop. Med. Parasitol. 29:35-62.

Khan, M.U. (1982) Interuption of shigellosis by hand washing. Trans. Roy. Soc. Trop. Med. Hyg., 76(2):164-168.

Khan, M.E. and Manderson, L. (1992) Focus groups in tropical diseases research Health Policy Planning; 7 (1):56-66.

Kightlinger, L.K., Seed, J.R. and Kightlinger, M.B. (1995) The epidemiology of Ascaris lumbricoides Trichuris trichiura, and hookworm in children in the Ranomafana Rainforest, Madagascar. J. Parasitol., 81(2):159-169.

Killewo, J.Z.J., Cairncross, S., Smet, J.E.M., Maikwano L.F. and vanAsten, H. (1991) Patterns of hookworm and *Ascaris* infection in Dar es Salaam. Acta Tropica, 48:247-249

Krishnamoorthi, K.P., Abdulappa, M.K. and Aniwikar, A.K. (1973) Intestinal parasitic infections associated with sewage farm workers with special reference to helminths and protozoa. In: Proceedings of Sympsium on Environmental Pollution. Central Public Health Engineering Research Institute, Nagpur, India. Abstracted in: Wastewater Irrigation in Developing Countries: Health Effects and Technical Solutions (eds. Shuval et. al.) Worlk Bank Technical Paper Number 51 (1986). Washington, D.C.

Lara Aguilera, R. (1984) Las geohelmintiasis en México y perspectivas de control. Sal. Públ. Méx. 26:573-578.

Ling Bo, Den Ting-xin, Lu Zhi - Ping, Mi Lov-wei, Wang Zhu-xuen, Yuan An-xiu (1990) Health assessment of nightsoil and wastewater reuse in agriculture and aquaculture. Final report. Chinese Institute of Environmental Health and Engineering. Beijing.

Linnemann, C.C. Jaffa, R., Gartside, P.S., Scarpino, P.V., Clark, C.S. (1984) Risk of infection associated with wastewater irrigation spray system used for farming. J. Occup. Med. 26: 41-45.

Long, E.G., Tsin, A.T. and Robinson, B.A. (1985) Comparison of the Faecal concentration System with the Formalin-Ethyl Acetate Technique for Detection of Intestinal Parasites. J. Clin. Microbiol. 22(2):210-211.

Manderson, L. & Aaby, P. (1992) Can rapid anthropological measures be applied to tropical diseases? Health Policy and Planning; 7 (1): 46-55.

Mara, D., (1983) Sewage trewatment in hot climates., J. Wiley & Sons, New York.

Mara, D.D. & Cairncross, S. (1989) Guidelines for the Safe Use of Wastewater in Agriculture and Aquaculture : Measures for public health protection. United Nations Environment Program. WHO, Geneva.

Mara, D.D. & Silva, S.A. (1986) Removal of intestinal nematode eggs in tropical waste stabilisation ponds. Journal of Tropical Medicine and Hygiene. 89, 71-74.

Martinez Garcia, M.C., Munoz, O., Peniche, A., Ramirez Grande, M.A., Gutierrez, G.1989. Epidemiology of acute diarrhea in a rural medical care system in Mexico. Arch. Invest. Med. (Mex.), 20:69-78.

Mata, J.L., Kromal, R.A. and Urrutia, J.J. (1977) Effect of infection on food intake and the nutritional studies. Am. J. Clin. Nutr., 30:1215-1227.

Mejia Maravilla, E. & Sánchez, R.R. (1993) Uso de aguas residuales en la agricultura, México, Ingeniería Ambiental, 6(20):19-33.

Metcalf & Eddy Inc. (1979) Wastewater Engineering: Treatment, Disposal Reuse. Second Edition. McGraw Hill, New York.

Monreal, J.C. (1994) Evolución de la morbilidad entérica en Chile, luego de la aplicación de medidas de restricción de cultivos en zonas regadas con aguas servidas. Report.

Ongerth, H.J. and Jopling, W.F. (1977) Water reuse in California. In Water Renovation and reuse. Ed. Shuval, H. G. Academic Press, New York

PAHO (1991) Informal consultation on Intestinal Protozoan infections. PAHO/CDS/IPI.91.2

Pickering, H., Hayes, R.J., Tomkins, A.M., Carsen, D. and Dunn, D.T. (1987) Alternative measures of diarrhoeal morbidity and their association with social and environmental factors in urban children in The Gambia. Trans. Roy. Soc. Trop. Med. Hyg., 81:853-859.

Pierce, V., Ascoli, W. and Leon R. et al. (1962) Studies of diarrhoeal disease in Central America, III: Specific etiology on endemic diarrhoea in Guatemalan children. Am. J. Trop. Med. Hyg., 11:395-400.

Pinfold, J.V., Horan, N.J. and Mara, D. (1991) Seasonal effects on the reported incidence of acute diarrhoeal disease in Northeast Thailand. Int. J. Epidemiol., 30(3): 778-786.

Porter, J.D.H., Gaffney, C., Heymann, D. and Parkin, W. (1990) Foodborne outbreak of Giardia lamblia. Am. J. Publ. Hlth., 80:1259-1260.

PRONAR (1986) Inventario Geoestadistico. Proyectos de aprovechamiento de aguas residuales. Documento Interno, Comision Nacional de Agua, Mexico.

PRONAR (1990) Programa de seguimiento. Proyectos de reutilizacion de agua residual. Documento Interno, Comision Nacional de Agua, Mexico.

Rahman, M., Aziz, K.M.S., Huq, E., and Rahman, M.M. (1985) Impact of environmental sanitation and crowding on infant mortality in rural Bangladesh. The Lancet, July 6, 28-31.

Rivera Arguero, F., Acevedo-Cruz, N.C. and Alcocer-Durán, J. (1985) Capacidad de eliminación de helmintos parasitos de una laguna de estabilización en Santo Tomás Atzingo, Estado de México. Rev. Latin. Micro. 27:335-340.

Rivera Ramírez, L. (1980) Effects on Public Health of the Use of Wastewater in Irrigation District 03 of the Western Area of Tula, State of Hidalgo. Master´s thesis, School of Public Health, Mexico City, México.

Romero Alvarez, H. (1995) Wastewater reuse in Mexico; Case-study.

Rose, J.B. and Gerba, Ch.P. (1991) Use of risk assessment for development of microbial standards. Wat Sci. Tech., 24(2):29-34.

Ruiz Palacios, G. & Pickering, L. (1991) Antibodies in milk directed against specific entheropathogens. In: Human lactation: maternal environment. H. Amosh & A. Goldman (eds.).

Sáenz Forrero, R. (1994) Lagunas de estabilización en el trópico: promoción del reúso sanitario de las aguas. Bol. Of. Sanit. Panam. 116 (3), 189-195.

Salas, W. and Hernández, F. (1988) Alteración de la Economía Agrícola debido a la Contaminación de aguas para riego. Ciencias Sociales. 39:111-117.

Sánchez Leyva, R. (1976) Use of Wastewater for Irrigation in District 03 and 88 and its Impact on Human Health. Master´s thesis, School of Public Health, Mexico City, Mexico.

Sawaya, A.L. & Amigo, H. (1990) The risk approach in preschool children suffering malnutrition and intestinal parasitic infection in the City of Sao Paulo, Brazil. J. Trop. Ped., 6: 184-188.

SARH (1987). Estudio de la calidad de las aguas residuales crudas con fines agrícolas. Instituto Mexicana de Tecnologia del Agua.

SARH (1989) Informacion geoestadistica del valle del Mezquital. 165pp.

SARH (1990) Informacion geoestadistica de los Distritos de Riego 03 y 100, Valle del Mezquital, Mexico 143pp.

Schlessmann, JJ (1982), Case-control studies. Design, conduct, Analysis. Oxford Univ. Press. New York

Scrimshaw, S.C.M. and Hurtado, E. (1988). Anthropological involvement in the Central American Diarrhoeal Disease Control Project. Soc. Sci. and Med. 27:97-105.

SSA (1990) Normas de calidad de agua para distintos usos. Agua de riego agricola, SDireccion de Regulacion ambiental, Mexico.

Sehgal, R. & Mahagan, R.C. (1991) Occupational risks in sewage work. The Lancet 338:1404-1405.

Shuval, H.I. (1964) The uses and misuses of environmental standards. Am. J. Publ. Health., 54:1319-1324.

Shuval, H.I. (1988) Rationale for Engelberg Guidelines. IRCWD News 24/25:18-19.

Shuval, H.I. (1991) Health guidelines and standards for wastewater reuse in agriculture: historical perspectives. Wat. Sci. Tech., 23:2073-2081.

Shuval, H.I., Yekutiel, P. and Fattal, B. (1986a) An epidemiological model of the potential health risk associated with various pathogens in wastewater irrigation. Wat. Sci. Tech. 18:191-198.

Shuval, H.I., Adin, A., Fattal, B., Rawitz, E. and Yekutiel, P. (1986b) Wastewater Irrigation in developing Countries: Health Effects and Technological Solutions. Studies in water supply and sanitation., Washington, D.C. : The World Bank.

Shuval, H.I., Wax Y., Yekutiel P. and Fattal B. (1989). Transmission of enteric disease associated with wastewater irrigation: A prospective Epidemiological Study. Am. J. Publ. Health, 79(7):850-857.

Sole, T.D. & Croll, N. (1980) Intestinal parasites in man in Labrador,Canada. Am. J. Trop. Med. Hyg., 29:364-368.

Spencer, H. *et al.* (1976) Endemic amoebiasis in an Arkansas community. Am. J. Epidemiol., 104:93-99.

Sprunt, K. et al. (1973) Antibacterial effectiveness of routine handwashing. Pediatrics, 52:264-271.

Stanton B.S. & Clemens, J.D. (1987) An educational intervention for altering water sanitation behaviours to reduce childhood diarrhoea in urban Bangladesh. Am. J. Epidemiol. 125(2): 284-291.

Stoopen, M. & Beltran, F. (1964) Caracteristicas epidemiologicas de las helmintiasis en Yankuitlalpan, Pue., Medicina (Mex). 44:28-33.

Stott, R., Ayres, R., Lee, D. and Mara, D. (1994) An experimental evaluation of potential risks to human health from parasitic nematodes in treated wastewater used for crop irrigation. ODA Research Numographs in Tropical Public Health Engineering, No. 6.

Strauss, M. (1988) Examples of Wastewater and Excreta Use Practices in Agriculture and Aquaculture. IRCWD News 24/25:13.

Strauss, M. & Blumenthal, U. (1989) Human waste use in agriculture and aquaculture. Utilization practices and health persepctives. IRCWD report 08/89.

Tomkins, A. (1981) The significance of Intestinal parasites in malnourished populations. Parasitol., 82: 38-40.

VanDerSlice, J. & Briscoe, J. (1991) All coliforms are not created equal: comparison of the effects of water source and in-house water contamination on infantile diarrhoeal disease. Water Resources Research.

Velazquez, E. (1991) Perfil socioeconomico de productividad en los Distritos de Riego 03 y 100, Mexico. Documento Interno, SARH/CNA.

Vitora, C.G. et al. (1988) Water supply, sanitation and housing in relation to the risk of infant mortality from diarrhoea. Int. J. Epidemiol. 17(3):651-654.

WHO (1973) Reuse of effluents: Methods of wastewater treatment and health standards. Technical Report Series No. 517., WHO Geneva.

WHO (1984) Guidelines for a sample survey of diarrhoeal disease morbidity, mortality and treatment rates. WHO Diarrhoeal Control Pragramme document, WHO/CDD/SER: 84-86. WHO, Geneva.

WHO (1985) Diagnostic Techniques for Intestinal Infections Applicable to Primary health Care Services. PDP/85.2, WHO, Geneva.

WHO (1987) Prevencion y control de las infecciones parasitarias intestinales. Technical Report Series No. 749., WHO, Geneva.

WHO (1989) Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture. Technical Report Series No. 778. WHO, Geneva.

WHO (1991) Informal consultation on intestinal protozoan infections. WHO/CDS/IPI/92.2.

Yañez, F.R. & Rojas, M.L. (1980) Evaluation of the San Juan stabilization ponds. Final Research Report of the first phase. Pan American Center for Sanitary Engineering and Environmental Sciences, Lima, Peru.

Yeager, B.A. et al. (1991) Transmission factors and socioeconomic status as determinants of diarrhoeal incidence in Lima, Peru. J. Diar. Dis. Res., 9(3):186193.

Zeitlyn, S. and Islam, F. (1991) The use of soap and water in two Bangladeshi communities: implications for the transmission of diarrhoea. Rev. Inf. Dis., 13(Suppl. 4): 259-264.

APPENDIX 1 SURVEY QUESTIONNAIRE

REVISADO

CENSO DE SALUD Y SANEAMIENTO

INSTITUTO NACIONAL DE LA NUTRICION

CLASIFICACION

1. INDEX No. [ ][ ][ ][ ][ ][ ][ ]

2. FECHA [ ][ ][ ][ ][ ][ ]

3. ENCUESTADOR [ ][ ]

4. SUPERVISOR [ ]

5. CUADRANTE [ ][ ]

6. NOMBRE DEL INFORMANTE ______________________

Apellido paterno Apellido materno Nombre (s)

LISTADO DE LAS VIVIENDAS.

** Encierre la respuesta en un círculo**

INDEX

Vamos a platicar de la higiene en esta casa.

8. Qué tipo de agua para beber es la que tienen en esta casa?
Garrafón de vidrio
Agua entubada dentro de la vivienda
Agua entubada fuera de la vivienda
Manantial
Pozo con tapa
Agua de pipa (Deposito comun)
Cisterna pública
Hidrante público
Pozo sin tapa
Canal de riego
Jaguey
Otros (describa) ____________________

9. Cuanto tiempo le lleva hacer un viaje completo para traer una cubeta de agua para beber o cocinar?

1. Menos de un minuto
2. De 1 a 4 min.
3. De 5 a 15 min.
4. Más de 15 min.

10. Donde guardan el agua para beber?

1. En una cisterna
2. En ollas o cubetas tapadas
3. Garrafón de vidrio
4. En ollas o cubetas sin tapar
5. Otras (describir) ____________________

11. Que le hacen al agua antes de beberla?

1. Siempre se hierve
2. Sólo a veces se hierve
3. Nunca se hierve
4. Otros (especifique) ____________________

12. En esta casa, cúal es el lugar donde los adultos van al baño?

1. Suelo de patio
2. Letrina
3. Baño con taza
4. Fosa septica
5. Canal
6. Otros (describa) ____________________

13. En esta casa, cúal es el lugar donde los niños van al baño?

1. Suelo de patio
2. Letrina
3. Baño con taza
4. Fosa séptica
5. Canal
6. Otros (Describa)

14. Tienen agua para lavarse las manos despues de que van al baño?

1. Si, siempre
2. Solo a veces
3. Nunca
4. Otros (Describa) ____________

15. Existe un recipiente o lugar especial para tirar la basura dentro de la casa?

1. Si
1. No

16. De donde vienen las verduras que se comen en esta casa?

1. Camioneta ambulante
2. Recaudería
3. Mercado local
4. Merced
5. Mercado fuera de la localidad
6. Otros (Describa) ____________

17. En esta casa siembran verduras?

1. Si (pase a la pregunta 18)
2. No (Pase a la pregunta 21)

18. Que tipo de verduras siembran?

1. ____________
2. ____________
3. ____________

19. Con que agua la riegan?

1. Con agua de canal (pase a la 20)
2. Bombeo
3. Otros (Especifique) ____________

20. De que canal recogen el agua para regar la hortaliza familiar?

1. ____________
2. ____________

21. Cuanto tiempo se hace caminando al canal de riego más cercano?

1. Menos de 5 min.
2. Entre 5 y 20 min.
3. Entre 20 y 60 min.
4. Más de una hora

** Encierre la respuesta en un círculo**

INDEX

22. Sabe usted el nombre del canal?

____

23. Que clase de agua corre por ahí?

1. Agua de la presa
2. Agua del río
3. Agua negra
4. Agua mezclada
5. Agua Endho
6. Otros (Describa) ________________

24. Cuantas personas a parte del jefe de familia contribuyen al gasto de la casa?

1. Número de adultos _______
2. Número de niños _______

25. En la semana pasada, cuantos días comieron pollo en esta casa?

26. En la semana pasada, cuantos días comieron carne en esta casa?

27. En esta casa donde ustedes viven es?

1. Propia
2. Rentada
3. Prestada
4. Otros (Describa) ________________

28. De que material es el piso de los cuartos para dormir?

1. Tierra
2. Cemento
3. Mosaico
4. Otros (Describa) ________________

29. De las cosas que le voy a mencionar, digame cuales tienen esta casa?

1. Estufa de gas
2. Radio
3. Televisión
4. Todas las anteriores

30. De las cosas que le voy a mencionar, digame cuales tienen esta casa?

1. Animales de yunta
2. Tractor rentado
3. Tractor propio

31. Tiene parcelas de su propiedad?

1. Si
2. No

TRANSLATION OF QUESTIONNAIRE

Health and Sanitation Survey
National Institute of Nutrition

Reviewed Y N

Classif.__________

Date.__________

Household's Index No. ______________

SupervisID______________

Respondent'sName____

LIST OF DWELLERS

page 1

8.-Drinking water supply I__I

List of options, all coded

e.g. 1. Piped water 2. Spring 3. Public taps

9.-Time to get a bucket of water I__I

List ranges, all coded

e.g. 1. < 1 min 2. 1-4 mins 3. 5 - 15 min

10.-Storage of drinking water I__I

List options, all coded

e.g. 1. Covered recipients 2. Unprotected recipients

11.-Boiling practices (drinking water) I__I

List and coded

e.g. 1. Usualy 2. Sometimes 3. Never

12.-Defecation practices adults I__I

List options, all coded

e.g. 1. Around the yard 2. Latrine 3. Flush-toilet

13.-Defecation practices children I__I

List of sites, all coded

14.-Washing hands habits I__I

List coded

e.g. 1. Usualy 2. Sometimes 3. Never

15.-Rubbish disposal practices I__I

List of possibilities, all coded

e.g. 1. Dustbin 2. Yard

16.-Source of vegetables diest I__I

List of possibilities, all coded

e.g. 1. Market 2. Shop 3. Family plot

17.-Cultivation of vegetables I__I

Open, but codes used (1= Cereals 2= Fodder 3= Vegetables

e.g.1_______________ 2__________________ 3_____________

18.-Irrigation source I__I

1. Spring 2. River 3. Canal 4. Rain 5.Other____________

19.-Type of crops harvested I__I

Open, but codes used (1= Cereals 2= Fodder 3= Vegetables

1_______________ 2__________________ 3_____________

page 2

20.-Irrigation points I__I

List of possibilities, all coded

21.-Irrigation of backyard vegetables I__I

List of possibilities, all coded

22.-Name of the canal I__I

List coded

23.-Type of water I__I

List of possibilities, all coded

24.-Number of wage earners in household I__I

List coded

25.- Number of days in last week chicken eaten I__I

26.-(Diet) days eat Meat. I__I

27.-Is the dwelling of their own? I__I

1. Yes 2. No, Rented, etc.

28.-Building materials (floor) I__I

Option coded

29.-Commodities (tv, radio, tec) I__I

List all coded

30.-Farming commodities (oxen,etc) I__I

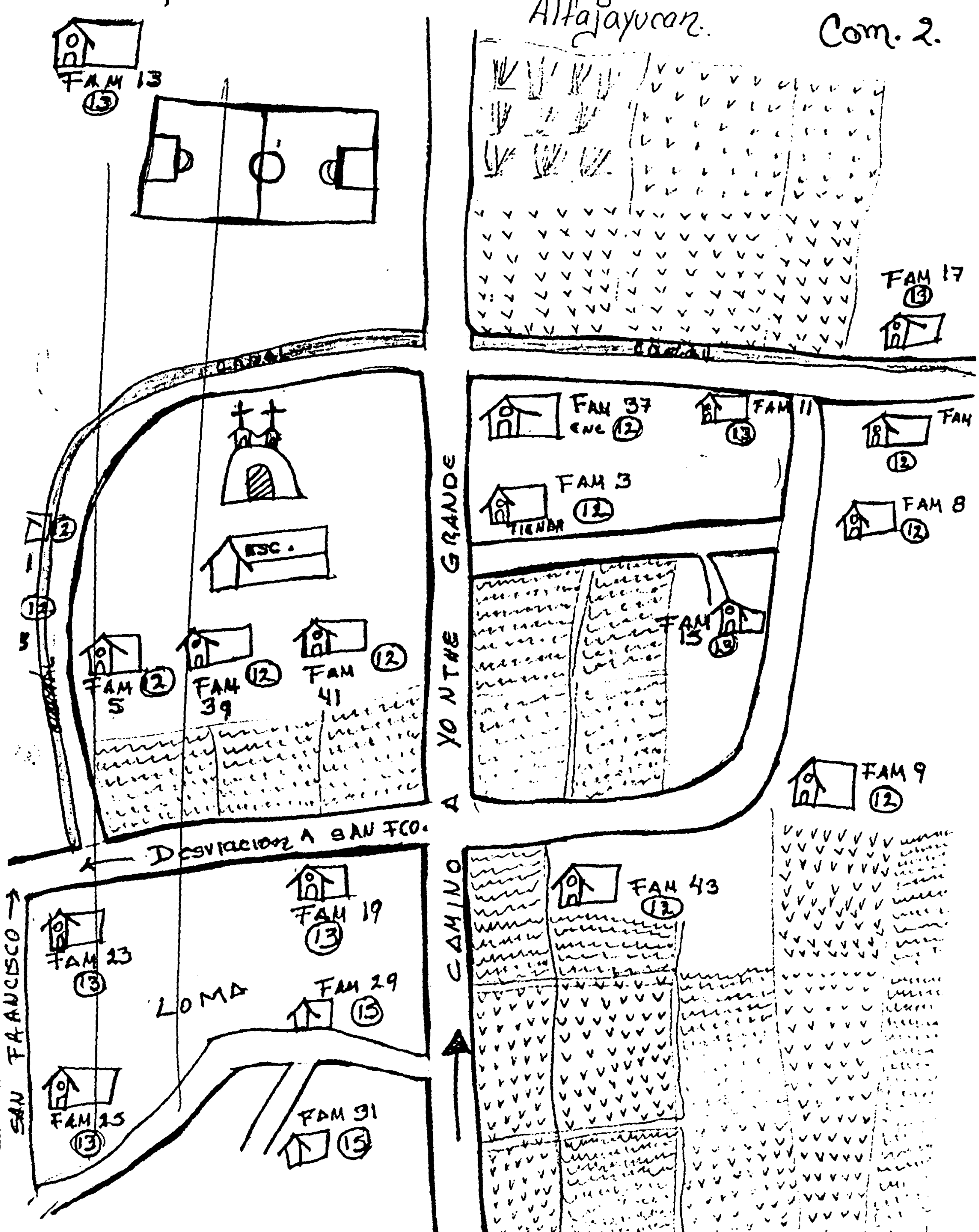
List, coded

31.-Agricultural plot owned. I__I

List, coded

APPENDIX 2 EXAMPLE OF VILLAGE MAP

YONTHE GRANDE



APPENDIX 3

ARTICLE SALUD PUBLICA DE MEXICO

Escenario epidemiológico del uso agrícola del agua residual: El Valle del Mezquital, México

Enrique Cifuentes, M.C., M. Med. Sci.,[1] Ursula Blumenthal, M. Sc., Ph. D.,[2]
Guillermo Ruiz-Palacios, M.C.,[3] Stephen Bennett, M. Sc., Ph. D.,[2] Anne Peasey M.Sc.[2]

**Cifuentes E, Blumenthal U,
Ruiz-Palacios G, Bennett S, Peasey A.
Escenario epidemiológico del uso agrícola del agua residual: El Valle del Mezquital, México.
Salud Publica Mex 1994;36:3-9.**

RESUMEN

En este estudio se presentan los resultados preliminares del impacto a la exposición ocupacional al riego con aguas residuales de los distritos de riego 03 y 100, del Valle del Mezquital. Esta región representa, de acuerdo al esquema de reuso de aguas residuales con aplicación agrícola, la más grande del mundo. La investigación tuvo como objetivo evaluar la prevalencia de enfermedades diarreicas e infecciones intestinales, mediante encuestas transversales realizadas en dos épocas diferentes del ciclo agrícola. Sólo se presentan los resultados de la primera, que se aplicó durante el periodo de lluvias, a 1 900 familias de agricultores; de éstas, 680 utilizan agua residual "cruda" para regar cultivos (alta exposición); 520 agua residual previamente almacenada en presas de retención (grupo de exposición intermedia) y el grupo control o de baja exposición lo integran 700 familias campesinas de las zonas temporaleras de la misma región. Los resultados de esta encuesta indican que el riesgo de infección por Ascaris lumbricoides *es más alto en el grupo de mayor exposición que en los otros dos grupos (IC 95%= 2.9-10.8). De acuerdo a los procedimientos utilizados, la infección por* Entamoeba histolytica *es más frecuente en los individuos menores de 15 años expuestos al agua residual sin tratamiento,*

**Cifuentes E, Blumenthal U,
Ruiz-Palacios G, Bennett S, Peasey A.
Epidemiological panorama for the agricultural use of wastewater: The Mezquital Valley, Mexico.
Salud Publica Mex 1994;36:3-9.**

ABSTRACT

Wastewater from Mexico City is used to irrigate over 85 000 hectares mainly of fodder and cereal crops in the Mezquital Valley. A cross-sectional study method is being used to test the impact of exposure to raw wastewater and wastewater from storage reservoirs on diarrheal disease and parasitic infections in farmworkers and their families. The study population in the rainy season survey included 1 900 households: 680 households where the farmworker is exposed to untreated wastewater (exposed group), 520 households exposed to reservoir water (semiexposed group), and 700 households where the farmworker practices rain-fed agriculture (control group). Preliminary analysis of the data from the rainy season study (dry season study in progress) has been carried out. Current information indicates that the risk of Ascaris lumbricoides *infection is much higher in the exposed group than in the control group (95% CL= 2.9-10.8). According to the procedures employed* Entamoeba histolytica *infection was more frequent among subjects aged 5 to 14 years from households exposed to raw wastewater, than among subjects of the same ages belonging to the control group (95% CL= 1.07-1.72). When diarrheal disease rates were analyzed, children under 5 years from exposed*

(1) Unidad de Proyectos Especiales, Centro de Investigación en Salud Pública, Instituto Nacional de Salud Pública, México.
(2) Departamento de Epidemiología y Ciencias de la Población, Escuela de Medicina Tropical e Higiene, Universidad de Londres.
(3) Profesor y Jefe del Departamento de Infectología, Instituto Nacional de la Nutrición "Salvador Zubirán", México.

Fecha de recibido: 2 de septiembre de 1992 **Fecha de aprobado:** 12 de marzo de 1993

El desarrollo industrial de las últimas décadas, el rápido crecimiento de las poblaciones en centros urbanos y las demandas en la producción de alimentos, crearon conflictos de enormes dimensiones entre las necesidades económicas y la salud pública. Mientras tanto, en muchos países el agua residual continuó empleándose en el riego agrícola, al margen de las regulaciones vigentes, pero en otros la práctica se abandonó casi por completo por considerar el estándar fuera del alcance financiero y técnico.

A mediados de los ochenta un grupo de científicos de diferentes disciplinas se reunieron en Engelberg, para evaluar los estándares vigentes y las implicaciones de los pocos estudios epidemiológicos disponibles en los que se indicaban cuáles eran los riesgos por usar el agua residual en la agricultura y la acuacultura. En la segunda mitad de esa década, diversos organismos internacionales, como el Banco Mundial y la Organización Mundial de la Salud (OMS), auspiciaron nuevas reuniones en las que se revisaron diversos aspectos microbiológicos, epidemiológicos, técnicos y sociales. Como resultado de estas reuniones, se propuso un modelo que describe los riesgos de la salud relacionados con el empleo del agua residual en la agricultura y la acuacultura. La poca información epidemiológica y el modelo citado, sugieren que el riego de cultivos con agua residual sin tratamiento se asocia con las infecciones por nemátodos intestinales, en consumidores de verduras y también en trabajadores agrícolas. Las infecciones bacterianas y virales constituyen, de acuerdo al mismo modelo y en orden descendente, riesgos adicionales. A finales de la década, la OMS resumió la información epidemiológica y microbiológica, y planteó que el riego de cultivos con agua residual tratada no aumenta los riesgos de infecciones por nemátodos, atribuibles al uso de agua residual.[4,7,8]

Los resultados de las reuniones de Engelberg y de la OMS proporcionaron las bases para sugerir nuevas medidas de protección para los trabajadores agrícolas y consumidores de productos regados con agua residual. Los estándares bacteriológicos se relajaron y se introdujo el criterio de monitoreo de huevecillos viables de helmintos, debido a que los nuevos parámetros se pueden alcanzar con relativa facilidad mediante el tratamiento del agua residual "cruda" en lagunas de estabilización. En estos sistemas el agua es sometida a procesos naturales en los que intervienen algas y bacterias. En sistemas bien diseñados, con intervalos de retención prolongados, se favorece la sedimentación de huevecillos, resultando en efluentes con $\leq$ 1 huevecillo de helminto por litro y con cuentas de coliformes fecales $\leq$ 1 000 por cada 100 ml, como lo recomienda ahora la OMS para el riego sin restricciones. Las lagunas de estabilización son alternativas técnica y económicamente factibles, ya que no requieren de equipo electromecánico ni de complicados manejos de operación y mantenimiento, además de que su capacidad de remoción de patógenos resulta más eficiente que los sistemas convencionales.

En virtud de que el uso del agua residual en la producción de alimentos es una realidad mundial, tanto el grupo de Engelberg como el que se reunió poco después bajo los auspicios del BancoMundial y la OMS, definieron áreas prioritarias de investigación aplicada. Entre estas últimas destacan las de carácter epidemiológico que contribuyan a evaluar los nuevos lineamientos. Otra área, de acuerdo a las recomendaciones, es la evaluación epidemiológica en escenarios agrícolas, en los que se aplican los nuevos lineamientos, así como la definición de los grupos de alto riesgo, como pueden ser los hijos de campesinos ocupacionalmente expuestos.

No obstante, estos escenarios son difíciles de encontrar, dado que se necesita que las poblaciones expuestas al riego con la calidad recomendada sean numerosas, y también otras poblaciones que, siendo similares en otros aspectos, no utilicen el agua residual para incluirlas como grupo de referencia. De localizar dichos escenarios, la investigación epidemiológica deberá complementarse con estudios de calidad microbiológica del agua residual.

MATERIAL Y MÉTODOS

El trabajo se realizó en el Valle del Mezquital, estado de Hidalgo. En este lugar se encuentran los distritos de riego más importantes del país, tanto por la superficie que abarcan como por el valor económico de su producción agrícola. De acuerdo a la información consultada, es el esquema de reuso de mayor tamaño en el mundo.[1-4]

La caracterización del área de estudio fue en términos del tipo de agua utilizada en la producción agrícola. De acuerdo con criterios básicos se definieron cuatro regiones:

1. Zona irrigada con agua residual "cruda" que llega directamente de la Ciudad de México.
2. Zona que utiliza agua residual almacenada por varias semanas en la presa Endho, cuyo efluente recibe aportaciones de agua "cruda" en su curso hacia las parcelas.

Al concluir las encuestas (noviembre 1991), los resultados de las pruebas de laboratorio fueron impresos y entregados a cada una de las familias, con una explicación verbal detallada; a los individuos con resultados positivos se les proporcionó gratuitamente medicamento antiparasitario.

Para el monitoreo de la calidad microbiológica del agua de riego, se recogieron muestras, con intervalos mensuales, en puntos previamente definidos de la red de canales de los distritos de riego 03 y 100. Los puntos de muestreo fueron definidos para dar una medida de la calidad del agua utilizada por los grupos de alta y mediana exposición y para medir los cambios de calidad después de su retención en las presas de almacenamiento. Los indicadores empleados fueron los coliformes fecales y huevecillos viables de helmintos.

RESULTADOS

La encuesta del periodo de lluvias incluyó a 9 433 individuos de 1 900 viviendas. Un total de 7 665 muestras de heces se recogieron y procesaron, lo cual representa una tasa de participación de más del 80 por ciento en los exámenes de laboratorio.

El cuadro I muestra que la prevalencia más elevada de infección por *A. lumbricoides* correspondió al grupo de mayor exposición en todas las edades; se observó también que las prevalencias disminuyen paralelamente con la exposición. En la categoría de los más pequeños, el grupo más expuesto tuvo una prevalencia más alta que los controles (RR= 5.6, IC 95%= 2.92-10.83), y las diferencias fueron aún mayores en los grupos de más edad (RR= 15 y 11, IC 95%= 8-30 y 5.2-24 respectivamente). Las prevalencias detectadas en el grupo de exposición intermedia no resultaron estadísticamente diferentes a las que se encontraron en el grupo de baja exposición.

Las prevalencias de infección por *G. lamblia* fueron similares entre los niños menores de 15 años de los tres grupos de exposición, pero entre la población adulta la prevalencia fue mayor en el grupo de exposición media (RR= 1.91 y IC 95%= 1.28- 2.85). Estos resultados se relacionan con las mayores carencias higiénico-sanitarias que caracterizan a muchas de las comunidades de esta zona.

Como se observa en el cuadro I, la prevalencia de infección por *E. histolytica* entre los niños de menor edad fue similar en todos los grupos de exposición. La prevalencia de infección por este protozoario fue mayor entre los niños de 5 a 14, afectando sobretodo a los grupos de mayor y mediana exposición (RR= 1.4, IC 95%= 1.07 - 1.72 y RR= 1.4, IC 95%=1.04-1.70). No se detectaron diferencias entre la población adulta.

El cuadro II muestra las prevalencias de enfermedad diarreica detectadas en la población. Las tasas más elevadas corresponden al grupo más expuesto, observándose que los niveles menores de exposición reducen dicha prevalencia. En los niños de 0 a 4 años, el grupo de alta exposición tuvo prevalencias significativamente mayores que los de menor exposición (RR= 1.3, IC 95%= 1.03-1.64); no se observaron diferencias importantes entre estos últimos y los de exposición media (RR= 1.1). De manera similar, en la categoría de 5 a 14 años, el grupo más expuesto tuvo prevalencias de enfermedad diarreica mayores que las detectadas en el grupo de menor exposición (RR= 1.7, IC 95%= 1.25-2.37); en éste

CUADRO I
Prevalencia de infecciones intestinales de acuerdo a la exposición y edad

	Grupos de exposición		
	Expuesto	Control	Semi-expuesto
Ascaris lumbricoides			
0 - 4 años	15.3 (59/396)	2.7 (10/368)	3.3 (11/335)
5 - 14 años	16.1 (132/817)	1.0 (9/862)	2.0 (15/733)
15 + años	5.3 (86/1614)	0.5 (7/1462)	1.2 (13/1088)
Giardia lamblia			
0 - 4 años	13.6 (47/345)	13.5 (60/443)	15.9 (66/416)
5 - 14 años	9.6 (21/219)	9.2 (106/1149)	10.8 (59/548)
15 + años	2.3 (17/733)	2.5 (49/1961)	4.8 (44/922)
Entamoeba histolytica			
0 - 4 años	7.0 (27/386)	7.3 (27/368)	5.4 (18/335)
5 - 14 años	16.4 (134/817)	12.0 (104/862)	16.1 (118/733)
15 + años	16.0 (257/1614)	13.8 (202/1462)	14.5 (158/1088)

REFERENCIAS

1. Strauss M. Examples of wastewater and excreta use practices in agriculture and aquaculture. IRCWD News 1988;13:24-25.
2. Strauss M, Blumenthal U. Health aspects of human waste use in agriculture and aquaculture. Utilization practices and health perspectives. Dubendorf, Switzerland: International Reference Centre for Waste Disposal, 1990.
3. Mara D, Cairncross S. Guidelines for the safeuse of wastewater and excreta in agriculture and aquaculture. Geneva:World Health Organization UNEP, 1989.
4. Shuval HI, Adin A, Fattal B, Rawitz E, Yekutiel P. Wastewater irrigation in developing countries: Health effects and technical solutions. Washington D.C.: The World Bank, 1986. Technical Paper No. 51, Integrated Resource Recovery Series, UNDP Project Management Report No. 6.
5. Bartone RC, Arlosoroff S, Shuval HI. Implementing water reuse. En: Proceedings Water Reuse Symposium IV AWWA. Denver: Research Foundation, 1988.
6. State wide standards for the safe direct use of reclaimed wastewater for irrigation and recreational impoundments. Berkeley, Ca.: California State Department of Public Health, 1968.
7. International reference center for wastes disposal IRCWD News 1985;23:11-18.
8. World Health Organization. Health guidelines for the use of wastewater in agriculture and aquaculture. Geneva: World Health Organization, 1989:74. Technical Report Series No. 778.
9. World Health Organization. Guidelines for a sample survey of diarrhoeal disease morbidity, mortality and treatment. Geneva: WHO Diarrhoeal diseases control programme document, 1984.