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PREVALENCE OF INTESTINAL PARASITES IN THE SOUTHERN PUNJAB, PAKISTAN

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SUMMARY

Introduction: Intestinal parasites are a major health problem for people living in developing countries. The high prevalence of these parasites is related to poverty, poor living conditions, poor hygiene and inadequate sanitation and water supply. The International Water Management Institute assesses the relationship between water and diseases in its 'health and environment' program. In Pakistan, studies were carried out in the South of the Punjab where access to water for domestic uses is limited, because the groundwater is brackish and rainfall very low. Therefore, people have to rely on irrigation canals as the main supplier of fresh household water. This study was carried out to investigate the prevalence of intestinal parasites in this environment and to identify risk factors that could be targeted with control activities.

Materials and methods: The study was undertaken in the irrigation command area of the Hakra 6R distributary, located in the Bahawalnagar District of the Southern Punjab. Of the 94 villages in this area, 10 were selected in 1998 for an epidemiological research project. Out of these 10 villages a random sample was made of 200 households. To study the intestinal parasites, stool samples of children below the age of 12 in the selected households were microscopically examined

Results: Out of the 430 children below 12 years of age in the study area, 373 children (87%) provided one or more stool samples. The prevalence of helminth infections was low (12.9%) compared to other developing countries, while the prevalence of protozoan infections was relatively high (54.4%). The prevalence of helminth infections was lower in children of families with a relatively high economic status and with educated parents. These families had significantly better sanitation facilities and had a better water supply to their houses. The prevalence of helminth infections was similar in all villages along the distributary. For protozoan infections the situation was different. The prevalence of these parasites decreased from villages at the head to the tail end of the distributary, while no relationship was found between the water supply and sanitation facilities and protozoan parasites at household level.

Conclusions: The prevalence of helminth infections was lower than expected in this area where quality and quantity of water for domestic use is a problem, education level is low and sanitation facilities are only available in 35 percent of the households. A reason for the low prevalence could be that the climatic conditions in this hot desert area are not suitable for helminth eggs to survive. Another reason could be the over-consumption of drugs in the area. Although self-medication with anthelminthic drugs could have played a role in the low prevalence, unregulated use of medicines is inefficient, leads to more side effects than necessary and could cause the development of resistance to different drugs. Contrary to the low prevalence of helminths, a high prevalence of protozoa (mainly *Giardia lamblia*) was found. This high prevalence is an indication of poor water quality, hygiene and sanitation. The prevalence of protozoa was the highest near the waterlogged head of the distributary. In this area more surface water was available for domestic use than in the downstream areas of the distributary. Cysts from *Giardia lamblia,* the most common protozoa, have the ability to survive very well in surface water and can therefore easily spread by this source, which is used for drinking without any treatment in all villages. Poor functioning of sanitation facilities due to high groundwater tables in the waterlogged areas are another problem that could have played a role in the high prevalence of protozoa near the head of the distributary. For both helminths and protozoa, improvements in the water supply for domestic use are important. To reduce the prevalence of protozoal infections the quality seems to be the most important factor while for the helminth infections the quantity of water available in the house is more important, as this ensures enough water for good hygiene practices. Since education of the mother and the availability of proper toilet facilities in the house also had a positive effect on helminth infections, improvements in the water supply have to be combined with health education and improvements in sanitation facilities.

1. INTRODUCTION

Intestinal parasites[1] are a major health problem for people living in developing countries, especially children, who are most severely affected. High worm loads and repeated infections with intestinal protozoa have influence on the nutritional status of children and can cause severe anemia and chronic diarrhea. This has negative impacts on growth, fitness and learning ability of children (Sakti et al. 1999; UNICEF 1997). Furthermore, annually about 150,000 children die due to intestinal obstruction and other abdominal complications caused by large adult worms. The high prevalence of infections with intestinal parasites in developing countries is related to poverty, poor living conditions, poor personal and environmental hygiene, inadequate health services and inadequate sanitation and water supply facilities (Montresor et al. 1998; Cook 1996).

The International Water Management Institute assesses the relationship of water with diseases in the 'health and environment' program. In Pakistan, studies were carried out in the canal-irrigated rural area Hakra 6R near the city of Haroonabad in the Punjab Province to investigate the prevalence of intestinal helminths and protozoa. In the study area the supply of water for domestic uses is problematic, because the groundwater is brackish and rainfall, very low (160mm/year). Therefore people have to rely on irrigation canals as the main supplier of fresh household water. Low quality and decreasing quantity of water supply for domestic use is a problem for the population (Van der Hoek, Konradsen and Jehangir 1999).

This report presents the results of this study. The introduction is followed by an overview of other studies on prevalence of intestinal helminths and protozoa in Pakistan. Chapter three describes the materials and methods used in the study, followed in chapter four by the results. Finally, in the last chapter a discussion and conclusions and recommendations are presented.

[1] See text block page 2 - 3

Intestinal parasites

Parasites are defined as organisms that obtain food and shelter from another organism, the host. Helminths and protozoa are natural parasites found in the intestine of man. In small numbers these parasites are harmless. However, in large numbers, they can cause morbidity and even mortality. Protozoa can cause chronic diarrhea and sometimes cysts in liver, lung and brain (for example *Entamoeba histolytica*). Large numbers of adult worms can obstruct the intestine or cause damage to the intestine wall. This damage can cause bleeding and poor absorption of nutrients. Especially in children this situation can cause malnutrition and chronic anemia, leading to growth problems (stunting), reduced fitness and reduced learning ability.

Most worms and protozoa do not duplicate in the human body. Their eggs need a stage outside the body, in the soil or an intermediate host, before larvae can develop. Therefore high worm loads will only be present in people living in a situation where helminths or protozoa are endemic and where re-infection occurs regularly. In countries with warm and humid climates the situation is ideal for the survival and development of the stages of parasites outside the human body. In developing countries, a combination of warm climate with poor socio-economic status and inadequate hygiene practices causes morbidity and mortality due to parasitic infections.

1. Helminths

The helminths can be divided in three groups, the nematodes or roundworms, the cestodes or tapeworms and the trematodes or flukes. In Pakistan only the first two groups are of importance. In the group of nematodes, about 12 species are natural parasites of human. The most important of these nematodes, the large roundworm *Ascaris lumbricoidus,* the whipworm *Trichuris trichiura* and the hookworms *Ancylostoma duodenale* and *Nector americanus* are soil transmitted. The eggs of these worms have a development stage in the soil before infecting man. Figure A shows the life cycle of these soil-transmitted nematodes. The nematode *Enterobius vermicularis* is also very common, but this worm contrary to the nematodes mentioned before, is not soil transmitted but spreads directly via the faeco-oral route from person to person.

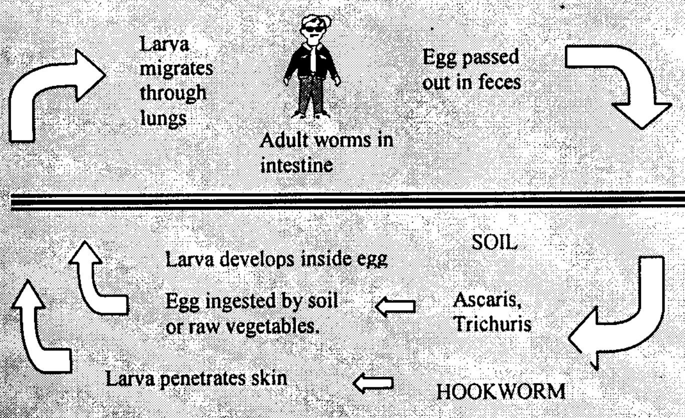

Figure A: Life cycle of soil transmitted helminths

Among the cestodes or tapeworms, only two species are important in Pakistan, the beef tapeworm *Taenia saginata* and the dwarf tapeworm *Hymenolepis nana*. *H. nana* is spread directly, while *T. saginata* needs cattle as the intermediate host. The life cycle of the last worm is shown in figure B.

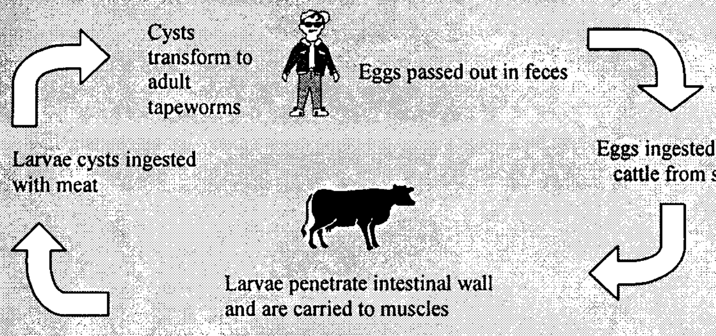

Figure B: Life cycle *Taenia saginata*

2. Protozoa

The protozoa relevant for this study and present in Pakistan are *Giardia lamblia* and *Entamoeba histolytica*. Both parasites are very common worldwide. Protozoa are spread from person to person with food items and drinks, which became contaminated with human faeces (figure C). For *G. lamblia* it is furthermore known that cysts can survive for a very long time in surface water. Infection can be caused by the use of this surface water for drinking without filtering, which is the only way to eliminate *G. lamblia* cysts.

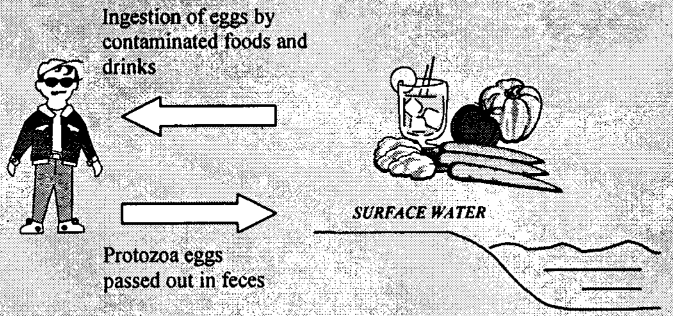

Figure C: Life cycle of protozoa

2. INTESTINAL PARASITES IN PAKISTAN

According to figures of WHO and UNICEF about 30 percent of the world population is infected with intestinal parasites. Most of these people live in the developing world. The most common parasite is the whipworm *T. trichiura.* However, the roundworm *A. lumbricoides* is associated with the highest annual morbidity (250 million) and mortality (60,000), closely followed by hookworms. Beside helminths, protozoa are very common in the developing world. Prevalence rates can reach up to 40 percent for *G. lamblia* and even up to 50 percent for *E. histolytica.* The protozoa *E. histolytica* is furthermore associated with mortality of about 40,000 – 100,000 people annually (Montresor et al. 1998; UNICEF 1997; Cook 1996).

Only a few studies have been carried out in Pakistan to investigate the prevalence of intestinal helminths and protozoa in the last 30 years (table 1). The studies took place in both rural and urban areas of Pakistan. The population under study varied from schoolchildren to hospital patients with gastric complaints or diarrhea and the results are therefore not comparable. The studies showed a prevalence of helminths varying between 10 and 35 percent, while the prevalence of protozoa varied between 7 and 28 percent. The studies carried out in the 1960's and 1970's showed same results as compared to studies carried out in the 1990's, indicating that there was no significant improvement during this period. In rural areas a slightly higher prevalence was found than in the urban areas. The roundworm *A. lumbricoides* was found to be the most prevalent helminth in Pakistan, followed by the dwarf tapeworm *H. nana.* Only in one study, carried out in 1968 with a special flotation technique, a much higher prevalence was found for *H. nana* infections. Infections with hookworms, *T. trichiura, E, vermicularis* and *T. saginata* were rarely seen. The most prevalent protozoa found in the most recent studies was *G. lamblia,* while in studies carried out in the 1960's and 1970's the protozoa *E. histolytica* was more common.

The prevalence of soil transmitted nematodes is low in Pakistan, compared with other developing countries. In recent published articles from developing countries in Africa, Asia and Latin America much higher prevalences were reported for the roundworm *A. lumbricoidus*, hookworm and *T. trichiura.* The prevalence of protozoa and the cestode *H. nana* found in studies in Pakistan are comparable with the prevalence found elsewhere in the world (Beach et al. 1999; Cook 1996, Miranda, Xavier and Menezes 1998; Sakti et al. 1999; Stoltzfus et all 1997).

Table 1: Studies on helminths and protozoa carried out in Pakistan between 1966 and 1999[2]

DETAILS OF THE STUDIES ON INTESTINAL PARASITES IN PAKISTAN						PREVALENCE OF INTESTINAL PARASITES[3]								
study place / author	*year of study*	*study population*	*Rural / urban*	*sample size*	*Techniques used*	*total % positive*	*protozoa*		*nematodes*				*cestodes*	
							E. Hist.	*G.. lamblia*	*A. lumbr*	*Hook worms*	*T. trich.*	*E verm.*	*H.nana*	*T. Sag.*
Villages (5) around Sheikhupura and Gujranwala *Haley JA, et al.*	1966/1967, published 1970	whole population house to house	Rural	1322 (from 1677)	direct smear of preserved samples	18.5% only protozoa	16.9%	14.3% (22.7% under 15 yr.)						
Doonga (vill. in Punjab) *Buscher HN, Haley JA*	1966-1968, published 1972	children 2-19 year	Rural	93 (from 116)	Flotation in brine solution	37-50% (only H.Nana)							37-50%	
Karachi *Bilqees FM, Khan A, Ahmed A*	1978-1979, published 1982	Pat. with gastric complaints from 3 hospitals	Urban	3249	direct smear of fresh samples	*30.4%* helminths 15.2% Protozoa 18.5%	17.8%	8.2%	3.6%	0.8%	0.7%	1.8%	0.7%	0.1%
Peshawar *Bano L,Begum Y*	Published 1981	Schoolchildren (5-16 years) 8 schools	Urban	1140	Preservative ?? Direct smear, centrif., flotation	*30.0%* helminths 12.4% Protozoa 19.0%	6.6%	10.3%	7.4%	0.2%	0.1%	0.1%	4.7%	
Abottabad and Haripur *Khan PS, Ullah R, Khan JA, Aziz F*	1987, published 1987	school children (8 schools) between 4-16 yr.	Urban	856 (703 F / 153 M)	??	*35.3%* helminths 35.0% protozoa 0.3%	0.2%	0.1%	28.0%	0.2%	2.8%	0.4%	5.7%	0.4%
Islamabad *Ahmed S, Maqbool S*	published 1988	schoolchildren of low income families	Urban	820 (out of 1000 stud.)	Direct smear of fresh samples	Giardia 18.3%		18.3%						
Lahore, Chaudry colony (slum) *Ahmad M*	published 1993	<5jr, select. criteria?? house to house??	Urban	276	Direct smear of fresh samples	69.5% only helminths			34.8%	5.4%	2.2%	6.5%	7.2%	11.2%
Kuri-Sher, village near Islamabad *Malik Z, Junejo F, Dil AS*	published 1993	all children below 6 years of the village	Rural	600	Fresh samples Method?	*53.1%* helminths 25% protozoa 8%	4.3%	23.7%	11.0%	5.3%		2.5%	7.0%	
Rawalpindi *Iqbal J, Munir MA, Khan MA*	1996., published 1999	children < 3jr with acute diarrhea + contr. hospital populat.	Urban	475 diarrhea	Direct smear of fresh samples	*54.3%* helminths 24.5% protozoa 19.5%	6.9%	12.6%	11.1%	2.5%			10.9%	
				150 control		*19.9%* helminths 10% protozoa 6.6%	2.0%	4.6%	4.0%	0.0%			6.0%	

[2] Details about methods used and study population were not always complete and this is marked in the table by a question mark. Information about the year of data collection was also not always given, in these cases only the year of publication is used in the table.

[3] Full names of the parasites are *Entamoeba histolytica, Giardia lamblia, Ascaris lumbricoides , Trichuris trichiura, Enterobius vermicularis, Hymenolepis nana and Taenia saginata.* The hookworms include: *Ancylostoma duodenale and Necator americanus.*

3. MATERIALS AND METHODS

3.1 Study Area and Population

The study took place in the irrigation command area of the Hakra 6R canal, located in Bahawalnagar District of the Southern Punjab, Pakistan (map 1). The irrigation system in this area is part of the largest irrigation system in the world, using water from the Indus River and its tributaries. The length of the 6R canal is 45 km. and the size of the command area is about 50,000 ha. The environment changes along the canal from severely waterlogged areas in the head-end to desert areas in the tail end.

In the area, 94 villages are registered, with an estimated population of 160,000 people. The majority of these people are occupied in agriculture and almost 100 percent of the families depend on agricultural incomes. About half of the families own land, while others are tenant farmers and casual laborers in the agricultural sector. The average landholding per household is 5 acres. The religion of the majority of people in the area is Sunni Muslim. The education level of the population is low, only 53 percent of the adult men and 23 percent of the adult women, of the families selected for this study, had received formal education. The educational level is however comparable with the situation in the whole country, as the adult literacy rate[4] is 54 percent for Pakistani man and 24 percent for Pakistani women (UNICEF 1999). Most households in the study area are large. The average number of people in a household registered for this study was 7.1. However, the actual number of people per household was even higher, since in a large number of households only the core family was selected for the study. The main reason for the large household size is the high total fertility rate[5], combined with the fact that most families live in an extended family system.

Most fresh water in the study area originates from the irrigation canals. Canal water is issued to village water tanks on a regular basis and this water is used for drinking either directly or after passing a simple filter. Other households use the water that has seeped out of the canals and that can be pumped out of the ground with a motor or hand pump. The majority (79%) of the families has a connection to their house from one of these sources, but other families (21%) have to carry the water to their homes from the village tank or hand pump. Long walking distances and unreliable water supply shows that most of the villagers use less water than recommended for good hygiene (Gleick 1998). Besides, problems in the water supply, there are also inadequate sanitation and wastewater disposal facilities. Only 34 percent of the villagers have access to toilet facilities. There is no sewerage system in the villages. Wastewater is collected in the streets or is dumped in the fields or canals.

Of the 94 villages in the Hakra 6R area, 10 were selected in 1998 for an epidemiological research project. These 10 villages were evenly distributed over the area (map 1). Villages were selected at the tail, middle and head of the Hakra 6R irrigation scheme. Out of these 10 villages a random sample was made of 200 households, proportionally distributed over the 10 villages (table 2). For households with more than 10 family members, only one core family was selected. Before the beginning of the epidemiological study in May 1998 all individuals were given identification numbers and basic demographic data of the households was collected. To study the intestinal helminths and protozoa in this area, the population of children below the age of 12 of the selected households was surveyed. The same identification numbers as in the epidemiological research were used.

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[4] Adult literacy rate: Percentage of persons aged 15 and over who can read and write.

[5] Total fertility rate: The number of children that would be born per woman if she were to live to the end of her childbearing years and bear children at each age in accordance with prevailing age-specific fertility rates. For Pakistan the fertility rate is 5.0, UNICEF 1999

Map 1: Study area with selected villages

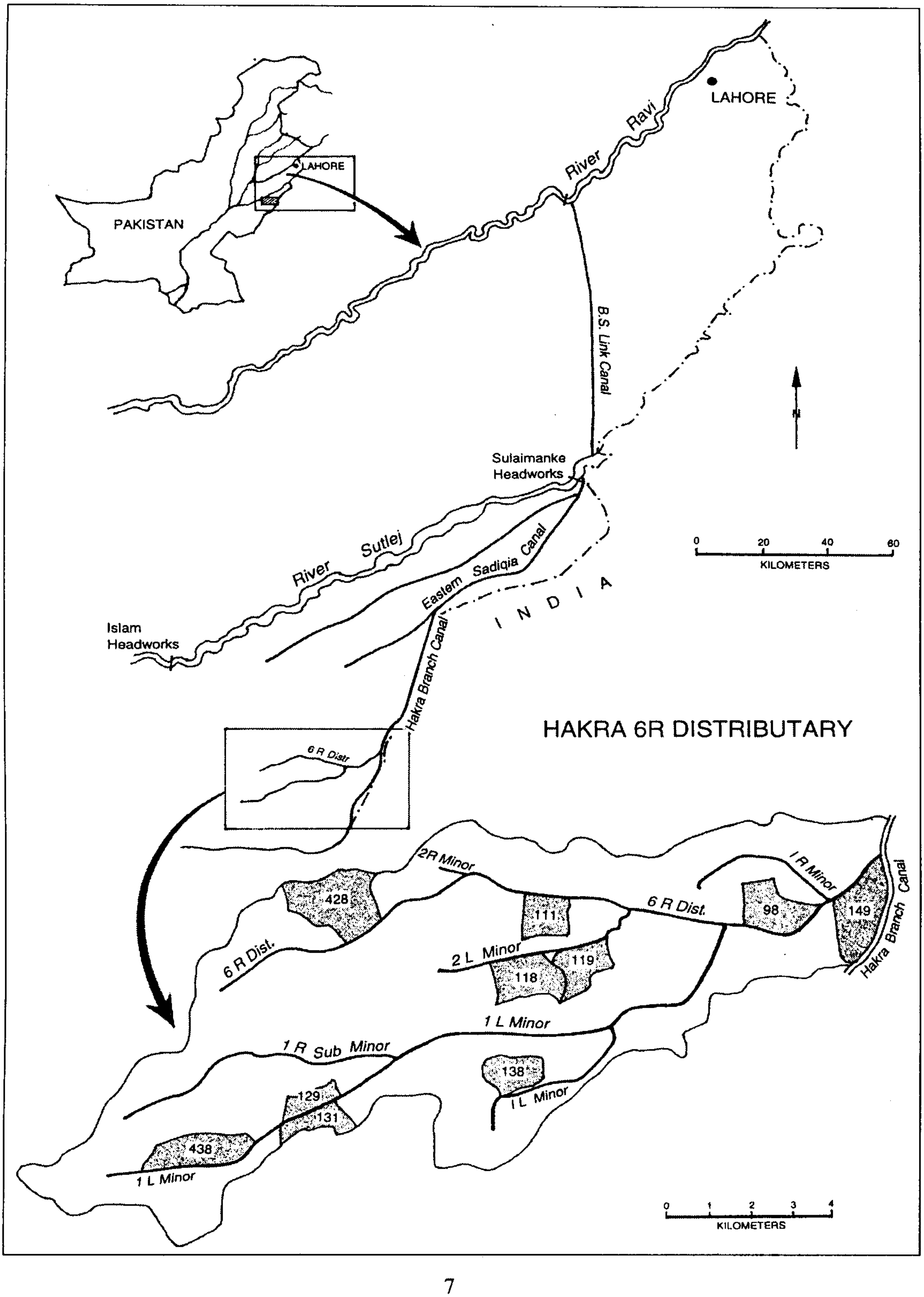


Table 2: Selected villages

Village name	Location in the system	Estimated Population	No. of sample households
98 –6R	Head	2030	29
111-6R	Middle	1534	29
118-6R	Middle	2100	16
119-6R	Middle	1619	22
129-6R	Tail	2350	27
131-6R	Tail	1388	9
138-6R	Middle	1500	15
149-6R	Head	900	11
428-6R	Tail	2700	33
438-6R	Tail	966	9
Total		17087	200

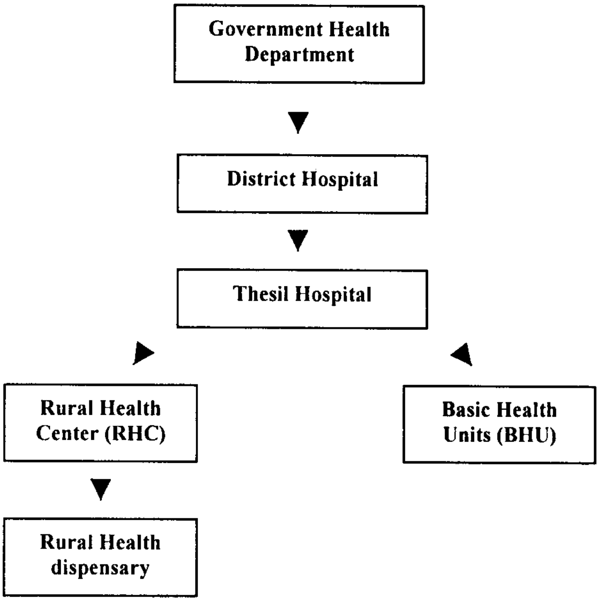
3.2 Health Facilities in the Study Area

The Government health facilities are organized per district (figure 1). The study area was situated in district Bahawalnagar, which is divided into four different thesils (administrative areas). The study area was part of thesil Haroonabad. The district hospital of the area was situated in Bahawalnagar city, while a thesil hospital was located in the city of Haroonabad. The thesil hospital had a capacity of 60 beds and 12 trained doctors, of whom 5 were consultants. The hospital had an operating theater, laboratory and X-ray facility. Within the study area, one Rural Health Center was present, located in the small town Faqirwali. This center had a capacity of about 10 beds and 3 trained doctors (2 male and 1 female) and a Lady Health Visitor. The Lady Health Visitor had a 2 year training to treat and advice women (mainly on family planning) and to perform antenatal tests and deliveries. The RHC had basic laboratory facilities, X-ray and a small operating theater used for minor surgery and for applying plasters.

There were 21 Basic Health Units in the Haroonabad thesil of which 8 had a trained doctor. Ten of these BHU's were located in the study area, but of these only two had a trained doctor. In all BHU's a dispenser was present and more than half of the BHU's had a Lady Health Visitor. In addition there were 4 dispensaries and a lot of medical shops that supplied the people in the area with medicines. These shops and dispensaries had a wide range of different drugs in stock and all these medicines were easy accessible to people in all villages, as medicines were relatively cheap and could be bought without a prescription from a medical practitioner. Since the start of the "Prime Minister Program for Family Planning and Primary Health Care" in this area in 1998, Lady Health Workers (LHW) were trained. They were living in the village or very near to the village and used a house in the village for their practice. They received three months training and one year for supervision to give health education (mainly on family planning) and to treat simple diseases in the village. They were provided with some medicines that are dispensed free of charge. As the first group of LHW"s in this area started work in 1998, only half of the villages in this area had a LHW at the time this study was conducted.

In each village there were also other than non-governmental health care providers, such as midwifes (with or without training), homeopathic healers, spiritual healers and herb doctors (Hakeem). In one of the villages a schoolteacher started a clinic. In the city of Haroonabad there were several private clinics.

Figure 1: Organization of the government healthcare facilities and referral levels



3.3 Collection of Stool Samples

Intestinal parasites were studied in stool samples of all selected children. There were three rounds of stool sample collection. Survey 1 took place in December 1998, survey 2 in May 1999 and survey 3 in August 1999. IWMI field-staff received one-day training before the start of the first survey and half-day refresher training before the second and third survey.

In a period of 8 days, the field-staff visited the selected households. They informed the families about the impact of helminth infections and about the survey. If they agreed to give stool samples of their children, plastic containers with a wooden spoon were provided for all children below the age of 12 in the household. An instruction was given to the mother on how to put some stool in the container with a wooden spoon. The plastic containers were marked with the identification-number of the child and the first letter or full name in Urdu-script of the child. For marking of the containers permanent ink was used. The next day the family was visited again to collect the samples. Every child, above the age of 6 months, who gave a stool-sample, was given the anti-helmintic drug albendazole. In table 3 the dosages used according to age are given. The team members were provided with suspensions and tablets albendazole of the brand Zentel to give according to the age and the capacity of the child. To measure the right amount of suspension a measure spoon was used. The children took the medicine under supervision of a team member.

Table 3: Albendazole (Zentel) doses

Age	Dose of Zentel
Below age of 6 months	no treatment
6 months – 2 year	200 mg
Above 2 year	400 mg

3.4 Preservation of the Samples

After collection in the villages, the samples were transported to the IWMI office in Haroonabad, where a technician of the Institute of Public Health preserved the samples. For the preservation a solution was used, containing formalin 10 percent, glycerin 20 percent and distilled water 70 percent. To each plastic bottle an amount of preservative between 10-20ml was added, according to the amount of stool. The technician made an estimation of the amount of stool and added about one milliliter preservative for each gram of stool. The samples were stored in a cool room. However, the temperature of the room reached about 30-35 degrees Celsius in the hot summer months.

3.5 Microscopic Examination

The preserved stool samples were transported to the Institute of Public Health in Lahore for microscopic examination. The direct smear technique was used (Cheesbrough 1992). A drop of saline was placed with a pipette on the left half of a microscopic slide and a drop of Lugol's iodine on the right half of the slide. With a stick a small portion of preserved faecal material was mixed with the drop of saline. The same stick was used to mix a second small portion with the iodine drop. A cover glass was placed over each drop. The smear contained the right amount of material when printed letters could just be read through it. The slide and the container with the stool sample were numbered with permanent ink. The slide was examined under the microscope with the 10x objective to scan the whole slide systematically. After scanning, the 40x objective was used to identify the parasites and to count the number of parasites per preparation. For reporting the counting of parasites the guidelines in table 4 were used. Before a slide was reported as negative, always 100 fields with the 40x objective had to be examined. The slides were seen by two senior microscopists. One of the authors (RH) checked and counted all slides.

Table 4: Counting of parasites (Cheesbrough 1992)

Classification	Number of parasites per preparation
Scanty	1 - 3
Few	4 -10
Moderate	11-20
Many	21-40
Very many	over 40

3.6 Treatment of Protozoal Infections

After the second survey in June 1999, all children who were positive for *G. lamblia* or *E. histolytica* received treatment with anti-protozoal drugs according to table 5. The drugs were given by the field-staff. The staff members watched the children take the medicines to make sure that the right child took the medicines.

Table 5: Doses of tinidazol/secnidazole for G. Lamblia and E. Histolytica infections

Age	Medicine
2 years and younger	½ secnidazole suspension 250mg
2, 3, 4 and 5 years	secnidazole suspension 500mg
6, 7 and 8 years	2 fasigyn tablets (1000mg tinidazole)
9, 10 and 11 years	3 fasigyn tablets (1500mg tinidazole)
12 years and older	4 fasigyn tablets (2000mg tinidazole)

3.7 Data Analysis

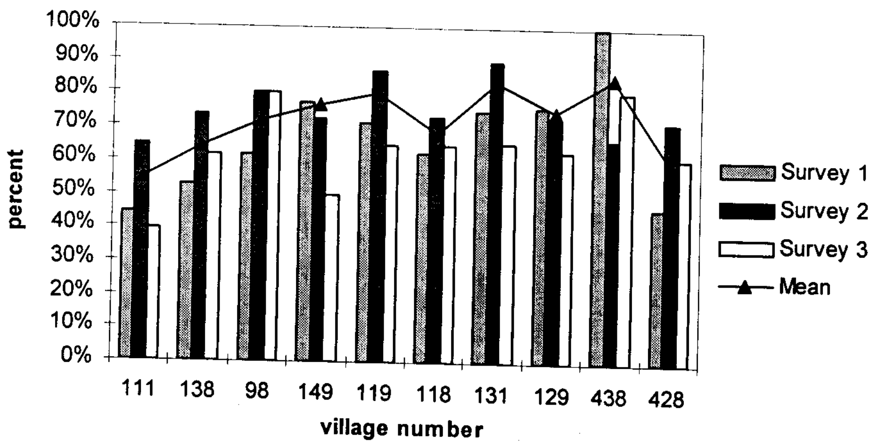
Data was analyzed using Microsoft Excel and Access and the statistical program SPSS and EPI info.

4. RESULTS

4.1 Characteristics of the Study Population

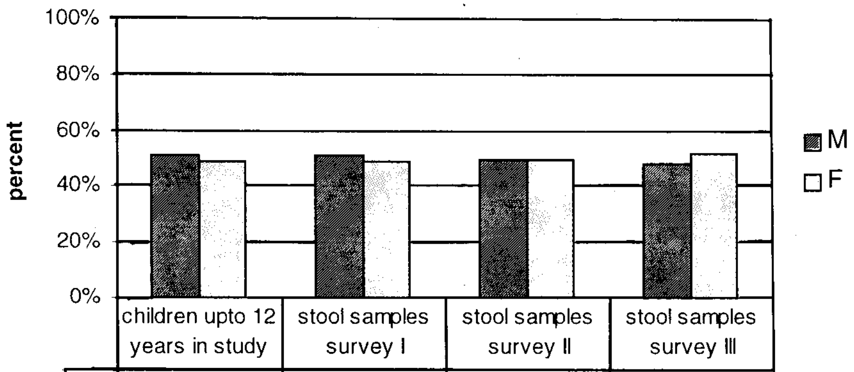
In the 200 selected households in Hakra 6R, 430 children below the age of 12 years were registered, 51 percent boys and 49 percent girls. During the first survey in December 1998, stool samples were collected from 265 children (61.4%). In the second and third round (May and August 1999), 316 (73.4%) and 269 (62.5%) samples were collected. Out of the 430 children below 12 years of age in the study area 373 children (87%) provided one or more stool samples. Of these children 180 (42%) gave a sample in every round. In figure 2 the coverage for each of the 10 villages is shown. The coverage in the different villages ranged between 55 and 80 percent.

Figure 2: Coverage per village



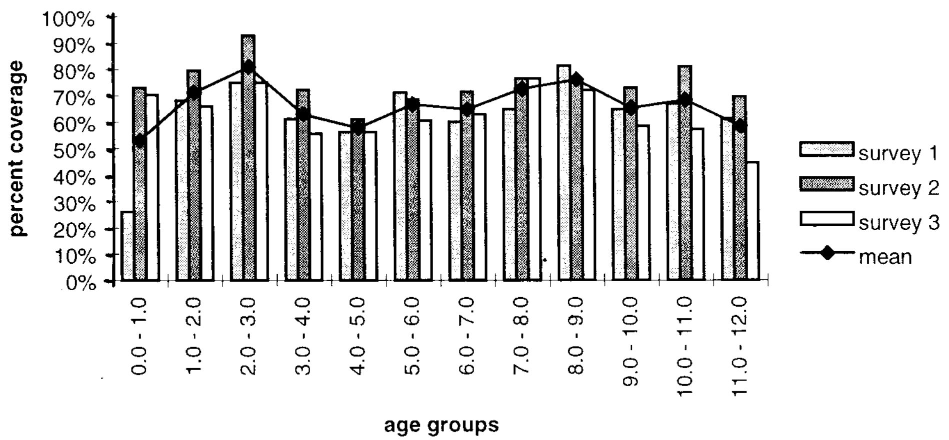
There was an equal distribution of boys and girls in the studied population (figure 3) and the children were also equally distributed among different age groups (figure 4). Only in the age group 0-1 year less stool samples were collected during the first survey. There were some very young children during the first survey, of which the mother refused to give a stool sample. During the second survey these children were some months older and the percentage of children who gave a sample in this age group became the same as in the other age groups.

Figure 3: **Male / Female distribution among the study population**



	children upto 12 years in study	stool samples survey I	stool samples survey II	stool samples survey III
M	0.51	0.51	0.5	0.48
F	0.49	0.49	0.5	0.52

Figure 4: **Coverage per age group**



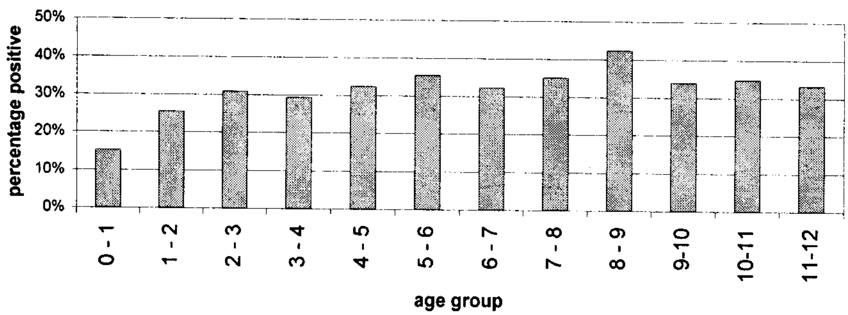
4.2 Prevalence of Intestinal Protozoa and Helminths in Hakra 6R

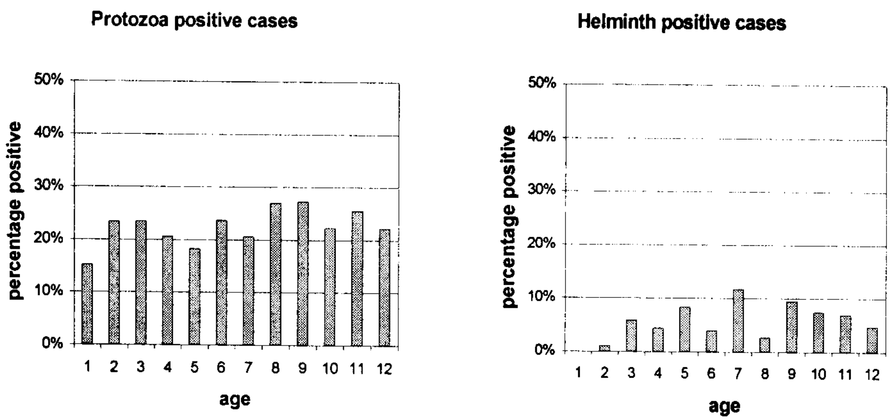
The number of positive stool samples were 132 (49.8%), 121 (38.3%) and 55 (20.4%) for the three surveys (figure 5). When all results are counted together 60 percent of the children (224 out of the 373 children who gave at least one sample) had one or more positive result. In table 6 the prevalence of intestinal protozoa in the different surveys are presented. Beside the total positive samples, the prevalence rate of the two different groups, helminths (nematodes and cestodes) and protozoa are presented. For the protozoa only the pathogenic protozoa *G. lamblia* and *E. histolytica* are counted.

Table 7: Prevalence of helminths and protozoa in the Hakra 6R area

Parasite	Prevalence
Protozoa (without Entamoeba coli)	54.4 %
Entamoeba histolytica	8.0 %
Giardia Lamblia	51.2 %
Entamoeba coli	30.8 %
Helminths	12.9 %
Hymenolepis nana (cestode)	10.5 %
Hookworm (nematode)	1.9 %
Ascaris Lumbricoides (nematode)	1.3 %
Trichuris trichiura (nematode)	0.5 %
Enterobius vermicularis (nematode)	0.3 %

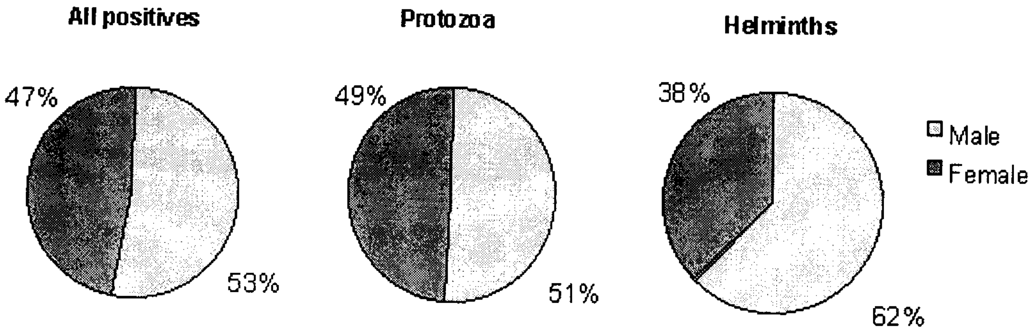
Figure 6: Age distribution of positive cases





While there were only minimal differences in prevalence between the different age groups, there was a larger difference between the sexes. There were more males then females among the positive cases (53% : 47%). The pattern was the same for the helminths and protozoa positive results (figure 7). Among the helminth positive cases the difference between male and female was the most obvious (62% : 38%), but this difference was not statistically significant (Chi-square test, P =0.102).

Figure 7: Male / female distribution of positive cases



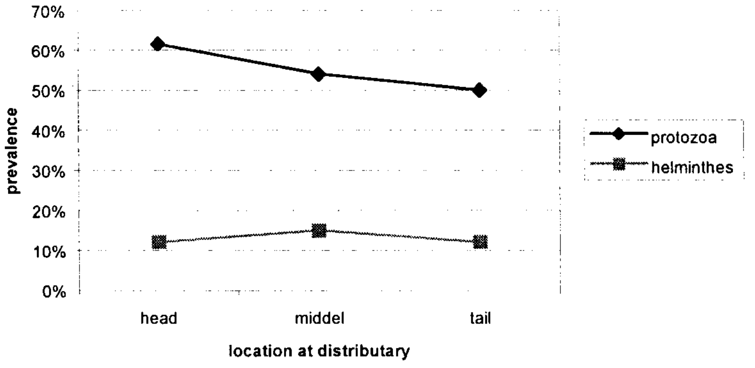
4.3 The Influence of Water and Sanitation on Parasitic Infections

There were differences between the villages in the amount of water that they received through the irrigation system. Villages at the head of the distributary received more water than villages located at the tail end of the Hakra 6R distributary. Therefore villagers at the head of the distributary had easier access to surface water then villagers living at the tail end of the distributary. Between different areas along the distributary there was no significant difference in the prevalence of helminth infections. The prevalence of protozoan infections however decreased from the head to the tail end of the distributary (table 8, figure 8).

Table 8: Analysis of the prevalence of helminths and protozoa by location along the distributary

Place along distributary	Number of children	Prevalence	RR	95% CI	Chi-square	p-value
HELMINTHS						
Head	91	12.09	1.00			
Middle	136	14.71	1.22	0.61 – 2.42	0.32	0.573
Tail	146	11.64	0.96	0.47 – 1.96	0.01	0.918
PROTOZOA						
Head	91	61.54	1.00			
Middle	136	54.41	0.88	0.65 – 1.02	1.13	0.287
Tail	146	50.00	0.81	0.71 – 1.11	3.01	0.083

Figure 8: Prevalence of helminths and protozoa by location along the distributary



At household level there were also differences in access to water. In houses with a pipe connection to the house from a water source, more water was available than in houses without this facility. In these cases the women had to fetch water from the central water tank or hand pump. Furthermore, some houses had a tank for storage of water. In times of water scarcity these houses could rely on their own resources. When analyzing the influence of water availability at household level on helminths and protozoan infections, it was found that the prevalence of helminth infections was much lower in children who lived in houses where a tank was available for the storage of water. However, the availability of a water connection to the house had no influence on the prevalence of helminth infections. Better access to water at household level had no positive effect on protozoan infections. Children living in houses with a storage facility even had a higher prevalence of protozoan infections than the ones without such a facility (Table 9). The availability of toilet facilities in the house were related to water connection and water storage facilities, as the available toilets were generally flush or pour flush toilets with a septic tank. Some houses lacking a flush toilet had a 'female' toilet, which is a designated place in the small room with a simple toilet for female members of the family, which directly drains into the street. However, in the analysis a distinction was made between families having a flush or pour flush toilet and those not having such a facility, which in most cases indicates no facility at all. The availability of a toilet had a positive effect on the prevalence of helminths, while an effect on the prevalence of protozoan infections could not be demonstrated (table 9).

Table 9: Analysis of the influence of water and sanitation on helminths and protozoa

	Number of children	Prevalence	RR	95% CI	Chi-square	P-value
HELMINTHS						
Water availability at household level						
Connected + storage	112	8.04	1.00			
Connected, no storage	182	15.38	1.91	0.94 – 3.91	3.40	0.065
Not connected	79	13.92	1.73	0.75 – 3.98	1.71	0.191
Sanitation facilities						
Toilet	129	9.30	1.00			
No toilet	224	14.75	1.59	0.86 – 2.94	2.24	0.135
PROTOZOA						
Water availability at household level						
Connected + storage	112	59.82	1.00			
Connected, no storage	182	50.55	0.85	0.69 – 1.04	2.40	0.121
Not connected	79	55.70	0.93	0.73 – 1.19	0.32	0.569
Sanitation facilities						
Toilet	129	53.49	1.00			
No toilet	224	54.92	1.06	0.84 – 1.25	0.07	0.792

4.4 The influence of Socio-economic Status on Parasitic Infections

To estimate the socio-economic status a score (1-13) was given to every family based on income, land under cultivation, possession of animals and quality of the house. To estimate the influence of socio-economic status on the prevalence of parasitic infections, 122 poor families who scored 3 or less points were compared with 107 better off families who scored 6 or more points. A much lower prevalence of helminth infections was found in the group with a higher economic status. For protozoan infections however, no difference was observed (table 10).

A higher score for socio-economic status was strongly related with the availability of a water connection and storage facilities in the house and it was also related with the availability of toilet facilities ($p<0.001$ and $p<0.001$, chi-square test). The socio-economic score also had a relationship with the educational level. The mean years of education of both father and mother were significantly higher for children in a household with a high (6 or above) socio-economic score ($p< 0.001$ and $p=0.01$, chi-square test).

Table 10: Analysis of the influence of socio-economic status on the prevalence of helminths and protozoa

Socio-economic status	Number of children	Prevalence	RR	95% CI	Chi-square	P-value
HELMINTHS						
High > = 6	107	10.28	1.00			
Low < = 3	122	18.85	1.83	0.94 – 3.58	3.31	0.069
PROTOZOA						
High >= 6	107	56.07	1.00			
Low < = 3	122	54.92	0.98	0.78 – 1.24	0.03	0.861

4.5 The Influence of Education on Parasitic Infections

The literacy level was very low in the study area. From the children included in this research only 53 percent of the fathers and 23 percent of the mothers had received some education. Education of the mother had a statistically significant effect on the prevalence of helminth infections. Education of the father had a weaker effect. In contrast, education of mother and father did not seem to be important for the prevalence of protozoan infections (table 11).

Table 11: Analysis of the influence of education on the prevalence of helminths and protozoa

Education	Number of children	Prevalence	RR	95% CI	Chi-square	P-value
HELMINTHS						
Mother educated	90	6.67	1.00			
Mother not educated	283	14.84	2.23	0.98 – 5.06	4.07	0.044
Father educated	198	11.12	1.00			
Father not educated	175	14.86	1.34	0.79 – 2.27	1.16	0.281
PROTOZOA						
Mother educated	90	57.78	1.00			
Mother not educated	283	53.36	0.92	0.75 – 1.14	0.54	0.463
Father educated	198	53.54	1.00			
Father not educated	175	55.43	1.04	0.86 – 1.25	0.13	0.714

4.6 Re-infection with Helminths after Treatment

All children were treated with the anti-helmintic drug albendazole after each round of stool sample collection. The cure rate of albendazole has been reported to be almost 100 percent for all nematode helminths while the cure rate for the cestode *H. nana* is fair (65%) (Jagota 1986; Pamba et al. 1989). Assuming a 100 percent cure, re-infection rates were calculated for 180 children who gave stool samples in all 3 surveys.

Re-infection did not occur among children positive for *A. lumbricoides, T. trichiura* and *E. vermicularis.* All children positive in the first survey were negative in the second and third survey. There was only one case of re-infection among the six children that were positive for hookworm

(17%) in the second survey, while re-infection was not seen for this helminth in the third survey. However, among children positive for the cestode *H. nana* in the first survey, 64 percent were found infected in the second survey. In the third survey 35 percent of the children positive for *H. nana* in the second survey were found infected again. Although the cure rate of albendazole for *H. Nana* is less than for the nematode helminths, this could not explain the large percentage of children infected again in the second and third round after treatment. Re-infection must have played an important role.

4.7 Re-infection of G. Lamblia and E. Histolytica after Treatment

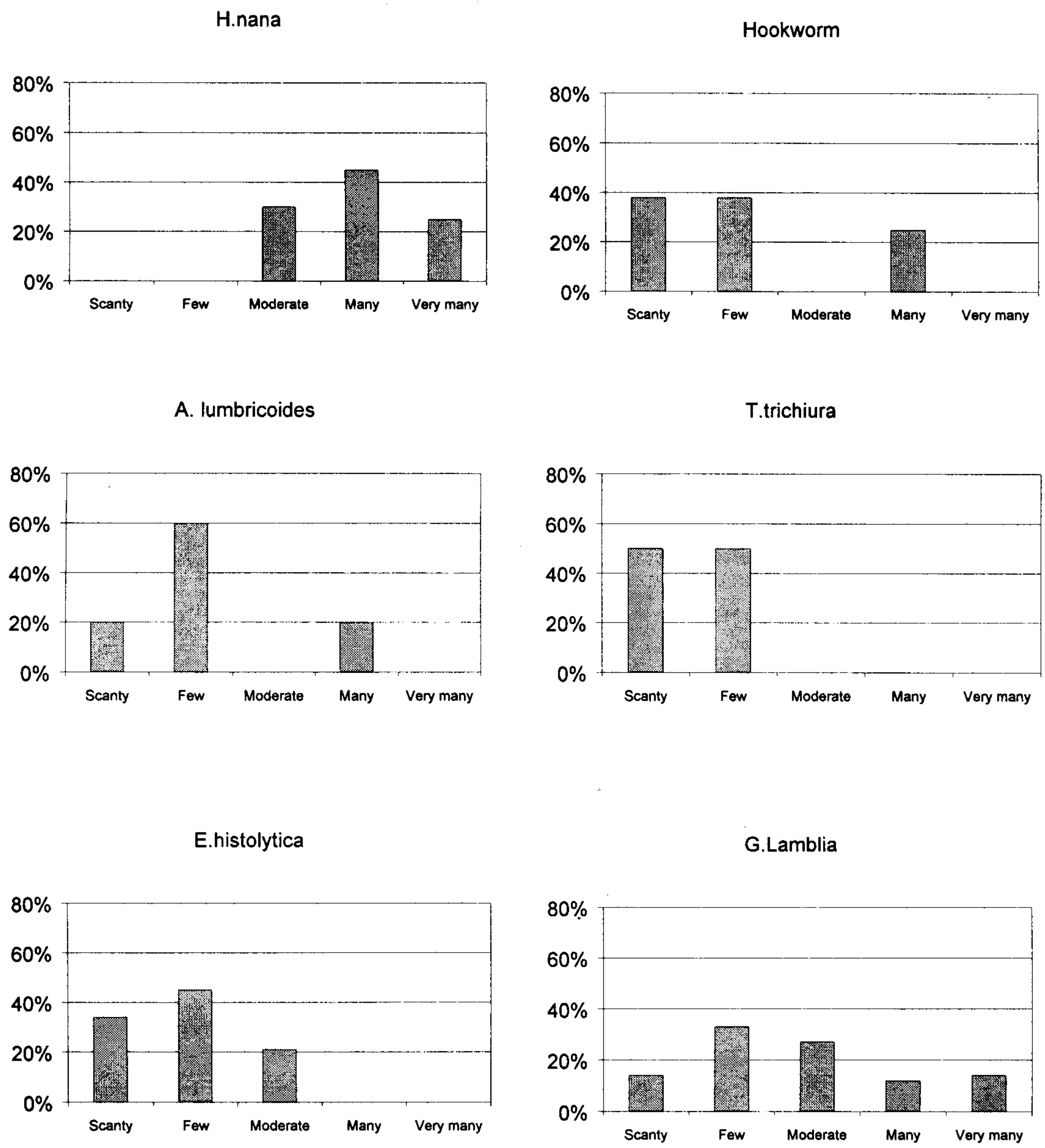
After the second survey all children positive for *G. lamblia* and *E. histolytica* were treated with anti-protozoan drugs as explained before. Among the children positive for *G. lamblia* during the second survey, 30 percent were re-infected in the third survey, despite treatment. Although no treatment was given to cases positive for *G. lamblia* after the first survey, an infection rate of only 28 percent was seen with the same protozoa among these children in the second survey.

The re-infection rate for *E. histolytica* in the third survey was 0 percent after treatment. Although no treatment was given after the first survey, there were also no positive cases in the second survey among those positive for *E. histolytica* in the first survey.

4.8 Intensity of Infections

The microscopic method used does not allow a very accurate assessment of the intensity of infection. The exact amount of preservative per gram of faeces was not known, which could have resulted in some samples being more diluted than others. Nevertheless this method gave an idea of the intensity of infection by dividing the positive samples in the categories scanty, few, moderate, many and very many as explained before (table 4). In figure 9 the percentage of positive samples in these categories for the different protozoa are shown.

Figure 9: Intensity of infections: number of parasites found by microscopic examination



5. DISCUSSION AND CONCLUSION

5.1 Study Population

In all IWMI health studies in the Hakra 6R area, people were very cooperative and coverage of almost 100 percent was reached. However, difficulties were faced in this study since people were reluctant to give stool samples due to cultural and religious factors. The same problem was observed in other health surveys in Pakistan (Khalil et al. 1993; Ahmed and Maqbool 1988). Finally we covered 61.4 percent, 73.4 percent and 62.5 percent of the population in survey 1, 2 and 3 respectively. Out of all 430 children in the study population 373 (87%) gave one or more stool samples during the whole survey. Of these children, only 42 percent gave a sample in all three rounds. Although coverage was not very high, the studied group was representative for the population below the age of 12 years. Male, female and age distribution of the sampled children was the same as for the whole population below this age.

5.2 Methods

There are some limitations to the methods used in this study for collection of the stool samples and microscopic examination. Bottles for the collection of samples were provided to the families at their home and collected the next day. Since there was no supervision with the actual collection of the sample, the possibility of mistakes was present, especially in families where more than one bottle was distributed and parents were illiterate. There were also limitations in the microscopic examination, as only preserved samples were examined. With this method there is a chance to miss infections, as motile trophozoites of protozoa can only be seen in fresh samples. Furthermore quite a large amount of preservative was added to all stools, diluting the samples. The chance to find low intensity infections was therefore less, as also no concentration technique was used.

The used methods nevertheless gave an idea on the prevalence of intestinal helminths and protozoa in the area. To compare the prevalence of intestinal helminths and protozoa in the Hakra 6R study area with other studies, the total prevalence from the three different studies was combined. This was the most useful, because a lot of children gave only one sample in one of the three rounds of collection. Furthermore, cysts are only sequentially excreted in the stool in case of a protozoa infection. When only one sample is examined from a person infected with *G. lamblia*, the chance to find a positive result is only 70 percent. When 3 samples are examined, this chance increases to 85 percent (Cook 1996).

5.3 Helminths

The total prevalence of helminths (12.9 %) found in Hakra 6R was low compared to other studies carried out in Pakistan. Only Iqbal, Munir and Khan. (1996) found a lower prevalence of 10 percent in Rawalpindi, but this prevalence was found in a non-comparable population of children without diarrhea in a hospital. Studies carried out at community level or among schoolchildren are more comparable. A study carried out in a village near Islamabad by Malik, Jujeno and Dil in 1993 showed 25 percent of the children positive for helminths. Studies carried out in schoolchildren in Abottabad (Khan et al. 1987) and Peshawar (Bano and Begum 1981) showed prevalence of 35 percent and 12 percent respectively. The last figure is comparable with the results of this study, the other results give a much higher prevalence.

The most common helminth found in the Hakra 6R study area was the dwarf tapeworm *Hymenolepis nana* with a prevalence of 10.5 percent. This helminth was also common in other studies carried out in Pakistan. With the same techniques as we used, this helminth was found between 4.7 and 10.9 percent in comparable populations of children in other areas in Pakistan. A comparable prevalence was found for this cestode in other parts of the world also (Cook 1996). Compared to other developing countries, especially the prevalence of the soil transmitted nematodes was very low in the Hakra 6R study area. However, the prevalence was also found relatively low in other studies in Pakistan, as described in chapter 2. Since the prevalence of helminths is related with sanitation and hygiene, a comparable prevalence of these nematodes would be expected, because the water supply and hygienic conditions in most of the villages in Pakistan are very poor. In the Hakra 6R area 65 percent of the households had no proper toilet facilities and water supply to the houses was limited.

The low prevalence of soil transmitted helminths in this study could partly be explained by the low sensitivity of the used microscopic techniques. Low intensity infections could have been missed by not using concentration methods and dilution of the samples with preservative. However the prevalence of nematodes was also found low in other studies carried out in Pakistan. It is likely that the climatic conditions in Pakistan are not suitable for the survival of helminth eggs in the soil. In Pakistan, the summer is long and extremely hot. From April to October, the day temperature is always above 40 degrees Celsius, with maximum temperatures of around 48 degrees Celsius. Rainfall is very limited in large parts of Pakistan. Laboratory studies on the survival of helminth eggs have shown that the larvae can not develop when the temperature is above 40 degrees Celsius and when the environment is not humid (Ghiletti et al. 1995; Gaspard, Ambolet and Schwartzbrod 1997). A study conducted in different countries in South Asia concluded that in most parts of Pakistan hookworm infections were not present due to the climatic conditions (von Hinz 1972). An extra argument for the theory that soil transmitted nematodes in Pakistan are low due to the climatic conditions, is the fact that in this study the prevalence of the cestode *H. nana* was found comparable with other developing countries, while the prevalence of nematodes was much lower. The cestode *H.nana* has, contrary to most nematodes, no development stages in the soil, and is therefore less influenced by climatic conditions (Cook 1996). Furthermore re-infections after treatment with albendazole were very rare for the soil-transmitted nematodes, while re-infection was high among the children infected with the cestode *H. nana* under the same circumstances.

Another reason for low prevalence of soil transmitted helminths in this study could be the over-consumption of drugs in the area. Medicines are easily available in all villages and used liberally. In a limited survey carried out by IWMI of village shops, dispensers and BHU's, it was found that 80 percent of the sampled villages had anti-helminthic medicines available at village level or at a nearby BHU. Therefore it can be assumed that anti-helmintic medication is used frequently. Studies in Africa have shown that the prevalence of helminth infections, especially roundworm and hookworm, declines when regular anti-helminth medication is used (Albonico et al. 1999; UNICEF 1997). Regular self-medication with anti-helmintics therefore can be the reason for the low prevalence of these infections found in our study.

The number of positive cases for helminths was low, therefore only a limited analysis could be done on causative factors. No relation was found between the availability of surface water and helminth infections. In villages near the head of the distributary, the same prevalence was found as in villages near the tail of the distributary. On household level however, differences in prevalence were found between houses of families of different socio-economic status. In families of higher socio-economic status, which had significantly better water supply to their houses and better toilet facilities, the prevalence of helminth infections was found lower. Another important factor of influence on helminth infections was the educational level, especially of the mother. If the mother was educated a lower prevalence of helminth infections was found.

5.4 Protozoa

The prevalence of protozoa was high in the Hakra 6R area compared with other studies in Pakistan and elsewhere in the world. This was mainly due to the prevalence of *G. lamblia,* 51.2 percent. In other comparable studies in Pakistan the prevalence of this parasite was found between 10.3 percent and 23.7 percent (Table 1). The prevalence of *E. histolytica* (8.0%) in the Hakra 6R area was in the same range as found in other studies in Pakistan, but low compared to elsewhere in the world.

A reason for the high prevalence of *G. lamblia* could be the poor quality of water used for domestic purposes in the study area. In the villages near the head of the distributary the prevalence of infections with *G. lamblia* was much higher than in villages at the tail end of the distributary. The head of the system receives more surface water than the more downstream areas along the distributary. Since cysts of *G. lamblia* have the ability to survive for long periods in surface water as described before, the abundantly available surface water near the head of the distributary could be a reservoir for *G lamblia* cysts. Surface water is used for drinking without any treatment in all villages. Besides by water, *G. lamblia* can also spread through food items and directly from person to person. Since sanitation facilities were more problematic in the waterlogged areas where groundwater tables were high, this could be another reason for the high prevalence of protozoa at the head of the distributary. There was no relation found in this study between the different causative factors on household level and the prevalence of *G. lamblia.* However, since *G. lamblia* was endemic in the study area and there are many ways to get infected, it could have been impossible to detect differences in prevalence between households within a village. Although there is no direct evidence that transmission can occur from animals to humans, a higher prevalence of *G. lamblia* in people living in close contact with animals is described and *G. lamblia* has been detected in specimens of animals (Cook 1996; Sullivan et al. 1998). Therefore, another reason for the high prevalence of protozoa can be close contacts of people in this area with animals.

Re-infection with *G. lamblia* was very high after treatment with anti-protozoan drugs. There was no difference in percentage re-infected after treatment and in infection rate without treatment. The re-infection rate was also found high in studies conducted in other parts of the world (Sullivan et al. 1988; Gilman et al. 1988).

5.5 Conclusions and Recommendations

The prevalence of helminth infections in this study was lower than expected in an area where water supply is a problem, sanitation facilities are limited and educational level is low. The low prevalence of helminth infections could be explained by the climatic conditions that are not ideal for the survival of helminth eggs. Another very important reason for the low prevalence of helminths could be the over-consumption of drugs in the area. A wide spectrum of medicines is easily available for the population without prescription and therefore used liberally. Although self-medication can reduce helminth prevalence, this unregulated use of medicines is inefficient, leads to more side effects than necessary and could cause the development of resistance to different drugs.

Although the prevalence of helminths was low, a relation was found between helminth infections and the availability of sanitation facilities, good water supply to the house and education of the mother. Improvements in the amount of water available for hygiene practices and sanitation facilities together with health education could reduce helminth infections. These improvements should be combined with a more rational use of anthelmintic drugs.

Contrary to the low prevalence of helminths, the prevalence of protozoa was relatively high. This high prevalence was mainly due to the protozoa *G. lamblia.* A high prevalence of *G. lamblia* in the population is an indication of poor water quality, hygiene and sanitation. Although the association between *G. lamblia* cysts accidentally found in the stool and symptomatic illness is weak, it is known that *G. lamblia* can cause chronic or acute diarrhea and intestinal malabsorption. It is therefore important to reduce the prevalence of this parasite. Drugs against *G. lamblia* have a good cure rate, but re-infection rates are very high in endemic areas. In our study there was no difference in the infection rates after treatment (third survey) and without treatment (second survey). Mass treatment of a population and treatment of accidentally found *G. lamblia* infections is therefore not recommended. Improvements in the quality of water supplied to the villages seem to be useful, as a clear relation was seen in this study between the availability of surface water for domestic use and prevalence rates of *G. lamblia.* Surface water is used in the villages for drinking without any treatment. In certain villages seepage water from canals is used which is of much better quality because the canal water has passed through a kind of slow sand filter. As filtering of water is the only way to eliminate *G. lamblia* cysts, the use of seepage water is a good option.

Improvements in the water supply are very important for both helmintic and protozoan infections. To reduce the prevalence of protozoa the quality seems to be the most important factor while for the helminth infections the quantity of water available in the house is more important, as this ensures enough water for good hygiene practices. Since education of the mother and the availability of proper toilet facilities in the house could reduce helminth infections, improvements in the water supply have to be combined with health education and improvements in sanitation facilities.

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