Human Echinococcosis in Palestine-The West Bank

By

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Under the Supervision

Of

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This Thesis was defended successfully on the 19th April 2000 and approved by

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DEDICATION

To My Beloved Family With Love

ACKNOLEDGMENT

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Abstract

Based on hospital records 390 cases of human CE were recorded during the period of January 1990 to January 1998 with a mean annual surgical incidence of 3.1 /100,000. Such findings indicate that CE is an endemic and widespread disease in the West Bank of Palestine. The incidence varied widely between governorates and was higher in rural areas. Most cases were among the age group 11-21 years. The highest MASI was observed in Hebron (5.1/100,000) and the hottest spot was Yata town (16.8/100,000). Up to age 20, the surgical incidence was similar between boys and girls, however, in adults the incidence increased markedly in women and peaks at age group 21-30. Differences in incidence rates between males and females were significant (147 v. 243, $P < 0.005$). The liver was the most common affected organ (69.9%), followed by the lung (25.9%). In age group less than ten, the lung was the most affected site (59.6%). Recurrent cases constituted (16.7%).

Seroprevalence rate of CE IgG antibodies among children in highly endemic area (Yata town) was 2.75%. Such high rate was also confirmed using IHA and ELISA test in a reference immuno-parasitology laboratory at Yarmouk University.

CHAPTER I

Introduction

1.1 Introduction

Echinococcus (unilocular echinococcosis) is an infection caused by the larval stage of tapeworm (*Echinococcus granulosus*) whose eggs are transmitted to human by direct contact with infected dogs or other canines, by ingestion of soil, vegetables, or water contaminated by the feces of infected animals. It has a worldwide distribution and most frequently found in sheep and cattle raising areas of South America, South Africa, the Soviet Union, and the Middle East (Dar F. K, Alkarimi T. 1997).

The human infection is characterized by hydatid cysts (a larval tapeworm occurring in fluid filled sac) found mostly in the liver and the lungs but also in other organs. The incubation period varies from months to years and is often difficult to determine because the majority of infection does not present any symptoms.

The hydatid cysts filled with protoscolices, each has the ability to grow into adult worm when ingested by a canine host. The tremendous reproductive potential poses a problem in the intermediate host (particularly in human). If the cyst breaks open, each protoscolex could grow into a new hydatid cyst.

Hyadtid disease has many features, which render diagnosis difficult. These include absence of discharges in any stage of infection and the slow development and growth of cysts. Ultra sound, X-ray and computed tomography have been used for diagnosis in human, while these procedures are suitable for well-

developed cysts, they are costly and non-specific. Consequently, serology remains the only convenient method for diagnosis, especially at early stages of infection. Several sero-diagnostic techniques have been employed for the detection of disease in man. These include the Casoni intra dermal test (Schantz *et al.*, 1975), Indirect Heam Agglutination test (Schantz and Gottstein 1985), Enzyme Linked Immuno-Sorbant Assay (Hoida *et al.*, 1995) and many other tests.

The prevalence rate is determined by epizootiological factors related to the size of stray dog population and its worm burden and to the infection rates in the intermediate host reservoir, livestock population. Socio-economic development and socio-cultural practices are considered important in the continued transmission of the disease (Dar, F.K., Alkarmi, T. 1997).

Governmental Hydatid Control Campaigns are usually the body that can actively work towards the control of hydatid disease. The aim of such campaigns is to implement long term on going programs to arouse and maintain public awareness in order to achieve complete control and ultimate eradication of hydatid disease (Wilkinson, F. *et al.*, 1997).

In Palestine, epidemiological studies are limited; however, they seem to indicate that hydatid disease is an endemic disease in the area (Al-Yaman, *et al.*, 1987). Therefore, the present study aims to achieve the following objectives:

1- To determine the mean annual surgical incidence of hydatid disease in the West Bank. Such data is expected to provide more information about the epidemiology and spread of the disease in various localities.

2-To determine sero-prevalence of CE IgG antibodies among high-risk populations.

1. 2 Biology and Life Cycle of *Echinococcus granulosus*

Dogs and other Candis are parasitised by the adult tapeworm. Gravid proglottids disintegrate in the dog's intestine. The eggs passed in feces are highly resistant, being able to survive freezing and drying conditions on the ground for up to a year (figure 1).

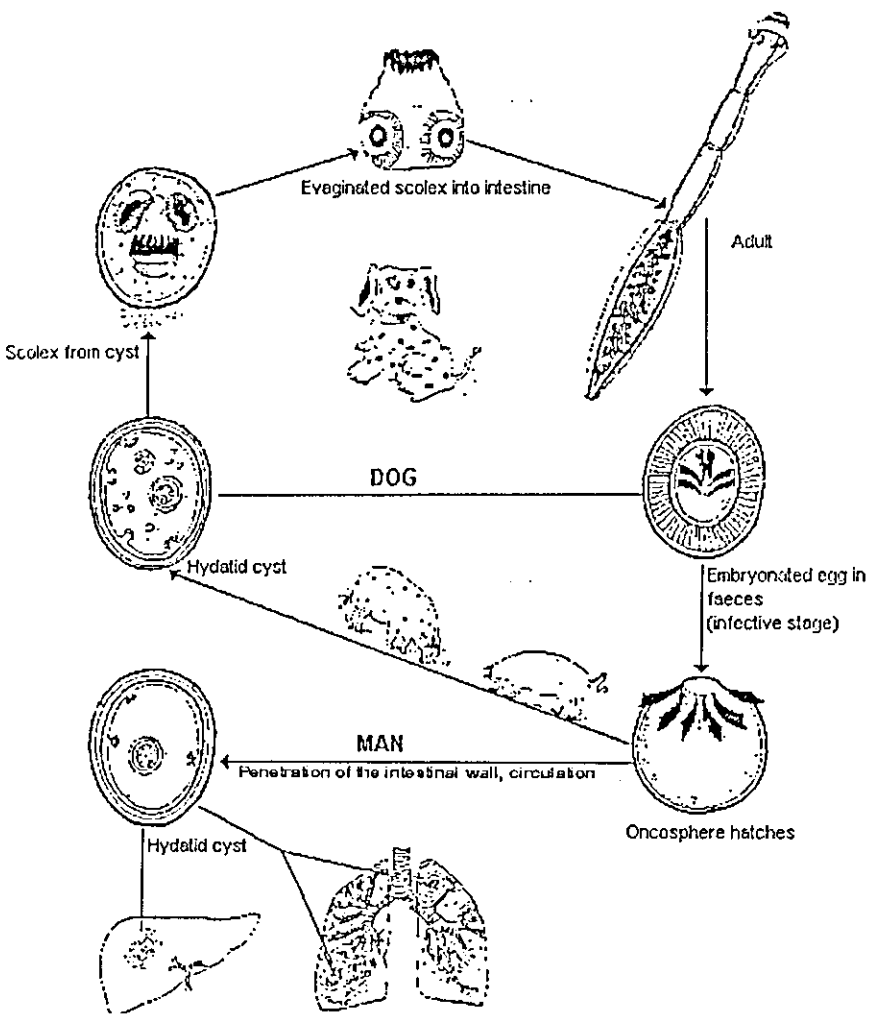

Fig. 1. Life cycle of ***Echinococcus granulosus***

Many mammals apart from man may act as intermediate hosts, in particular sheep and horses. The situation is highly complex, as at least 9 subspecies have been identified, all with different host specificity. For example; *E. g. granulosus* - adult form in most canids apart from the red fox, hydatids in sheep, pigs, cattle, man and many wild ruminants. *E. g. equinus* - adults in canids, hydatids in horses and other Equidae, but probably not man. *E. g. canadensis* - adults in canids, hydatids in caribou, reindeer and man. *E. g. borealis* - adults in canids, hydatids in many cervids and man. In addition, in parts of Kenya there is a strain or subspecies that is particularly adapted to transmission between man and domesticated dogs exist (Thompson, R.C., *et al.,* 1988). Other un-characterized strains or subspecies may also exist (Thompson, R.C., *et al.,* 1988). The egg enters the host by ingestion either from contaminated grass (infections of herbivorous ruminants), or in the case of man by contamination (dog licking face after it has been cleaning itself), or other examples of bad hygiene followed by transfer to the mouth. The egg then hatches in the intestine, penetrates the gut wall, and travels via the lymphatic or blood system throughout the body till they lodge within the body's tissues. The cysts may develop anywhere within its intermediate host's body, but as the circulatory blood stream passes from the mesenteric blood vessels to the liver, it is in the liver that the majority of the cysts (in about 65% of cases) are found. Next in frequency of infection is the lungs (~20%), brain (1%), peritoneal cavity (8%), kidneys (3%) and bone marrow or other organs. Development of the cysts to produce infective protoscolices takes approximately 1 to 2 years (Schantz 1982).

Following the death of the intermediate host, either directly by predation on the part of the dog, or by the scavenging of the dead cadaver, the protoscolices are able to survive in carrion for several weeks and the cyst is usually ingested along with the offal. The cyst wall is then digested, liberating the protoscolices which quickly evaginate, penetrating deeply into the crypts of Lieberkuhn, and developing to adult worms in approximately 7 to 9 weeks. Due to the presence of many protoscolices in each hydatid cyst, dogs may be infected with many *E. granulosus* tapeworms.

The larvae, these metacestodes (Hydatids) are large, roughly spherical, fluid filled hollow bladders containing numerous protoscolices (forming the so-called hydatid sand). Brood capsules and daughter cysts are identical in form to their parent cyst. The cyst wall itself consists of an outer laminated hyaline wall supporting the whole cyst. Beneath this there is a nucleated germinal layer, studded with developing brood capsules which may eventually break off to float freely in the fluid filled cyst. The protoscolices are formed within the brood capsules, which may rupture to give the free protoscolices in the hydatid fluid. They vary considerably in size depending on where in the body they form which may be almost any organ of the body. Those found in the liver (most common affected organ) may be approximately 20cm in diameter, but those found in the peritoneal cavity may sometimes be very much larger containing several litters of fluid.

The adult parasites in the dog represent one of the smallest of the tapeworms. They measure between 3 - 9 mm in length, and usually consist of only 3 proglottids: an immature, a mature, and

a gravid proglottid. The scolex is globular in shape and has a prominent rostellum armed with a double row of between 30 - 36 hooks. The eggs are very similar to those of the genus Taenia, and measure between 30 and 40μm in diameter (Thompson *et al.,* 1988).

1. 3 Pathology of Infection

In domesticated animals clinical signs appear to be uncommon, whilst in man they vary in their seriousness depending on where in the body the hydatid develops and how large it grows. Sometimes the infection is asymptomatic and the only evidence of infection being the presence of calcified cysts on autopsy due to an unrelated cause. The major pathology is due to the size of the cyst giving rise to pressure related injury. A complication may arise if the cyst is ruptured, possibly due to blows to the body, muscular strain, or during operations. In this case the contents of the hydatid cyst is released into the body's circulatory system, and the liberated protoscolices may give rise to numerous secondary cysts throughout the body. In addition the hydatid cyst fluid is highly allergenic and cyst rupture may result in anaphylactic shock and rapid death (Placer *et al.,* 1988).

Adult tapeworm is usually non-pathogenic to its canine hosts, although sometimes in very heavy infections there may be some inflammation of the intestinal wall.

1. 4 Immuno Diagnosis of Human Cystic *Echinococcosis*

Cystic *Echinococcosis* is among the few parasitic infections where the basis for laboratory diagnosis is primarily serology (Craig *et al*; 1995b). Imaging methods for detection of space

occupying masses (i.e; X-ray, Ultra sound, CT scan or MRI) are the primary approaches for clinical diagnosis of CE (Schantz and Cottstein, 1986; Sinnner, 1991). Even when cyst structures suggestive of E. granulosus (e.g., daughter cysts and laminated layer) can be imaged, conformation by serology is still frequently requested. On the other hand, in many cases characteristic cyst structure does not present as clear image or absent (Rogan, *et al.*, 1990). In the later cases immuno-diagnostic confirmation are extremely important.

1. 4. 1 Antibody Detection

Despite years of research and useful developments, there is still no standard, highly sensitive and specific serologic test for CE antibody detection. The most abundant detectable specific antibody in serum of human CE patients is of the IgG isotype. Studies on IgG subclasses in human CE showed significant increase in all subclasses and in particular IgG1 and IgG4. IgG3 is considered as the most discriminatory tool between CE and other non-CE patients (Hira, *et al.*, 1990). Such findings were confirmed by Wen and Craig 1994 as they report that subclasses 1 and 4 antibodies were predominant in the sera of advanced CE patients.

The Enzyme-Linked Immuno-Sorbent Assay (ELISA) is one of the best practical and sensitive methods for the measurements of antibody class and subclasses responses.

Antigen preparations from crude hydatid fluids are usually derived from sheep, cows and horses. Such antigens are most frequently employed in immuno-diagnostic tests. The fluid

represents a mixture of parasite derived secretion and excretion or degradation products and other host components, notably albumin and Ig. The high sensitivity of ELISA using these antigens may be at the cost of reduced specificity (Craig, R. 1986). For clinical confirmation of suspected individuals, a high degree of specificity is required. The most specific test is the arc5 precipitin test (Capron, *et al;* 1967) which has proved extremely useful for such cases (Varila–Diaz, *et al.,* 1975a; Pinon, *et al.,* 1987).

Gel precipitation of arc5 antigens is mostly taeniid specific, as cross reactivity appear to occur only with alveolar and poly-cystic hydatid cases (Moro, *et al.,* 1992). Arc5 precipitation is however, not very sensitive (50-60%) and particularly low for pulmonary cases (Rickard, 1984). Clinical serologic confirmation of CE antibodies should therefore, undertaken by combination of two or more tests, usually to include sensitive assay like ELISA or IHA as primary test with arc5 as confirmatory test.

More than 10 antigen are present in the hydatid fluid and the somatic tissue of the metacestode two of them originally demonstrated Ag5 and AgB (Capron, *et al.,* 1967) have the highest immuono-reactivity with patient sera and they have been characterized by SDS-PAGE and immuno-blotting.

SDS PAGE analysis of *Echinococcosis granulosus* hydatid cyst fluid has identified the main sub unit composition of Ag5 and B (Di Felice, *et al.*, 1986; Shepherd and McManus, 1987; Lightowlers, *et al.,* 1989). Under reducing conditions, 38-39, and 25 KDa sub units of the heat labile Ag5 and 8-12, 16, and 20-24 KDa sub unit molecules of heat stable antigen B (also

present under non reducing condition) were predominantly recognized by sera from proven CE patients. The 38 KDa sub unit of Ag5, though highly antigenic, appeared also to be recognized non-specifically by apportion of sera from heterologous infection and from normal controls (Shephard and McManus, 1987; Maddison, *et al.*, 1989). Western blots, however, recognizes the lower molecular weight sub-units of Ag B, more specifically. The 8 KDa sub unit of hydatid cyst fluid bound specific antibodies in 91% of proven CE patients, with false positive only for sera from alveolar (*E.multilocularis*) and ploycystic (*E.vogeli*) echinococcus cases, and small proportion of *T. solium* cysticercosis patients (Maddison, *et al.*, 1989).

There may be greater echinococcus specificity associated with recognition of the 16 and 20-24KDa bands, and also greater sensitivity (80%) for all B sub unit bands with hepatic CE compared to pulmonary cases, 56% (Verastegui, *et al.*, 1992). Using immuno-blot, Wen and Craig (1994) showed that Ag B sub units bound predominantly IgG4 antibodies, while those of Ag5 more reactive with IgG1.

1. 4. 2 Circulating Antigens and Immune-Complexes

Detection of circulating antigens and immune complexes seems to have additional benefit for laboratory diagnosis (Craig, 1986). Serum antigens is less affected by the location of hydatid cysts, thus should have greater correlation for post treatment surveillance compared to antibodies (Craig and Nelson, 1984).

Antigen detection usually depends on the binding to specific monoclonal or polyclonal antibodies to parasite antigen present

in serum or other fluids. Antigen assays must be as sensitive as possible, as concentrations of circulating antigens are usually low unless cyst rupture has occurred. Immune complexes are usually precipitated with polyethylene glycol.

1. 5 Transmission Patterns

Echinococcus granulosus in the Levant countries as well as other Middle Eastern countries belong to the European bio type of *E. granulosus* which became adapted to synathropic host as a result of animal husbandry practices (Raush, 1995).

The elucidation of transmission patterns in a focal, endemic, or highly endemic area requires the identification of the various definitive and intermediate hosts, their geographic distribution, and the prevalence of the disease in each. Various domestic ungulates may serve as intermediate hosts for *E. granulosus*, in the Levant sheep, goats, cattle, camels and donkeys have been repeatedly found infected with *E. granulosus*. However, the relative importance of each in perpetuation of the life cycle has not been carefully studied. In addition to the study of infection rates, examination of fertility and viability of hydatid cysts in each animal type must also be considered. Sheep are major intermediate host in all countries or area studied in Levant. Multiple, sometime massive, infection with cyst that localize predominantly in the liver and lungs have been described in Jordan (Al-Yaman, *et al.,* 1988; Kamhawi, *et al.,* 1995) and in Lebanon (Pipkin *et al.,* 1951). Old infected animal may show fertile cysts in the spleen and kidney as well as in the liver and lungs (Al-Yaman, *et al.,* 1987; 1988; Abdel-Hafez and Al-Yaman, 1989). In addition, increase age of ewes slaughtered has

proven significantly enhance the risk of transmission (Abdel-Hafez, *et al.*, 1986; Kamhawi, *et al.*, 1995).

Overall, goats showed a significantly lower infection rate than other animals (Abdel-Hafez, *et al.*, 1986 a, 1986 b; Kamhawi, *et al.*, 1995), this was attributed to the different feeding habits of goat (Cousi, 1951).

Variable infection rates of cysts have been reported for cattle, camels and equines in Levant countries with camel showing the highest rate of infection in Lebanon and Syria (Al-Yaman, *et al.*, 1987, 1988; Abu-Shehada, 1988; Kamhawi, *et al.*, 1995). However, their role in transmission of CE to human appear to be insignificant (Thompson, 1995).

1. 6 Cystic Echinococcosis in Palestine

Nehmias, *et al.*, (1993) reviewed reports of infection rates with *E. granulosus* in intermediate hosts in Palestine and Israel since 1922. As many as 80% of the -animal slaughtered in the country in 1922 were infected. Pipkin, *et al.*, (1951) reported the finding by Witenburg in 1933 of a 12% infection rate in cattle in Jerusalem. During 1927-1930, 7.4% of 141,246 slaughter animals were found infected. In 1935, infection rates of 4 - 37.5 % were reported among sheep, cattle and goats (Pipkin *et al;* 1951; Nahmias *et al.*, 1993). Subsequently, infection rates in slaughtered animals dropped to only 0.8-0.9%-infected livestock during the period 1954-55. This was attributed to the establishment of new laws such as implementation of abattoir surveillance, importation of frozen meat, and initiation of anti- rabies campaigns in which 43,000 stray dogs and 816 jackals were destroyed (Nahmias, *et al.*, 1993). Nehmias *et al.*,

(1991) reported an infection rate of 10% in sheep slaughtered in Yirka, an Arab- Druze village in northern Israel. Moreover, Furth *et al.* (1994b) reported an infection rate of 10.1% in 255 sheep compared to non-in 19 cows slaughtered in an abattoir from the same village. The majority of infected sheep came from the West Bank.

Palestinian Autonomy areas and Israel have undergone and are still going through major demographic changes, which had and will probably continue to have a considerable effect on the prevalence of CE in this region. Yarrow, *et al.*, (1991) and Nahmias *et al.*, (1993) gave accounts of the history of human CE in this area leading up to today's. The earliest record of human prevalence was of 129 cases reported by Schmidt from the Holly Land during 1922-1935 with an estimated annual incidence of 1/100000 (Yarrow, *et al*; 1991). The incidence of CE increased over the years reaching 5/100000 in 1959 (Yarrow, *et al.*, 1991) and as high as 53/100000 during 1980-1989 in Yirka, northern Israel (Goldsmith, *et al.*, 1991).

Comparative studies showed that the incidence among Arab Druze communities was 70 time higher than that of the native Jews. Within the Arab Druze communities, the incidence of CE was highest 45.9/100,000 and lowest in Muslims 7/100,000 with an intermediate incidence of 22.5/100,000 in Christians (Yarrow, *et al.*, 1991).

Published data on the prevalence of C.E in Palestine are limited. Studies on surgical records from Al-Makased Hospital in East Jerusalem, showed that out of 140 patients who have surgery from 1991- 95, the mean annual incidence was estimated at 1.76 /100,000. Thus, decreasing from 2.2 /100,000 in 1991 to

1.3 and 1.4/100000 in 1994 and 1995, respectively (Abdel-Hafez and Kamhawi, 1997).

1. 7 Treatment of Hydatid Cyst

Small calcified cysts in sero-negative patients need no treatment, however surgical treatment is most commonly used, as most cases do not respond to drug administration. Therapy consists of removal of cyst contents without contaminating the patient. Surgical resection of the cyst partial hepatoctomy has been the traditional treatment of echinococcal cyst of the liver. It is considered as an invasive procedure, which is not always possible or effective. It may increase the risk of dissemination of protoscolices and result in secondary echinococcosis (Ghilevich, *et al.*, 1980).

Pericystectomy or dissecting the cyst from the surrounding uninvolved parenchyma may be considered when hepatoctomy is not feasible. Bile and vascular leaks are major complications associated with this procedure.

More conservative approaches include complete evacuation of cyst contents followed by internal or external tube drainage. Marsupialization (suturing of the cyst wall to the abdominal wall) has been largely abandoned due to the high complication rate after cyst evacuation. Other therapeutic alternatives to surgery are now available; percutaneous aspiration of the cyst under ultra sound guidance followed by injection of a scolicidal agent has been successful in selected patients. This alternative is particularly attractive for patient with single small cyst of the liver or for patients in whom abdominal surgery would be poorly tolerated.

The benzimidazole compound is anti-helminthes that have been used successfully to treat in operable cases of echinococcosis. Albendazole appear to be superior to mebendazole and it appear more active that mebendazole (81.3% to 56.2%) considering liver, abdominal cysts (De Rosa, F.A *et al.*, 1995).

Combination of surgery with the use of benzimidazole derivative (mebendazole or albendazole) seems to prevent the development of protoscolices into hydatid cysts and allow cysts to dry and membrane to collapse if the drug is given before surgery (El-Mufti, *et al.,* 1993).

Toxicity of benzimidazoles is most commonly seen clinically as transient elevation of trasaminases, leukopenia, allergic reaction, therefore, treatment with benzimidazole requires constant medical supervision and regular monitoring of transaminases level and number of leukocytes.

1. 8 Economic Consideration

The cost of this disease in animals is expected to be high and in heavily affected areas may significantly influence the national economy; in this sense it must be understood that echinococcosis control may be more than simply an investment in health.

The cost of the disease in man should be determined by the price of surgical treatment plus hospital costs per patient (over approximately two months) and the pressure placed upon the hospital service. The patients also lose approximately four months working time with consequential loss of productivity. There are some deaths and varying degrees of permanent disability.

1.9 Control of the Disease

A control program for echinococcosis in Palestine requires political commitment and decisions: of fundamental importance is the establishment of a long-term surveillance program as an indication of progress. Experience in other countries (Gemmell, 1978) suggests four important functions for a control authority. The first concerns operational funding and it's planned expansion over many years. The second is the selection of personnel and their training in health education and other relevant matters. The third, requiring collaboration with other authorities, involves the collection and evaluation of baseline and subsequent surveillance data; the fourth concerns the direction of continuing education.

The measures to be taken in echinococcosis control arise from the life cycle of *echinococcus*. In most cases the dog is the final host of the parasite, man, sheep, goats, cattle and camels being intermediate hosts. Stray dogs are the principal of environmental contamination and so must be eliminated.

Domestic pet dogs may become infected and pass the infection to the family, particularly the younger children. These dogs must be examined regularly and treated as necessary, it is important to prevent dogs from eating offal and animal waste at municipal abattoirs, village slaughter houses and on farms.

Health education should be aimed at the general public, and special emphasis on school, using leaflets and the powerful instrument of radio and television.

CHAPTER II

MATERIALS AND METHODS

2. 1 Antigen Preparation

Antigen preparation was carried out as described by Moosa and Abdol Hafez, (1994) and were used for the detection of IgG anti-bodies for *E. granulosus* with certain modification. The following is a summary of this method.

Hydatid fluid was collected from fertile sheep liver cysts from the Municipality slaughterhouse of the City of Nablus. Cysts with viability more than 70% were used and the hydatid fluids were then pooled centrifuged at 2000 rpm for 30 minutes and the supernatant was lyophilized and stored at -20°C until used. One gram of lyophilized fluid was then dissolved in 4ml distilled water and dialyzed against phosphate buffer pH 7.2. The sample was then centrifuged at 25,000 rpm and total protein concentration was determined using the method Bradford. Protein final concentration was adjusted to 20µg/ml using Bicarbonate buffer pH 9.6.

2. 2 Enzyme Linked Immuno Sorbant Assay

This method is used for the detection of IgG antibodies for *E.g.* hydatid fluid antigen. The method was carried out as described by Moosa and Abdel-Hafez (1994). The following is a brief description of this method:

1. Flat bottomed plates (Greinerf ELISA, Nurtinger, West Germany) were coated with 100µl of (20µg/ ml CSHF antigen), that was diluted in 0.05M Carbonate Bicarbonate buffer pH 9.6.
2. The plates were kept over night at 4°C on an automatic rocker.
3. The wells were then washed three times with 0.05% Tween-20 in Phosphate buffer saline (0.15M, pH7.2).

4. Wells were then blocked with 100µl of 5% skimmed milk (Regilait, France) protein in PBS for one hour at room temperature.

5. Wells were then washed for three times as in step 3.

6. A 100µl of diluted sera (1:100) in 1-% Skimmed milk in PBS buffer was added to each well and the plates were then incubated for one hour at room temperature.

7. The wells were then washed as described in step 3.

8. Diluted (1:2000) horse radish peroxidase, conjugated rabbit IgG fraction against human IgG (Cappel, Durham NC, USA), was added to each well and the wells were incubated for one hour at room temperature.

9. The wells were then washed as described in step 3.

10. A 100µl of substrate solution, containing O-phenyl diamine (Sigma, St. Louis, USA), and H_2O_2 (C.B.H, U.K) in 0.1M Citrate buffer pH 4.5, was added to each well.

11. The plates were incubated at RT for 15 minutes and were read at 490nm using micro-ELISA auto reader (Dynatech, Torrance, California, USA).

Positive and negative control sera were included (2 positive and 6 Negative). The out side marginal well for each plates were excluded. All sera samples were tested in duplicates. Cut-off values = mean of negative control OD + 3SD

2. 2. 1 Buffers used for ELISA System

1. Carbonate-bicarbonate buffers 0.05M (5.3 gm $NaHCO_3$, 4.2gm $NaHCO_3$, dissolved in one litter D.W) adjusted to pH 9.6 and used as coating buffer.

2. Phosphate buffer saline 0.15M (NaCl 8gm, NaH_2PO_4 0.2gm, Na_2HPO_4 0.456 gm, KCl 0.2gm in 1L of D.W, adjusted to pH 7.2 using 0.1 M NaOH or KCl).

3. Phosphate buffer pH 7.2.

Solution A Na_2HPO_4 9.5gm/L

Solution B NaH_2PO_4 9.2gm/L

72.2 ml of solution A and 27.8 ml of solution B added to 900ml of D.W.

4- Citrate buffer (0.1M, pH4.5)

This buffer was made up of two solutions:

Solution A (0.1M) was prepared by dissolving 29.4 tri sodium citrate in IL of D.W solution B (0.1M) was prepared by dissolving 24g citric acid in IL of D.W then 23.3 ml of solution A was mixed with 26.7 ml of solution B to prepare citrate buffer. The pH was adjusted to 4.5.

5- PBS Tween-20 (0.05% PBS-T)

This solution was prepared by adding 0.5ml Tween 20 (Sigma St. Louis USA) in one litter of PBS. (0.15M pH 7.2)

6-Substrate solution

This solution contains 50 mg of OPD, 20 µl of 30% H_2O_2 in 50 ml of citrate buffer.

7- Skimmed milk-blocking solution (5% protein).

This solution made by dissolving 12.5gm Skimmed milk in 100ml PBS.

8. Skimmed milk (1% protein).

This solution was prepared by taking 10ml of 5% skimmed milk solution and added to 40ml PBS. This solution is used to dilute human sera and HRP.

2. 3 Melotest Echinococosis - EIA

Melotest Echinococosis is based on the principle of an enzyme immunoassay (EIA). The assay system utilizes an inactivated specific antigen, which is coated on the microtitration wells, and an anti human IgG antibody is contained in the antibody-enzyme (horseradish peroxidase) conjugate solution. Test samples and controls are allowed to react with the solid phase specific antigen. In the presence of specific antibodies, they will bind to the well surface. After 15 minutes incubation at room temperature, the wells were washed with the washing solution to remove unbound material. In the second step, the Anti-human IgG/ HRPO conjugate is added to the wells resulting in the specific antibody being sandwiched between the solid phase antigen and enzyme conjugate antibody. After 15-minutes incubation at room temperature, the wells were washed with the washing solution to remove unbound material. Substrate / chromogen solution is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of the stop reaction solution and the color turns yellow.

The yellow color is measured spectrophotometrically at 450nm. The concentration of specific IgG Antibody is directly proportional to the color intensity of the test sample.

Test Procedure

1. All reagents brought at room temperature before used.
2. Samples were diluted 1:25 using sample diluent (8µl of sample in 192µl of sample diluent). Controls included in the kit are already pre diluted and ready for used.
3. A 50µl of each diluted sample and controls were added to the appropriate well in the micro well plate.
4. The plates were incubated for 15 minutes at room temperature.
5. They were then washed 5 times using 300µl of the diluted washing solution.
6. A 50µl of conjugate solution was then added to each well.
7. The plates were incubated for 15 minutes at room temperature.
8. The plates were then washed as described in step 5.
9. A 50µl of substrate solution A (TMB) and 50µl of substrate solution B (H_2O_2) were then added to each well.
10. Plates were then incubated in the dark for 15 minutes at room temperature, and the reaction was stopped by the addition of 50µl of (H_2SO_4 2N).

The cut off value was calculated as follows:
Cut off value = The average O.D of the negative control + 0.15.

2. 4 Indirect Heamagglutination

The indirect heam-agglutination test (IHA) was performed as described by Moosa and Abdel-Hafez 1994.

2. 4. 1 Formalinization of Sheep Red Blood Cells

1- Sheep blood was collected and mixed with anti-coagulant (EDTA1g/L) to avoid clotting.

2-Blood was then washed in PBS 0.15M, pH 7.2, suspended in the same buffer to a final concentration of 7%. The samples were then centrifuged at 1500rpm for 15 minutes at 4°C several times to remove plasma and puffy coat.

3- When supernatant becomes clear, RBC's were then re-suspended in PBS (7%) and mixed with equal amount of 7% formaldehyde (40%) in PBS.

4-Blood samples were then incubated at 37°C for 1 hour with frequent gentle shaking.

5-A three-quarters of the rest of 7% formaldehyde in PBS were added to the blood and incubated for at least 24 hour at 37°C with gentle shaking in first few hours.

6-On next day, formalized SRBC were washed, 8-10 times with PBS, and centrifuged at 1500 rpm for 10 minutes each time.

7- The blood was re-suspend as 2.5% in PBS and then 0.02% sodium azide were added and finally the formalized blood was stored at 4°C. Samples can be stored for up to two years.

2. 4. 2 Tanning of Sheep Red Blood Cells

1. 1:40,000 solution of Tannic acid in PBS 0. 15M, pH 7.2 was prepared and 1:1 v/v of tannic acid solution was mixed with 2.5% formalinized SRBC.
2. This was then followed by incubation at 37°C for 15min and was then centrifuged at 900rpm for 15 min.
3. Tanned formalinized cells were washed 2 times with PBS and centrifuged for 20min each time.

4. The cells were then re-suspend to 2.5% concentration in PBS.

2. 4. 3 Coupling Tanned SRBC with antigen:

1- Crude Sheep hydatid fluid was diluted into 300µg/ml with PBS 0.15M, pH 7.2.

2- 2.5 % suspension of tanned cells solution was divided into two fractions: one without antigens but, diluted to 1:1 ratio of PBS (negative control for Ag's), and the second portion was mixed with 1: 1 v/v Ag's solution.

3- Preparations were then incubated in water bath at 37°C for 30min and were then centrifuged at 900rpm for 10 min followed by 2 times washing with PBS.

5- The cells were then re-suspend to 2.5% concentration in PBS-0.02 Sodium Azide solution and were stored at 4°C until used

2.4.4 Test Procedure

1- All tested sera were incubated in water bath at 56°C for 30 minutes.

2- The U shaped microtiter plates were used.

3- Sera was diluted into 1:16 (12.5 µl sera in 187.5 µl PBS, .pH7.2).

4- 50µ l of the diluted sera were taken to the first well, which contain 50µl of the coupled SRBC with the antigen.

5- Samples were diluted to 1:25.600

6-Incubate for 12-24 hour at 4°C on rocket shaker.

7-Positive and negative sera are included in each plate.

8-Positive sample will form latice.

The highest titer obtained when sera from patients with other parasitic infections were tested in the IHA was 1:64, which was considered to be the cutoff point for hydatid positive sera in this test.

CHAPTER III

A Retrospective Study of Surgical Cases of Cystic Echinococcosis in the West Bank of Palestine Between 1990-1997

3.1 Introduction

Cystic echinococcosis constitutes a major public health problem of global dimension. It is prevalent in most regions where sheep raising is a major industry and where there is increased contact between man and the dog definitive host of the parasite. The disease is endemic in many countries in the world including the Middle East (Mattosian, *et al.*, 1977; Schantz *et al*, 1995; Abdel-Hafez and Kamhawi, 1997). The most commonly used index of human infection is the annual incidence of hospital cases. Despite the limitation of this method, data on annual rates proved to be of great value for defining the public health importance, providing new information about the epidemiology of the disease, documentation or spread of the disease in various localities and identification of groups at risk. In northern Israel, a resurgence of cystic echinococcosis was noted beginning in the early 1970. Between 1960 and 1989, the mean annual surgical incidence rose five folds (Gold Smith, *et al.*, 1991). Analysis of the ethnic origin of the patients revealed that the great majority of cases occurred on member of the Arab Druze population. In Palestine, the earliest records on human incidence was 1/100,000 during the period of 1922-1935, and the incidence increased over the years reaching 5/100,000 during 1959 (Yarrow *et al.*, 1991). In 1995, surgical records from Al-Maqased hospital (Jerusalem) shows an estimated annual incidence rate of 1.76/100,000 (see Abdel-Hafez and Kamhawi, 1997). Determining infection rates in livestock has not been attempted so far.

The current study is a retrospective study of all surgical cases of CE recorded in West Bank hospitals between January 1990 to December 1997.

3.2 Source of Data and Analysis

Compared to most of the surrounding countries, Palestine is relatively small with a total population of 2.896 million, of which 1.873 millions reside in the West Bank area and the rest are in Gaza Strip (Palestinian Central Bureau of Statistics). The West Bank is divided into nine governorates, and most of these areas are served by governmental and private hospitals. In this study, a survey of all surgical hospitals in the area (5 private; Al-Itihad, Saint Lukes, Al Ahli, Al-Maqased, Augista, Victoria and 7 governmental; Rafeedia, Jenin, Tulkarem, Ramalla, Alia, Jericho, Al-Hussein). During the period January – September 1998, patients records were reviewed after a preliminary study on the suitability of the filing systems in these hospitals between January 1990-December 1997. Patients files and records were reviewed for confirmed surgical cases of cystic echinococcosis. As in all retrospective studies, some data were not available in the files examined, and consequently those records were not included in the epidemiological analysis. Thus, the present data represent the minimum baseline information for the surgical incidence of CE in the West Bank of Palestine. Data regarding place of residence, age, gender, previous history of disease and cyst location, number and size were recorded. The total number of major surgical operations was also deduced from hospital filing systems. The completed data was analyzed using SPSS software program.

3.3 Annual Surgical Incidence of Cases

A total of 390 surgically confirmed CE cases were found in the records of surveyed surgical hospitals of the West Bank of Palestine. The number of cases ranged between 29-63 pre year (Table 1). On the basis of a population estimate of the West Bank (1.873 million in 1997) and an estimated growth rate of 4.9% (source: Microsoft Encarta, 1998), the mean annual surgical incidence (MASI) ranged between 2.2-3.7 per 100,000, corresponding to 1990 and 1995, respectively. The overall MASI was 3.1 per 100,000 (Table 1).

Table 1. Annual Surgical Cases of Cystic Echinococcosis

Year	Population X 10^6	No.of CE Cases	MASI /100,000
1990	1.318	29	2.2
1991	1.386	41	3.0
1992	1.457	49	3.4
1993	1.532	46	3.0
1994	1.611	47	2.9
1995	1.694	63	3.7
1996	1.781	60	3.4
1997	1.873	55	2.9
	Mean - 1.582	Total - 390	mean 3.1

The Mean Annual Surgical Incidence was calculated on the basis of population estimate according to 1997 census. For the other years on the basis of annual growth rate of 4.9% (Microsoft Encarta, 1998).

The MASI for each governorate is shown in Table 2. Evidently, the MASI was highest in Hebron (5.1 per 100,000). Out of the 140 cases reported in Hebron governorate, 35 (25%) were for patients living in the town of Yata. This town had an estimated mean population during the 8year period of 26,000. Thus, the MASI of CE in this town was calculated at 16.8/100,000.

Table 2. Mean Annual Surgical Incidence of CE in the Governorates

Governorate	No. of Cases	Mean No.cases/year	Population X 10^3	MASI /100000
Hebron	140	17.5	342	5.1
Jericho	011	1.4	28	5.0
Bethlehem	045	5.6	116	4.9
Jenin	059	7.4	202	3.7
Ramallah	051	6.4	180	3.6
Nablus	037	4.6	261	1.8
Tulkarm	015	1.9	113	1.7
Qalqilia	005	0.6	61	1.0
Jerusalem	020	2.5	277	0.9
Un-known	007	0.9	---	---
Overall	390	48.8	1582	3.1

According to 1997 census with a growth rate of 4.9% (Microsoft Encarta, 1998)

Jericho and Bethlehem governorates showed similar MASI to that of Hebron (5.0 and 4.9/100,000, respectively). Jenin and Ramallah governorates also showed significantly high MASI rates of 3.7 and 3.6/100,000, respectively. The lowest MSAI was observed in Jerusalem and Qalqilia at 0.9 and 1.0/100,000, respectively.

Data presented in Table 3 shows the annual reported cases in the various governorates of the West Bank between 1990-1997. Evidently, Hebron population was the most affected accounting for 36.6% of all cases recorded. In addition, Jenin, Ramallah, Bethlehem and Nablus governorates showed the highest number of cases recorded during this period. Little fluctuations in the number of cases were observed in all governorates throughout most of the period of study. On the other hand, Qalqilia governorate did not have any CE patient during the last 4 years of study. All in all, a stable endemicity of the disease is observed in the region as a whole.

Table.3 Yearly Distribution of 383 Surgically Confirmed CE Cases in Patients from Various West Bank Governorates of Palestine.

Governorate	No of cases recorded during the study year									
	90	91	92	93	94	95	96	97	Total	%
Hebron	11	15	24	11	12	22	22	23	140	36.6
Jenin	7	8	0	10	10	9	9	6	59	15.4
Ramalla	2	4	11	5	8	10	7	4	51	13.3
Bethlehem	2	5	5	7	6	6	5	9	45	11.7
Nablus	1	2	3	5	4	9	8	5	37	9.7
Jerusalem	3	6	3	2	1	0	0	5	20	5.2
Tulkarm	1	0	2	1	1	3	5	2	15	3.9
Jericho	0	0	1	1	3	2	2	2	11	2.9
Qalqilia	1	1	1	2	0	0	0	0	5	1.3
All	28	41	50	44	45	61	58	56	383	100
%	7.3	10.7	13.1	11.5	11.7	15.9	15.1	14.6	100	

The origin of the other seven cases was not recorded.

3-4. Age and Sex Distribution of Surgical Cases

Age groups 11-20 and 21-30 were the most affected groups and constituted 27.4% and 21.5% of the total number of cases, respectively. Significantly lower rates (6.4-11.8%) were observed among the rest of the other age groups. Females constituted 62.3% of the total cases. The overall gender difference was statistically significant ($P< 0.005$). Evidently, CE is contracted at an early age of < 10 years as manifested in 12.1 of the total number of surgically confirmed cases. Moreover, up to 39.5 and 61.0% of the cases were seen in patients of 20 and 30 years or less, respectively. A further significant observation is the lack of gender difference in the number of cases during early ages of 20 years or less (Table 4). Beyond that age, more surgically confirmed cases were consistently observed among females than males.

Table 4. Distribution by Age and Sex of Recorded Human Cystic Echinococcosis Cases.

Age	Male	Female	Total
<10	23	24	047(12.1%)
11-20	52	55	107(27.4%)
21-30	30	54	084(21.5%)
31-40	09	37	046(11.8%)
41-50	06	19	025(6.40%)
51-60	08	33	41(10.50%)
> 60	19	21	40(10.30%)
Total	147	243	390(100%)

3-5. Location of Hydatid Cysts in Surgical Cases

The data presented in Table 5 show that the liver was the most common site of infection (58.2% alone and 11.7% in combination with other sites). The lung was the second mostly involved site of infection (20.8% alone and 5.1% in combination with the liver).

Table 5. Distribution of Cyst in Various Sites

Site	No of cases	Percent
Liver alone	227	58.2
Lung alone	081	20.8
Liver and Lung	020	5.10
Liver and Kidney	011	2.80
Liver and other organ	015	3.80
Kidney	003	0.80
Brain	004	1.00
Spleen	004	1.00
Other organ *	006	1.50
Un specified	019	4.90
Total	**390**	**100**

*(Ovary, Spinal Cord, Thyriod gland, Peritonial cavity, Diaphram, Mesocolon)

To investigate whether there is a delineation of lung cysts to be in younger age groups. The lung involvement in CE cases was calculated in the various age groups (Table 6). Evidently, younger

age groups manifested significantly higher percentages with lung cyst involvement than older ones. Up to 27.7% and 30.7% of the patients from the age groups 10 years or less and 11-20 years, respectively. A significant decrease in the number of patients with lung hydatid cysts was observed in the age groups over 30 years (Table 6).

Table 6. Reported Lung Hydatid Cyst among Age Groups

Age group	Lung	%	Liver and Lung	%	Total	%
<10	20	24.7	08	40	28	27.7
11-20	26	32.1	05	25	31	30.7
21-30	12	14.8	04	20	16	15.8
31-40	05	6.2	02	10	7	6.9
41-50	08	9.9	01	5.0	9	8.9
51-60	08	9.9	00	0.0	8	7.9
>60	02	2.4	00	0.0	2	2.0
Total	81	100	20	100	101	100

Regardless of locality of the cyst in the patient, single solitary hydatid cysts were encountered in 42.3% of the cases (Table 7). The occurrence of multiple cysts was significantly high in surgically confirmed cases, as they constituted one third of the total number of patients.

Table 7. Number of Recorded Cysts in the Studied Files

Number of Cysts	No. and % of Cases
Solitary	**165 (42.3)**
Multiple	**130 (33.3)**
Un known	**95 (24.4)**
Total	**390 (100)**

3.6 Recurrence and Cyst Size in Surgical Cases

Most of the patients (72.5%) in this survey had surgeries for the extraction of hydatid cysts for the first time. However, at least 16.7% of the cases had recurrences of hydatid cysts that warranted surgery (Table 8).

Table 8. Reported Recurrent Cases

	No of Cases	Percent
Recurrence cases	65	16.7
Single	283	72.5
Un-known	42	10.8
Total	390	100

Hydatid cysts that measured over 5cm in diameter were found in 69.7% of the cases whose cyst size was estimated. The largest recorded cyst was 20 x 20 x 20 cm in size. Small cysts with a mean diameter of less than 5cm constituted only 6.9% (Table 9).

Table 9. Recorded Cyst Size

Size	No of cases	Percent
Less than 5 cm	027	06.9
Greater than 5cm	272	69.7
Un- known	091	23.3
Total	390	100

3.7 Discussion

The present study on the surgical incidence of CE in the West Bank of Palestine represents the first comprehensive investigation in this area in recent years. The overall MASI of 3.1/100,000 as reported here (Table 1) represents an underestimation of the actual incidence for several reasons. First, this incidence does not take into account patients who underwent surgeries for CE in both Israel and Jordan. Secondly, not all patients infected with CE undergo surgery, as there are several a symptomatic cases for each surgically confirmed case. In this respect, sero-epidemiology and imaging techniques using ultrasound are better indicators of the actual incidence (Shambesh *et al*; 1999). Thirdly, hospital records may not account for all surgeries for CE due to poor or limited filing system.

In spite of the above-mentioned limitations of the present study, CE appears to be a significant endemic disease in the West Bank. The MASI was consistently high during the eight-year period of study ranging between 2.2- 3.7/100,000 (Tables 1,2). This incidence contrasts a lower MASI of 1.76/100,000 reported earlier for cases recorded at Al-Makased General Hospital in Jerusalem (see Abdel-Hafez and Kamhawi, 1997). It is similar to surgical incidences reported in nearby countries such as Jordan (2.9/100,000) and Lebanon (3.6/100,00) as well as other Arab countries such as Kuwait (3.6/100,000) (Scwabe and Abou-Daoud, 1961; Shweiki et al. 1990; Kamhawi, 1995). Localitywise, residents of Hebron, Jericho, Bethlehem, Jenin and Ramallah are considered at higher risk of contracting CE than the other localities of the West Bank (Table 2). The town of Yata in Hebron

governorates appears to be the hottest spot for CE with an MASI of 16.8/100,000. There is need to investigate the various parameters and conditions that rendered Yata town to have such high incidence. This applies to all of the Governorates that showed high incidence. Evidently, several of these governorates rely on agriculture and animal husbandry that rendered their population at higher risk of infection with *E. granulosus* than others. Knowledge, attitudes and practices of residents of these governorates warrant detailed investigation through questionnaires and interviews. Apart from Qalqilia governorate where no surgical case detection was recorded over the last 4 years of the study, all other governorates consistently showed case detection with little fluctuation during the study period (Table 3). This observation reinforces the endemicity or hyperendemicity of the disease in all these governorates.

In the West Bank, CE appears to be contracted during early ages as most of the cases were detected in the age groups 11-20 and 21-30 years. As the cysts are known to develop slowly over several years, early infection would lead to symptomatic disease during the above mentioned age groups. Another indication of early infection is the relatively high surgical rates with the very young age group of < 10 years (Table 4). At the young ages, no significant difference in surgical incidence was noted between males and females. However, more females were found infected throughout the ages beyond 20 years of age (Table 4). This can be explained on the bases of occupational roles that males and females play in Palestinian society. At this age and beyond,

Women are more exposed to the eggs of the parasite than men. This is consistent with observation made by Kamhawi (1995) and Abdel-Hafez and Kamhawi (1997) in Jordan and other Middle Eastern countries. A further evidence for the early exposure to the parasite comes from the significant number of lung cases detected particularly during the younger age groups under 20 years (Tables 5,6). It appears that patients contracting the infection during childhood are at higher risk of lung than liver involvement. The present data reinforces this observation which was also noted by Chaouachi *et al.*, (1989) among Tunisian children. The finding of 69.8% of the cases with cysts greater than 5 cm in diameter is an indicator of slow growth of the parasite to reach a symptomatic size that warrants surgical intervention. Late diagnosis and/or lack of awareness about the possible complications associated with the disease are also contributing factors to having cysts of large size. Evidence in support of such assumption can be deduced from the findings of three reported cases with trauma resulted in anaphylactic shock due to rupturing of cysts and the finding of a patient with hundreds of cysts distributed within the visceral cavity. Recurrent cases constituted 16.7%, which is higher, compared to reports from Turkey (Kama *et al*; 1998). This also supports the previous assumptions for late diagnosis and lack of awareness. The limited use of imaging techniques including CT scan and computer axial tomography, or misinterpretation of the results of such techniques may also account for such findings. Thus, the need for the use of better diagnostic techniques and health care to assume early diagnosis is essential.

Chapter IV

Prevalence of *Echinococcus granulosus* Among High Risk Groups

4. 1 Introduction

Cystic ecchinococcus (CE) is chronic zoonotic parasitic helminthes disease due to the infection with the larval stage (hydatid) of the small dog tape worm *Echinococcus granulosus.* The parasite has global distribution but is particularly prevalent in rural areas where it is transmitted in cycle between the dog definitive host and the sheep and other livestock as intermediate hosts. Human CE is often considered as an occupational public health problem for sheep farmers, ranchers, or shepherds in endemic areas (Cohen *et al.*,1997).

In the Middle East, *E. granulosus* is endemic and significant public health problem in several countries including Jordan, Iraq, Lebanon, Egypt, and most of Arabian countries in Northern Africa (Dar FK, Alkarmi T.1997, Abed-Alhafez and Kamhawi, 1997).

In 1991, Furth *et al.*, reported that the West Bank is a highly endemic area based upon their findings in the slaughterhouse of Yirka, an Arab-Druze village in Israel. Such suggestion was based on the fact that most of the infected animals came from the West Bank area. Hydatidosis is reported to be highly endemic in Jordan, where Abu-Shehada (1993) found 2.8% seropositive rate among school children. Highly endemic areas in Libya (Shambesh *et al.,* 1999), show seropositivity rate of 10.5% using AgB ELISA serology in age group 0-14 year, also in the same country (Gebreel *et al.,* 1983) found a seropositve of 8.38% in the EL Abair area and 12% in local ruler area.

In the West Bank of Palestine, our retrospective study revealed a MASI 3.1 /100,000), they also shows that Yata Town (Hebron governorate) has the highest MASI 16.8/100000.

In Palestine, traditional animal husbandry and the practice of domestic slaughter of animals contribute to the maintenance of *E. granulosus* cycle. Evidence from other countries suggests that changes in traditional practice may affect transmission dramatically and there is a need to study community cultural habits in order to define points, which might be pertinent in lowering the incidence of echinococcosis (Gebreel ; *et al* 1983).

Nothing is known about the prevalence of hydatid cyst in animals in Palestine. According to the 1996 census, about 1271 cattle, 270.825 sheep, 3.707 donkeys, and 1146 camels were reported in Hebron district. This represents 32.8 % of the domestic animals in the West Bank area (Ministry of Agriculture, Veterinary Department, 1996). The most obvious source of infection is sheep and dogs in rural areas, and stray dogs in urban or semi urban areas. Thus, contamination of pastures, by infected dog feces, is expected to occur continuously. Such situation is expected to increase the risk of infection for both humans and other animals through ingestion of contaminated food.

The present investigation focused on seroprevalence of C.E in Yata town, the hottest foci for the disease in the West Bank of Palestine. Moreover, lifestyles and practices of the town population, which led to a high surgical incidence, have been investigated.

4. 2 Study and Community

Palestine has an area of 27,000 Km^2, and the West Bank has an area of 5556 Km^2 and population of 1.873 million. The town of Yata has population of 30,800 according to 1997 census. The town is a rural area and most of its inhabitants raise domestic animals including sheep and cattle. Stray dogs, although their number is not estimated, are abundant in the vicinity of the town.

The data presented in chapter three, showed that in the West Bank population, ages < 20 years showed a high percentage of surgically confirmed cases. Indeed, age group 11-20 years showed the highest (27.4%) of all the surgical cases among all studied age groups. This finding stimulated further investigation to determine the seroprevalence among this age group in the town of Yata. For this purpose, two randomly selected schools were chosen from the town namely, Riqah girl school, and Yata primary school for boys. A total of 190 female and 137 male were included in this study. All students agreed to be apart of the sample. A specially designed questionnaire was used to collect demographic data pertaining to the practices and lifestyles of the inhabitance of Yata town as pertaining to CE. The questionnaire was also designed to obtain details of the relationship between human and dogs, and to determine retrospectively the number of previous surgical operation for CE in the student examined or their family members. Data regarding water supply was also included. The research worker fills each questionnaire as students were asked the questions.

A 2.5 ml venous blood samples were collected by vein puncture, sera were separated and stored at -20°C until used.

Sera samples were assayed for the presence of anti *E. granulosus* IgG antibodies using EIA through a commercial kit (Melo Kit). Seropositive samples were further confirmed using both ELISA and IHA tests with crude sheep hydatid fluid as target antigen. This was done at reference laboratory (immuno-parasitology Laboratory, Yarmouk University, Irbid, Jordan).

4. 3 Results

4. 3. 1 Behavior and Lifestyle of Town Inhabitants

Based upon the data presented on the questionnaire answered by 203 female from Yata town about family practices, 60% of the students came from families that raise domestic animals and 29.4% have dogs at their homes. Stray dogs were reported in the vicinity of the town by 75.1% of the students Table 10.

Table 10. Percentage of Students who Answered the Questions Pertaining to Behavior and Life style at Yata Town.

Animal raising	60.4%
Dogs	29.4%
Stray Dogs	75.1%
Family throws dead animals in the open	80.3%
Family burns dead animals	8.3%
Family bury dead animals	11.4%
Drinking water from municipality pipes	34.8%
Drinking water from rain water collecting wells	60%
Drinking water from springs	5.2%

Percentages were based on a questionnaire filled after interviews of 208 female students. Those who did not give definite answers were excluded.

Regarding the method of handling dead and or infected animals, 80.3% of the students reported that their families threw dead animals and offal of animals which is not suitable for human consumption in the open (table 10). Only 8.3% and

11.4% of the students report that their families bury or burn dead animal or contaminated offal, respectively.

With respect to water supply, 60% of the student's families depended on collected rainwater and wells, as a main source of household water supply, while 34.8% depend on water supplied by the municipality and 5.2% depend on water collected from nearby springs.

4. 3. 2. Seroprevlence

Tables 11 and 12 show the seroprevalence of anti hydatid cyst IgG among 327 blood samples collected from 137 male and 190 female students whose ages ranged between 7-14 years. A commercial ELISA kit (Melo Tec) Spain was initially used to assess seroprevalence and confirmation of results was carried out in a reference laboratory in Yarmouk University, Irbid - Jordan utilizing the ELISA and IHA techniques. In all tests males showed a high seroprevalence than females (Table 11). Using the Melo commercial kit, a seroprevalence of 2.9 and 2.6 was observed among males and females students, respectively. Using the ELISA test at Y.U the seroprevalence drop to 2.19 and 2.1 among male and female students, respectively. The over all seroprevalence was 2.75, 2.14 and 0.61 using the Melo kit, Y.U.ELISA, Y.U.IHA, respectively.

Table 11. Serodiagnosis of Student Samples from the Town of Yata .

		No. and % of seropositive cases					
		Melo. ELISA		Y.U.ELISA		Y.U. IHA	
Gender	No.tested	No.	%	No.	%	No.	%
M	137	4	2.9	3	2.19	1	0.73
F	190	5	2.6	4	2.1	1	0.53
Both	327	9	2.75	7	2.14	2	0.61

Table 12. Correlation Between Seropositive Serum Samples of Students Using Three Different Tests.

Sample No.	Gender	Melo. ELISA	Y.U, ELISA	Y.U. I.H.A
1	F	±	+	–
12	F	+	–	–
67	F	+	–	–
141	F	±	–	–
173	F	+	+	–
188	F	±	–	–
233	F	+	+	+1:800
282	F	+	+	–
288	M	+	+	+1:800
317	M	±	–	–
321	M	+	–	–
337	M	+	+	–
349	M	±	–	–
351	M	+	+	–
Total		9	7	2

± Equivocal

Table 12, shows that out of 327 assayed blood samples (190 female and 137 male), 9 sera samples were positive and 5 were with equivocal values using the EIA (Melo Test). ELISA and IHA were used as Confirmatory tests (Yarmouk University, Jordan). ELISA revealed that only 6 out of our 9 positive samples were positive and one of the 5 equivocal was also identified as positive. Indirect Heam Agglutination Test showed that only 2 of the 9 positive sera (Yarmouk University) were positive by this method, however, none

of the equivocal samples was positive. Non of seronegative samples assayed by Melo. Kit was positive in either of the used confirmatory tests.

4.4. Discussion

The present investigation is the first to report on the sero-epidemiology of hydatidosis in an endemic area of the West Bank. Both the ELISA and IHA are known to be sensitive tests for the detection of antibodies in serum of patients with cystic hydatid disease. Sensitivity rates vary from 60 to 90%, depending on the characteristic of the cases (Lightowlers, *et al*; 1995). Crude hydatid cyst fluid is generally employed as antigen. At present, the best available serologic diagnosis is obtained by using combination of tests EIA or IHA for screening purposes. Although, immunoblot assay or one of the varieties of gel diffusion assays that demonstrate the echinococcal Arc5 or antigen B subunits are used as confirmatory tests, they usually give false positive reaction with the sera of patient with cysticercosis and with alveolar echinococcosis (Verastegui *et al*; 1992). Since such parasites are not endemic in Palestine, one should exclude the possibility of false positive affecting our findings.

In the present study, utilizing the ELISA, a seropositivity rate of 2.75 among age group7-14 years was obtained. Differences in seropositivity rates among male and female students were of no significance (P = 0.876). The fact that seropositivity among male students slightly exceeds that of the female is a reflection of greater exposure of male to eggs of the parasite than female in this age group. This finding is in agreement with the findings of Abdel-Hafez (personal communication) in Jordan. Why males show higher seropositivity to CE require careful analysis of habits and duties carried by both sexes in the town. Occupational, social habit practices and poor

hygiene principal associated with children habits may account for such slightly higher rate of infection. It noteworthy, that certain families have more than 18 family members, as polygamy is common. Such situation is most likely to be associated with poor hygiene habits due to over crowding. The abundance of stray dogs in the vicinity of residential areas (Table 10) and the poor hygiene levels go hand by hand to explain such high seroprevalence. In this context, the infection rate of dog's with E. granulosus in a representative sample of stray dogs in Yata town is of great importance. This will reflect accurately the degree of endemicity of the disease in this area. Necropsy or detection of coproantigens in the dog fecal samples can answer the actual prevalence of E. granulosus in stray dogs.

The differences in the seropositivity rates obtained by the ELISA, using the commercial kit, and Y.U can be attributed to differences in the preparation of the detecting antigen. Also, it should be noted that the Melo kit was the first used to test the sera for anti hydatid IgG antibodies. The sera were freshly prepared. In contrast, the same sera were carried to Y.U from the West Bank. It may be that some activity was lost in some of samples as they were transferred from the West Bank to Jordan. Moreover, it is difficult to explain why the IHA result dropped the seroprevalence to only 0.61 in which two sera samples were positive. Sera sample from a surgically confirmed case was used and scored a very high positivity in all employed tests.

Five of the seropositive samples came from families with a long history of animal raising specially, sheep. Three of them have sheep dogs and all of them reported to see stray dogs in their residential areas. Three reported that their water house supply

depends on rainwater collected in wells, and non-of them report the occurrence of the disease in any of their family members. Such findings raise and emphasize the strong association between animals and transmission of the disease to humans. However, such data require further investigation.

Recommendations and Suggestions for Further Studies on Echinococcosis in Palestine

1- To determine the prevalence of infection and distribution of echinococcosis among animals and man and the relationships between them.

2- To ascertain whether animal / human echinococcosis is caused by a particularly virulent strain or sub species of *E.granulosus.*

3- To study the role of water-borne infection: in water holes, pools, and storage tanks.

4- To carry out surveys in all alleged endemic areas.

5- To discover whether unknown reservoir exist.

6- A control program for echinococcosis in Palestine requires political commitment and decisions: of fundamental importance is the establishment of a long-term surveillance program as an indication of progress.

7- As in most cases the dog is the final host of the parasite and stray dogs are the principals of environmental contamination, so they must be eliminated.

8- Domestic pet dogs may become infected and pass the infection to the family, particularly the younger children. These dogs must be examined regularly and treated as necessary.

9- It is important to prevents dogs from eating offal and animal waste at municipal abattoirs, village slaughter houses and on farm.

10- Health education should be aimed at the general public, and special emphasis on school, using leaflets and the powerful instrument of radio and television.

Abdel-Hafez ,S. K. and Al-Yaman, F. M..(1989). Spleen hydatidosis in sheep from North of Jordan. *Veterinary Parasitology* 30:191-196.

Abdel-Hafez, S.K., Kamhawi, S.A.(1997). Cystic Echinococosis in the Levant countries (Jordan, Palestinian Autonomy, Israel, Syria, and Lebanon). Compendium on Cystic Echinococcosis in Africa and in Middle Eastern Countries with Special Reference to Morocco. (eds) Anderson, F.L. Ouhelli, H., Kashani, M. Brigham Young University.

Abdel-Hafez, S. K., and Said, I. (1986a). Further studies on prevalence of Hydatidosis in slaughtered animal from North Jordan. Zeitschrift fur Parasitenkunde 72:89-96.

Abdel-Hafez, S. K; Said, M. I. and Al-Yamman F. M.(1986b). Comparative aspects on the fertility and viability of hydatid cysts in sheep from North Jordan. *Japanese Journal of Parasitology* 35:491-496

Abu-Shehada, M.N. (1993). Research note. Some obsarvation on Hydatidosis in Jordan.Journal of Helminthology. 67, 248-252.

Abu-Shehada, M. N.(1988). Prevalence of hydatidosis in donkeys from central Jordan. *Veterinary Parasitology* 30:125-130.

Al-Yaman, F. M; Abdel-Hafez, S. K and Saliba. E, (1988) Hydatidosis: Global and local importance. part 11. Hydatidosis in Jordan. *Jordan Medical Journal* 21:119- 132.

Al-Yaman, F. M; Abdel-Hafez, S. K and E. Saliba, E, (1987) Hydatidosis: global and local importance part 1: Global importance. *Jordan Medical Journal* 21:119-132.

Bonifacino, R; Malgor, R; Barbeito, R; Balleste, R; Rodrigeuz, M.J; Botto, C. and Klug, F.(1991). Seroprevalence of *Echinococcus granulosus* infection in a Uruguayan rural human population. *Transaction of the Royal Society of Tropical Medicine and Hygiene.* 85, 769-772.

Capron, A; Vernes, A. and Biguet, J.(1967). Le diagnostic immunoelectrophoetique de l'hydatidose.'Le Kyste hydatique du foie'.In Journaux Lyonnaises d' Hydatidologie, SIMEP, Lyon, France, p.27-40.

Chaouachi, B; BenSalah, S; Lakhoua, R; Hammou, A; Garbi, H.A. and Saied, H. (1989). Hydatid cysts in children. Diagnostic and therapeutic aspects. Apropos of 1195 cases. *Annals Pediatrics*, Paris, 36 (7):441-444, 447-449.

Cohen, H; Paolillo, E; Bonifacino, R; Botta, B; Parada, L; Cabrera, P; Snowden, K; Gasser, R; Tessier, R; Dibarboure, L; Wen, H; Rogan, M.T; Craig, P.S.1998. Human Cystic Ecchinococcosis in Uruguayan Community: Sonographic, Serologic, and Epidemiologic study. . *American Journal of Tropical Medicine and Hygiene.*59 (4) . pp.620-627

Cousi, D.(1951). Lechinococcose en Tunisie. Afrique Francaise Chirurgicale. 4:379-386.

Craig, P.S.(1986). Detection of specific circulating antigen, immune complexes and antibodies in human hydatidosis from Turkana (Kenya) and Great Britain, by enzyme -immunoassay. *Parasite Immunology* 8:171-188.

Craig, P.S, and Nelson, G,S.(1984). The detection of circulating antigen in human hydatid disease. *Annals of Tropical Medicine and Parasitology* 78:219-227.

Craig, P.S; Rogan, M.T. and Allan, J.C.(1995b). Hydatidosis and cysticercosis-larval cestodes. In Medical Parasitology- a practical approach. Oxford University Press, IRL series, p.209-237.

Craig, P.S; Zeyhle, E. and Romig, T. (1986a). Hydatid disease: research and control in Turkana.11. The role of immunological techniques for the diagnosis of hydatid disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 80:183-192.

Dar F K., Alkarmi T.(1997). Public health aspect of cystic *Echinococcosis* in the Arab Countries. *Acta Tropica*, 15; 67(1-2): 125-32.

Derosa, A.,Teggi, A., Lastilla, M.G. (1995). Our experience in treatment of hydatid cyst with Mebendazole and albendazole. International congress of hydatidology. Abstract number A102.Limassol-Cyprus.

DiFelice, G; Pini, C.; Afferni, C; and Vicari, G. (1986). Purification and partial characterization of the major antigen of *E.granulosus* (antigen5) with monoclonal antibodies .*Molecular and Biochemical Parasitology* 20:133-142.

El-Mufti, M., Kamag, A., Ibrahim, H., Taktuk, S., Swaisi, I., Zaidan, A., Sameen, A., Shimbish, F., Bouzghaiba, W., Hassi, S. (1993). Albendazole therapy of hydatid disease :2 year follow-up of 40 cases. *Annals of Tropical Medicine and Parasitology* 87:241-246.

Furth, M. J. (1994b). The development of new foci of *Echinococcosis* in northern Israel: prevalence in domestic animals. *Journal of Helminthology* 68:45-47.

Gebreel, A.O; Gilles, H.M. and Prescott, J.E.(1983). Studies on the sero-epidemiology of endemic disease in Libya.*Echinococcus* in Libya.

Annals of Tropical Medicine and Parasitology, Vol. 77, No.4, 391-397.

Gemmell, M.A. (1978).Prespective on options for Hydatidosis and Cystecercosis control. Veternary Medical Review. 1, 13-48.

Ghilevich, Y.S.(1980). Recurrence of hydatid disease. Vestink Khirurgii (Russian) 124:39-45.

Goldsmith, R., Nahmias, J., Schants, P., Peleg, H., Shtamler, B., El-On, J.(1991). Resurgence of hydatid disease (echinococcosis) in comminutes in Northern Israel. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 85:89-100

Hira, P.R; Bahr, G.M; Shweiki, H.M. and Behbehani, K.(1990). Diagnostic value of anti arc5 IgG antibody and analysis of IgG sub classes in sera of patient with cystic hydatid disease. Sero-diagnosis and Immunotherapy in Infectious disease 4:285-293.

Hoida G; Furth M; Malshy Y; Greenberg Z; Craig P.S; Schantz P.M. and El-On, J.(1995). Prevalence of E.granulosus in human and animals in Temra, Israel.XV11. International congress of Hydatidology.

Kama, N.A; Sahin, M; Gomen, E; Bayrak, M; Kolaoclu, H; Akat, A. (1998). The Result of surgical techniques in hepatic Hydatidosis : Treatment with drainage versus treatment with out drainage - a 6 year experience. *J. R. coll. Surg. Edinb.* 43 PP 254-256 .

Kamhawi, S. (1995) A retrospective study of human cystic echinococcosis in Jordan. *Annals of Tropical Medicine and Parasitology.* 89: 409-414.

Kamhawi, S. and Abdel-Hafez S. K.(1995). Cystic echinococcosis as a major public health problem in Jordan. XV11 International congress of Hydatidology, Limassol, Cyprus. Abstract No.A17.

Kamhawi, S; Hijjawi, N.; Abu-Ghazaleh, A. and Abbass, M.(1995). Prevalence of hydatid cyst in livestock from five regions in Jordan. *Annals of Tropical Medicine and Hygiene* 89:621-629.

Leggat, G.R; Yang, W. and McManus, D.W.(1992). Serological evaluation of the 12 KDa sub unit of antigen B in *Echinococcus granulosus* cyst fluid by immunoblot analysis.*Transactions of the Royal Society of Tropical Medicine and Hygiene* 86:189-192.

Lightowlers, M.W, Gottstein, B. (1995). *Echinococcus* -hydatidosis: Antigens, immunological and molecular diagnosis. In: Thompson RCA, Lymberg A.J.,(eds). *Echinococcus* and hydatid disease. Wallingford, U.K: CAB International, 355-410.

Lightowlers, M.W; Liu, D; Haralambous, A. and Rickard, M.D (1989). Sub unit composition and specificity of the major cyst fluid antigens of *Echinococcus granulosus* . *Molecular and Biochemical Parasitology* 37:171-182.

Maddison, S.E; Slemenda, S.B; Schantz, P.M, Fried, J.A., Wilson, M. and Tsang, V.C.W. (1989). A specific diagnostic antigen of Echinoccus granulosus with an apparent molecular weight of 8 KDa. *American Journal of Tropical Medicine and Hygiene* 40:377-383.

Moosa, R. A; and Abdel-Hafez, S. K (1994). Serodiagnosis and seroepidmiology of human unilocular hydatidosis. *Parasitology Research* 80:664-671.

Moro, P.L; et al (1992). Immunoblot (Western blot) and double diffusion (DD5) tests for hydatid disease cross-react with sera from patient with cysticercosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 86: 422-423.

Motossion, R.M., Rickard, M.D. and Smyth, J.D.(1977).Hydatidosis: A global problem of increasing importance. *WHO Chronicle* 55: 499-507.

Nahmias, J, R. and Goldsmith, N., Schantz, P; Siman, M; El-On, J. (1994). High prevalence of human hydatid disease (*echainococcosis*) communities in northern Israel: Epidemiologic studies in the town of Yirka. *Acta Tropica* 50:1-10.

Nahmias, J; Goldsmith, R; Greenburg, Z. and Elon, J.(1993). Hydatid disease in Israel. *Harefuah* 124:529-534.

Pinon, J.M, (1987). Value of isotype characterization of antibodies to *Echinococcus granulosus* by enzyme linked immunofiltration assay. *European Journal of Clinical Microbiology* 6:291-295.

Pipkin, A. C; Rizk, E and Balikian, G. B.(1951). *Echinococcus* in the Near East and its incidence in animal hosts. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 45:253-260

Placer, C; Martin, R; Sanchez, E, Soleto, E. 1988. Rupture of abdominal hydatid cysts. *British Journal of Surgery*. 75:157.

Rausch, R. L. (1995) Life cycle pattern and geographic distribution of *Echinococcus* species. In *Echinococcus* and hydatid disease, Thompson, R.C.A. and Lymbery, A.J. (eds.). CAB International, Wallingford, U. k; p.89- 134.

Rickard, M.D.(1984). Serological diagnosis and postoperative surveillance of human hydatid disease. 1. Latex agglutination and immunoelectrophoresis using crude cyst fluid antigen. *Pathology* 16:207-210.

Rogan, M.T., Morris, D.L., Pritchard, D.I and Perkins, A. C.(1990). . *Echinococcus granulosus*: the potential use of specific radio-

labelled antibodies in diagnosis by immuno-scintigraphy. *Clinical and Experimental Immunology* 80:225-23.

Schwabe, C.W; and Abou-Daoud, K. (1961). Epidemiology of Echinococcosis in the Middle East:1.Human infection in Lebanon, 1949 to 1959. *American Journal of Tropical Medicine and Hygiene* 10:374-381.

Shambesh, M.A; Craig, P.S; Macpherson, C.N.L; Rogan, M.T; Gusbi, A.M. and Echtuish, E.F. (1999). An extensive ultrasound and serologic study to investigate the prevalence of human cystic Echinococcosis in Northern Libya. *American Journal of Tropical Medicine and Hygiene:* 60(3).pp 462-468.

Schantz, P.M., Chai, J., Craig, P.S., Eckert, J., Jenkins, D.J., Macpherson, C.N.L., Thakur, A. (1995). Epidemiology and control of hydatid disease. Thompson, R.C.A. and Lymbery, A. (eds.). In *Echinococcus* and hydatid disease, Wallingford: United Kingdom: CAB International, p.233-331.

Schantz, P.M. and Gottstein, B. (1986). Echinococcosis (hydatidosis). Immunoserology of parasitic disease. Vol.1: Helminthic disease, K.F.Walls and P.M.Schantz (eds). Academic Press, New York, p.69-107.

Schantz, P.M and Gottstein, B.(1985). *Echinococcus* (Hydatidosis). In immunology of parasitic disease. K.F.Walls and P.M.Schantz (eds).New York: Academic press.

Schantiz, P.M. (1982). Echinococcosis. In CRC handbook series in zoonoses, section C, J.H.Steel (ed.). Parasitic zoonoses. CRC press, Boca Raton, Florida, USA. 1:231-277.

Schantz, P.M; Ortiz-Valqui, R.E and Lumbercas, H; (1975). Non specific reactions with the intradermal test for hydatidosis in

person with other helmenth infection. *American Journal of Tropical Hygiene*. 24:849-852.

Shepherd, J.C; and McManus, D.P.(1987). Specific and cross reactive antigens of *Ecchinococcus granulosus* hydatid cyst fluid. *Molecular and Biochemical Parasitology* 25:143-154.

Shweiki, H.M., Hira, P.R. and Behbehani, K.(1990). Cystic hydatid disease: aspects of the incidence in man in Kuwait, Arabian Gulf. *European Journal of Epidemiology* 6:15-19.

Sinner, W.N. Von. (1991). New diagnostic signs in hydatid disease: radiography ultrasound, CT and MRI correlated to pathology. *European Journal of Radiology*12: 159-159-.

Thompson, R.C.A, Lymberg, A.J and Constanine, C.C.(1995). Variation in *Echinococcus*: towards a taxonomic revision of the genus. *Advances in Parasitology* 35:145-176.

Thompson, R.C.A; Lymberg, A.J.(1988). The nature, Extent and Significance of Variation within the Genus Ecchinicoccus. *Advances in Parasitology*. (Academic press, New York) Vol. 27,pp 210-258.

Varela-Diaz, V. M; Guisantes; J. A.; Ricardes, M. I;.Yarzabal, L. A and Coltorti, E. A. (1975a). Evaluation of whole and purified hydatid fluid antigens in the diagnosis of human hydatidosis by immunoelectrophoresis test. *American Journal of Tropical Medicine and Hygiene* 24:298-303.-

Verastegui, M., Moro, P., Guevara, A., Rodriguez, T., Meranda, E., Gilman, R.H.(1992). Enzyme-Linked immuno electro-transfer blot test for diagnosis of human hydatid disease. *Journal of clinical Microbiology*.30:1557-1561.

Wen, H; Craig, P.S. (1994). ImmunoglobinG subclass responses in human cystic and alveolar *Echinococcosis. American Journal of Tropical Medicine and Hygiene* 51:741-748.

Wilkinson, F.C; Scherender, S.(1997). Hydatid Disease: Biology and Control.

Yarrow, A; Slater, P. E; Gross, E. M. and Costin, C. (1991). The epidenuiology of echinococcosis in Israel. *Journal of Tropical Medicine and Hygiene.* 94:261-267.

الملخص

في دراسة ميدانية لملفات أقسام الجراحة في مستشفيات الضفة الغربية تم العثور على ٣٩٠ حالة إصابة لمرض الأكياس المائية الكلابية وذلك في الفترة الزمنية ما بين كانون ثاني ١٩٩٠ ولغاية كانون ثاني للعام ١٩٩٨. بناء على ذلك فقد كان معدل الإصابة السنوي ٣,١ لكل ١٠٠٠٠٠ مواطن. وتدل هذه النتائج على ان هذا المرض من الأمراض الوبائية ذات الانتشار العالي نسبيا في الضفة الغربية. وقد لوحظ كذلك بان هنالك فروقا ذات قيم إحصائية بين المحافظات المختلفة وقد كانت أعلى نسبة للإصابة في محافظة الخليل (٥,١ لكل مائة ألف) بالتحديد في بلدة يطا (١٦,٨ لكل مائة ألف).

ولقد كانت اكثر الإصابات في الفئة العمرية ما بين ١١-٢١ عام، وكانت نسبة الإصابة متماثلة ما بين كل من الذكور والإناث وذلك في الفئات العمرية لغاية ٢٠ عاما بينما وجدت زيادة ملحوظة للإصابة لدى الإناث في الفئات العمرية التي تزيد عن ذلك. وقد كانت الفروقات بين كلا من الذكور والإناث ذات قيم دالة إحصائيا. وقد بينت الدراسة كذلك بان الكبد هو من اكثر الأعضاء إصابة (٦٩,٩ %) في حين كانت الإصابة في الرئات بنسبة ٢٥,٩% وتتناسب هذه النسب مع ما هو مذكور عالميا. ومن الجدير بالذكر

بان الإصابات الرئوية احتلت أعلى النسب (٥٩,٦%) في الفئة العمرية ما دون عشرة سنوات. وتبين كذلك بان تكرار الإصابة قد بلغ نسبة ١٦,٧%.

بناء على النتائج السابقة وبالتحديد التي لوحظت في بلدة يطا فقد قمنا بدراسة عينات مصلية في الفئة العمرية ٧-١٤ سنة بهدف تحديد نسبة تعرض هذه الفئة للإصابة وذلك بالاعتماد على وجود المضادات لانتجينات هذا الطفيلي وذلك من خلال استخدام طريقة ال ELISA وتم تأكيد النتائج من خلال مختبر مرجعي في جامعة اليرموك. وبينت هذه الدراسة أن ما نسبته ٢,٧٥% من الأطفال كانت نتائج امصالهم إيجابية بالنسبة للمضادات من نوع IgG.