Sudan University of Science and Technology
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Echinococcus Granulosus of Camel Origin: Experimental Infection and Immunodiagnosis in Dogs

المشوكة الحبيبية من الابل: الاصابة التجريبية والتشخيص المصلي في الكلاب

By

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Dedication

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Abstract

The present study was designed to experimentally study the development and infectivity of *Echinococcus granulosus* of camel origin in the definitive host the dog in the area of Tambool, Butana Province, Sudan. Also to evaluate the potentiality of immunological technique,Enzyme linked immunosorbant assay (ELISA) in the diagnosis of echinococcosis in the definitive host.

The development of *E granulosus* of camel origin was studied in seven dogs experimentally infected with protoscolices originating from hydatid cysts isolated from camels *(Camelusdromedarius*). Dogs were necropsized 15, 21, 28, and 35 days post-infection (p.i.); all dogs had a low worm burden (12-37 worms). At day 35 p.i., majority of the parasites had developed three segments, while the embryophore of egg was not detected and the oncosphere was not completed.

The results of post mortem examination for sixteen dogs shot by the police in Tambool town, showed that 5 dogs were found harboring *E. granulosus* adult worms. From these dogs, 8 were found infected with *Taenia hydatigena*. Mixed-infection with *E. granulosus* and *T. hydatigena* was observed in only one dog.

When tested by ELISA out of these 16 Sera, 7 were found positive. Four out of five dogs which were positive at postmortem were positive at ELISA test. Only one dog which was found positive at postmortem was found negative by ELISA. Two dogs negative for E. granulosus and positive for T. hydatigena were found positive by ELISA test. One dog negative at postmortem for both, *E. granulosus* and *T. hydatigena* was positive of ELISA.

Sera collected from dogs experimentally infected with E. granulosus were negative for ELISA until the last week of the experiment (35 days post infection). Although the readings of sera from experimentally infected dogs were below the cutoff point,

we have observed that the antibody titre of the samples was slightly increasing during experiment.

The results suggested that the use of ELISA in the diagnosis of canine echinococcosis could be used successfully in epidemiological surveys of echinococcosis in Sudan.

The detection of *E. granulosus* in dogs at Tambool area was found 25% (5 out of 20 dogs were found harboring adult worms at postmortem). The high prevalence reported in dogs is an indication of hazard to human in this area.

A mass dog eradication programme with a mass drug treatment programme of house dogs, coordinated with an educational campaign, could be effective to control the disease if implemented in this area.

الملخص

صممت الدراسة الحالية لدراسة تطور ومدى قدره المشوكة الحبيبية (Echinococcus granulosus)مصدرها الإبل في عدوي العائل النهائي ,الكلب ,في منطقة تمبول ، محافظة البطانة ، السودان. وأيضًا لتقييم إمكانية استخدام(اختبار الأليزاELISA)في تشخيص داء المشوكات في المضيف النهائي.

تمت دراسة تطور*Echinococcus granulosus* من أصل إبل في سبعة كلاب مصابة تجريبياً بالبروتوسكوليسيس من أكياس عدارية معزولة من الإبل.(*Camelusdromedarius*)تم تشريح الكلاب بعد 15 و 21 و 28 و 35 يومًا من الإصابة. وجد ان؛ كل الكلاب لديها اصابة منخفضة بالدودة (12-37 دودة). في اليوم 35 عقب الاصابة ، طورت غالبية الطفيليات ثلاث قطع ، بينما لم يتم الكشف عن الجنين في البيضة ولم يكتمل الغلاف الخارجي.

وأظهرت نتائج تشريح ستة عشر كلبا في مدينة تمبول ، أنه تم العثور على 5 كلاب تأوي الديدان البالغة من نوع *E. granulosus*. من بين هذه الكلاب ، تم العثور على 8 مصابة بـ *Taenia hydatigena*. لوحظ وجود عدوى مشتركة*E. granulosus* و*T. hydatigena* في كلب واحد.

عندما تم اختبارها بواسطةاختبار الأليزامن بين 16 مصل ، تم العثور على 7 إيجابية. أربعة من خمسة كلاب كانت إيجابية عند التشريح كانت إيجابية في اختبارالأليزا

تم العثور على كلب واحد فقط كان إيجابيًا عند التشريح سلبيًا بواسطة اختبارالأليزا .كما تم العثور على كلبين سلبيين لـ*E. granulosus* ولـ*T. hydatigena* عند التشريح كانا موجبين بواسطة اختبار الأليزا.

كلب واحد سلبي عند التشريح لكلا الدودتين ، *E. granulosus*و*T. hydatigena*كان إيجابيا للالأليزا.

الأمصال التي تم جمعها من الكلاب المصابة تجريبياً ب*E. Granulosus*كانت سالبة لـالأليزاحتى الأسبوع الأخير من التجربة (35 يوم بعد الإصابة). على الرغم من أن قراءات الأمصال من الكلاب المصابة تجريبياً كانت أقل من نقطة القطع ، فقد لاحظنا أن عيار الجسم المضاد للعينات كان يزداد قليلاً أثناء التجربة.

أشارت النتائج إلى أن استخدام في تشخيص داء المشوكات في الكلاب يمكن استخدامه بنجاح في المسوحات الوبائية لمرض المشوكات في السودان.

وجد ان نسبة الاصابة في الكلاب في منطقة تمبول بلغت 25% (5 من كل 20 كلاب وجدت مصابة بالديدان البالغة عند التشريح). معدل الانتشار المرتفع في الكلاب هو مؤشر على وجود خطر على الإنسان في هذه المنطقة.

يمكن أن يكون برنامج الاستئصال الجماعي للكلاب مع برنامج العلاج الجماعي للكلاب المنزلية ، بالتنسيق مع حملة تعليمية ، فعالاً في السيطرة على المرض إذا تم تنفيذه في هذه المنطقة.

Introduction

Echinococcosis is an important zoonotic parasitic disease caused by a tapeworm of the genus *Echinococcus* (family taenidae), and is one of the most important helminthic diseases throughout the world (Altinta.2003), (Budke*, et al.,* 2006).

Like all tapeworms the life cycle involves two animals. A carnivore is the definitive host where the adult worms live in the intestines and almost any mammal including humans can be the intermediate host, where the worms form cysts in various organs. These cysts contain the larvae (protoscolices) are most located in the liver or lungs. The disease is called hydatid cysts infection or Hydatidosis in the intermediate host specially in human. Some sings are poor growth, reduced quality and yield of milk and meat, fertility and the value of fleece is reduced due to infection (Budke *et al*., 2006).

In humans, the disease can be severe occasionally fatal and the treatment is expensive and difficult. In carnivorous (definitive host) the disease is known as Echinococcosis. There are five currently recognized species of the genus *Echinococcus* which are regarded as valid taxonomically: *Echinococcus granulosus* (Batsch1786). *Echinococcus multilocularis* (Leuckart, 1863) *Echinococcus oligarthrus* (Diesing, 1863), *Echinococcus vogeli* (Rausch and Bernstein1972) ; Rausch 1975) and 1995) ; Thompson *et al*. 1995) and *Echinococcus shiquicus* (Eckert and Thompson 1997); Xiaon *et al* 2006).

Justification:

A better knowledge of the camel strains of *E. granulosus* which circulate in our country will help to understand the epidemiology of the disease. Thus, in order to adapt a control strategy, it is necessary to study the interaction between this strain and its host. Therefore, the aim of this study was to determine the infectivity,

morphological and developmental characteristics of the adult stages of *E. granulosus* of camel origin in experimentally infected dogs.

The present study was also, proposed to obtain some base line information concerning the epidemiology of echinococcosis in dogs in a selected area (Tambool, Butana Province, Sudan).

Also to evaluate the efficacy of the ELISA technique in detecting dogs with *E. granulosus* infection, in an endemic area of Sudan, and comparing serological data with parasitological data obtained from the dogs at necropsy.

Objectives of this study:

1. To study the development of *E. granulosus* in dogs infected experimentally with hydatid cysts isolated from camels.
2. To determine the prevalence of *E. granulosus* infections in stray dogs in Tambool area.
3. To evaluate *E.granulosus* adult worm as a source of antigen, to be used in Enzyme Linked Immunosorbent Assay (ELISA).
4. To assess the efficacy of indirect ELISA test in the diagnosis of echinococcosis in dogs, using the necropsy technique as standard.

Chapter one

1-Literature review

1.1. Taxonomy of the Genus Echinococcus

At the present time four species, *Echinococcus granulosus, E. multilocularis, E.vogeli and E. oligarthrus* are considered to be valid taxonomically. The status of a fifth species*, E.cruzi,* has been in doubt (Rausch *et al*(1978). However,(Rausch *et al*., (1984) compared paratype material of *E. cruzi* with the two species, *E. vogeli* and *E. oligarthrus*, and found that *E.cruzi* was synonymous *with E. oligarthrus*.

The taxonomy of the genus Echinococcus at both specific and subspecific levels has been a matter of controversy and some confusion. The current situation has been comprehensively reviewed (Thompson and Lymbery,(1988); Eckert and Thompson, (1988); Thompson *et al*., (1994). A total of 16 species and 13 subspecies have been described in the genus Echinococcus, but only the four species referred to the above, have been recognized as valid taxonomically. The reasons for dismissing the remainder as taxonomic entities have been discussed in depth (Rausch,1967; Thompson and Lymbery,1988; Thompson *et al.,*1994).

***Echinococcus granulosus* (Batsch, 1786).**

Classification:

Echinococcus granulosus was classificated as follows:

Kingdom	Anamalia
Sub/Kingdom	Metazoa
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Taeniidae

Genus Echinococcus

Genus Echinococcus:

Species granulosus

Currently there are six species recognized within the genus Echinococcus (Jenkins *et al.,* 2005), these are *E. granulosus, E. multilocularis, E. vogeli, E. oligarthus, E. ortleppi and E. equinus*. Seventh species, *Echinococcus shiquicus*, was recently described (Xiao *et al.,* 2005) based on morphology, host specificity and molecular characteristics (Pearson *et al.,* 2002; McManus and Thompson, 2003). The *E.granulosus* complex is divided into three species and eight defined strains. The present recognition of echinococcus species reflects a series of largely host adapted species that are maintained in distinct cycles of transmission (Thompson,(2001); Thompson and McManus, (2002). These are characterized by the principal intermediate hosts which are; sheep, horses, cattle, camels and different species of rodents. Although these cycles of transmission may overlap in some geographical areas, the parasites involved have been shown to maintain their genetic identity (Thompson *et al.,*(1995); Thompson and McManus, (2002); McManus and Thompson , (2003); Haag *et al,*. (2004). *Echinococcus granulosus* is the most widely distributed species which exists as a series of genetically distinct strains/genotypes, some of these genotypes are likely to warrant species status in the future, particularly those in pigs, camels and cervids (Harandi *et al*.,(2002); Thompson and McManus, (2002); Lavikainen *et al*., (2003). However, more researches are required to determine their host and geographic ranges and whether their genetic characteristics are conserved between different endemic regions (Jenkins *et al.,(*2005).

Although the most frequent strain associated with human cystic echinococcosis (CE) appears to be the common sheep strain (G1), other strains such as the Tasmanian sheep strain (G2), camel strain (G6), pig strain (G7/G9) and cervid

strain (G8) occur in a significant number of cases in some locations. *E. ortleppi* and *E. equinus* were previously characterized as cattle (G5) and horse (G4) strains of *E. granulosus* respectively (Le *et al*., 2002); McManus *et al.,* 2002); Thompson and McManus, 2002). *E. multilocularis* species recognition occurred as late as 1953 when the parasite and its life cycle in foxes and rodents were described by(Rausch, 1954) and Vogel, 1957).

Although a number of *E. multilocularis* isolates have been described from different geographical areas, their genetic variability remains undetermined (Jenkins *et al.,* 2005). The description of *E. vogeli* and *E. oligarthus* was relatively recent (Thatcher and Sousa, 1966; Rausch and D, Alessandro,1978).

1.2. Biology of *E. granulosus*:

Echinococcus granulosus is found in the small intestine of carnivores (particularly the dog) and the metacestode (hydatid cyst) is found in a wide variety of ungulates and man (Soulsby, 1968).

The parasite has a cosmopolitan distribution. Adults are 2-7mm long and usually possess three or four proglottids (rarely up to six). The penultimate proglottids are mature and the terminal proglottid is gravid and is usually about half the length of the worm. The rostellum has two rows of hooks. The ovary is kidney –shaped. Genital pores alternate irregularly and normally open in the posterior half of the mature and gravid proglottid (Soulsby, 1968).

The uterus of the gravid proglottid has well-developed diverticulitis. The gravid proglottid usually disintegrates in the intestine so that only eggs and not proglottids are found in the faeces. The eggs are typical taeniid eggs and measure 32-36 by 25-30µm (Soulsby, 1968).

1.3. Geographical distribution and prevalence :

Echinococcus granulosus has a world wide geographic distribution and occurs in all continents. There are not many countries in the world where cystic

echinococcosis (CE) has not been recorded (Macpherson and Craig, (2000). *Echinococcus granulosus* is found in Africa, Europe, Asia, the Middle East, the Mediterranean and Central and South America. Highest prevalence is found in populations that raise sheep. In North America, *E. granulosus* is rarely reported in Canada and Alsaka, and a few human cases have also been reported in Arizona, New Mexico ,California and Utah in sheep-raising areas (Craig *et al.,(* 2007a).

Distribution of (CE) is the most wide spread disease due to adaptation of echinococcus strains to a wide variety of host species and repeated introduction and movement of domestic animals throughout the world had made possible its broad geographical distribution (Schantz *et al.,* 1995).

In Europe, zoonotic members of the *E. granulosus* complex have been reported in every country with the exception of Ireland, Iceland and Denmark. They are most intensely endemic in the Mediterranean areas and parts of Eastern Europe such as Bulgaria (Torgerson and Budke, (2003).

In the UK, the parasite has a restricted distribution, being found mainly in mid and southern Wales. In Asia, the parasite is endemic in large parts of China and is an important reemerging zoonosis in the former Soviet Republics in Central Asia (Torgerson *et al*.,(2002b). Echinococcus are also found throughout the Indian Subcontinent and the Middle East. In USA, the Echinococcus are very sporadic with just a few foci such as certain communities in Utahand California. In South America the parasite is extensive, particularly in Argentina, Uruguay and Peruvian Andes. In Australia, the Echinococcus is common due to a sylvatic cycle between dingoes and wallabies with over 25% of dingoes and up to 65% of macropod marsupials infected (Jenkins and Morris, 1995; Jenkins, 2002). Risk factors for human infection include uncontrolled dogs living closely with people uncontrolled slaughter of livestock and unsanitary living conditions.

Cystic Echinococcsis remains highly endemic in pastoral communities, particularly in regions of South America, the Mediterranean littoral, Eastern Europe, East Africa, the Near and Middle East, Central Asia, China and Russia, with several millions of humans infected. It is responsible for approximately1% admissions to surgical wards in some countries such as Iran. It is estimated that CE results in annual economic losses of several billion dollars in livestock sector due to low performance, morbidity and/or mortality of infected animals, and condemnation of infected organs of slaughtered animals.

Due to the lack of representative and well documented data from many countries, only an incomplete and preliminary picture of the geographical distribution of echinococcosis can be provided (Eckert *et al.,* 2001).

The incidence of the disease is particularly high in rural areas, where there is increased contact between man and dog. Human (CE) is often considered an occupational public health problem for sheep farmers, ranchers and shepherds in endemic areas (Cohen *et al.,* 1997). *Echinococcus granulosus* is the species most widely distributed throughout the world and occurs in all continents including circumpolar, temperate, subtropical and tropical zones (Craig *et al.*, 1996; Eckert *et al.*, 2001; FAO, 1982). It causes serious public health problems in certain parts of the world (Schantz, 1990).

The wide variety of animal species that can act as intermediate hosts and the domestication and spread of some of these animals from Europe to other parts of the world have given the parasite a worldwide distribution. High parasite prevalence is found in the Mediterranean region, Russia, China, Africa (northern and eastern) and South America (Eckert *et al.,* 2001; Menghebat *et al.*, 1993); McManus and Smyth, 1986). It is also common in parts of the United Kingdom, Europe and Australia (Cook, 1989); Schantz, 1990); Arambulo, 1997); Eckert *et al.,*2000); Lightowlers *et al.*, (2000). In some European countries the annual

incidence (IA) rates of hospital cases of cystic echinococcosis (CE) vary between <1.0 and > 8.0 per 100,000 populations. In China, the average hospital cases were 8.7 per 100,000 in 1990. High incidence rates or prevalence have also been recorded from countries in northern and eastern Africa (prevalence up to >3%) and in South America (example: Uruguay) of 6.5 per 100,000 populations in 1997. A few islands are free of *E. granulosus* (Iceland, Greenland) or "temporary free" as New Zealand, Tasmania and southern Cyprus. Hydatid cysts were recovered to be 35.2% of camels in Iran. The organ distribution of cysts was 49.4% in lungs alone, 30.0% in both liver and lungs, 14.6% in liver only and 6.0% in other organs. Therefore, the lungs were the predominant sites of the hydatid cyst. The range in the number of cysts was 1-48 in infected animals. There was a direct relationship between the rate and intensity of infection and host age. The fertility rate of lung cysts (69.7%) was higher than that of liver cysts (58.7%) and other organs (50.0%), while the viability rate of protoscolices of liver fertile cysts (80.3%) was significantly higher than that of lung cysts (55.8%) and other organs (57.1%) (Ahmadi, 2005).

In Hadhramout, Yemen, the prevalence was 3.21% in sheep and 2.13% in goats (Baswaid, 2007). All the positive animals were males. The liver was the predominant site of infection in both animals. In relation to the age-group (years) the rate of infection increased with the animal's age. There was a slight difference in the fertility of the cysts in sheep (46.6%) and goats (55.2%), while the same observations were noted between liver and lungs in the two animal species.

1.4 Hydatidosis In Sudan:

In Sudan, animal echinococcosis was first reported in 1908 in a camel slaughtered in Khartoum (Anon 1908). AbdelMaliek, 1959) made a chick list of the parasites of domesticated animals in Sudan, including a single case of liver hydatidosis in cattle, 4 cases of *E. granulosus* in dogs and 3 cases in camels. Likewise, (Eisa *et*

al., 1962) reported a serious zoonosis in Southern Sudan that affected 21% of the cattle examined in Equatoria and upper Nile province. The authors revealed that only 31.3% of the hydatid cysts were fertile but other were calcified. Similarly about 33% and 9.4% infection rates amongst sheep and goats were reported, respectively (Eisa *et al.,* 1962). Another survey carried by Eisa 1962) in Malkal and Rank district revealed over all infection of 2.7% among cattle. Cahill *et al.,* 1965) conducted serological study on 152 individual in Southern Sudan. The study result indicated a positive rate of 13.3% of hydatidosis cases. He noted that 50% of human cases of hydatidosis that occurred in Uganda were immigrants from Southern Sudan. Elkhawad *et al.,* 1976) survey the helminthes parasite of cattle, sheep and goats in Southern regions of Sudan and documented a prevalence of 6.2% and 9.5% in cattle and sheep, respectively. In central Sudan a study by (Eisa *et al.,*1977) revealed a prevalence of 6% of *E. granulosus* in dogs. Elkhawad *et al.,* 1979) reported infection rates of 25%, 12% and 10.3% among cattle, sheep and goats of Kordofan in Western Sudan, respectively. New information was collected on cystic echinococcosis in livestock (camels, cattle and sheep) and humans in the central region of Sudan (Elmahdi *et al.,* 2004). The livestock data were collected in abattoir-based surveys in the towns of Omdurman, Tambool and Wad Madani, between 1998 and 2001, and covered a total of 8205 animals. The highest prevalence of infection was found in the camels (44.6% of 242 infected), followed by the sheep (6.9% of 5595) and cattle (3.0% of 2368).

A survey of cystic echinococcosis in livestock was conducted by(Omer *et al*., 2010) in central, western and southern Sudan. Hydatid cysts were present in 59% (466/779) of camels, 6% (299/4893) of cattle, 11% (1180/10,422) of sheep and 2% (106/5565) of goats, with little variation among different geographical areas. A number of 532 of these cysts were examined by PCR and could be over whelming (98.7%) allocated to *Echinococcusc anadensis* G6/7 (all of 215 cysts from camels,

112 of 114 cysts from cattle, 134 of 138 cysts from sheep, and all of 65 cysts from goats); the genotype G6 was identified by sequencing 13 of these isolates. Only 2 cysts from cattle belonged to *Echinococcus ortleppi*. The mean number of cysts per infected animal was much higher in camels (5.1) than in the other species (1.0–1.3), and cyst fertility was higher in camels and cattle (74% and 77%) than in goats and sheep (31% and 19%). Fertile cysts from five human patients from hospitals in Khartoum and Juba belonged to *E. canadensis* (G6). The authors confirmed the predominance of the 'camel strain' in Sudan and the infectivity of this strain for humans.

In another study by (Elmahdi *et al.,* 2013) they found that, all isolates (158) in Nyala and Tambool (areas in Sudan) genotyped were clearly demonstrate similar band pattern reported for G6/7 genotypes and G5 genotypes. With both molecular techniques used for genotyping in this study (RFLP-PCR & specific G5/6/7 PCR) 156 *E. granulosus* isolates were G6 genotypes and only 2 isolates were found to be G5 genotypes (*E. ortleppi*). *Echinococcus canadensis* G6 was found to be the most prevalent Echinococcus species of stray dogs in the study area (Tambool&Rofaa) are present first report in Sudan of *E. granulosus* (sheep strain) (Elmahdi *et al.,* 2013).

1.5 Morphology:

1.5.1 Adult

The adult worm consist of three part : the head, neck or middle segment and mature and gravid segment. The adult worm varies between 2 mm-7mm in length (rarely up to 11 mm) and usually possesses three or four segments (rarely up to six). In head, the scolex has four muscular suckers and a rostellum with two rows of large and small hooks (Thompson and McManus, 2001), they are tightly inserted into the crypts of Lieber-kǜhn in the gut mucosa. The rostellum contains a

rostellum gland which secretes a substance, which appears to be a lipoprotein (Smyth, 1964 b).

The penultimate segment is mature, and the genital pore normally opens posterior to the middle of both mature and gravid segments. The gravid uterus is characterized by well-developed lateral sacculations (Thompson and McManus, 2001, 2002). The second type is a fully developed adult which is composed of immature, mature and gravid segments; the number of segments in fully developed adults never exceeds three. The characteristics of *E. shiquicus* are summarized in, together with other related morph-species. The strobila of *E. shiquicus* adults is extremely small and its rostellar hooks are smaller than those of the other species. The location of the genital pore and the number of eggs in gravid uterus are also useful for differentiating *E. shiquicus* from the other species. (Xiao *et al.,* 2005).

1.5.2Eggs:

The eggs closely resemble those of other Taenia species found in dogs from which they were considered to be morphologically indistinguishable (Eckert *et al.,*(2003). But this problem has been solved by (Craig *et al.,* 1986) with the development of a specific Echinococcus anti–oncospheral monoclonal antibody, which specifically identifies Echinococcus eggs.

Eggs are ovoid (30 - 40 um) each consisting of keratinized embryophore which gives the egg a dark striated appearance.

Echinococcus eggs contain an embryo that is called an oncosphere or hexcanth. The name of this embryo stems from the fact that these embryos have six hooks. The eggs are passed through the feces of the definitive host and the ingestion of these eggs lead to infection in the intermediate host (John and William, 2006).

1.5.3 The metacestode of *Echinococcus granulosus:*

Metacestode stage is a fluid-filled bladder usually unilocular but communicating chambers also occur .The cyst wall consists of an inner germinal or nucleated layer

supported externally by a tough, elastic, a cellular laminated layer of variable thickness, surrounded by a host-produced fibrous adventitial layer. Typically, E. *granulosus* produces a single-chambered unilocular cyst in which growth is expansive by concentric enlargement. Asexual proliferation of the germinal layer and brood capsule formation takes place entirely endogenously. Pouching of the cyst walls may occur giving rise to secondary chambers communicating with the central cavity. Sometimes, the central cavity may be partly separated from the secondary chambers by incomplete septa. Occasionally, cysts may abut and coalesce, forming groups or clusters of small cysts of different size. In some hosts, particularly man, where unusually large cysts may develop, daughter cysts may form within the primary cyst (Thompson and McManus, 2001, 2002)

Filled bladder surrounded by inner cellular layer (germinal layer) from where brood capsules arise through inward budding in the inner surface of these brood capsules, protoscoleces develop by asexual reproduction reaching number as high as hundreds or thousands in one single hydatid cyst (Thompson and McManus, (2001); Diaz *et al.,* (2011). Protoscolices are only present in fertile cyst and each has the potential to evolve into a sexual mature adult parasite. They are invaginated immature forms of the adult worm and already present hooks and four suckers. Released protoscolices and ruptured brood capsules precipitate in the hydatid fluid creating hydatid sand (David de Morais,(1998). The structure of metacestodes is a key element to distinguish between different species of *Echinococcus*. In *E. granulosus* the hydatid cyst is a single chamber which grows concentrically. Although some incomplete septa may develop, separating the primary cyst from secondary chambers, the metacestod is unilocular and asexual reproduction occurs internally (Thompson and McManus, 2001). The outer surface of the germinal layer is covered with microtriches which are microvillus that increase contact area, enhancing the nutrient uptake and other metabolic exchanges between the parasite

and the intermediate host. This layer is also responsible for the synthesis of an external cellular layer that separates the hydatid cyst from the host tissue. This laminated layer has about 3 mm thickness in *E. granulosus* and protects metacestode against host immune system (Diaz *et al.,* (2011).

1.5.5 Life cycle

Basic life-cycle pattern (General life-cycles):

Echinococcus *spp*. require two mammalian hosts for completion of their life-cycles. Segments containing eggs (gravid proglottids) or free eggs are passed in the faeces of the definitive host, a carnivore. The eggs are ingested by an intermediate host, in which the metacestode stage and protoscolices develop. The cycle is completed if such an intermediate host is eaten by a suitable carnivore.

The definitive hosts of *E. granulosus* are primarily dogs and other canids, *E. multilocularis* are primarily foxes, also other canids and cats, *E oligarthrus* are wild felids and *E. vogeli* bush dog. Intermediate hosts of *Echinococcus granulosus* are primarily ungulates, also marsupials, primates and humans*, E. multilocularis* arvicolid rodents, also other small mammals and humans, *E. oligarthrus* rodents, agoutis, paca, spiny rats, humans and *E. vogeli* primarily agoutis, also other rodents, humans (Thompson *et al.,* (1995). Echinococcus adult worms develop from protoscolices and are typically 6mm or less in length and have a scolex, neck and typically three proglottids, one of which is immature, another of which is mature and the third of which is gravid (containing eggs) (John, *et al* (2006). All disease-causing species of Echinococcus are transmitted to intermediate hosts via the ingestion of eggs and are transmitted to definitive hosts by means of eating infected, cyst-containing organs. When thinking about transmission, it is important to remember that humans are accidental intermediate hosts that become infected by handling soil, dirt or animal hair that contains egg (Eckert; *et al* 2010).

Eggs are passed in faeces of the carnivore, they are immediately infective and on ingestion by ungulates the oncosphere penetrates an intestinal venule or lymphatic lacteal (Health, (1971) to reach the liver or lungs (although other organs can be infected). The hydatid cyst develops slowly over several months. Hydatid cyst are commonly 5-10cm in diameter although larger ones have been recorded, principally in man; for example a cyst 50cm in diameter and containing about 16 liters of fluid has been recorded. The hydatid cyst is usually unilocular and is composed of fairly thick concentrically laminated membrane and within this is a granular germinal membrane. From this, brood capsules, each containing protoscolices, develop about five months after infection. At this time the cyst is infective for definitive host. The brood capsules may become detached and float free in the cyst fluid and being called hydated sand. Occasionally daughter cyst develops within the hydatid. Protoscolices and brood capsules can develop into other external daughter cyst. External daughter cyst may also be formed if a piece of germinal membrane becomes enclosed a laminated layer. The life cycle is completed when a dog ingests protoscolices. These evaginate, penetrate deeply between the villi into crypts of Lieberkühn and develop to maturity in about 47 days. Dogs may remain infected for about two years. In heavy infections the intestine becomes carpeted with worms. Not all hydatid cysts produce brood capsules or protoscolices. Thus, they may be sterile For instance, (Thompson,(1977). He found 27% of horse hydatid cysts and 51% of sheep hydatid cysts to be sterile. Cysts in cattle and pigs are frequently sterile Sterility of cysts is also associated with the age of the host upon infection. Hydatid cysts are found primarily in the lungs of sheep where they are frequently multilocular. They are found in both the liver and lungs of the pigs, but primarily in the liver of horse and cattle. In horse the hydatid cysts are usually unilocular. In man hydatid cysts are found in wide variety organs. Hydatid cyst fluid is pale yellow with 17-200 mg

protein/100 ml, has a striking similarity to the serum of the host and it contains immunoglobulin. In particular anti complementary substances are found in cyst fluid and calcareous corpuscles (Kassis and Tanner, 1976). The hematological changes like Eosinophilia is often associated with helminth infections in dogs (Misra and Cens 1971); Saror *et al*., 1979).

1.6 Diagnosis of Echinococcosis in the definitive host(dogs)

The Gold Standard technique of Echinococcus infection in the definitive host is the recovery of adult parasites in the intestine after necropsy. The best known method is described by(Eckert *et al.,*2001) as the "sedimentation and counting technique" (SCT). This is based on examining the faeces in the small intestine of the dogs with a microscope and counting the number of adult parasites. Although this method is 100% sensitive and specific, it is also time consuming and bio-hazardous and can only be used on dead animals. Before starting, it is important to remember that these experiments involve a potential risk of infection for humans, so safety precautions should be taken when handling this material. Eggs can be deactivated by freezing at -80°C for at least 4-7days or by heating to 60°C for 5 min (Eckert *et al*.,2001).

1.6.1 Arecoline purging:

Ante mortem diagnosis of canine echinococcosis has traditionally been performed by purging with arecolinehydrobromate (Eckert *et al.,* 2001). Arecoline is a parasympathomimetic drug that, when given to dogs in tablet or liquid form at doses between 1.75- 3.5 mg/kg body weight, purges the entire intestinal contents, increases intestinal peristaltic movements, and paralyses the tapeworms. These can then be collected and identified. This technique has been used in many control programs all over the world in recent decades. It has got 100% of specificity, however it has certain limitations. For example, its sensitivity is limited since not all dogs respond to the purge, and not all infected dogs eliminate *Echinococcus*

granulosus, it is also biohazardous and time-consuming and must be administered by trained personnel (Eckert *et al*., 2001) . In situations where endemic rate of *Echinococcus granulosus* in the dog population is low, the predictive value of the test diminishes as the percentage of infected dogs decreases (Schantz, 1973).

This is an unpleasant technique but is the only quantitative technique that can be used on living dogs and it continues to play an important role in epidemiological studies. Most epidemiological data, and the models developed from them, come from the resu1ts of this method (Torgerson and Budke, 2003).

1.6.2 Macroscopic and microscopic examination

Adult parasites or proglottids, can be detected and identified by macroscopic examination of faeces, but unless purging is used the chances of finding the adult cestoda are extremely inconsistent. In addition copro-microscopic examination (sedimentation and flotation technique) to detect *Echinococcus granulosus* eggs is, unfortunately, not a useful method of diagnosing for this parasite. *Echinococcus granulosus* eggs are morphologically indistinguishable to those of other taeniid cestoda, emission of eggs is variable and inconstant (and naturally not present in the prepatent period). Copro-microscopic examination may be used successfully if it is combined with other more specific techniques, such as PCR examination of DNA from the isolated eggs.

1.6.3 Detection of serum antibodies

Specific antibodies against oncosphere and protoscolex antigens can be readily detected in the serum of infected dogs (Heath *et al.,* 1985); Jenkins and Rickard, 1985).

The presence of Echinococcus -specific serum antibodies in canine echinococcosis was first convincingly demonstrated using ELISA in carefully designed experimental studies with helminth-free reared dogs (Jenkins and Rickard, 1985). Crude antigens were prepared from excretory / secretory (ES) or somatic extracts

of *E. granulosus protoscolices* and were able to detect serum antibodies by two weeks post -infection on and thereafter (Jenkins and Rickard, 1986).

Assessment of a similar ELISA, standardized with a somatic antigen preparation from protoscolices, for immunodiagnosis of natural canine echinococcosis in south east Australia resulted in a 72.7 % (16/22) sensitivity with a 19.8% specificity rate (Gasser *et al*., 1988). Further assessment of protoscolex antigen -ELISA with natural dog infections in North West Turkana (Kenya) were disappointing, as only 40% (20/50) of Echinococcus -infected dogs were correctly identified (Jenkins *et al*., 1990).

The specificity level was better at 70% (25 apparent false positive from 93 non -Echinococcus -infected dogs) but still below the previous expectations (Jenkins *et al*., 1990). Recently, the protoscolex antigen -ELISA for serum antibodies (IgG) was tested against arecoline-purged dogs in Uruguay and gave a serologic sensitivity of only 35% for *E.granulosus* (Craig *et al.,*(1995).

1.6.4 Copro -antigens detection:

Copro-antigens detection is an alternative to arecoline testing, based on a faecal antigen-detection using antibody sandwich enzyme-linked immunosorbent assay (ELISA) which has been developed recently. This has shown particular promise, as copro-antigens can be detected shortly after infection (10-14 days) and their level declines rapidly following expulsion of the worms (within 3-4 days) (Malgor *et al*., 1997). The test is based on a parasite-specific layer of captured IgG antibodies which retains antigens from faecal supernatants. Copro-antigen detection ELISA tests have been developed that uses polyclonal antibodies to *Echinococcus granulosus* excretory/secretory (ES) antigens. Post mortem examination of naturally infected dogs showed 56% sensitivity and 96% specificity (Deplazes *et al*., 1992).

(Allan *et al.,* 1992), using antiserum to some antigens in copro-antigen detection, the result was 88% sensitive in naturally infected dogs. False negative results in these studies have been attributed to low worm burden. Indeed, when the results were compared with those of post mortem examination, overall sensitivity was 63% but this increased to 92% in dogs with more than 100 worrns (Deplazes *et al.,* 1992). Copro-antigens can be detected prior to the release of the eggs by Echinococcus worrns, and therefore are not related to egg antigens (Deplazes *et al.* ,1992; Sakashita *et al.*,(1995). Positive ELISA results were obtained during the prepatent period in dogs as early as 5 days post infection (Deplazes *et al.*,(1992); Sakashita *et al.,*(1995); Nonaka *et al.*, (1996) . This has the advantage of being able to detect prepatent infections.

ELISA copro-tests can also detect heat-stable antigens. They have been used in a number of studies in the Middle East, Wales, Southern and Eastern Europe, and South America (Deplazes *et al.,* 1992); Sakashita *et al.*1995); Eckert *et al.*, 2001). The high sensitivity of monoclonal antibodies (MAb) to parasite specific antigens could increase the reliability of copro-antigen detection. Some sandwich ELISA systems have been evaluated for their ability to detect *E. granulosus* copro-antigens. They used a monoclonaI antibody produced against somatic extract of *E. multilocularis* (Sakai *et al.*, 1995); Malgor *et al.*,1997). Although the test was very sensitive in naturally and experimentally infected animals, there were also cases of cross-reactivity with *Taenia hydatigena* (Malgor *et al.,*1997). Recently the first MAbs for *E. granulosus* coproantigen detection were produced: two IgM murine monoclonal antibodies (MAbs), EgC l and EgC3, against the excretory/secretory (E/S) products of *E. granulosus* adult worms (Casaravilla *et al.*, 2004) . A copro-antigen capture ELISA was developed using a rabbit polyclonal antibody against E/S products from adult tapeworms as catching antibodies, and each of the MAbs as detecting antibody. The assays detected 7 out of 8 (EgC1), and 8 out of 8

(EgC3) experimentally infected dogs (worn numbers ranging from 61 to 75,500), and none (n=8) of the negative control samples. Faecal samples from 2 dogs experimentally infected with *E. multilocularis* were not recognized by the EgC 1 assay. This suggests that this is a potential species-specific diagnostic tool for discriminating *E.granulosus* and *E. multilocularis* infections (Casaravilla *et al*.,(2004). These advances made in applying MAbs in ELISA tests for coproantigen detection suggest that this may be an interesting research line inorder to develop new and more sensitive kits for diagnosing echinococcosis in dogs.

1.6.5 PCR protocols:

It would be useful to develop more specific techniques in cases where the presence of the parasite in the dog population is relatively low (Christofi *et al.*, 2002) , as well as for discriminating between dogs with Echinococcus and those with other *taeniid* infections. Several PCR tests were developed for detecting *E. granulosus*-specific DNA (Cabrera *et al*., 2002; Abbasi *et al.*, 2003; Dinkel *et al.,*2004; Stefanic *et al*.,2004) .

Two different protocols could be used for isolating the DNA: one extracts DNA from the total amount of faeces, and the second isolates and concentrates first taeniid eggs by combining sequential sieving with flotation solutions (Mathis *et al.* ,1996). This second protocol seems to be most useful because: faeces could contain substances that inhibit DNA amplification, only a limited amount of material can be processed in DNA extraction, and laborious purification of the DNA is often indispensable (Stefanic *et al.*,(2004). DNA is obtained by alkaline treatment (to lyse the eggs) , neutralisation, proteinase K digestion and DNA purification using organo-solvent extractions and/or DNA adsorbing matrices or, more recently, by the use of commercially available kits designed for DNA isolation from faeces (Abbasi *et al.,* (2003); Stefanic *et al.,* (2004). The available protocols mentioned above for detecting *E. granulosus* are all designed to detect G1 *E.granulosus*

(Sheep strain). The protocol evaluated by(Dinkel *et al*., 2004) describe primers that detect strains G5 (Cattle strain) , G6 (Caprine strain) and G7 (Pig strain) , With the additional possibility of strain typing by means of a second PCR. Only the protocols of (Abbasi *et al*., 2003) and Stefanic *et al*., 2004)) were tested on faecal or environmental material, while (Cabrera's *et al.,* 2002) had a sensitivity limit of at least 100 eggs per gram of faeces when applied to infected dogs (Abbasi *et al.,* 2003) .

The PCR test used by (Abbasi *et al.,* 2003) found 100% sensitivity and specificity using DNA samples extracted from 0.3 mI of faeces from 34 infected and 18 non-infected dogs, and found seven positive result when the sample contained only two *E. granulosus* eggs.

1.7 Diagnosis of hydatid diseases (intermediate host):

Infection by *E.granulosus* induces an immune response directed against the parasite antigens in the intermediate host, and specific antibodies of the IgM, IgG, IgA and IgE classes (Matossian *et al.,* 1976) have been detected in the sera of patients affected by hydatidosis. The immunodiagnosis of hydatidosis is based on the detection of humoral or cellular immune responses of the host against the parasite antigens.

The most widely used antigen for serological diagnosis of hydatidosis in human and livestock is hydatid cyst fluid of various intermediate hosts. About ten or more distinctive antigens of parasite origin are present in hydatid fluid together with many components of the host's serum, (Pozzuoli *et al.,* 1974).

Two major parasite antigens have been purified from hydatid cyst fluid: the thermo labile lipoprotein antigens 5 (Pozzuoli *et al.,*1975), and the thermo stabile lipoprotein B (Oriol *et al*., 1971). Antibodies to antigens 5 have been demonstrated by immunoelectrophoresis and shown to be diagnostic for hydatid disease, the importance of this antigen and its high degree of specificity have been confirmed

by various authors (Varela-Diaz *et al*., 1975). But this specificity cannot be regarded as absolute since antibodies against antigen 5 have been demonstrated also in sera of patients infected with larval *E. multilocularis, E. vogeli or Taenia solium.* However, antigens 5 can still be considered the best antigen in terms of the classic cystic disease caused by *E. granulosus.*

1.7 Serologic Tests of hydatid disease

Many serological techniques have been evaluated for hydatid disease. However, because sera from patients can be positive in some tests but negative in others, it is now widely accepted that two or more method must be simultaneously applied to improve sensitivity and specificity of the immunodiagnosis of hydatidiosis.

The methods include: -

1.7.1 The Haemagglutination Test (HA)

The HA test is based on the principle that adsorption of hydatid antigen on tanned erythrocytes causes their subsequent agglutination upon contact with specific antibodies. The test has proved to be a sensitive and specific diagnostic method for human hydatidiosis (Garabedian *et al*., 1957; Kagan, 1968; Matossian and Araj, 1975).

The use of formalized (Kagan, 1968) or pyruvic-aldehyde treated erythrocytes (Matossian, 1977) has prolonged the storage time of sensitized cells.

A slide HA tests, using chromic-chloride treated erythrocytes, sensitized by exposure to farmalinized hydatid antigen has produced result comparable to the tube test (Matossian *et al.,* 1976).

1.7.2 The latex Agglutination (LA) test:

Latex particles, coated with hydatid antigen, are agglutinated upon contact with corresponding antibody. Introduced by Fischman 1960) the LA test has been found to be an easy, specific and sensitive slide agglutination test, useful in hydatid

detection and in population surveys (Varela-Diaz *et al.,* 1976). Confirmation of the diagnosis is obtained by immunoelectrophoresis (IEP).

1.7.3 Intradermal (ID) Test

An immediate hypersensitivity reaction produced by the intradermal injection of minimal amounts of hydatid antigen has been used for diagnostic and epidemiological purposes. The test has a high sensitivity, but its low specificity has made the interpretation of the results impossible (Kagan, 1968).

1.7.4 The complement fixation test

The CF test has met with a mixed reception. Variation in its sensitivity, ranging from 36-93% of the known infected cases, have often discouraged its use (Kagan, 1968). Standardized, potent antigens and adequate controls have improved the accuracy of the method (Bradstreet, 1969). The CF test is of value in detecting recurrent illness (Matossian and Araj, 1975).

1.7.5 The Counter-Immunoelectrophoresis CIE) Test:

CIE has been adopted in the diagnosis of viral, bacterial, mycotic, protozoan and helminth infections (Draper, 1976). It produces counter migration, through a gel, of antigens and immunoglobulins, in an electric field, and on a narrow front, so that the maximal amounts are driven towards each other and form a line of precipitate.

1.7.6 The indirect-Fluorescent-Antibody (IFA) test:

The use of particulate antigens made visible through immunoflurescence has helped in the diagnosis, therapeutic follow-up and sero-epidemiological surveys of hydatidosis (Matossian, 1977). The antigen, when absorbed on a glass surface, forms a complex with specific antibody. Addition of an anti-human globulin conjugate induces fluorescence (Matossian *et al.,* (1972) described IgG, IgM and IgA antibodies detectable by this method. Further studies revealed the inconsistent detection of IgM and IgA hydatid antibodies. The IFA test is sensitive, but

occasional reactivity of samples in patients with hepatic disorders may hinder its interpretation.

1.7.7. The Immunoelectrophoresis (IEP) Test

In this test there is an initial separation, by electrophoresis, of the fractions present in an antigenic complex. The separated antigens are subsequently made to react with serum to determine the formation of precipitation bands, caused by the antigen-antibody reaction. (Capron *et al.*, 1967) reported that the diagnosis of human hydatidosis was possible with IEP through the demonstration of an arc of precipitation that was specific for *E.granulosus*.

The antigen corresponding to this arc described as (Fraction 5) was subsequently purified from hydatid fluid (Bout *et al.,* 1974). The formation of *E. granulosus* arc 5 by a serum is considered as the only specific diagnostic sign for the disease (Varela-Diaz *et al.,* 1976). The need for concentrated serum and antigen has limited the wide scale use of the procedure.

1.7.8 The Enzyme linked Immunosorbent Assay (ELISA)

ELISA pioneered by(Engvall and berlmann, 1972), has been successfully applied to the diagnosis of viral, bacterial and parasitic infections (Voller *et al*.,(1976). The test is conducted by coupling an antigen to a solid surface in a tube. Serum, when incubated with the sensitized carrier, forms an antigen-antibody complex. An enzyme-Labeled antiglobulin, when added, compiled with the complex and cannot be washed away.

The amount of residual conjugate is measured photometrically by the quantity of substrate it degrades. (Farag *et al.,*(1975) applied ELISA to the diagnosis of human hydatidosis. When used with a purified antigen, ELISA was found to be a simple, specific and sensitive diagnostic procedure.

1.7.9. The Radio-immuno Assay (RIA)

RIA uses tracer amounts of labeled antigen to determine the percentage of unlabeled antigen bound by specific antibodies. The percentage binding of the labeled antigen by the serum is compared with that of the standard sample, making the calculation of the unknown serum possible (Self *et al.,* 1976). (Musiani *et al.,* 1974) described the use of solid-phase RIA in the diagnosis of human hydatidosis. (Dessaint *et al.,*1975) isolated specific hydatid antibodies by the use of an immunosorbent. These were subsequently measured by RIA to evaluate the IgE class of antibodies. Elevated levels of IgE antibodies were observed in 60% of the patients.

1.7.10. Lymphocyte Transformation (LT)

Lymphocytes collected from animals previously sensitized to a wide variety of antigens undergo proliferation when in contact with the relevant antigen in vitro (Matossian, (1977). (Miggiano *et al.,* (1966) produced blastic transformation and mitosis of blood lymphocytes from hydatid patients, upon exposure to *E.granulosus* antigens. Yusuf *et al.,* (1975) observed a significant increase in the stimulatory indices of lymphocytes of hydatid patient incubated with hydatid fluid. Araj *et al*., (1977) described the time-related increase in thymidine uptake of spleen cells from syngeneic mice with secondary hydatidosis . The LT assay requires additional supportive data before its routine application. It may be of value in pulmonary hydatidosis where circulating antibodies are often absent.

Ultrasonography imaging is the technique of choice for the diagnosis of both cystic echinococcosis and alveolar echinococcosis. This technique is usually complemented or validated by computed tomography (CT) and/or magnetic resonance imaging (MRI) scans.

Cysts can be incidentally discovered by radiography. Specific antibodies are detected by different serological tests and can support diagnosis. Biopsies and

ultrasound-guided punctures may also be performed for differential diagnosis of cysts from tumors and abscesses.

1.8 Diseases In Human

Humans become infected when they swallow eggs in contaminated food. The infection is carried to the liver, where cysts form. Cysts can also form in the brain ,bones, kidney, lungs, skeletal muscles, and spleen.

Echinococcosis occurs in form of cystic echinococcosis, also known as hydatid disease or hydatidosis, caused by infection with *Echinococcus granulosus*; alveolar echinococcosis, caused by infection with *E. multiloculari*, polycystic echinococcosis, caused by infection *with E. vogeli*; and unicystic echinococcosis, caused by infection with *E. oligarthrus*.

The two most important forms, which are of medical and public health relevance in humans, are cystic echinococcosis and alveolar echinococcosis.

Humans are so-called accidental intermediate hosts, acquiring the infection in the same described above for the intermediate hosts, but are not able to transmit the disease.

Cystic echinococcosis is principally maintained in a dog–sheep–dog cycle, yet several other domestic animals may be involved, including goats, swine, horses, cattle, camels and yaks.

Alveolar echinococcosis usually occurs in a wildlife cycle between foxes, other carnivores and small mammals (mostly rodents). Domesticated dogs and cats can also be infected.

1.8.1 Signs and symptoms

Cystic echinococcosis / hydatid disease

Human infection with *E. granulosus* leads to the development of one or more hydatids located mainly in the liver and lungs, and less frequently in the bones, kidneys, spleen, muscles, central nervous system and eyes.

The asymptomatic incubation period of the disease can last many years until hydatid cysts grow to an extent that triggers clinical signs. Non-specific signs include anorexia, weight loss and weakness. Other signs depend on the location of the hydatid(s) and the pressure exerted on the surrounding tissues.

Abdominal pain, nausea and vomiting are commonly seen when hydatids occur in the liver. If the lung is affected, clinical signs include chronic cough, chest pain ,shortness of breath., Pain in the upper right part of the abdomen. Bloody sputum Fever and Severe skin itching.

1.8.2 Alveolar echinococcosis

Alveolar echinococcosis is characterized by an asymptomatic incubation period of 5–15 years and the slow development of a primary tumour-like lesion which is usually located in the liver. Clinical signs include weight loss, abdominal pain, general malaise and signs of hepatic failure.

Larval metastases may spread either to organs adjacent to the liver (for example, the spleen) or distant locations (such as the lungs, or the brain) following dissemination of the parasite via the blood and lymphatic system. If left untreated, alveolar echinococcosis is progressive and fatal.

1.9 Distribution

Cystic echinococcosis is globally distributed and found in every continent except Antarctica. Alveolar echinococcosis is confined to the northern hemisphere, in particular to regions of China, the Russian Federation and countries in continental Europe and North America.

In endemic regions, human incidence rates for cystic echinococcosis can reach greater than 50 per 100 000 person-years, and prevalence levels as high as 5%–10% may occur in parts of Argentina, Peru, East Africa, Central Asia and China. In livestock, the prevalence of cystic echinococcosis found in slaughterhouses in hyperendemic areas of South America varies from 20%–95% of slaughtered

animals. The highest prevalence is found in rural areas where older animals are slaughtered. Depending on the infected species involved, livestock production losses attributable to cystic echinococcosis stem from liver condemnation, reduction in carcass weight, decrease in hide value, decrease of milk production, and reduced fertility.

Globally, most human cases of CE are caused by the sheep strain (G1) of E. granulosus. Information on the infectivity of the lion strain and the buffalo strain is not available. Currently, there is no evidence that the horse strain is infective to humans (Thompson, 1995; Eckert and Thompson, 1997;Thompson and McManus;2002). This strain is widespread and common in Ireland, but up-to- date original cases of human CE have not been observed.

The camel strain has recently been identified in human CE cases in Argentina, Nepal and Iran (Fasihi Harandi *et al.,* 2002; Rosenzvit *et al.,* 1999; Thompson and McManus, 2002). In Sudan , cattle strain (G5) occurs less frequently in cattle and sheep strain (G1) is currently absent in central Sudan (Elmahdi *et al*., 2004). Camel strain was reported to be the most prevalent strain in Sudan (Omer *et al*., 2004; Osman *et al.,* 2007). It was found that Sudanese breeds of sheep and goats are known to harbor calcified hydatid cysts (Saad and Magzoub, 1989b).

According toThompson & McManus (2002) and Le *et al*., (2002), special features revealed by genetic comparisons and phylogenetic analyses would justify recognition of the horse and the cattle strains of *E. granulosus* separate species, namely, *E. equinus* and E. *ortleppi,* respectively.

Chapter two
Materials and Method

2.1 Study area

The study was conducted at Tambool town in Butana Province, in central Sudan. Tambool area is located at the coordinates 14°56′0″ North and 33°24′0″ East in Eastern Gezira locality, Gezira State. The samples were collected from Tambool slaughterhouse which is located near the market. Slaughtering of camels along with other animal species was performed early in the morning at 3:00 a.m. on each Saturday and Tuesday every week while the ante-mortem was performed the day before.

2.2 Natural infections of dogs with Adult *Echinococcus granulosus*:

A survey was carried out to examine the prevalence of *E. granulosus* infections in stray dogs in Tambool. Stray dogs were collected in cooperation with local authorities and Butana University at Tambool town, where stray dogs were reported as a problem. A total of twenty adults (dogs and bitches) were shot by the police authorities as part of the normal dog control. Immediately after shooting, blood samples for serum preparation were collected from each dog directly from the heart using a syringe, and transferred to a plain containers and labeled with unique code.

Dogs were then, transported to the College of Veterinary Medicine , Albutana University, at Tambool for necropsy and samples collection.

Necessary safety measurements were taken during handling dogs, and their intestines, which were possibly containing infective *E. granulosus* eggs. Laboratory coats, gloves, were used as precautions to prevent contamination with

infectious *E. granulosus* and other dog's pathogens as described by (Eckert and Deplazes, 2004; Eckert *et al*., 2000).

After death, dogs were transported to the laboratory, the small intestines of the dogs were removed in a tray and, after rinsing in normal saline to remove any external blood, the intestines were opened under fresh saline in large black-bottomed trays .(Soulsby 1982).

If no *E. granulosus* were seen, the intestinal mucosa was scraped and examined by washing and decanting with fresh saline. Intestines were examined by eye only. Any worms found were relaxed for 30 minutes in saline, and they were then either preserved in phosphate buffer saline for antigen preparation or fixed in 70% ethanol or in 10% formal saline. Objects which appeared on gross examination to be either whole worms or segments of *E. granulosus* were kept for microscopic examination later. Morphological identifications of adult Echinococcus parasites found were based on earlier reports (Soulsby 1982). In all cases where adult worms were visible, their position along the intestine was measured from the pylorus. Other parasites present in the small intestine were processed in the same way as the *E. granulosus* specimens. Many of these were later identified microscopically and the *Taenia spp.* were identified using the classifications of Verster, (1969).

2.2 Experimental infection of dogs:

2.2.1Collection of hydatid cysts for experimental infection:

Cysts were collected from camels slaughtered at Tambool town in central Sudan. Majority of animals slaughtered there, were originated from western Sudan. The liver, lungs and internal organs of each camel were examined visually and palpated for the presence of cystic lesions. Hydatid cysts when found, were carefully excised and taken to the laboratory in ice chamber, for further examination. The laminated membrane, germinal layer, cyst fluid and protoscolices were carefully removed. Cyst fluids, including protoscolices, were removed by aspiration. The

remaining laminated membrane including the germinal layer was carefully washed to recover any remaining protoscolices. Protoscolices were allowed to sediment and then counted. For small cysts, total protoscolices count was determined by examination of the cyst contents and counting in a Petri dish under a binocular microscope. Only fully developed, invaginated protoscolices were counted. For larger cysts, a proportion of the total sediment was examined and total protoscolices were estimated by extrapolation to the entire sediment volume.

2.2.2 Infection of puppies with *E. granulosus*:

The experimental infection of dogs with hydatid cysts of camel origin was carried out on seven, local breed dogs (approximately 2–4 months old). Infection of dogs with protoscolices was done as described by Saad and Magzoub, (1988).

Scolices were administered by mouth; they were counted, mixed with milk and fed to the dogs. Puppies given 10 000 scolices from camels hydatid cysts. Dog number 7 was kept as uninfected control. The infection was conducted in the Central Veterinary Research Laboratory, Soba, khartoum. Lack of facilities for the safe handling of dogs harboring gravid worms prohibited maintaining infected animals for longer than 35 days .Clinical observations were recorded daily throughout the period of the experiment; dogs were fed on boiled milk ,bread, and water.

2.2.3 Blood samples collection

Blood, for serum preparation, was collected from the infected dogs weekly starting at week zero (before infection). 3ml of blood were taken from cephalic vein in plain tube left over nigh ,centrifuged at 4000 rpm for 5 min, and supernatant was taken in a clean1.5ml tube, labeled and store at -20ºC until used.

Dogs were necropsied after euthanasia by injecting a saturated solution of magnesium sulphate into the heart. Puppies were starved for 18 hours prior to autopsy.

2.2.4 Autopsy of dogs

Dog number one was autopsied at 15 days post-infection (p.i.), and dog number two died due to infection with diarrhea at day 14 and was dissected together with dog number one. Dog number three was autopsied 21 days (p.i.), dog number four at 28 days (p.i.), and dogs number 5, 6 were autopsied at 35 days (p.i.).

Dogs after necropsy, the whole small intestine was removed. Tight, double adjacent ligatures were made at both ends of the intestine to secure the contents.

2.2.5 Worm examination:

The development of the adult *E. granulosus* of Sudanese camel origin was examined by studying the growth, segmentation and maturation in the definitive host dogs. Parasites of *E. granulosus* collected were examined under microscope to study organs morphology in different infective stages. The procedures and criteria employed were the same as those used in similar studies (Thompson, (1977b); Kumaratilake; *et al* (1983). All measurements were carried out on 10 specimens for each period. The growth, strobilization, development and morphological characteristics of parasites at each stage were investigated, the morphological features comprised, scolex, hooks, suckers, rostellum, neck, immature and mature segments. The developments of male and female genitalia were also described.

2.3 Detection of Antibodies against *E. granulosus in* infected dogs.

2.3.1 Excretory and Secretory (E/S) products Antigen

Adult *E. granulosus* were collected from infected dogs, and washed until they were free of any visible contaminants. This was achieved by washing for six times in warm (37^0C) sterile phosphate buffered saline (PBS). The washed worms were placed into tissue culture flasks containing PBS. The flasks containing the worms were incubated at (37^0C) for 24 h. After which, the medium was placed in (– 70^0C) for ten days to insure that all the eggs of *E. granulosus* were destroyed. The PBS was centrifuged at 2000 g, for 30 min. The supernatant was collected and the

protein concentration was determined. The protein concentration of the E/S products was estimated by the method previously described by (Smith *et al.*,1985) using Thermo Scientific™ micro-BCA™ Protein Assay Kit. The solution containing the excretory and secretory products, then aliquoted into 1 ml volume and stored at (-20^{0}C) until used.

2.3.2 Measurement of antigen protein concentration:

Protein concentration in the harvested antigen was measured using Thermo Scientific™ micro-BCA™ Protein Assay Kit. The Thermo Scientific™ Micro BCA™ Protein Assay Kit is a detergent-compatible bicinchoninic acid formulation for the colorimetric detection and quantitation of total protein. An adaptation of the Thermo Scientific™ BCA Protein Assay Kit (Product No. 23225), the Micro BCA Kit has been optimized for use with dilute protein samples (0.5-20µg/mL). The method uses bicinchoninic acid (BCA) as the detection reagent for Cu+1, which is formed when Cu+2 is reduced by protein in an alkaline environment. A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu+1). This water-soluble complex exhibits a strong absorbance at 562nm that is linear with increasing protein concentrations.

2.3.2.1.Kit Contents:

Micro BCA Reagent A (MA), 240mL

Micro BCA Reagent B (MB), 240mL

Micro BCA Reagent C (MC), 12mL

Bovine Serum Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules containing bovine serum albumin (BSA) at 2.0mg/mL in a solution of 0.9% saline and 0.05% sodium azide

2.3.2.2 Preparation of Standards:

The standard protein reagent supplied in 2mg/ml concentration. To use in the standard assay procedure the reagent was diluted first to 200µg/ml by adding distilled water. Then the standard was serially diluted two fold for 10 times.

2.3.2.3 Preparation of the Micro BCA Working Reagent (WR):

The WR was prepared by mixing 25 parts of Micro BCA Reagent MA and 24 parts Reagent MB with 1 part of Reagent MC (25:24:1, Reagent MA:MB:MC). 150µL of WR is required for each sample in the Microplate Procedure.

2.3.2.4 Microplate Procedure:

For use in the micro assay the kit was used as described by the manufacturer.150µL of each standard or unknown sample replicate was pipetted into a microplate well. Then 150µL of the WR were added to each well and the plate was mixed thoroughly on a plate shaker for 30 seconds. The plate was then covered using aluminum foil, and incubated at 37°C for 2 hours. Then the plate was cooled to room temperature (RT). The absorbance was measured at or near 562nm on a plate reader. The average absorbance reading of the blank standard replicates was subtracted from the reading of all other individual standard and unknown sample replicates.

A standard curve was prepared by plotting the average reading for each BSA standard vs. its concentration in µg/mL, using Microsoft EXCEL software, and the standard curve was used to determine the protein concentration of each unknown sample.

2.3.3 Dogs' sera

Three groups of sera were used. Group one (test sera): 15 sera from helminth-free dogs collected from the police dogs unit. Group two (test sera): 16 sera collected from dogs experimentally infected with *E. granulosus* of camel origin .

Group 3 (test sera): 16 sera from stray dogs from an endemic hydatid cyst region with or without *E. granulosus* worm burdens at necropsy. Sera were stored at(-20°C).

2.3.4 Procedure of *i*ndirect Enzyme linked immunosorbant assay (ELISA)

The assay was optimized as described by (Gasser *et al.*,1993) with some modifications. Briefly; the antigen was diluted in coating buffer at optimal concentration. Optimal concentration was determined after preparation of different antigen concentrations and different dilutions of sera and conjugate (Chequer-board titrations). The optimal concentration was 5 µg/ml coating buffer for the E/S antigen, and 1:200 the dilution of sera, 1:20 000 for the conjugate. Micro titration plates (Nunc, Maxisorp, Thermo Fisher Scientific, USA) were coated overnight at (4°C) with 5/µg ml protoscolex somatic antigen diluted in carbonate-bicarbonate buffer, pH 9.6 (Sigma, USA, Cat No: C3041-100CAP) for IgG assay. Plates were washed three times to get rid of excess unbound antigen, with PBS buffered,(Sigma, USA, Cat No: P4417-50TAB) pH 7.2 (PBS) containing 0.05% v/v Tween 20(TWEEN® 20 Sigma, USA, Cat No: P5927-100ML) (PBS-T).To get rid of the remaining free binding sites ,plates were blocked with 200 µl/well,(PBS pH 7.2) (PBS) containing 0.05% v/v Tween 20 (PBS-T) and 5% w/v skim milk powder (Sigma, USA, Cat No: 70166) (PBS-TB) for 2 hrs. at(37°C). Plates were emptied, and washed three times with washing buffer (PBS-T). Hundred microliters of serum, diluted 1:200 in PBS were added to the plate and incubated in duplicates for1 h at 37°C. Plates were emptied, and washed four times with PBS-T and 100 µl/well of anti-dog IgG (whole molecule)-peroxidase antibody produced in rabbit, (Sigma Aldrich A9042) diluted 1/20000 in PBS were added to the plate and incubated for 1h (37°C). Plates were subsequently washed four times with PBST. 100 µl/well Substrate solution containing Tetramethyl Benzidine (TMB) (Microwell Peroxidase Substrate System (2-C) Product code 50-76-00.

KPL) was added and incubated for 30 min at(37 ºC). The reaction was stopped by adding 50µl per well of 1 M sulphuric acid.

The absorbance at wavelength 450 nm was determined using a Bio Teck ELISA reader**.** Positive-negative cut-off absorbance value of 0.512 was based on three standard deviations above the mean optical density (OD) value for 15 sera of uninfected dogs (Gasser *et al.,* 1993).The reference positive serum originated from one of the dogs found positive for *E. granulosus* at necropsy.

Chapter Three

Results

3.1. Experimental infection of dogs:

Six puppies were infected orally with protoscolocies while one puppy was left uninfected as a control. To monitor the success of the infection and to determine the development of the parasite in experimentally infected puppies, they were euthanized at different stages of the infection.

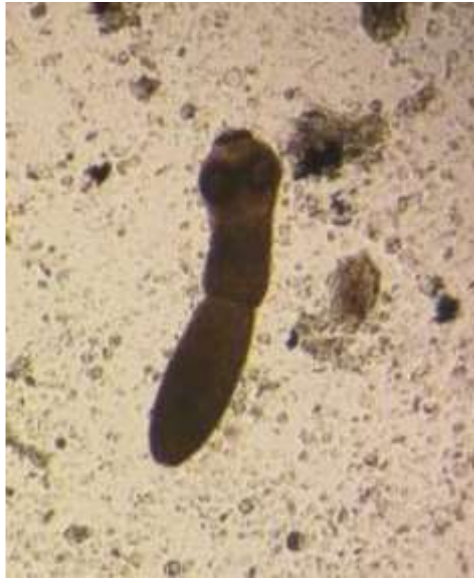
Result showed that numerous *E. granulosus* worms were found at post mortem. The total number of worms collected from dogs ranged from 12-37.

On the 15th day(p.i.) dog No. one was euthanized and dissected with dog number two, numerous small young cestodes were found in the duodenum and anterior part of the jejunum extending over about 30 cm. The intestine showed excessive discharge of mucus, associated with inflammation of the mucous membrane. Most of cestodes were found buried in the mucus. They could be easily removed from the intestinal wall. The cestode of this stage consists of a scolex and one proglottid (fig 2). However, in few specimens, with younger parasites, the borderline between the scolex and first proglottid was not clear (fig 3). Male and female genitalia of the parasite doesn't appear at this stage. Uterus, cirrus sac and testes could not be also observed at this stage in all specimens collected. The large and small rostellar hooks are alternately arranged in two rows. Their shape is similar in appearance to that of the scolices within hydatid cyst as shown in (fig 1). The suckers are also similar to those of adult worms. The appearance of the excretory canal is not clear.

Dog number three was euthanized after 21 days of pi. The cestode of this stage consists of a scolex and two proglottids (fig 4). Several worms were noted with an incomplete third row of tiny hooks and showed developing testes and a definite development of the uterus.

Dog number four was euthanized after 28 days pi. The strobila consists of a scolex and two proglottids in most of the specimens tested (fig 5), although few worms have three proglottids. This stage is characterized by the presence of common genital pore, cirrus sac, winged (lung shape) ovary, central blind tube-shaped uterus, vitelline gland and testes. Testes were clearly visible distributed all over the terminal segment.

On the 35th day pi, dogs No five and six were euthanized and dissected. Many cestodes were found in the duodenum and the anterior part of jejunum extending over about 30cm. Catarrh was found in the intestine. The strobila consists of a scolex and three proglottids as that of adult worm (fig 6). The last proglottid has grown larger and it shows the typical form of *Echinococcus* tapeworm. However, the development of the egg is not completed. The uterus has fairly well developed, and it harbors many immature eggs.



plate(1*).Echinococcus granulosus* collected from dog No.one on15 days post infection. (Borderline between the scolex and first proglottid is clear).

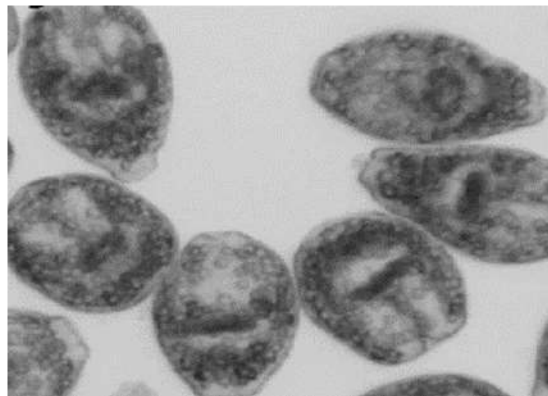


plate (2). Protoscolices collected from camel hydatid cyst

plate (3*). Echinococcus granulosus* collected from the dog No.on 15 days post infection. The body consists of scolex and neck (borderline between the scolex and first proglottid is not clear).

plate (4): *Echinococcus granulosus* collected from dog No three on 21 days post infection.

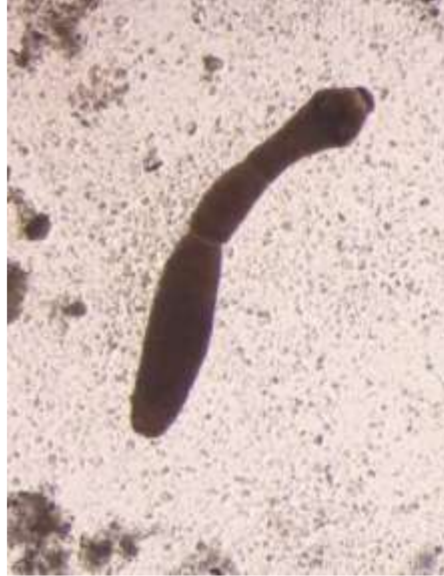


plate (5). *Echinococcusgranulosus* collected from the dog No. four on 28 days post infection.

plate (6): *Echinococcus granulosus* collected from the dog No. six at 35 days post in fection.

3.2 Results of Immunodiagnosis:

The chequerboard assays were evaluated by calculating the differences between (OD) values measured for the reference sera at different of the antigen concentrations, sera and conjugate dilutions tested. This evaluation determined the concentration of *E granulosus* antigen to be optimal at 5 μg/ml protein. For antigen coating of the ELISA plates 0.06 M carbonate buffer (pH 9.6) appeared to be the best choice.

Saturation of free binding sites on the ELISA plates was most effective when 5% SM-TPBS was used as the blocking agent. Moreover, background reactions on the ELISA plates could be reduced to some extent by diluting the sera and conjugate with 5% SM-TPBS instead of PBS alone. The optimal dilution of the secondary serum was found to be 1: 100 and that of the conjugate to be 1: 1000.

The cutoff point was calculated by adding two stander deviations to the mean optical density readings of the negative controls. The negative controls were fifteen sera from helminth-free dogs, collected from the police dogs unit. The cutoff point was determined at 0.512.

Specific IgG antibodies against *E. granulosus* adult worm antigen were detected by enzyme-linked immuno sorbent assay (ELISA) in sera from dogs experimentally or naturally infected with *E. granulosus*.

The results of post mortem examination for 16 dogs shot by the police in Tambool town, showed that five dogs were found harboring *E. granulosus* adult worms. From these dogs, eight dogs were found infected with *Taenia hydatigena*. Mix infection with *E. granulosus* and *T. hydatigenia* was observed in only one dog.

When tested by ELISA out of these 16 Sera, seven were found positive. Four out of five dogs which were positive at post mortem were positive by ELISA test. Only one dog which was found positive at postmortem was found negative by ELISA test. Two dogs negative for *E. granulosus* and positive for *T. hydatigena* were

found positive by ELISA test. Only one dog negative at postmortem for both, *E. granulosus* and *T. hydatigena* was positive for ELISA, (Table 1).

Sera collected from dogs experimentally infected with *E. granulosus* were negative for ELISA until the last week of the experiment (35 days post infection). Although the readings of sera from experimentally infected dogs were below the cutoff point, we have observed that the antibody titre of the samples was slightly increasing during experiment (fig.8).

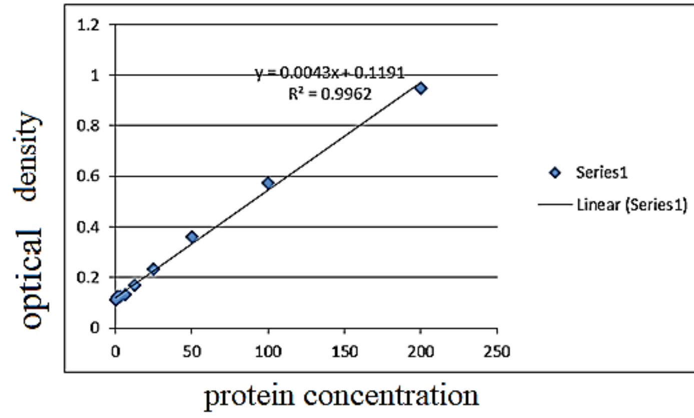


Figure7. Standard curve prepared by plotting the average reading for each BSA standard vs. its concentration in µg/mL.

		Results at postmortem		ELISA result
No	**ELISA OD value**	***Echinococcus granulosus***	***Taenia hydatigena***	
1	**0.419**	+ **ve**	-ve	-ve
2	**0.6445**	+ **ve**	+**ve**	+**ve**
3	**0.538**	+**ve**	- ve	+**ve**
4	**0.558**	+ **ve**	-ve	+**ve**
5	**0.53**	+ **ve**	-ve	+**ve**
6	**0.52**	-ve	-ve	+**ve**
7	**0.398**	-ve	-ve	-ve
8	**0.4085**	-ve	+**ve**	-ve
9	**0.3435**	-ve	-ve	-ve
10	**0.3985**	-ve	+**ve**	-ve
11	**0.3445**	-ve	-ve	-ve
12	**0.3805**	-ve	+**ve**	-ve
13	**0.351**	-ve	+**ve**	-ve
14	**0.4125**	-ve	+**ve**	-ve
15	**0.5195**	-ve	+**ve**	+**ve**
16	**0.5141**	-ve	+**ve**	+**ve**

Table1: Comparison of postmortem and ELISA results

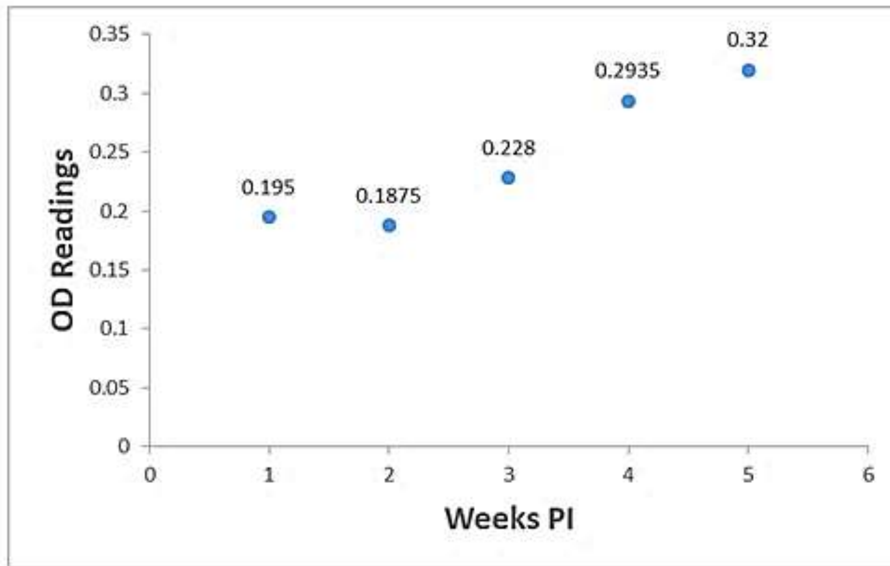


Figure 8: Mean optical density(OD)reading of experimentally infected dogs.

Chapter Four

Discussion

The camel is an important intermediate host for *E.granulosus* in Sudan. Knowledge of morphological changes in the development of *E.granulosus* in the definitive host the dog is very important, as this will help in understanding differences between strains circulating in the area, and thus the epidemiology of the disease.

In this study, the camel protoscolices were infective to all puppies. *Echinococcus* worms were concentrated in the proximal third of small intestine.

The morphological observations in different developmental stages of *E. granulosus* from camel in dogs on the 15th, 21th, 28th, and 35th day post infection of the larval cestode were described. The cestodes occur in the duodenum and anterior part of jejunum with length of about 30 cm. Specimens of the tapeworm on the 35th day post infection, showed that the parasite has fully developed, while the embryophore of egg is out of sight and the oncosphere is not completed.

In this experiment, the growth and development of the tapeworms in the six dogs fed with protoscolices of Sudanese camel origin were similar to those recorded by (Eckert *et al.,* 1989).

The collected number of adult worms from dogs in this experiment was very low. This was also observed by Saad and Magzoub (1988), and Slepnev *et al*., (1976), with worms of camel origin. They attributed this low recovery to the storage of the cysts in the refrigerator for three days before they were administered to the experimental dogs. In our experiment, the cysts were fed to the dogs two days after their collection from the slaughter house.

Lack of facilities for the safe handling of dogs harboring gravid worms prohibited maintaining infected animals for longer than 35 days, so the time required by

scolices to develop to maturity was not calculated in this study. Saad and Magzoub, (1988) reported that for scolices derived from camel hydatid cysts, the period was 37-49 days (average 43 days), while for those from cattle hydatid cysts the period was 40-51 days (average 45 days). While Slepnev *et al.,* (1976), reported a range of 54-97 days (average 75 days). Eckert *et al*., (1989) found that at day 41 post infection, more than 50% of the parasites contained mature eggs (embryonated eggs with fully developed, "thick-shelled" embryophores).

The experimental infections revealed that the dogs appeared to be readily and uniformly susceptible to infections with protoscolices of camel origin. Further work based on experimental infections, is needed to clarify important relationships involving the strains of *E. granulosus* and then the intermediate and final hosts.

Accurate detection of *E. granulosus* infection in the definitive host, the dog, is important to establish baseline data on prevalence, and in surveillance of hydatid control programs (WHO, 1981). The eggs of taeniid cestoda are extremely similar, and thus identification by microscopic examination of the feces is risky and non-specific. The Gold Standard technique of Echinococcus infection in the definitive host is the recovery of adult parasites in the intestine after necropsy (Benito &Carmens, 2005).

In the present study, post-mortem confirmation of immunodiagnostic results was carried out. Sixteen sera from dogs confirmed positive or negative at postmortem examination were tested by ELISA, seven were found positive. Four out of five dogs which were positive at postmortem were positive at ELISA test. Only one dog which was found positive at postmortem was found negative by ELISA test.

Two dogs negative for *E. granulosus* and positive for *T. hydatigena* were found positive by ELISA test, this could be due to cross reactive nature of antigen with other parasitic antigens. This could possibly due to sharing of common epitopes between closely related species. The study conducted by (Gasser *et al*., (1988)

demonstrated that 25–60 % of the serum from dogs infected with *E. granulosus* did not show significant levels of specific antibody and revealed cross reactivity with other parasite species. Similarly Jenkins *et al*., (1990) evaluated somatic antigen of *E. granulosus* for sero-diagnostic purpose and found variable diagnostic sensitivity and high cross reactivity with antigens from other parasitic species.

One dog which was negative at postmortem for both, *E. granulosus* and *T. hydatigena* was positive for ELISA, It is possibly that, this sero positive dog had had previous infection which was removed spontaneously. Recent studies on canine taeniasis have indicated that serum antibodies can persist for several weeks after removal of worms (Jenkins and Rickar,(1985); Heath, Lawrence &Oudemans,(1988).

Sera collected from dogs experimentally infected with *E. granulosus* were negative for ELISA until the last week of the experiment (35 days post infection). This result must be interpreted with some caution, however, because the *E. granulosus* infections were terminated at only 35 days. This result is in agreement with the results obtained by Gasser *et al.,* (1988), they reported that, samples collected from experimentally infected dogs were only reactive for antibody detection after 60 days post infection.

For further studies to evaluate the production of detectable antibodies by experimentally infected dogs, the experimental infection should be performed during longer period than what we have done here in this work, which need a well prepared facilities to prevent contamination with infectious *E. granulosus* and other dog's pathogens.

In this study, detection of infected dogs by ELISA was 80 % sensitivity (4/5). Despite this, serum antibody prevalence has been shown to reflect the relative parasitological prevalence on a population basis (Gottstein and mowatt, 1991).

Which indicates that antibody detection ELISA is useful as an epidemiological tool to rapidly screen canid populations for the presence of echinococcosis.

More research must be done to increase the sensitivity and reduce cross reaction with other parasites, this could be done by using purified antigens.

Echinococcus granulosus in dogs at Tambool area was detected, 25% (5/20 dogs were found harboring adult worms at postmortem). (Saad and Magzoub (1989a) conducted a survey for *E. granulosus* in dogs in this area, they found that out of 49 dogs examined, 25 (51%) harbored *E. granulosus*. They concluded that there is an on-going cycle of dog-camel-dog. The findings also suggest that there is potentially a high degree of risk to the local human population.

To our knowledge no attempt was made to assess the prevalence of infection in man. It is very important to assess the infection in man, and to find out if this correlated with the known high prevalence of the disease in camel and dogs in this area.

Recommendation:

The need for a control programme in Tambool is paramount, but there are many inherent difficulties in implementing a hydatidosis control programme in this area. Not the least are the nature of the people at this area, the lack of good abattoir facilities and the very low literacy rate. In addition, there is the huge dog population with whom the people have a very close contact.

Because of these problems, a unique control programme for this area has to be developed. It is our opinion that, any efforts to control hydatid disease in Tambool area should be directed parallel in two lines; increase the positive role of abattoir in preventing stray dogs' access to infected offal and against the dogs. It has been evident during our surveys that many dogs are stray dogs rooming freely around the slaughter house place and feed from condemned, improperly disposed offal. A mass dog eradication programme with a mass drug treatment programme of house dogs, coordinated with an educational campaign, could be effective to control the disease if implemented in this area.

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