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EVALUATION OF LABORATORY METHODS USED FOR DIAGNOSIS OF INTESTINAL PARASITES

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ABSTRACT

The direct wet mount (direct method), used for stool examination in laboratories in Sirte area was compared with the centrifugal sedimentation method (sedimentation method) as a standard method; and the data obtained was used for calculation of intestinal parasitic infection.

76% of samples included in the study (700 samples) were detected positive by macroexamination and by both of the used methods. Only 47.4% were positive by the direct method alone and 75.3% were positive with the sedimentation method alone ($P < 0.0001$). Multiple infections were found in 45.3% of the positive samples and 56.3% of the total number of parasites detected were non-pathogenic. *Entamoeba histolytica* was the commonest infection detected (35.9%), followed by *Blastocystis hominis*. Trophozoites and cysts form the majority of detected stages with ova and adults were few. *Giardia lamblia* was better detected by the sedimentation method.

Children in the age group 0-10 years were the most commonly infected than the other age groups and most of infected individuals were below age of 30 years. Among the total positive cases, abdominal pain and diarrhea were the most common presentations. 20.9% of the positive samples were from stool of abnormal consistency, but only 14.3% of negative samples had abnormal consistency. Occult blood was positive in 166 samples (68.7%) with *Entamoeba histolytica* infections and only with 4.8% of *Giardia lamblia* infections.

The relatively low sensitivity of the direct method (72.7%) means that more than a quarter of the samples will be false negative. The high specificity of this method (99%) means that it is less likely to diagnose negative samples as a positive, which is less important clinically than missing an infected sample.

The apparent high rate of parasitic infection, which has been shown in this study, can be explained by that samples used were obtained from individuals referred for stool analysis mainly on the bases of their complaint from symptoms, such as abdominal pain and diarrhea, which may associate with some parasitic infections.

The high frequency of protozoal infection can be due to simple life cycle and transmission of these parasites, especially in conditions of lack of sanitation, absence of clean water supply and availability of take-away food from unhygienic places. The low level of helminthic infections is probably due to the less use of human waste as a fertilizer in agriculture and the low moisture of soil in Sirte area. The low rate of infection with *Giardia lamblia* is probably because proper detection needs examination of the duodenal aspirates. Also the low infection with *Enterobius vermicularis* is probably because Scotch adhesive tape is needed for the collection of ova from perianal area.

Finally, the conclusion is that the sedimentation method should replace the direct method when a single method for analysis has to be used, or should be applied on samples shown to be negative with the direct method. Education, improvement of sanitation, clean water supply, proper disposal of waste and control of the intermediate host and vector insects are required to minimize parasitic infections.

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1: General considerations:

Parasitology is the science, which deals with organisms that live, temporarily or permanently, on or within other living organisms for the purpose of obtaining food. So parasitism includes any reciprocal association in which a species depends upon another for its existence. (Neva & Brown, 1994). The term parasite is determined from a word which means "one who eats at the table of another". Accordingly, all infectious microorganisms can be considered parasites, since they depend on the host organism for essential nutrients and cause some degree of harm. Nevertheless, by convention, parasitic infections refer to those caused by protozoa and helminths (David & Liu, 1994). The term parasite is applied to a weaker organism which obtains food and shelter from another organism and derives all the benefit from the association, because parasites often lack the necessary organs for utilizing raw food materials and depend upon the host for food. The harbouring species known as the host, may show no harmful effects or may suffer from various functional and organic disorders (Neva & Brown , 1994).

What distinguish the eukaryotic parasites are complex life cycles and long-lived chronic infections of the human host. Non human hosts involved

in the parasite life cycles include insects, mollusks and mammals. Transmission by these vectors or infected intermediate hosts is crucial for parasitic infections (David and Liu, 1994).

Protozoa are unicellular animals that occur singly or in colony formation. Each protozoon is a complete unit capable of performing the physiologic functions that in higher organisms are carried on by specialized cells. For the most part they are free-living but some are parasitic, having adapted themselves to an altered existence inside the host (Neva & Brown, 1994).

Protozoal and helminthic infections have caused enormous human suffering and death through the ages, and their global impact on human health remains considerable today. Over one billion people harbour intestinal nematode parasites, including Ascaris, hookworms and Trichuris (David & Liu, 1994).

Many traditional control measures have failed to curb the magnitude of parasitic infections (David & Liu, 1994).

1.2: Global distribution:

Human parasites still account for large loss of life, widespread morbidity and the retardation of economic development in many countries. It has been reported that the prevalence rates of parasitic diseases are high and the total number of protozoan and helminthic infections currently existing

worldwide outnumbers the total world population, because of the multiple infections (World Health Organization, 1986). Intestinal parasitic infection is considered to be one of the most common tropical diseases in developing countries and the prevalence in these countries ranges from 30-60% (World Health Organization, 1987).

In **Southeast Asia**, the prevalence of intestinal parasites among 1364 laborers from Thailand working in **Taiwan** was 18% (Peng et al., 1993) and in **Korea**, the overall positive rate of intestinal parasites was 30.2% in one study (Hong et al., 1990) and 6.5% in another study (Lee et al., 1994).

Nationwide survey of the distribution of human parasites in **china**, revealed an overall infection rate of 63.2%; polyparasitism was found among 43% of those infected; and the highest incidence of infections was among those living in rural areas, particularly among peasants and farmers (Yu et al., 1994).

In **South America**, the prevalence of intestinal parasites among the users of the Health Centre of Sousas district, Campinas, Sao Paulo, **Brazil**, during the period from 1986 to 1990, was 18.4% with a prevalence of *Ascaris lumbricoides* of 48.2%, *Giardia lamblia* of 30.7%, *Trichuris trichiura* of 18.4% and *Enterobius vermecularis* of 9.6% (Gioia, 1992); and in another study in **Brazil**, the prevalence of parasitic infections in patients attended the Outpatient Service of the Municipal Health Centre and Sao Vicente Public Hospital in Sao Jose da Bela Vista, Sao Paulo, from Jan.

1992 to Dec. 1996, was 44.4% (Travares-Dias & Grandini, 1999). In **Brazil** also, 87.6% had one or more parasites. These parasitic infections had a direct correlation with the lack of sanitation and clean water supply (Bioa et al., 1999). In a study among Schoolchildren from Lautaro, the IX region, **Chile**, the infection rate by intestinal protozoa and helminths was 85.4% (Biolley et al., 1990); also in **Chile**, infection with intestinal parasites was found in 55.6% of stool specimens taken from 200 persons from the riverside population of Villarrica Lake (Oberg et al., 1993).

Survey of intestinal parasites in stool specimens obtained from 327 individuals from a low socioeconomic level community from Mara, Zulia State, **Venezuela**, showed an overall parasitic infection rate of 92%; with multiple infections present in 89% of samples (Chacin-Bonilla et al., 1990). In **El Salvador**, in a study of the prevalence of intestinal parasites, stool specimens of 210 children with diarrhoea were examined; intestinal parasites were found in 49% of them, of which 53.4% showed a single infection, 31.7% double, 3% triple and 1% quadriple infection (Reinthaler et al., 1988a).

In the **Subsaharan Africa**, in a study to detect intestinal parasitic infections in schoolchildren from one rural area and one urban area in Pemba Island, Zanzibar, **Tanzania**; all subjects were found to be infected with helminths, with more than 97% of them were infected with more than one species and there was no significant difference between results from

the Urban area and the Rural area (Albonico et al., 1993). In another study in the same island, 94.4% of 413 stool samples, randomly collected from apparently healthy population, were positive for pathogenic intestinal parasites. The high moisture of soil and rain distribution during the year could be favouring the high prevalence of *Trichuris trichiura* and *Ancylostomatidae* (Pampiglione et al., 1987c). The same author had studied intestinal parasites in the **Democratic Republic of Sao Tome and Principe**. He analyzed 1050 stool specimens collected from apparently healthy subjects chosen at random. He found that the prevalence was 43% for *Entamoeba coli,* when protozoan were studied and 87.7% for *Trichuris trichiura* when helminthes were studied (Pampiglione et al., 1987b). In another study, the same author and co-workers had collected 289 stool specimens in East Boe and 288 stool specimens in Canhabaque Island, both are in **Guinea-Bissau**, from apparently healthy subjects, during the period of 1982-1983. The prevalence of intestinal parasites in East Boe was 68.9% for *Entamoeba coli*, 24.6% for *Endolimax nana* among protozoan infections, and among the helminthic infections the prevalence was 69.2% for *Ancylostomatidae*, 38.4% for *Trichuris trichiura*. The prevalence in Canhabaque was 85.1% for *Entamoeba coli*, 14.9% for *Iodamoeba buetchlii* among protozoan infections, and among helminthic infections the prevalence was 87.9% for *Ancylostomatidae*, 9.4% for *Trichuris trichiura* (Pampiglione et al., 1987a). The epidemiology of intestinal parasites in

Ogun State, **Nigeria**, was studied in 479 stool specimens submitted for examination at a hospital in Abeokuta during the rainy season in June 1986. Intestinal parasites were present in 62%. 41.1% of specimens showed a single infection, 34% showed double, 20% triple and 5% quadriple infection. The most commonly found worm was *Ascaris lumbricoides* (in 40% of specimens) (Reinthaler et al., 1988b). In the **Republic of Ginea**, it was found that the prevalence of intestinal protozoa was 58.5% to 76.5% and of helminths 10.4% to 47.7% in some areas of Futa Djalon, during the rainy season of 1989; that the level of oro-faecal transmission is higher in the Rural areas than in the Urban areas; most of the protozoan parasites detected were non-pathogenic while hookworms were the most prevalent helminths and about one third of the infected cases had multiple infection (Bosman et al., 1991).

In the **Arab nations**, stool specimens from 1282 school boys (age 5 to 13 years) in the city of Abha, southwestern of **Saudi Arabia**, were examined for the presence of intestinal parasites. Of these samples, 313 (24.4%) were found infected with one or more species of intestinal protozoa and helminths. The most common pathogenic protozoa was *Giardia lamblia* (10.9%), followed by *Entamoeba histolytica* (4.1%); and *Hymenolepis nana* was the commonest intestinal helminth (3%). Prevalence of *Entamoeba histolytica* was found to increase with age whereas *Giardia* infections were less strongly associated (Omar et al,

1991). Twenty percent of Muslim pilgrims for Haj and Omra were infected with parasites (Sarwaut & Al-Shaiby, 1993). In another study of intestinal parasitic infections among patients attending King Abdulaziz University Hospital in Jeddah, **Saudi Arabia**, of 2765 samples of stool examined, 1402 were from Saudis and the rest from expatriates; the overall prevalence rate was 31.3%. The saudis had a prevalence rate of 27.7% compared to 40.3% in non-Saudis. Symptomatic patients had higher prevalence rates than asymptomatic ones (AL-Fayes & Khogheer, 1989). In an epidemiological study on intestinal parasites in Northern **Morocco** (provinces of Tangier, Tetuan and Larache), 4643 stool samples were examined, of which 2637 samples were positive for intestinal parasites and the total number of detected parasites was 4816 (Jimenez-Albarran & Odda, 1994). In another study in **Palastine**, in Gaza strip, the overall prevalence of intestinal parasitic infections in examined stool specimens from 489 schoolchildren, in an overcrowded area with improper sewage disposal system and low socioeconomic standards, was 27.6%. *Giardia lamblia* was the most frequent (62.2%), followed by *Ascaris lumbricoides* (20.1%) and *Entamoeba histolytica* (13.3%) (Yassin et al, 1999). In a study of **Iraqi** children in Baghdad, it was found that 73% of children from Al-Thawra district (low socioeconomic level) were infected compared to 61% of children from University district (middle socioeconomic level); concluding that intestinal parasitic infections are more prevalent among

low socioeconomic classes (Al-Jebbori & Shafiq, 1976). Another study in the same country, **Iraq**, found that the prevalence of infection with intestinal parasites is higher among children from a village around the city of Mosul than children from the city itself (Al-Hanoon & Hayatee, 1980). A high rate of infection (62.7%) was also reported among primary school children from the city of Arbil in northern **Iraq** (Molan & Farag, 1989). Close high rates of infection were also reported from countries of the Middle East and the nearby area such as **Jordan** (Abdel-Hafez & Abdel-Hafes, 1984) and **Iran** (Farid & Jalays, 1987).

In **Libya**, intestinal parasites of 10 different species were found in 27.6% of the 631 children examined in Benghazi City. Overall, the most common protozoal infection was *Giardia lamblia* (11.4%), followed by *Entamoeba coli* (11.3%) and the commonest helminthic parasite was *Hymenolepis nana* (7.0%). Incidence of *Entamoeba histolytica*, *Ascaris, Strongyloides* and hookworms was low (Dar et al., 1978). In another study in Benghazi, stool samples of 188 children (age 1-4 years) suffering from diarrhoea were examined for parasitic infection. Rate of infection was about 18.6%, with *the Entamoeba histolytica* was the highest, followed by *Giardia lamblia, Cryptosporidium spp* and *Entamoeba coli*, respectively (Bugharara et al, 1999).

In **North America**, low rates of infection were reported from areas with good hygienic standards such as in Maryland, **USA** (Kuntz, 1958)

and Colorado, USA (Gleason et al., 1970). Also in the USA, it has been reported from Centers for Disease Control and Prevention (CDC)-Intestinal Parasite Surveillance Program, that parasitic forms were found in 15.6% of the stool samples examined (Centers for Disease Control, 1978). *Giardia Lamblia* was found in 3.8% of all stool specimens, *Trichuris trichiura* ova in 2.7%, *Ascaris lumbricoids* ova in 2.3%, *Enterobius vermicularis* ova in 1.6% and *Entamoeba histolytica* in 0.6% of all stool specimens. In another study of intestinal parasites including a six month survey of stool specimens examinations in outpatients treated at Olive View Medical Center (1350 samples) and Harbor General Hospital (493 samples) in Los Angeles, *Giardia Lamblia* was found in 14.5% and 8.7%, *Endolimax nana* in 13% and 8.5%, *Entamoeba coli* in 10.5% and 7.7%, *Entamoeba histolytica* in 4.5% and 5.3%, *Ascaris lumbricoides* in 3.9% and 2%, *Hymenolepis nana* in 3.3% and 1.4% and *Dientamoeba fragilis* in 2.1% and 2.8%, respectively; and other protozoa were identified in about 3% of all stool samples included in this study; other *Nematodes* in 3% and other *Cestodes* in 0.5% (Bruckner et al, 1979). In another study in **USA**, results of 216275 stool specimens examined by the state diagnostic laboratories in 1987, were analyzed. Parasites were found in 20% of samples, with highest percentages for the protozoans: *Giardia lamblia* 7.2%, *Entamoeba coli* 4.2%, *Endolimax nana* 4.2%, *Blastocystis hominis* 2.6% and *Entamoeba histolytica* 0.9%. The most commonly identified

helminths were the *Nematodes*: hookworm 1.5%, *Trichuris trichiura* 1.2% and *Ascaris lumbricoides* 0.8%. In the same study, data for 1991 was analyzed; parasites were found in 19.7% of the 178786 specimens and *Giardia lamblia* was found in 5.6% (Kappus et al., 1994). The medical profiles of 1967 refugee claimants to Montreal, Quebec, **Canada**, from January. 1987 to July 1987 were reviewed to evaluate the importance of imported intestinal parasitic infection. An overall infection rate of 29.3% was obtained for pathogenic parasites, where helminths were four times more frequently found than the protozoa *Entamoeba histolytica* and *Giardia lamblia* (Godue & Gyorkos, 1990).

In **Europe**, to obtain the most frequent enteropathogenic parasites found in practice throughout Switzerland, the results of 23276 stool samples were analyzed. Protozoa were found in 32% of samples (4.6% pathogenic, 24% facultative pathogenic protozoa and 3.4% non-pathogenic protozoa) and helminths (15 types) were demonstrated in 2.9% of stool samples with *Trichuris trichiura* predominating (one third) (Rohrbach et al., 1992). The prevalence of intestinal parasites in a random sample of immigrants and Italians employed in the food industry in Turin, **Italy**, was 20.8% among immigrants and 5.6% among Italians. Helminths were more common in Indochinese, but protozoa were more common among Africans. The prevalence of intestinal protozoa was significantly associated with the year of immigration, showing higher levels for new immigrants (Rosso and

Miotti, 1991). A study for evaluation of routine stool screening for 4592 refugees and asylum seekers entering the Stockholm area, **Sweden**, during the period 1987-1988, showed presence of intestinal parasites in 17% of samples. Protozoa, mainly *Giardia intestinalis* were found in 10% and helminths, mainly *Nematodes* in 7%, of samples. Most of the positive samples were obtained from the Indian subcontinent, Southeast Asia and Africa (Persson & Rombo, 1994). In another study in **Sweden**, the results of routine screening for intestinal parasites in 1377 refugees and asylum seekers, within 2 weeks of arrival in Sweden, showed that protozoa, mainly *Giardia intestinalis*, were found in 17% and helminths, mainly hookworms, in 19% of them; with higher rates in refugees coming from Southeast Asia, Africa and Latin America than in those from Eastern Europe and the Middle East (Benzeguir et al, 1999).

Behavioural patterns of living, dietary habits and ancient practices of animal husbandry continue to provide animal parasites with opportunities for survival and proliferation (Crompton & Savioll, 1993). For example, even in well developed countries, such as West Germany, the prevalence of *Taenia saginata* infections remains high with about 900.000 people are affected, giving an annual prevalence of 1.5%; on the other hand infections with *Taenia solium* are rare, because of improved methods of breeding and keeping pigs (Hinz, 1991). Infections with *Taenia spiralis*, *Taenia saginata* and *Taenia solium* are in the rise in Southeast Asian Countries, as changes

in eating habits now include ingestion of raw pork, beef and wild game (Kamiya & Ooi, 1991; Soh, 1991).

In a study of 274 **homosexual men** in Chicago with symptomatic diarrhoea, 39% of the stool specimens contained *Endolimax nana*, 26% *Entamoeba histolytica*, 14% *Entamoeba coli*, 9% *E. hartmanni* and 8% *Giardia lamblia* (Peters et al:, 1986). *Entamoeba histolytica* and *Giardia lamblia* are also found with increasing frequency in male homosexuals; however, these are often avirulent strains, and symptoms are minimal or absent (Ketstone et al., 1980). **AIDS** increased the prevalence of parasites which are rarely recognized in humans, such as *Cryptosporidium* species (Vermund et al., 1986).

Stool specimens from febrile Malaysian children with **cancer** receiving chemotherapy were examined, parasites were found in 42% of them. The most common helminths were *Trichuris trichiura* (24%), followed by *Ascaris lumbricoides* (22%) and hookworms (2%); and the most common protozoa were *Giardia lamblia* (6%), followed by *Blastocystis hominis* (41%) and *Cryptosporidium parvum* (2%) (Menon et al., 1999).

1.3: Routes of intestinal parasitic invasion and transmission

The transmission of parasites involves three factors:- a source of infection, a mode of transmission and the presence of a susceptible host.

The combined effect of these factors determines the prevalence of the parasite at any given time and place. Parasitic transmission is effected through direct and indirect contact, food, water, soil, vertebrate and arthropod vectors, and rarely from mother to offspring (Neva & Brown, 1994).

Transmission and the life cycle of intestinal and luminal protozoa are relatively simple. The parasites pass from host to host directly or through food and water after an extracorporeal existence. In most instances the cyst, which is capable of resisting adverse environmental conditions and the digestive juices of the upper gastrointestinal tract, is the infective form. The structure containing the sporozoites (oocyst) is the infective form of the intestinal sporozoa. Its resistant covering provides greater protection than is required by the sporozoites that are passed directly from insect vectors to humans (Neva & Brown, 1994).

The chances of infection are increased by environmental conditions favouring the extracorporeal existence of the parasite and by lack of sanitation and communal hygeine (Neva & Brown, 1994). Intestinal parasites can enter the human body by only a few infective routes, including oral ingestion.

Drinking untreated water can be particularly hazardous and because most intestinal parasites withstand freezing, contaminated ice water is equally unsafe. Hot tap water, if temperature exceeds the 43°C, is relatively

safe because the infective forms of most intestinal parasites are heat-sensitive. The ingestion of fresh, unpasteurized milk should be avoided in endemic areas (Koneman et al., 1997). In a study to determine the possible risks associated with raw wastewater reuse for agricultural purposes, the prevalence of helminthic infection was found to be significantly higher among randomly selected sample of 740 children from regions using raw wastewater for agriculture in Morocco, compared with 603 children randomly selected from regions that do not use such water; concluding that wastewater reuse may lead to public health risks of transmission of intestinal helminthic infections following consumption of raw vegetables (Habbari et al., 1999). In this study, it was found that 30.8% of children from areas of wastewater use are infected, as compared to only 5.6% among children living in the control areas; and the leading parasitic helminths caused infection in the area exposed to wastewater irrigation were *Ascaris lumbricoids*, *Trichuris trichiura*, *Enterobius vermicularis*, *Hymenolepis nana* and *Taenia saginata*. In different studies in India, it was found that 20% to 40% of sewage-farm workers were positive for ascariasis, as compared to only 13% for control groups (Shuval et al., 1986; Srivastava & Pandey, 1986).

Undercooked meat or raw fresh water fish can transmit flukes, tapeworm, and nematodes. Raw vegetables are relatively safe if peeled

before eating, however, lettuce and other leafy vegetables are particularly difficult to rid of infective parasitic eggs and cysts (Koneman et al., 1997).

Virtually any host organ or tissue can become parasitized, however, most parasites are highly specific in the selection of host sites in which they become established. Parasitic protozoa and helminths have evolved a variety of exquisite adaptations to recognize, invade, and survive in specific host cells and tissues. Extracellular protozoal parasites such as *Entamoeba histolytica*, which use an adherence protein (a galactose-inhibitable lectin) that binds to colonic mucins, to become attached to host cell membrane. Other extracellular parasites have developed specialized structures, such as the adhesive ventral disk of *Giardia* trophozoites, to affix themselves to host tissues (David & Liu, 1994). Helminth parasites elaborate a complex variety of enzymes and other substances which allow them to penetrate host tissues. Invasive larvae of nematodes and trematodes, such as hookworm larvae, secrete proteolytic enzymes which help to penetrate the skin. Once inside the host, helminth parasites use a variety of mechanisms to remain fixed in their preferred anatomic locale. *Trichuris* whipworms, for example, thread themselves into the superficial colonic epithelium, whereas *Ancylostoma* hookworms use buccal teeth to fasten onto small bowel mucosa. Worms which live in gut lumen, such as *Ascaris* roundworms, presumably "swim" by active motility to resist being swept downstream. Once parasitic helminths do invade the host, they

unveil a sophisticated array of mechanisms to evade host immune responses (David & Liu, 1994).

Since parasitic infections often tend to run a chronic course with few or no symptoms, an infected individual may become a "carrier" without showing clinical manifestations, thus serving as a potential source of infection to others; thus infection does not always result in disease (Neva & Brown, 1994,).

1.4: Pathology of intestinal parasitic infections

The pathogenesis of disease associated with parasitic infections depends upon several factors, such as the number of parasites and the various specific mechanisms of tissue damage. Protozoa in contrast to worms are capable of multiplying inside their hosts, so disease can result from infection initiated by only few organisms. Worms, generally, do not multiply in human host, so the likelihood of disease by helminths is related to intensity of infection. Pathologic changes are due to invasion and destruction of cells or tissues by the parasite itself or its products. Tissue damage secondary to immune response, or immunopathology, may occur. Generalized systemic symptoms e.g. fever and signs like splenomegaly and lymphadenopathy are common (Neva & Brown, 1994).

After entering its host, the parasite migrates to those parts of the body where conditions are suitable for temporary or permanent residency. The actual mechanisms by which parasites can damage the host include:

1-Mechanical effects, such as pressure from an enlarged cyst or obstruction of vessels or hollow viscera.

2-Invasion and destruction of host cells by the parasite itself.

3-Inflammatory reaction to the parasite or parasite products.

4-Competition for host nutrients. (Neva & Brown, 1994).

Investigations showed that intestinal parasitic infections adversely influence the health and nutritional status of people (Tripathy et al., 1972; Araya et al., 1985; Cooper et al., 1986).

1.5: Resistance and Immunity to intestinal parasites

Different stages of the same parasite usually occupy different sites in the body, and each stage displays a distinct repertoire of antigens so that the parasite represents a moving target to host immune system. Because of their size and metabolic diversity and because different stages of the same parasite usually occupy different sites in the body, parasites are antigenically complex.

Multiplicity of antigens favours the likelihood that there will be common antigens among related forms, making specific diagnosis of parasitic infections by serological tests difficult because of cross-reactions. Helminths often produce secretory and excretory products which are also antigenic. The persistence of the parasites as foreign antigen(s) in the host

results in immunological consequences which may involve the parasite as well as the host. Protective immunity to protozoa infections involving the blood and tissues often develops but is less effective or absent with protozoa of the intestinal tract or luminal surfaces (Neva & Brown, 1994).

Age may be a factor, with adults generally more resistant than infants or children. Natural resistance may be lowered by malnutrition, concurrent disease or immunosuppressive drugs (Neva & Brown, 1994).

IgE antibodies (reaginic or skin sensitizing antibody) binds to the surface of circulating basophils or tissue mast cells and mediates degranulation of these cells when exposed to specific antigens. Histamine and other products of degranulation contribute to pathology of some parasitic infections and are responsible for immediate hypersensitivity skin reactions (Neva & Brown, 1994).

Serum complement (another component of the immune system) plays an important role in some host-parasite interactions involving both helminths and protozoa and may participate in immunopathologic processes. T-lymphocytes also form a part of host reactions to parasitic infections (cell-mediated immune reactions or delayed hypersensitivity). When T-lymphocytes encounter an antigen to which they or their progenitors were previously exposed, they generate substances called lymphokines or interleukines which in turn can initiate a variety of immunologic events (Neva & Brown, 1994).

Eosinophilia is one immunologic manifestation traditionally associated with parasitic infections. The eosinophil response often involves the tissues as well as circulating blood and is almost always related to infection with helminths rather than with protozoa. The eosinophilia of parasitic infections is a response to tissue invasion by the parasite, not simply to their presence in the gut (Neva & Brown, 1994).

With parasites, it may be very difficult to evaluate the relative contribution of humoral versus cell-mediated defense mechanisms to immunity and often an interaction of both components is required (Neva & Brown, 1994).

Whereas in many instances, immunity to viruses and bacteria can be complete and lifelong after only a single exposure to the pathogen, such sterilizing immunity is rare among protozoa and non existing for helminths. The functional adaptations required of a parasite to live in completely different environments results in parasitic stages which are morphologically and biochemically distinct from one another. These adaptations make the parasites, compared with viruses and bacteria, antigenically complex. Each developmental stage is antigenically different, which has direct implications for the nature of immune responses in parasitic infections (David & Liu, 1994).

For many protozoal and helminthic infections, protection from serious infection is rarely, if ever, complete, becomes maximal only after

repeated infection, and declines in the absence of further exposure. For certain parasites such as the intestinal nematodes, naturally acquired immunity does not appear to develop at all (David & Liu, 1994).

Parasites have evolved numerous ways of escaping host defenses. In a typical chronic parasitic infection, the host mounts a series of immunologic responses which are unable to eliminate the parasite and in some cases lead to immunopathologic damage to host tissues. Few if any of these diverse host immune responses may prevent reinfection or limit the expression of clinical disease (David & Liu, 1994).

Scientific understanding of parasitic protozoa and helminths has lagged behind that of viruses and bacteria in part because their complex structures and life cycles make parasites difficult to study in the laboratory, furthermore, most serious parasitic infections are largely confined to the developing countries of Africa, Asia and South America. The enormous public health impact of parasitic infections and failure of classic control programs have stimulated a search for better ways to treat and control these diseases using modern immunologic and molecular biology methods (David & Liu, 1994).

1.6: Prevention of parasitic infections

Almost every parasite at some stage in its life cycle is susceptible to special exterminative measures, thus barriers such as sanitary excreta disposal may be established by breaking such weak links in the life cycle as

may exist at the departure of the parasite or its eggs from its host, during extracorporeal existence or at the time of its invasion of the human host. The control of the parasitic diseases includes the following procedures:

1- Reduction of the sources of infection in infected cases by therapeutic measures.

2- Education of the general public in personal prophylaxis to prevent dissemination of infection and to reduce opportunities for exposure.

3- Sanitary control of water, food, living and working conditions, and wastes disposal. Food handlers who may be carriers require careful supervision and training in personal hygiene.

4- Destruction or control of reservoir (intermediate hosts and vectors).

5- Erection of biological barriers to the transmission of parasites. (Warren & Mahmoud, 1976).

1.7: Clinical manifestations of intestinal parasitic infections

The most common symptom of intestinal parasitic infections is diarrhoea, which may be bloody or purulent. Cramping abdominal pain may be a prominent feature in these diseases in which the bowel mucosa or wall is invaded by the parasite, such as in infections with hookworms or intestinal flukes. Heavy infection with *Ascaris lumbricoids* can result in small-bowel obstruction. Patients with tapeworms may be asymptomatic except for weight loss despite increased appetite and food intake. Bloating

and steatorrhoea may be seen in patients with giardiasis (Koneman et al., 1997).

Peripheral blood eosinophilia is one of the most important markers for parasitic infections, although its absence does not preclude the diagnosis of parasitic infection. Space occupying cystic lesions of the liver, brain and other organs can be found in amaoebiasis and cysticercus (larval stage of *Taenia solium*) infections (Koneman et al., 1997). Transient pneumonitis may be experienced during the larval migratory phases of *Ascaris* or hookworm infections. Focal itching of the skin may occur at the sites of penetration of hookworm larvae (Koneman et al., 1997).

Chronicity is a prominent characteristic of infections with protozoa and helminths and is related to the remarkable ability of parasites to counteract or evade host immune responses. Immediate mortality is unusual in intestinal parasitic infections. Pathological and clinical disease may take years to develop in most helminthic infections (David & Liu, 1994).

1.8: Diagnosis of intestinal parasitic infections:

The cornerstone for the diagnosis of parasitic infections is a careful history of the patient's illness (David & Liu, 1994). The diagnosis can be in many instances, suspected on clinical grounds, but clinical manifestations of parasitic diseases can sometimes be so general that in most instances diagnosis based upon symptomatology alone is inadequate. Many

infections, chiefly of helminthic origin, give few and indefinite symptoms and often are clinically indistinguishable. Final diagnosis requires the identification of the parasite in intestinal contents in the laboratory or by appropriate serological tests (Neva & Brown, 1994).

In addition to selecting the correct diagnostic procedures, physicians also must ensure that the specimens are collected properly and arrive at the laboratory promptly; for example, motile erythrophagocytic trophozoites of *Entamoeba histolytica* are fragile and will not be detected in faecal specimens that arrive at laboratory several hours after collection (David & Liu, 1994).

Epidemiologic aspects of the illness are especially important because the risks of acquiring many parasites are closely related to occupation, recreation or travel to areas of high endemicity (David & Liu, 1994). The incidence of disease is not the only criterion which must be considered when determining where to concentrate efforts in diagnostic parasitology; for example the parasitic forms of *Entamoeba histolytica* must be identified without fail, although clinical correlation is needed to determine the clinical significance of any given isolate because most of those infected may be harbouring commensal strains of *Entamoeba dispar.* Krogstad and associates, 1978, discovered that segmented neutrophils, when observed in saline mounts of stool specimens, were often misidentified as amoebic cysts by inexperienced laboratory personnel, so stained smears of faeces

and concentrated stool preparations should always be examined to differentiate suspicious amoebic forms from inflammatory cells.

Cyclic shedding of most parasites in the faeces requires examination of a minimum of three samples collected on alternative days (David & Liu, 1994) or two obtained in successive days during normal bowel movement and a third after a Fleet's phosphosoda or magnesium sulphate purge (Koneman et al., 1997). A total of six specimens, collected on successive days, may be required if intestinal amaoebiasis or giardiasis is suspected. Post-therapy specimens should be examined 3 to 4 weeks after treatment of patients with protozoan infections and 5 to 6 weeks after therapy for taenia infections. The stool specimens should be visually examined for the presence of barium, oils or other materials that may render them unacceptable for further processing. Patches of blood or mucus should be specifically selected for microscopic study because they may be derived directly from ulcers or purulent abscesses where the concentration of amoebae may be highest (Koneman et al., 1997).

Because trophozoites disintegrate rapidly after defaecation and do not encyst, watery or loose stool specimens are more likely to contain them (David & Liu, 1994) and therefore should be examined within 30 minutes after collection (not 30 minutes after receipt in the laboratory). Semiformed stools should be examined within 60 minutes, to detect mobile trophozoites, particularly in suspected infection with *Entamoeba*

histolytica. Formed stools, in which trophozoites are not expected (Koneman et al., 1997), but more likely to contain cysts and all stages of helminths (David & Liu, 1994), may be examined up to 24 hours after passage. Stool specimens should never be frozen and thawed or placed in an incubator because parasitic forms may deteriorate rapidly (Koneman et al., 1997).

Microscopic examination of faeces is not complete and negative results for ova and parasite should not be accepted until direct wet mounts, concentration techniques, and permanent stains have been examined. Some intestinal parasites are more readily detected in anatomic locations other than faeces; for example the string test or its commercial substitutes to sample duodenal contents is sometimes necessary to detect *Giardia lamblia* and *Cryptosporidium*. The "Scotsh tape" technique to detect pinworm ova on the perianal skin will sometimes reveal ova of *T. saginata* deposited perianally when the motile segments disintegrate (David & Liu, 1994).

Two routine solutions are used to make wet mounts for the identification of the various life stages of helminths and protozoa: physiologic saline for trophozoites, cysts, ova and larvae and dilute iodine solution for staining protozoal cysts and ova. Iodine solution must never be used to examine specimens for trophozoites because it kills the parasites and destroys their characteristic motility (David & Liu, 1994).

Formed stools are unlikely to contain trophozoites, thus wet mounts are usually unnecessary and only concentrates need to be prepared and the sediment should be examined for helminth eggs and larvae and protozoan cysts. The preparation of stained smears is helpful in identifying cysts found in wet mounts (Melvin & Smith, 1979).

The two most common concentration procedures for detecting small numbers of cysts and ova are formalin-ether sedimentation and zinc sulphate flotation. The formalin-ether technique is preferable because all parasites sediment, but not all float. Permanently stained slides for trophozoites should be prepared before concentration. Additional stained slides for cysts and ova may be made from the concentrate (David & Liu, 1994).

Permanent fixation of stool specimens, that should be sent to reference laboratories for analysis and when delays in transport to laboratory are unavoidable, can be done by addition of a preservative such as 10% formalin-saline (100ml formaldehyde in 900ml 0.85% sodium chloride) or polyvinyl alcohol (PVA) to preserve the protozoal trophozoites. The latter is preferred, because formalin is unsuitable for the performance of concentration procedures and preparation of permanent-stained smears (Koneman et al., 1997). Refrigeration also will preserve trophozoites for few hours and protozoal cysts and helminths for several days (David & Liu, 1994).

In many instances, especially in differentiation of *Entamoeba histolytica* from other *amoebae*, identification from wet mounts or concentrates must be considered tentative. Permanently stained smears allow study of the cellular detail necessary for definitive identification. The iron-haematoxilin stain is excellent for crucial work but the trichrome stain is a satisfactory alternative that also stains parasites in specimens preserved in PVA fixative (David & Liu, 1994).

The development of humoral antibodies (IgM, IgG, IgA and IgE) to components of the infecting parasite can be demonstrated by various tests (such as 1-Complement fixation, 2-Haemagglutination, 3-Agar-gel precipitation, 4-Fluorescent antibody, 5-Enzyme-linked immunosorbent assay (ELISA) (Neva & Brown, 1994) and 6- the polymerase chain reaction (PCR) (Gasser, 1999)).

OBJECTIVES OF THIS WORK

1: To evaluate the direct wet mount method used for detection of intestinal parasites in stool samples in laboratories of Ibn-Sina hospital in Sirte and in the Sirte polyclinic, compared with the centrifugal saline sedimentation method as a standard method.

2: To use data collected for the calculation of intestinal parasitic infection among those attending the outpatient department of Ibn-Sina hospital in Sirte and in the Sirte polyclinic.

Studies of intestinal parasitic infections in the different regions of Libya are scarce and none has been done before in Sirte region (Appendex I), therefore a study of this type will be useful and productive.

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1: The collection of stool samples:

Stool specimens were collected in a clean, wide mouthed container with a tightly fitted lid and the time of collection was recorded on the container, which was properly labeled.

The following type of samples were excluded from examination for the purpose of this study (David & Liu, 1994; Koneman et al., 1997; Melvin & Smith, 1979).

a-Specimens that were admixed with urine or water (e.g. contamination from the toilet bowel or bed pan may introduce free-living protozoa and trophozoites may lose their motility or undergo lysis in water), were discarded (Melvin & Smith, 1979).

b-Samples collected from patients ingested barium or other contrast agents for radiological procedures (barium enema may prevent excretion of organisms in stool for at least one week following the enema)

c-Samples collected from patients taking antidiarrhoeal agents (cathertics with an oil base retard motility of trophozoites and distort the morphology of the parasites), antiacids (because these substances change the consistency of the faeces and interfere with microscopic detection of

parasites). And other medications containing mineral oil, bismuth, antibiotics, antimalarials (compromise the detection of intestinal protozoa).

To maintain adequate moisture the lid of each container was tightly fitted immediately after collection (Koneman et al., 1997).

A questionnaire was prepared (Appendix II) and was filled for every case included in the study.

2.2: Techniques used for stool examination:

2.2-1: Direct method (direct wet mount technique, saline and iodine smears) (Noble 1944):

a) Materials:

-Saline solution 0.9% (B. Braun Melsungen AG).

-Lugol's iodine solution (BDH laboratory supplies, Poole Dorset, UK).

-Plastic applicator sticks.

-Microscopic glass slides 75x38mm.

-Cover slips 22x22mm.

-Ordinary microscope.

b) Procedure:

Saline and iodine smears were prepared on one glass slide and the following was done:

1- One drop of saline was put in the middle of the left half of the slide and a drop of iodine solution was put in the middle of the right half.

2- About 2mg of the stool sample (forming a cone on the lower end of the wooden stick) were taken from inside the faecal sample and from the surface which contains mucus or blood and then mixed with the drop of saline on the slide. A second portion of stool was mixed with the drop of iodine then both were covered with glass covers and examined microscopically.

Examination of the slide was started from the top left hand corner, the slide was moved across the microscopic stage and the field was examined until the objective reached the other edge of the field. At first examination started by using 5x or 10x eye-piece to have a general view of the slide, then more comprehensive examination, when required, was done using 40x eye-piece. The procedure was repeated when indicated.

Each field examined by the 40x objective to check for the presence of protozoa. The use of 5x or 10x eyepiece is satisfactory.

2.2-2: Centrifugal saline sedimentation technique (Baroody and Most, 1946):

a) Materials:

-Plastic centrifugation tubes (10 ml).

-Saline solution 0.9% (B. Braun Melsungen AG).

-Pasteur pipette.

-Plastic applicator sticks.

-Wooden applicator sticks.

-Lugol's iodine solution (BDH laboratory supplies, Poole Dorset, UK).

-Microscopic glass slides 75x38mm.

-Cover slips 22x22mm.

-Ordinary microscope.

b) Procedure:

2-3 gm of the fresh stool was added to 4 ml of normal saline solution in a plastic centrifugation tube. The lid of the tube was tightly fitted and the contents were shacked thoroughly (the wooden applicator stick was used to break down the formed or semiformed stool). The contents were then centrifuged at 2500 rpm for 3 minutes. The supernatant was separated into a plain tube leaving the sediment in the bottom of the centrifugation tube, then a smear was prepared from the concentrated sediment and examined microscopically in the same way as in the direct wet mount examination.

Other Materials:

-Gloves.

-Masks.

-Gauze and paraffin film.

-Centrifuge (4000 rpm).

2.3: Occult blood technique:

A Commercial kit for detection of occult blood in faeces (Sentinel CH. Milan – Italy) was used. Each kit contains 25 slides and 25 plastic tubes which contain the reagent.

Procedure:

The provided plastic stick was plunged in the stool specimen and removed (usually about 2-3 mg of stool sticks on the plastic stick), then the stick was dipped in the provided reagent in the tube and stirred to mix the attached stool with the reagent. 3-4 drops of the mixture were put on the provided occult blood slide and the result was read after 3 minutes. Appearance of two red lines on the slide indicates a positive test.

2.4: Data analysis : (Maxwell, 1983).

Most of the data obtained by the two methods were compared as percentages, and the significance of the difference between results of the two methods was tested by Chisquare (χ^2) method of statistical analysis, where the frequencies expected under the null hypothesis were written down in the 4-cells contingency table. Value of χ^2 was obtained and checked for significance in the χ^2 table with a degree of freedom 1. P value less than 0.05 meant that there is significant difference between data obtained by the two methods.

Validity is the extent to which the method is accurately detects what it supposed to find. The two components of the validity (sensitivity and specificity) of the direct wet mount method were determined. Because it is impossible to get healthy control group who is not infected with parasites as some people may appear healthy but may have asymptomatic infection or are carriers, the results of the centrifugal sedimentation method were considered as the control for the calculation of sensitivity and specificity of the direct wet mount method.

Sensitivity of the direct wet mount method, which is the ability of the method to detect samples which have parasitic infection, was determined as follows:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + False negative}} \times 100$$

True positive Positive samples by macroexamination, centrifugal sedimentation method and direct wet mount method.

False negative Samples negative by direct wet mount method but positive by macroexamination and centrifugal sedimentation method.

Specificity of the direct wet mount method, which is the ability of the method to detect samples which do not have parasitic infection, was also determined as follows:

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + False positive}} \times 100$$

True negative Samples negative by macroexamination and centrifugal sedimentation method.

False positive: samples positive by the direct wet mount method.

Agreement between the two methods was determined by:

$$\frac{\text{No. of +ve by both methods + No. of –ve by both methods}}{\text{Total No. of samples}} \times 100$$

Measure of agreement between the two methods was also determined by using the Fourfold point (phi) correlation.

3. RESULTS

Sensitivity (proportion of true positives correctly identified) of the direct wet mount method (Table 1 & 2) $= \frac{532}{532 + 200} \times 100 = 72.7\%$

This means that more than quarter of the samples which can be positive for parasitic infection with the centrifugal sedimentation method will be negative by the direct wet mount method.

Specificity (proportion of true negatives correctly identified) of the direct wet mount method (Table 1 & 2) $= \frac{168}{3 + 168} \times 100 = 98.2\%$

If the 3 samples which were positive by the direct wet mount method but negative by macroexamination and the centrifugal sedimentation method are considered actually positive, as parasites have been seen visually under the microscope, then none of the samples will be false positive and specificity will be 100%.

The high specificity means that almost all of the samples which can be shown to be negative with the centrifugal sedimentation method are going to be negative with the direct wet mount method.

Predictive value for a positive test by the direct wet mount method

$$\frac{532}{532 + 3} = 0.99$$

This means that the sensitivity obtained by the direct wet mount method (72.7%) was low compared with the predective value for a positive test.

Predictive value for a negative test by the direct wet mount method

$$\frac{168}{200 + 168} = 0.46$$

This means that the value obtained by the direct wet mount method (98%) was very much higher than the predictive value for a negative test.

Agreement between the two methods (Table 3)

$$\frac{\text{No. of +ve samples by both methods + No. of --ve samples by both methods}}{\text{Total No. of samples}} \times 100$$

$$= \frac{297 + 170}{700} \times 100 = \frac{467}{700} \times 100 = 67\%$$

The fourfold correlation between the two methods (r) (Table 3)

$$\frac{bc - ad}{\sqrt{(a+b)(c+d)(a+c)(b+d)}}$$

$$= \frac{(297 \times 170) - (3 \times 195)}{\sqrt{(3 + 297)(170 + 195)(3 + 170)(297 + 195)}}$$

$$= \frac{49905}{\sqrt{9320202000}} = \frac{49905}{96541} = 0.52$$

The total number of samples included in this study was 700 samples; 532 samples of them (76%) were detected positive for parasitic infections with macroexamination and microexamination by both methods (Table 2 & Fig. 1). Number of positive samples detected by the direct wet mount method was 332 samples (47.4% of the total number of samples) and by centrifugal sedimentation method was 527 samples (75.3% of the total number of samples) (Fig. 2). The total positive samples detected by the centrifugal sedimentation method was significantly higher than those detected by the direct wet mount method ($p < 0.0001$) (Table 2). Details of types of parasitic infections detected are shown in tables 4 and 5, and Fig. 3.

The total number of parasites seen by the centrifugal sedimentation method (894) was higher than that seen by the direct wet mount method (409) (Table 4). None of the ova of helminthes were detected by the direct method, and trophozoites and cysts form the majority of the detected stages with the ova and adults were few (Fig. 4).

Multiple infections (more than one parasite) were found in 45.3% of the positive samples (Fig. 5 and Table 6) and 56.3% of the total number of parasites were non-pathogenic (Fig. 6 &Table 7).

Entamoeba histolytica was the commonest parasitic infection detected (35.9%), followed by *Blastocystis hominis* (29.6%) (Fig. 3 and

Table 5). Figure 7 shows the microscopic features of both trophozoite and cyst forms of *Entamoeba histolytica.*

Giardia lamblia was better detected by centrifugal sedimentation method , cystic forms were more frequently seen than trophozoites and samples positive for both forms were few (Fig. 8 & 9, & Table 4).

Entamoeba coli, which is non-pathogenic protozoa, was detected clearly with both direct and centrifugal methods either in both forms (cyst and trophozoite) or in cystic form alone (Fig. 10). *Entamoeba hartmanni* is another non-pathogenic protozoa which was also seen in both trophozoite and cystic forms (Fig. 11). *Endolimax nana* was better detected by centrifugal sedimentation method and it was seen in both forms (trophozoite and cyst Fig. 12). Figure 13 shows the trophozoite of *Blastocystis hominis*.

Among the helminthic infections, 5 cases of *Enterobius vermicularis* were seen, two of them were seen in both adult and ova forms (Fig. 14). In two cases the adult form was seen and in another case the ova only was seen.

Our result also revealed that children in the age group below 10 years were most commonly infected by parasites than the other age groups. *Entamoeba histolytica* and *Blastocystis hominis* were the most frequent parasites seen in children (Fig. 15 & Table 8). Overall, most of the infected individuals were below age of 30 years.

The percentage of the total protozoal and helminthic infections was almost equal in males and females (Table 9).

Table 10 shows the most common symptoms obtained from the patients stool examination requests. 42.3% of symptoms were unrelated to parasitic infections or stool analysis was requested just for check up. Among the total positive cases, abdominal pain and diarrhea were the most common presentations which constituted 32.3% and 23.7%, respectively.

Table 11 shows that abnormal stool consistency was observed in 19.3% of all samples. 20.9% of the positive samples were from stools of abnormal consistency, and only 14.3% of negative samples have abnormal consistency. 109 cases with protozoal infection, (20.5% of the total positive samples) had abnormal stool consistency, but only 2 cases of helminthic infection (0.4%) had abnormal stool consistency.

Mucus, pus cells and positive occult blood were observed in 44%, 50.7% and 23.7% of the total samples,. respectively (Fig. 16). Occult blood was positive in 68.7% of *Entamoeba histolytica* infections and only in 4.8% of *Giardia lambilia* infections (Table 12).

Most of the collected samples were from children (0 –15 years) (276 samples out off 700) and the percentage of the positive samples in this age group was 39.8 of the total positive samples (Table 13).

Table 1: A contingency table relating the true positive, false positive, true negative and false negative results of the direct wet mount method and the centrifugal sedimentation method.

		Sedimentation method (B)		
		Positive	Negative	
Direct wet mount method (A)	Positive	(a)= true positives= 532	(b)=fals positives = 3	(a + b) = total positives by (A) & (B)= 535
	Negative	(c)= false negatives = 200	(d)= true negatives = 168	(c + d) = total negatives by(A) & (B)= 368
		(a + c) = total true positives = 732	(b + d) = total true negatives = 171	(a+b+c+d)= grand total = 903

Table 2: Sensitivity and specificity of the direct wet mount method and the results of the statistical analysis of results obtained by the direct wet mount method and the centrifugal sedimentation method.

	Direct wet mount method	Centrifugal sedimentation method	X^2	Degree of freedom	P value
Positive samples	332 (47.4%)	527 (75.3%)	114.6	1	< 0.0001
Negative samples	368 (52.6%)	173 (24.7%)			
Total no. of parasites	409	894			
True positives	532				
False positives	3				
True negatives	168				
False negatives	200				
Sensitivity of the direct wet mount method	72.7%				
Specificity of the direct wet mount method	98%				

* Total positive: 76% and total no. of parasites: 899.

Table 3: A contingency table relating the positive and the negative results of the direct wet mount method and the centrifugal sedimentation method.

		Sedimentation method		
		Negative	Positive	Total
Direct wet mount method	Positive	(a) = 3	(b) = 297	(a + b) = 300
	Negative	(c) = 170	(d) = 195	(c + d) = 365
	Total	(a + c) = 173	(b + d) = 492	(a+b+c+d)=665

Figure 1: Percentage of total positive and negative samples

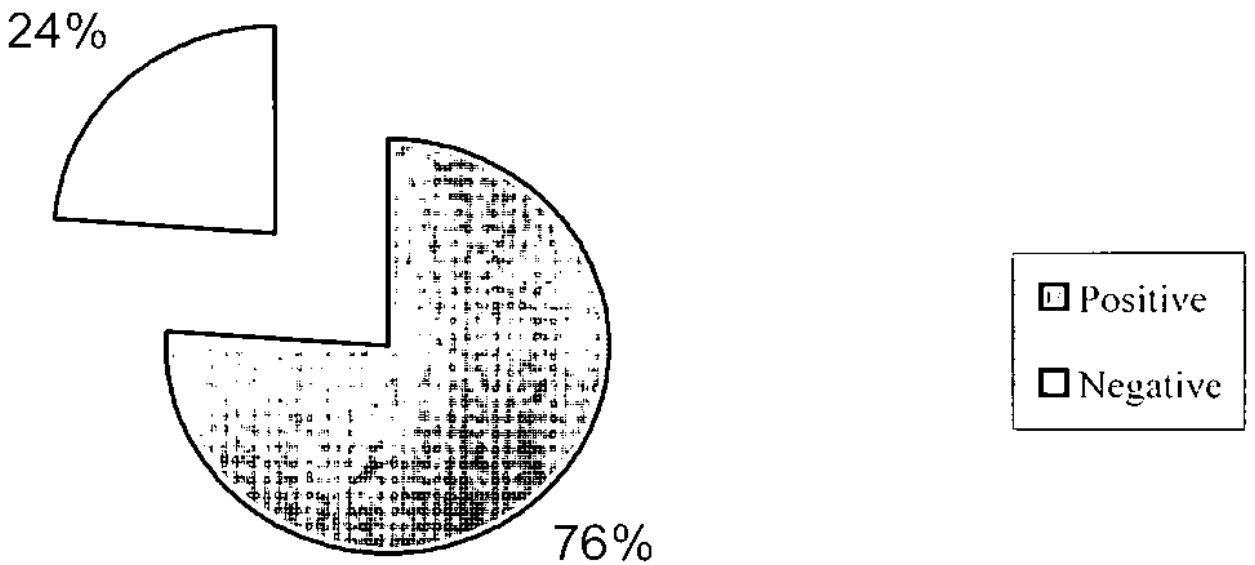


Figure 2: Percentage of positive and negative samples obtained by the two methods

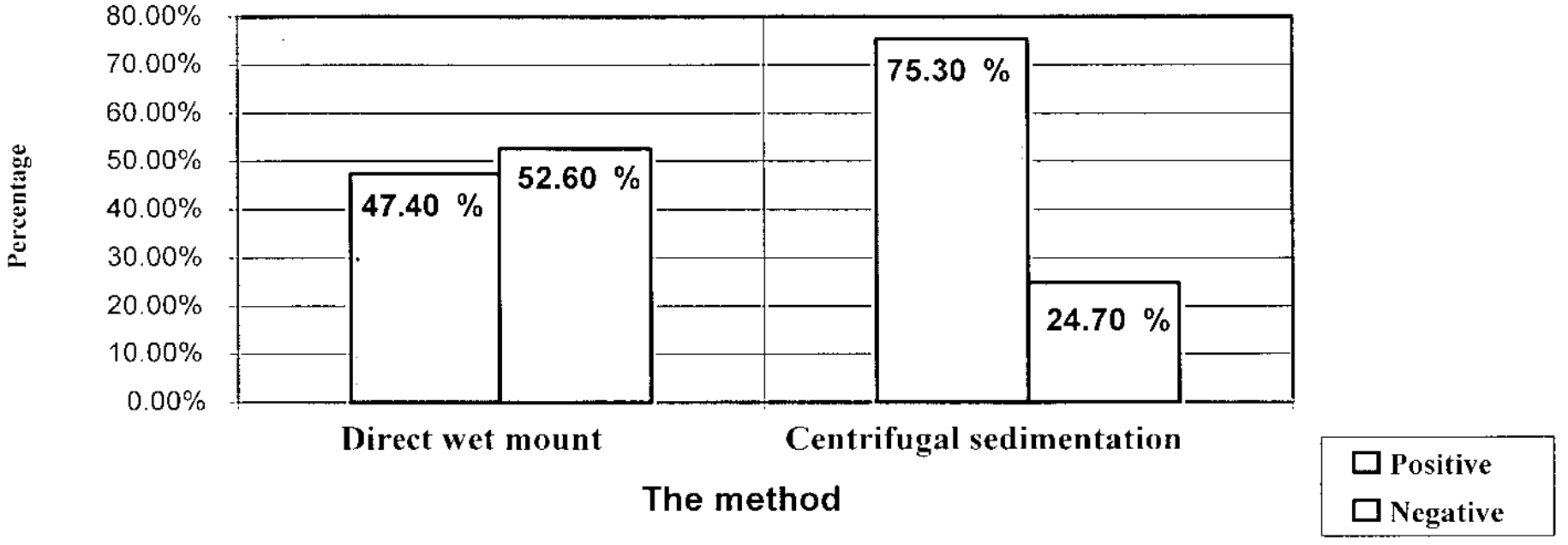


Table 4: Details of the results of parasitic infections obtained by the direct wet mount method and the centrifugal sedimentation method.

	Direct Wet mount method	Centrifugal Sedimentation Method
Positive samples	332	527
Negative samples	368	173
Total no. Of parasites	409	894
Entamoeba histolytica	199	323
Trophozoite and cyst	22	25
Cyst	177	294
Trophozoite	0	4
Giardia lamblia	34	64
Trophozoite and cyst	9	10
Cyst	22	51
Trophozoite	3	3
Entamoeba hartmanni	29	82
Trophozoite and cyst	29	71
Cyst	0	11
Endolimax nana	21	61
Trophozoite and cyst	21	61
Entamoeba coli	6	42
Trophozoite and cyst	1	9
Cyst	5	32
Trophozoite	0	1
Blastocystis hominis	105	263
Trichomonas hominis	13	52
Isospora belli	2	4
Enterobius vermicularis egg.	0	3
Adult female: ***Enterobius vermicularis***	4 cases by macroexamination	

Table 5: Type of parasitic infections obtained by the direct wet mount method and the centrifugal sedimentation method.

The parasite	No.	%
Entamoeba histolytica	323	35.9
Blastocystis hominis	266	29.6
Entamoeba hartmanni	82	9.1
Giardia lamblia	64	7.1
Endolimax nana	61	6.8
Trichomonas hominis	52	5.8
Entamoeba coli	42	4.7
Enterobius vermicularis	5	0.6
Isospora belli	4	0.4
Total no. of parasites	899	100

Figure 3: Percentage of the parasitic infection in the positive samples

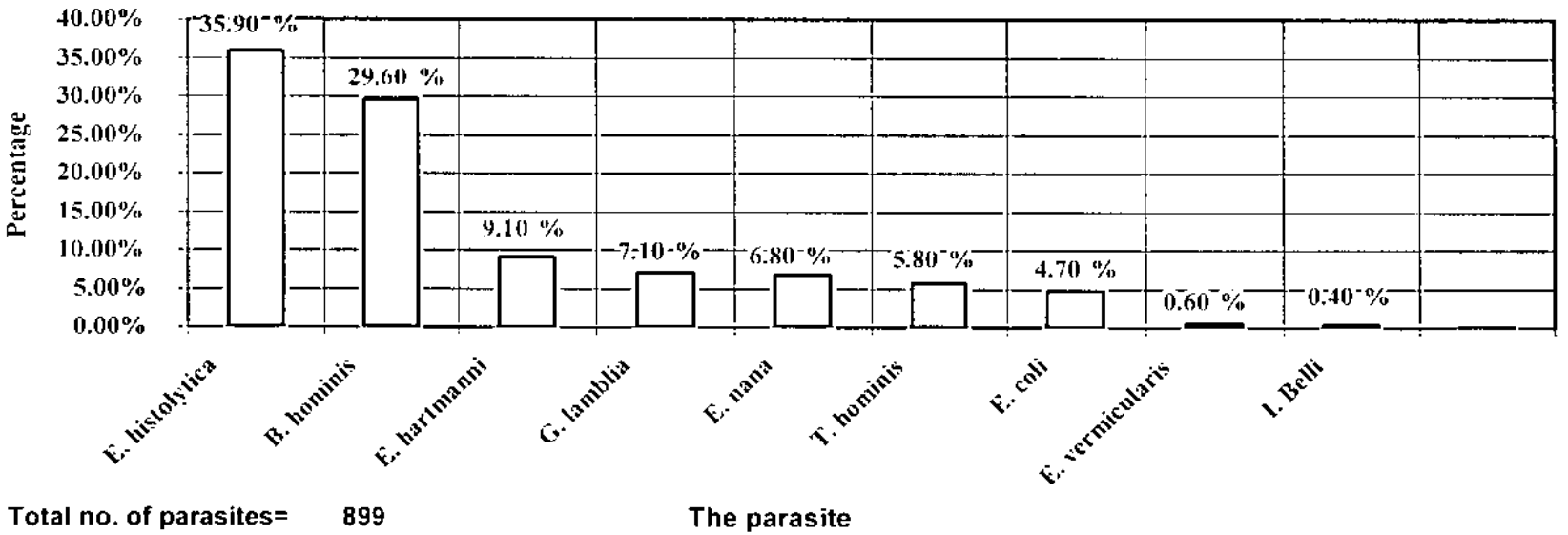


Figure 4: Relationship between the methods used and the stage of parasite

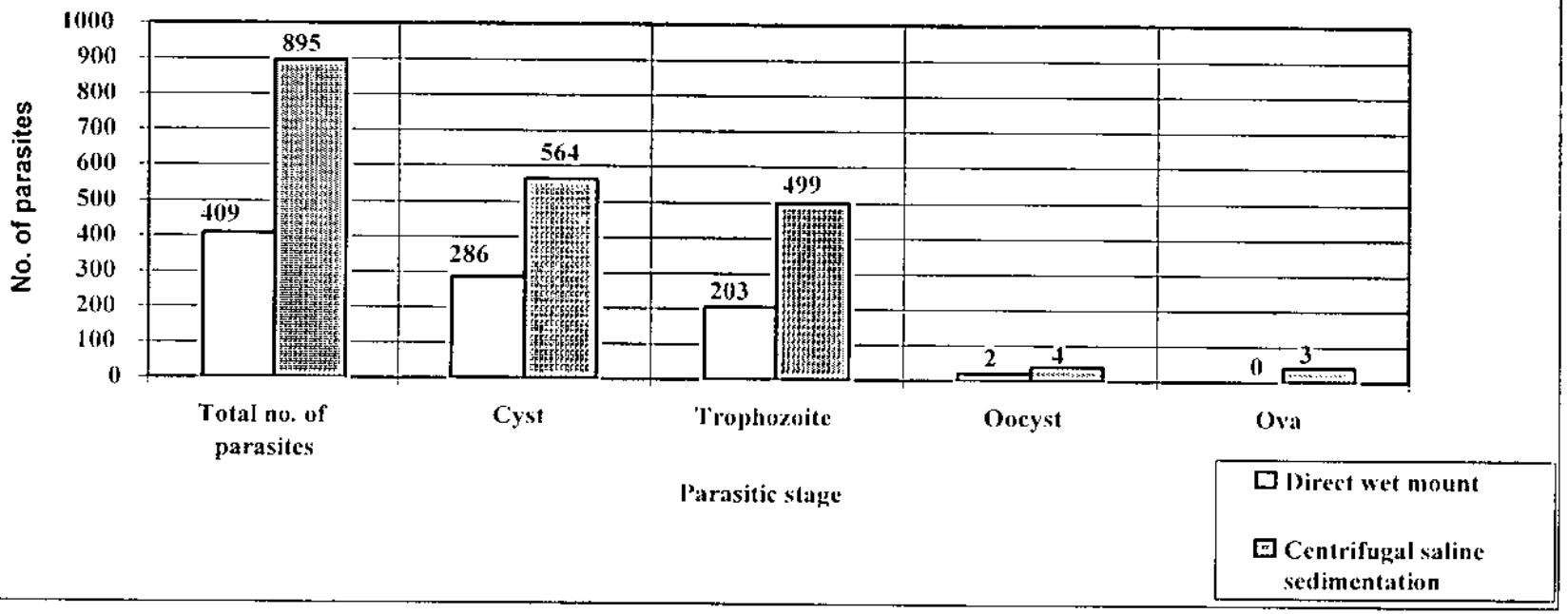


Figure 5: Percentage of Single and multiple infections in the positive samples

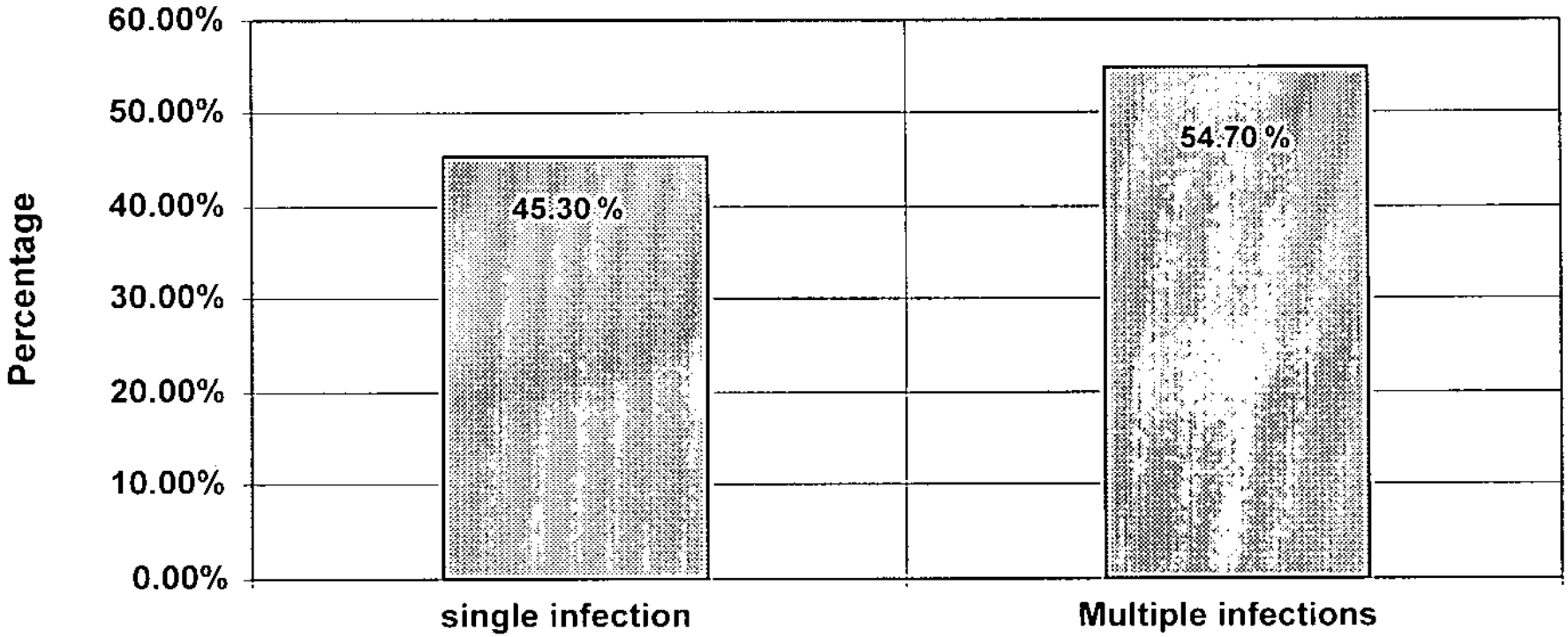
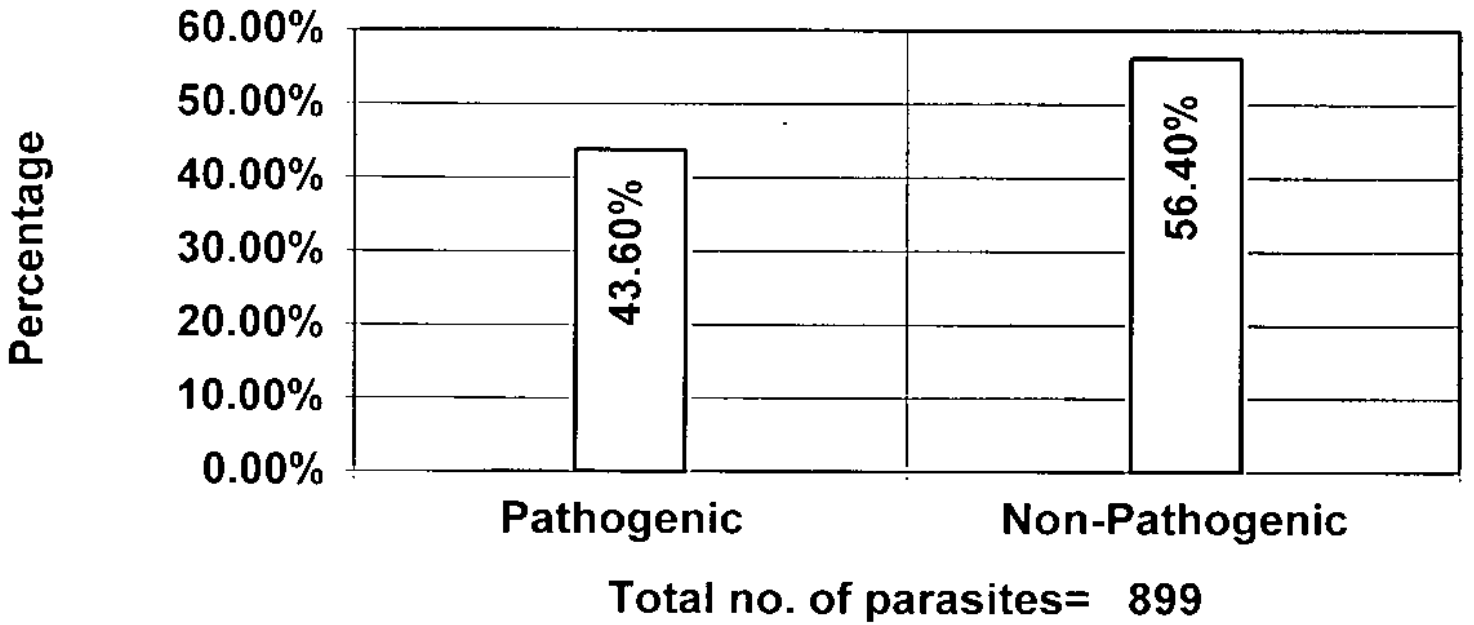


Table 6: Details of the single and multiple parasitic infections which have been seen in the positive stool specimens.

	No. Of samples		No. Of parasites
Single infection	242	45.5%	242
Double infection	217	40.8%	434
Triple infection	69	13%	207
Quadruple infection	4	0.7%	16
Total	532	100	899

Figure 6: Percentage of pathogenic and non-pathogenic parasites



Total no. of parasites= 899

Table 7: The percentages of pathogenic and non-pathogenic parasites which have been seen in the positive stool samples.

Total no. of parasites 899	Pathogenic parasites [1]		Non-pathogenic parasites [2]	
	No.	%	No.	%
	392	43.6	507	56.4

1-Pathogenic parasites: *Entamoeba histolytica, Giardia lamblia and Enterobius vermicularis.*

2-Non-pathogenic parasites: *Blastocystis hominis. Endolimax nana, Entamoeba hartmanni. Trichomonas hominis, Entamoeba coli and Isospora belli.*

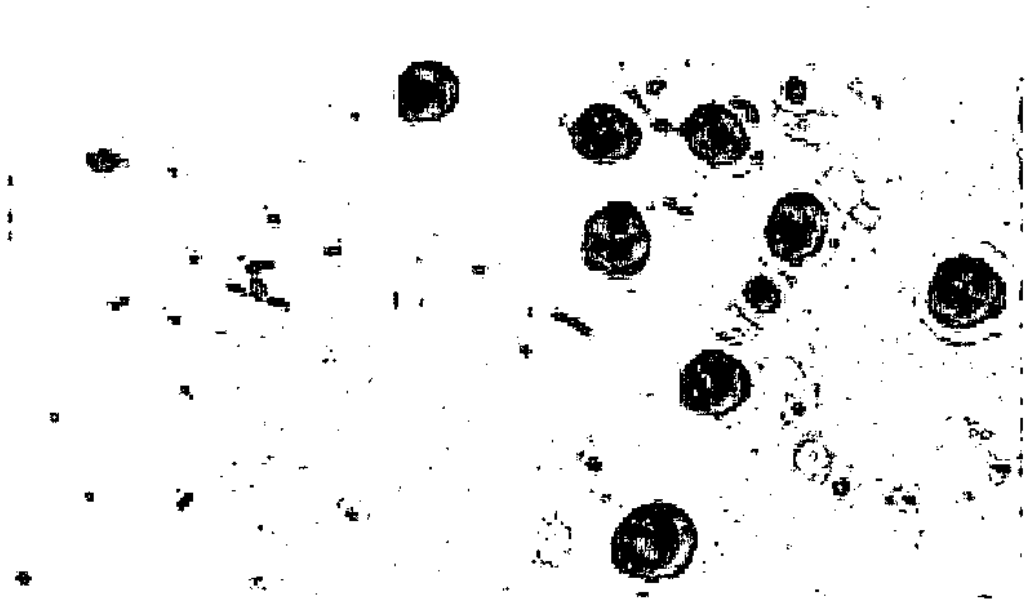


Figure 7: Cysts of *Entamoeba histolytica.*

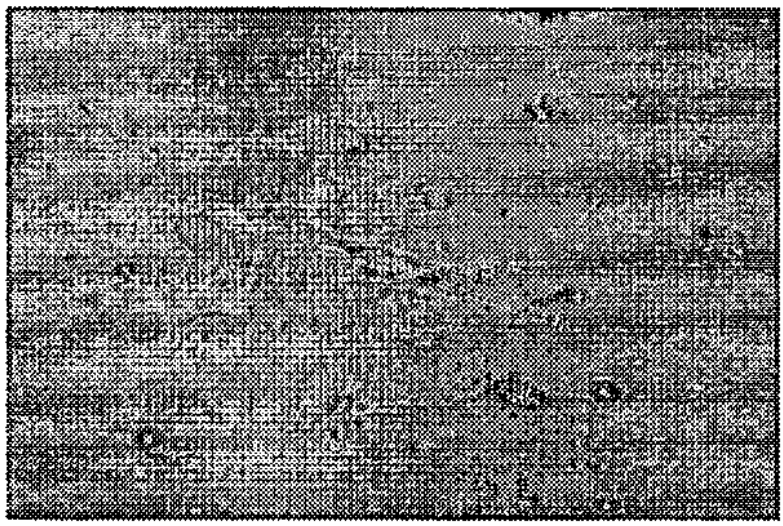


Figure 8: Cyst of *Giardia lamblia.*

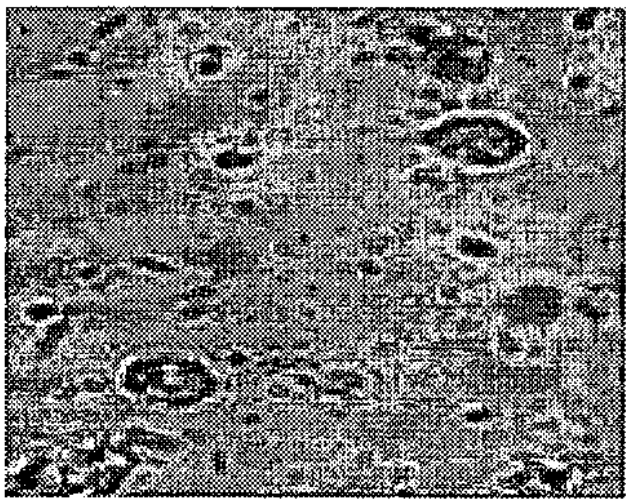


Figure 9: Trophozoites of *Giardia lamblia.*

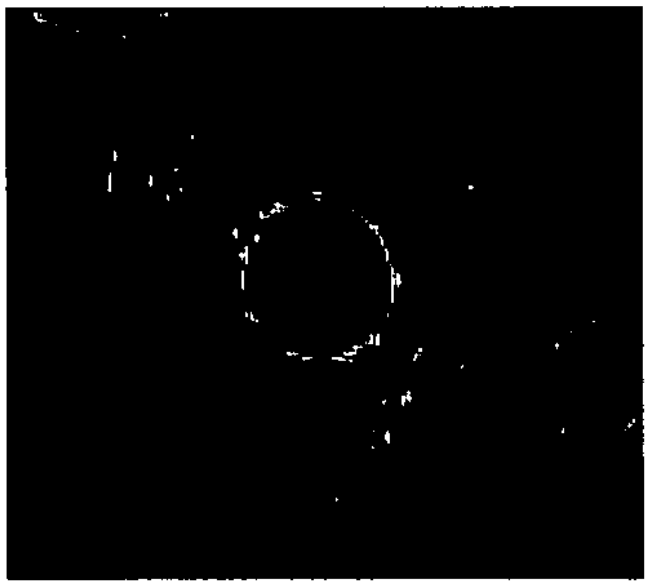


Figure 10: Cyst of *Entamoeba coli.*

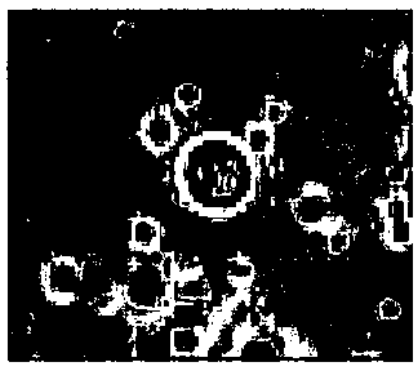


Figure 11: Cyst of *Entamoeba hartmanni.*

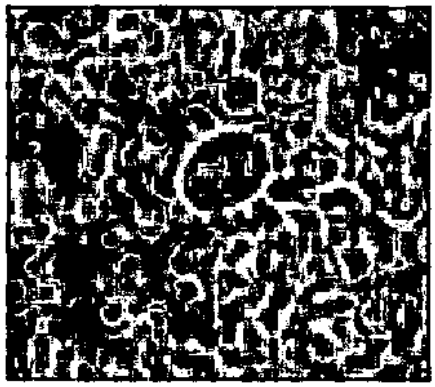


Figure 12: Cyst of *Endolimax nana.*

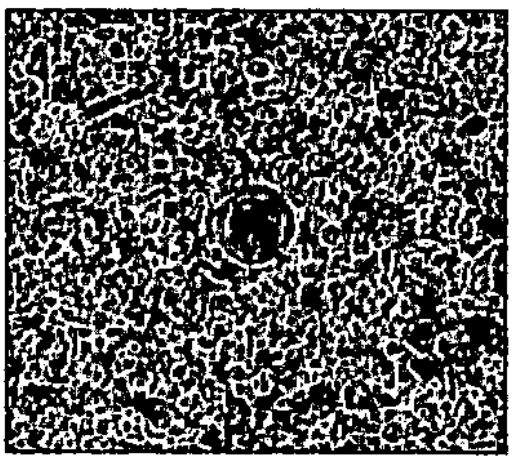


Figure 13: B*lastocystis hominis.*

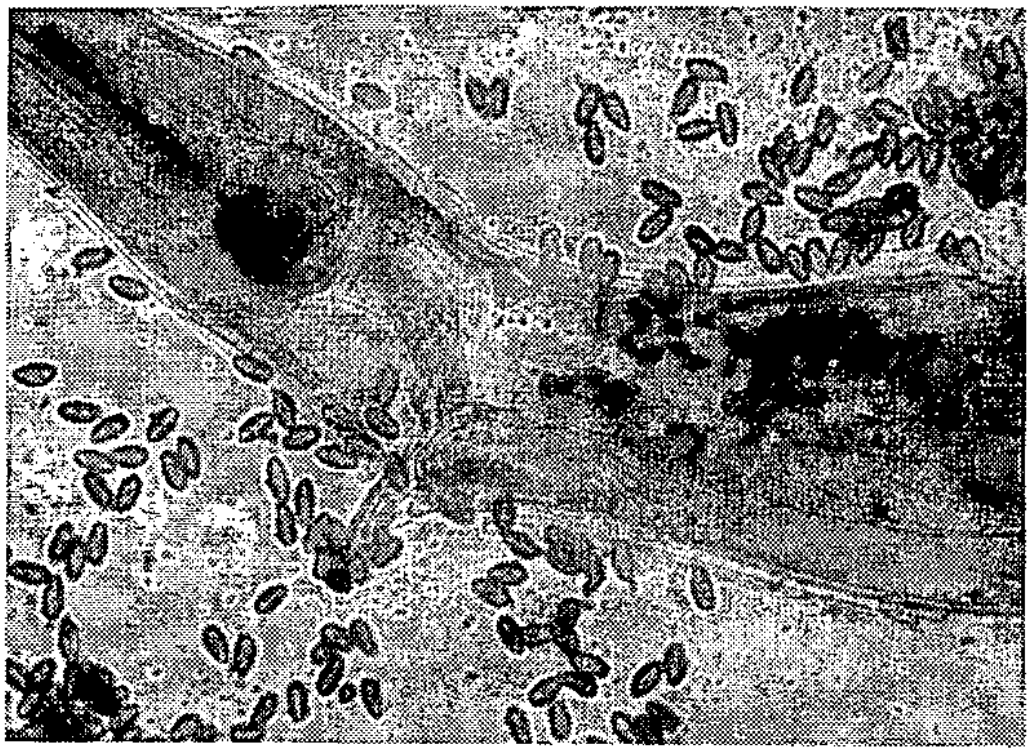


Figure 14: Adult of *Enterobius vermicularis* with ova.

Figure 15 : Positive and negative samples in relation of age groups

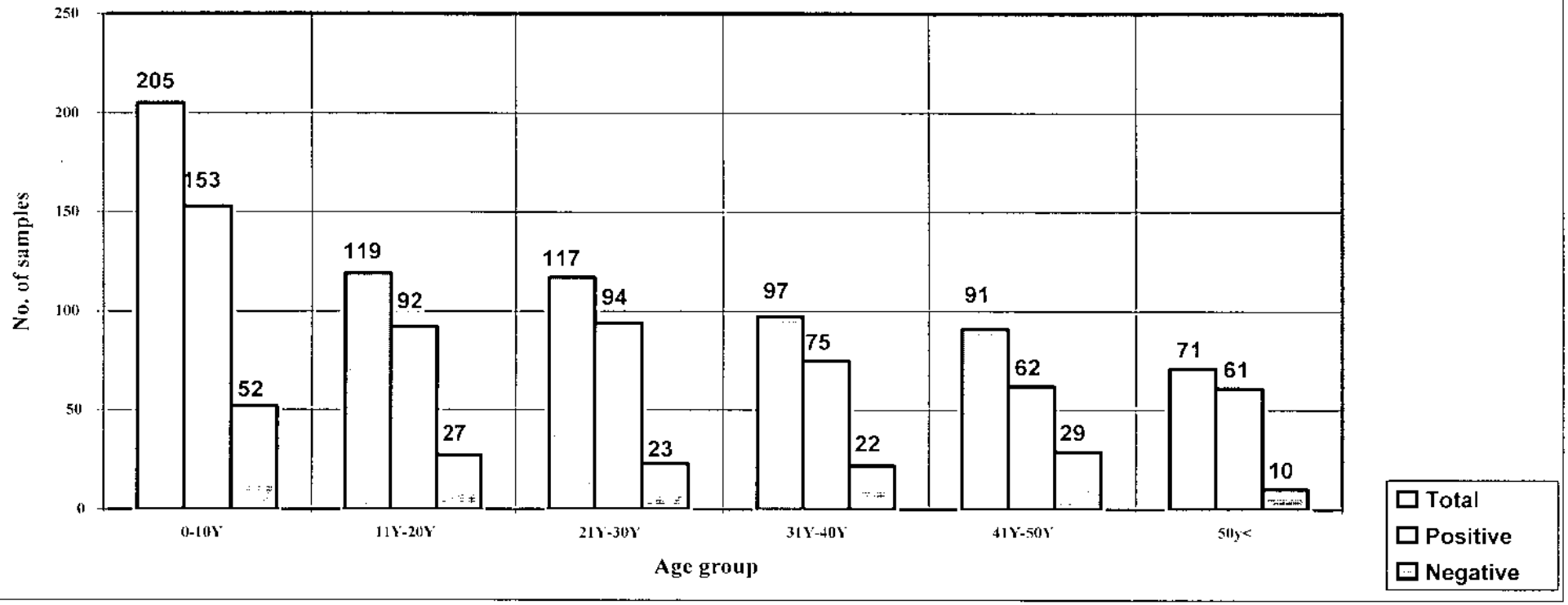


Table 8: Relationship of the parasitic infections to the different age groups.

Age Group / The parasite	0 – 10Y *	11Y – 20Y	21Y – 30Y	31Y – 40Y	41Y – 50Y	51Y <	Total
Entamoeba histolytica	96	58	51	40	38	40	323
Blastocystis hominis	72	56	53	29	26	30	266
Entamoeba hartmanni	23	17	15	13	8	6	82
Giardia lamblia	24	10	11	11	4	4	64
Endolimax nana	21	9	11	10	5	5	61
Trichmonas hominis	10	11	10	8	10	3	52
Entamoeba coli	15	9	10	2	4	2	42
Enterobius vermicularis	3	0	2	0	0	0	5
Isospora belli	3	1	0	0	0	0	4
Total	267	171	163	113	95	90	899

*** Y: Year.**

Table 9: Relationship of the parasitic infections to sex and nationality.

Nationality	Sex	No.	No. of +ve samples 532	%	Samples infected by protozoa	Samples infected by helminthes
Libyan	M*	332	254	76.5	252	2
	F*	310	236	76.1	233	3
	M+F	642	490	76.3	485	5
Non-Libyan	M	52	38	73	38	-
	F	6	4	66.7	4	-
	M+F	58	42	72.4	42	-
Total	M=384 F=316	700	M=292 F=240	M=76 F=75.9	527	5

* M: male & F: Female.

Table 10: Distribution of samples results by symptoms and the positivity of their stool samples.

Symptoms / Total Samples 700	Abdominal pain		Diarrhoea		Vomiting		Tenismus		Constipation		Others *	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
700	226	32.3	166	23.7	3	0.4	3	0.4	6	0.9	296	42.3
532 +ve samples	166	31.2	144	27	3	0.6	3	0.6	6	1.1	210	39.5
168 -ve samples	60	35.7	22	13.1	-	-	-	-	-	-	86	51.2

* Check-up & nil.

- Patients symptoms as obtained from stool examination request.

Table 11: The relation between stool consistency of the samples and parasitic infection.

	Negative samples	Positive samples	Samples infected by protozoa	Samples infected by helminthes
Normal * 565	144	421	418	3
Abnormal ** 135	24	111	109	2
Total	168	532	527	5

* Normal consistency: formed, semiformed.

**Abnormal consistency: liquid, loose.

Figure 16 : Percentage of stool containts among the total samples

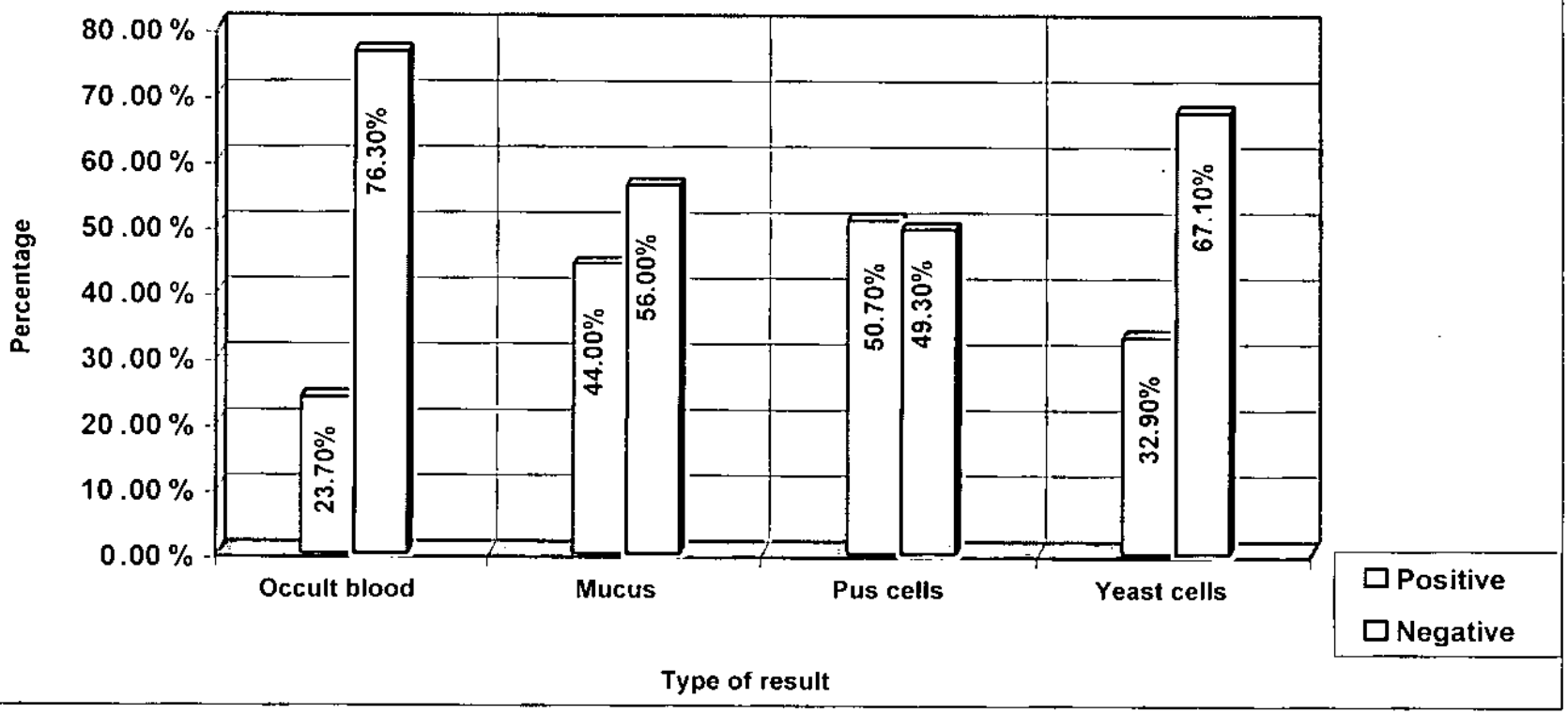


Table 12:The relation between presence or absence of occult blood to parasitic infections.

Total no. of Positive Occult Blood	Positive Occult blood in negative samples		Positive occult Blood in positive samples							
166	26	15.7%	140				84.3%			
			E. histolytica *		*G. lamblia* *		*E. histolytica* & *G. lamblia* *		Others **	
			114	68.7%	8	4.8%	6	3.6%	12	7.2%

* Some of them mixed with non-pathogenic parasites.

** *Entamoeba hartmanni 3, Blastocystis hominis 2, Trichomonas hominis 2, Entamoeba coli 2, Entamoeba coli and Entamoeba hartmanni2, Entamoeba hartmanni and Blastocystis hominis 1,*

Table 13: Details of the results of the whole samples according to the occupation of the patients.

Total	Children 1m-5y *		Students						Employed		Self Employed		House Wife		Retired		Unemployed	
			Primary & Preparatory		Secondary		University											
700	128		148		29		23		165		54		122		14		17	
Positive samples 532	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
	89	16.7	123	23.1	21	4	19	3.6	125	23.5	36	6.8	101	19	13	2.4	5	0.9

* M: Month & Y : Year

CHAPTER 4

DISCUSSION AND CONCLUSION

4. DISCUSSION AND CONCLUSION

Detection of parasitic infection in stool specimens depends largely on the method applied for preparation of the sample for microscopic examination and on the experience of the laboratory personnel. The applied method should be cheap, quick, sensitive, specific and reliable (Cowan & Olano, 1997).

The direct wet mount method, which commonly used in hospitals and clinics in Sirte, has a sensitivity of 72.7% which means that more than quarter of the samples will be false negative, and hence many of the infected people will not be treated. The high specificity (98%) of this method means that it is less likely to diagnose a negative sample as a positive, which is clinically less important than missing an infected sample. This conclusion has been supported by the high predictive value for positive test in the face of relatively low sensitivity of the direct wet mount method., and the low agreement (67%) between this method and the centrifugal sedimentation method (standard method). The fourfold correlation between the two methods was also low (0.52).

Although the direct wet mount method is less time consuming but a lot of time can be then spent on the repetition of stool analysis of the missed cases, because according to the negative result they will not be treated and

therefore most likely will come again for consultation and stool analysis; beside they will act as a source of infection which will spread it to others.

Intestinal parasitic infection was found in 532 specimens (76% of the whole 700 samples examined). Multiple infection was found in 54.7% of the total positive samples, with the number of protozoal infections largely exceeds the number of helminthic infections. High rates of parasitic infections were also reported in a number of developing countries such as Brazil (Bioa et al, 1999), Tanzania (Pampiglione et al, 1987c), Nigeria (Rienthaler et al, 1988b) and Iraq (Mahdi et al, 1993). Low rates of infection has been reported in the developed countries such as USA (Kappus et al, 1994).

The apparent high rate of parasitic infection which has been shown in this study can be explained by that the samples used were obtained from individuals referred for stool analysis mainly on the bases of their complaint from a symptoms (abdominal pain and diarrheoa) which can be associated with a parasitic infection.

Compared with the protozoal infections, helminthic infections were low, probably due to less use of the human waste as a fertilizer in agriculture and low moisture of soil in Sirte area. It has been reported that the use of raw sewage and wastewater for agricultural purposes has caused epidemics of

hookworms and ascariasis in Morocco (soil transmission) (Habbari et al, 1999).

The high frequency of the protozoal infection can be due to the simple life cycle of these parasites and the simple way of transmission, especiall in the presence of lack of sanitation, absence of clean water supply and take-away food from unhygienic places.

The high frequency of infection with *Entamoeba histolytica* shows that this protozoa is more troublesome in this area than other parasites and is the main cause of referral for stool analysis. Among the total number of samples, 23.5% were positive for the occult blood test and most of the positive cases were found in samples positive for *Entamoeba histolytica*. High rates of infection with *Entamoeba histolytica* has been shown in studies in Egypt (Mohamed et al, 1985 & Mohamed et al, 1988).

The low rate of infection with *Giardia lamblia* is probably due to that the proper detection of this parasite should be done by the examination of the duodenal aspirates. It has been shown that sensitivity of microscopical methods for detection of *Giardia lamblia* fluctuates between 46 and 95% (Janoff et al, 1989).

Infection with pin worms (*Enterobius vermecularis*) is prevalent in lower socioeconomic class groups and has the widest geographical

distribution than any other helminthic infection (Neva & Brown, 1994), but infection with this parasite has been shown to be very low in this study probably because a special method is required (Scotch adhesive tape) for collection of ova from the perianal area (Schantz & Mcanley, 1991); and eggs can be present in only about 5% of the usual stool samples of individuals infected with the parasite (Neva & Brown, 1994).

Blastocystis hominis which is commonly seen in stool of healthy and symptomatic individuals (Jacobs et al, 1990) has also been shown to be present in a considerable extent in stool samples included in this study.

Infection with parasites which are transmitted by eating raw fish and meat has not been detected in this study, because eating raw meat and fish is not a common custom in Libya in general.

Further studies are required to study:

1-Prevalence of Entrobiasis in Sirte.

2-Prevalence of the parasitic infections in school children in Sirte area.

Finally, the most important points concluded from this study and the recommendations are as follows:

1- Centrifugal sedimentation method should replace the direct wet mount method as a procedure for preparation of stool samples for microscopic examination to detect parasitic infections. Whenever complete replacement is

not possible, those samples which are shown to be negative by the direct wet mount method should be re-examined by the centrifugal sedimentation method. Staining, such as with Lugol's iodine, can enhance identification of parasitic forms, differentiation between different parasites and distinguishing some parasitic forms from white blood cells. Also special procedures are important for collection of samples for examination and for detection of some parasitic infections like *Enterobius vermicularis* and *Giardia lamblia.* Recently, The polymerase chain reaction (PCR) methods are available for diagnosis of some parasitic infections, but their expense limits their use which is reserved for certain cases and certain diseases.

2- Strict hygienic environment should be available in restaurants and take-away places and stool examinations, using proper methods, should be regularly done for personnel working in these places.

3- Control of the insect vector such as flies, to reduce transmission of parasitic infections.

4- Proper sewage draining and proper and clean water supply should be made available for public.

5- Quality control of technitions in the laboratories 0f Ibn-Sina hospital and in the Sirte polyclinic by continous trianing courses and supervion.

6- Public education through radio and television.

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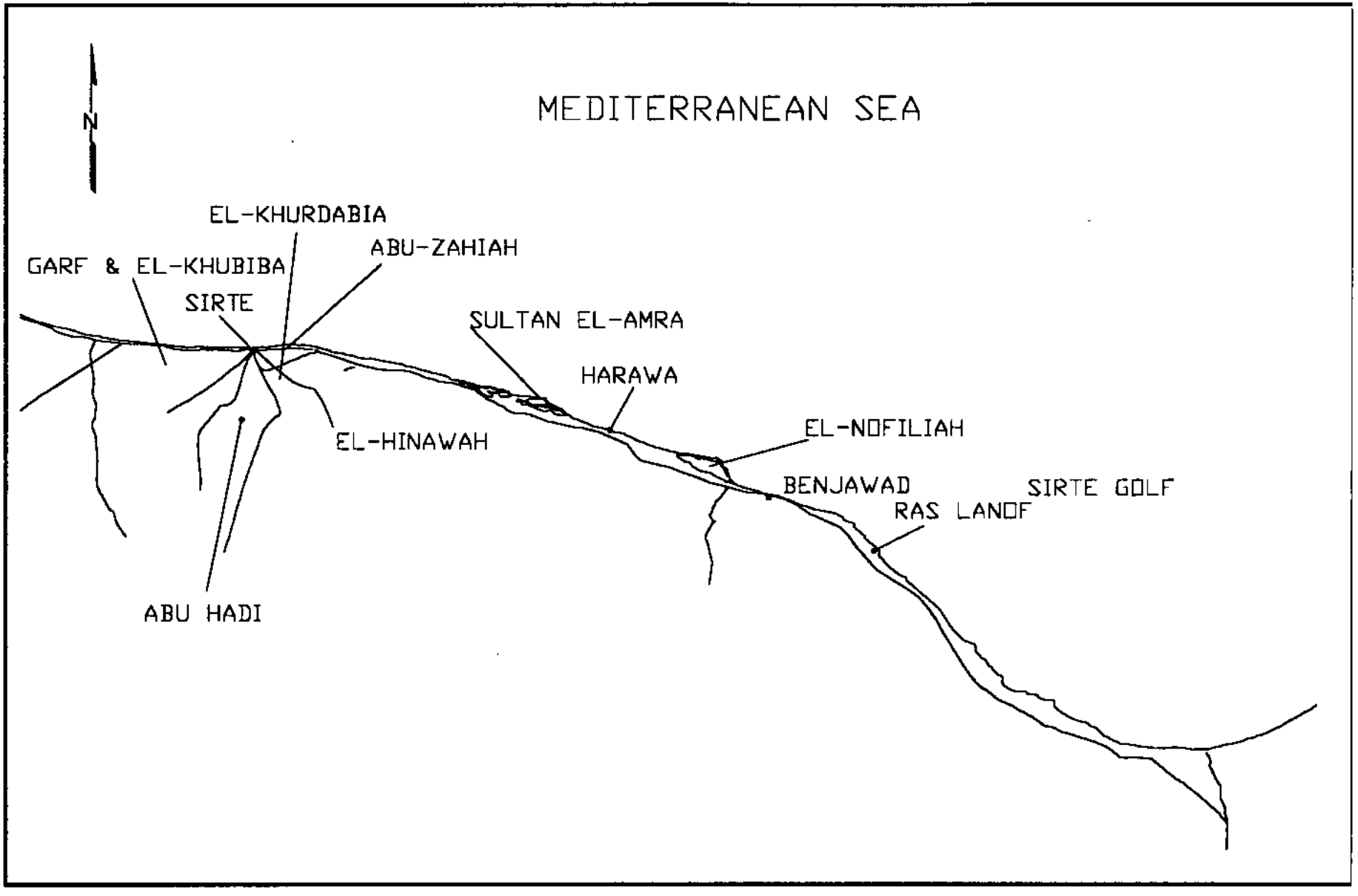
APPENDIX



APPENDIX T: THE MAP OF THE NORTH PART OF LIBYA SHOWING THE LOCATION OF SIRTE.

Appendix (II): The questionnaire, which was filled by every patient participated in this study.

Sirte Polyclinic Ibn-Sina Hospital

Date..... Sample no. .....

Patient's name..... Age..... Sex M F

Nationality..... Area or Village the patient comes from .....

Qualification..... Occupation..... No. of family members.....

Domestic animals present in the patient's surroundings: Sheep, Goat, Camel, Cow, Cat, Dog, Others.....

Reason for patient referral for stool examination: Vomiting ( ), Diarrhea ( ), Dysentery ( ), Abdominal pain ( ), Others .....

Macroexamination of the stool sample:

Color: ..... Consistency: .....

Mucus: present absent Occult blood +ve ( ) -ve ( ), Others: .....

Microexamination of the stool sample:

a) General: Puss cells: present absent R.B.Cs.: present absent. Others: .....

b) Microexamination by the direct wet mount method:

- Parasite ..... Stage ..... - Parasite ..... Stage .....

c) Microexamination by the centrifugal sedimentation technique:

- Parasite ..... Stage ..... - Parasite ..... Stage .....

الملخص العربي

في هذه الدراسة الحالية التي أجريت في منطقة سرت–ليبيا ، تم إختبار 700 عينة براز ، لأشـــخاص ترددوا علي العيادات الخارجية بسبب أمراض التلوث الطفيلي ، ومقارنة النتائج المعملية المتحصل عليها من إستخدام طريقة التحضير المباشر(Direct method) ، المستعملة فـــي المعامل الطبية بمدينة سرت ، والنتائج المعملية المتحصل عليها من طريقة تركيز الراسب بواســـطة جهـــاز الطرد المركزي (Sedimentation method) بالإضافة إلي الكشف العيني للعينات (Macroexamination). ووجد أن (76%) من هذه العينات مصابة بطفيليات معوية (إيجابية التحليل الطفيلي).

أظهرت نتائج هذه الدراسة أن هناك فروقاً ذات دلالة إحصائية بين إستخدام الطريقتين، حيث كانت نتيجة إستخدام طريقة التحضير المباشر (47.4%) أما طريقة تركيز الراسب فكانت نتيجتها (75.3%) من العينات التي تم إختبارها. وجد أكثر من طفيل (multiple infection) في (45.3%) من العينات إيجابية التحليل الطفيلي، وشكلت الطفيليات الغير مرضية (Non-pathogenic) نســـبة (56.3%) مـــن مجموع الطفيليات المتحصل عليها. وكان طفيل الأميبا المتحولة الحالة للنسج الأكثر تواجداً في العينات المصابة حيث شكل نسبة (35.9%) من هذه الطفيليات تلاه مباشرة طفيل (*Blastocystis hominis*).

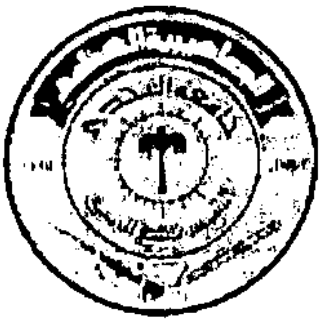
كـــانت الفـــئة العمرية (0–10 سنوات) الأكثر عرضة للإصابة الطفيلية ، وبشكل عام كانت أغلب الحالات المصابة عند فئات الأعمار الواقعة تحت (30 سنة) ، وكانت أغلب الحالات إيجابية التحليل الطفيلي تعاني من إسهالات معوية وآلام في البطن.

أمـــا إختبار الـــدم الخفي فقـــد أعطي نتيجة إيجابية مع (166) عينة من المجموع العام (68.7%) مع وجود إصابة بالأميبا المتحولة الحالة للنسج و(4.8%) مع الجياردیا اللمبلية.

أظهـــرت نـــتائج هـــذه الدراسة أن درجة الحساسية (Sensitivity) لطريقة التحضير المباشر ، منخفضة بشكل نسبي (72.7%) ويعني هذا أن أكثر من ربع العينات إيجابية التحليل الطفـــيلي لا يتـــم إكتشافها بهذه الطريقة ، أما درجة التمييزية (Specificity) فكـــانت عالـــية (99%) ويعـــني أن هـــذه الطريقة ذات قدرة منخفضة لكشف العينات إيجابية التحليل الطفيلي، ويمكنها تشخيص الحالات الإيجابية علي أنها سلبية ، الأمر الذي يفقدها أهميتها التشخيصية لأنه من الضروري إكتشاف الحالات المحتوية علي الطفيليات.

النَسـبة العاليـة للتَـلوثِ الطفيلي الظَاهرة في هذه الدراسة يُمكنُ أنْ تعزي إلي أن كل العينات قد جمعت من أشخاص يعانون من أعراض تلوث طفيلي ، كالإسهال وآلام في البطن ، وقد تم إحالتهم للمعمل للتأكد من وجود هذه الإصابة. ويمكن أن يعزي كثرة الإصابة بالطفيليات الأوليـــة ، إلـــي بساطة دورة حياتها ، وسهولة نقلها والإصابة بها ، خاصة في غياب التوعية الصحية العامة ، وتلوث مياه الشرب، وكثرة تناول الأطعمة في مطاعم الوجبات السريعة التي لا تـــتوفر بهـــا الشروط الصحية. أما فيما يتعلق بالعدد القليل للحالات المصابة بالديدان المعوية ، خاصة الديدان التي تنتقل عن طريق تلوث التربة ، فيمكن أن يعزي ذلك إلي عدم إستعمال مياه المجـــاري والمخلافات البشرية في العمليات الزراعية ، وعدم توفر درجة الرطوبة اللازمة في الـــتربة بمـــنطقة سرت . ويمكن أيضاً أن يعزي إلي عجز الطرق المعملية المستخدمة في هذه الدراســـة للكشف علي بعض أنواع الديدان الطفيلية مثل الدودة الدبوسية *(السرمية الدويدية)* التي تتطلب إستخدام طريقة الشريط اللاصق للكشف عليها.

ومـــن خـــلال نـــتائج هذه الدراسة نستنتج أن طريقة تركيز الراسب أفضل من طريقة التحضـــير المباشـــر فـــي الكشف علي الطفيليات المعوية ، الأمر الذي يستدعي إستخدامها في المعـــامل الطـــبية إذا كان المعمل يعتمد علي طريقة واحدة فقط ، وكذلك يتطلب إستخدامها مع العينات التي أظهرت نتيجة سلبية بإستخدام طريقة التحضير المباشر. الإهتمام بالتوعية الصحية للمواطـــنين وإيجـــاد بيئة صحية ومكافحة الحشرات التي قد تعمل كعائل وسيط لنقل الإصابات الطفيلية وتزويد السكان بمياه صحية صالحة للشرب.



الجماهيرية العربية الليبية الشعبية الإشتراكية العظمي

جامعة التحدي – سرت

كلية الطب

قسم الطفيليات

تقييم بعض الطرق المعملية المستعملة في تشخيص الطفيليات المعوية

بحث مقدم من الطالب / سالم رمضان علي السريتي

كجزء من متطلبات الحصول علي درجة الماجستير في علم الطفيليات

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2001 ف

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قدمت هذه الرسالة إستكمالاً لمطلبات الحصول علي الإجازة العالية "الماجستير" في علم الطفيليات

مقدمة من

سالم رمضان علي السريتي

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