SEROLOGICAL DIAGNOSIS OF HYDATIDOSIS IN ANIMALS SLAUGHTERED IN KANO ABATTOIR, KANO STATE, NIGERIA

BY

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A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

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DECLARATION

I hereby declare that this thesis entitled ‘’**Serological diagnosis of hydatidosis in food animals slaughtered in Kano abattoir, Kano state, Nigeria’’** is my personal work and has not been presented in any form for another qualification at any other university or institution. The information derived from other sources has been duly acknowledged by way of references.

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CERTIFICATION

This thesis entitled ''**Serological diagnosis of hydatidosis in food animals slaughtered in Kano abattoir, Kano state, Nigeria'** by Mrs Sodangi Abdulkarim Luka meets the regulations governing the award of the degree of Doctor of philosophy (PhD) of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To God Almighty, the author of knowledge,

To my husband, Mr. Abdulkarim Luka and children Rahila, Esther, Isaac and Freda with love

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ABSTRACT

The epidemiology of hydatidosis in animals including camels slaughtered in the Kano abattoir was investigated. Antigenic profiles and serum antibody responses from domestic animals naturally infected with *Echinococcus granulosus* were examined in Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting (WB) and Enzyme Linked Immunosorbent Assay (ELISA) using antigens prepared from *E. granulosus* hydatid cyst fluid. Available 10-year (1993-2002) hospital records on the disease in man were examined from four major hospitals in Kano municipal in order to determine the level of prevalence recorded in man.

A total of 21 hydatid cysts were collected from goats (8), sheep (6) and camels (7). Five hundred and fifty one (551) serum samples were collected from sheep (138), goats(130), cattle(147) and camels(136) during the study period.

The major antigenic components in the hydatid cyst fluid (HCF) antigen preparations were protein molecules of 12,45,50-66,118-160 kDa. Camel purified HCF showed prominent bands of 32,66,118,150 and 160 kDa.Goat purified antigen preparation showed prominent bands of 45-52,66,100 and118 kDa.The sheep HCF contained the 8,16,42,32-52,66,96,118 and 150 kDa bands respectively. The most consistent demonstrable protein in *Echinococcus granulosus* occurred as a complex in the 52-62 kDa region. Common antigenic bands occurred mainly between 30 and 67 kDa in electrophoresed cyst fluids of the animals.

The overall prevalence of *Echinococcus granulosus* antibody response by ELISA was 32.6%. The highest prevalence was recorded in camels (38.9%), sheep (36.2%), cattle (31.2%) and goats had the least (23.8%). Higher prevalence was recorded in older

animals than younger ones. There were no statistical significant differences between the prevalence rate of young and old cattle and camels ($\chi 2 = p > 0.05$), and no significant association between age and disease prevalence ($p > 0.05$). Significant differences were recorded between young and adult sheep and goats ($p < 0.05$). There were significant associations between age and prevalence in the sheep and goats ($p < 0.05$). Antibodies to *Echinococcus granulosus* was encountered in both males and females with no statistical significant difference in prevalence between males and females among all the animals and no association between the sex of the animals and prevalence of antibodies to *Echinococcus granulosus* ($p > 0.05$).

Western blot analysis showed that 110 sera from all the animals representing 19.9% of the 551 serum samples bind at least one of the 8, 24, 30,36,38,42,48,52,66,92,118 or 150 kDa *Echinococcus granulosus* specific antigenic bands which served as criteria for determination of positivity. Bands of 66, 118 and 150 kDa persisted throughout the study among infected animals while the 8-24 kDa bands fluctuated.

The abattoir was properly fenced, there were no stray dogs seen around the abattoir but the level of hygiene of the abattoir, method of disposal of animal wastes and condemnation of offals were very poor. Based on the hospital records, no egg (0.0%) or segment of *Echinococcus* was recovered in stool of patients that attended the four hospitals during the study period (ten year-record). Hospital surgery records showed that a hydatid cyst was recovered from the lung of a 30 year old male patient at the Mallam Aminu Kano Teaching Hospital in 1999. The study concludes that hydatidosis is prevalent in animals in the Kano area with potential health consequences in man.

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ABREVIATIONS

&	=	and
A.B.U	=	Ahmadu Bello University
APS	=	Ammonium per Sulphate
BSA	=	Bovine Serum Albumin
CmCF	=	Camel hydatid cyst fluid
dd H2O	=	Distilled deionized water
ELISA	=	Enzyme Linked Immunosorbent Assay
Etc	=	Etcetera
GtCF	=	Goat hydatid cyst fluid
HCF	=	Hydatid Cyst Fluid
H2O	=	Water
HPLC	=	High Pressure Liquid Chromatography
KDa	=	Kilo Dalton
Min	=	Minutes
NaCl	=	Sodium Chloride
^{0}C	=	Degrees Centigrade
OD	=	Optimal Density
OR	=	Odds Ratio
PBS	=	Phosphate Buffered Saline
PBS/T20	=	Phosphate Buffered Saline containing Tween-20
SB/ME	=	Sample Buffer containing 2-Mecaptoethanol

SD	=	Standard Deviation
SDS-PAGE	=	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
ShCF	=	Sheep hydatid cyst fluid
T/20	=	Tween- 20
TBS	=	Tris Buffered Saline
TBS-HCl	=	Tris Buffered Saline containing Hydrochloric Acid
T-TBS	=	Tween 20 in Tris Buffered Saline
TTBS-BSA	=	Tris Buffered Saline containing Bovine Serum Albumen
UNESCO	=	United Nations Educational Scientific and Cultural Organization
WHO	=	World Health Organization
µl	=	Microlitre

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Hydatidosis/Echinococcosis is a global animal and human health problem of increasing importance (Lightowlers *et al.,* 2000). It is a helminthic zoonosis caused by the larval stage (metacestode) of the canid tapeworm of the genus *Echinococcus* (Maddison *et al.,* 1989). It is termed one of the most important helminthic diseases worldwide (Altintas, 2003; Craig *et al*., 1991). *Echinococcus* is a small endoparasitic flatworm belonging to the class Cestoda, subclass Eucestod. A total of 16 species and 13 subspecies have been described but only 4 species have been recognized as valid taxonomically. These include: *Echinococcus granulosus, E. multilocularis, E. oligarthrus* and *E. vogeli* are recognized to be associated with the disease (Eckert *et al.,* 2001; Khuroo, 2002). The disease is common in sheep raising areas with high dog populations (Anyanwale *et al.,* 1982; Poretti *et al.,* 1999; Lightowlers *et al.,* 1989). The first outbreak of the disease was in Iceland in the mid 19th ^{century} Schwabe and Aboudoud. (1969) where one in every six persons harboured hydatid cysts. *Echinococcus granulosus* causes cystic echinococcosis (CE) in humans and ruminants while alveolar echinococcosis (AE) is caused by *E. multilocularis; E. vogeli* and *E. oligarthrus* both cause polycystic echinococcosis.

The definitive host of *Echinococcus* are carnivores (Thompson, 1995; Soulsby, 1982) which include dogs and other domestic and wild canids (Lightowlers, 1990) harboring the adult tapeworm in their small intestines. The domestic/urban form of the disease is caused by *E. granulosus* which utilizes goats, sheep, cattle, camels and horses as intermediate host. (Craig *et al.*, 1991). Man is usually an accidental intermediate host through direct contact with dogs or from environmental contamination. The sylvatic/forest forms are caused by *E. multilocularis* which has been isolated from species of wild rodents, pigs, horses and man (Tamas *et al.*, 2003). Eggs from the tapeworms are passed out into the environment with dog faeces and infect the intermediate hosts directly or indirectly via contaminated food stuffs and/or water (Torgerson *et al.*, 2003). In the intermediate hosts, the larval stage of the parasite forms large fluid filled cysts in the liver, lungs, brain, bone and other organs (Craig *et al*., 1988; 1995). Dogs and other canids become infected with the tapeworm by eating the infected viscera of the intermediate host.

Hydatidosis is primarily characterized by slow growing fluid filled cystic or vesicular lesions in the liver. The clinical manifestation of the disease depends primarily on the organ affected (Lightowlers, 1990), site of organ involvement (Ammann and Eckert, 1995), stage of cyst development and viability of the cyst contents (Khuroo, 2002). Majority of the cysts produce no symptoms and are detected as incidental findings on routine imaging or at autopsy. The symptoms of the disease are related to expanding mass pressure on adjacent structure, infection

and rupture of cyst contents into surrounding body cavities which leads to anaphylaxis and sudden death (Todorov and Stojanov,1979;Todorov and Boeva, 1999).

Cystic echinococcosis is geographically widespread affecting many countries on all continents (Eckert *et al.,* 2000) and its distribution closely parallels the areas of the world where pastoralism is the main occupation especially in poor rural areas where raising of sheep is very popular. The parasite has at least six genetically distinct strains two of which are relevant to human infection (Heath, 1999).

The sylvatic strain is maintained in wolves and wild ungulates in north Alaska, Canada, Scandinavia and Eurasia. The pastoral strain is maintained in dogs and domestic ungulates throughout the world (Willingham, 2003). The disease is considered a serious health problem of the nomadic pastoralists tribes of east Africa i.e. Kenya, Tanzania, Uganda, Southern Sudan, Somalia (Macpherson *et al.,* 1986). Other endemic areas include Asia, Europe, Australia and the Mediterranean (Turgut, 2001). While cystic hydatidosis persists in many parts of the world and is re-emerging in some regions (Jenkins, 1998). New Zealand has operated a national eradication campaign since 1959 and has successfully eradicated the disease (Heath, 1999). However, live animals imported into the country will have the potential of re-establishing the disease and needs to be monitored.

1.2 Statement of the problem

Hydatidosis –echinococcosis (*Echinococcus granulosus)* is a very old and still existent problem in different parts of the world. To date, inspite of the significant progress achieved in the field of research and control, human and animal cystic echinococcosis remains a considerable problem for public health and the livestock economy where sheep pastoralism is common and dog population high (Jenkins *et al.,* 1998; Heath, 1999; Larrieu *et al.,* 2001; Sotiraki *et al.,* 2003).

Agricultural practices, indiscriminate home slaughtering, and poor disposal of cysts from livestock, lack of adequate control policy, uncontrolled movement and commerce of animals and their products and difficulty in early diagnosis enhances the distribution of the disease (Arene, 1985; Ajogi and Adamu; 1998; Dada and Belino;1979). The ability of *E. granulosus* to adapt to a wide variety of host species and repeated uncontrolled introduction of food and domestic animals from endemic areas to other parts of the world has contributed to the broad distribution of the parasite and the spread of the disease.

In Nigeria, the prevalence of the disease is underreported (Dada *et al.,* 1979; Anyanwale *et al.,* 1982; Ajogi *et al.,* 1995; Ajogi and Adamu, 1998). There are scanty reports on the status of the disease and where available, only a few detailed surveys of its prevalence in animals mostly in parts of northern Nigeria have been conducted and low incidence has been reported. (Dada *et al.,* 1979).

In Kano State Nigeria, the uncontrolled movement and commerce of camels and other domestic animals from the neighboring Niger and Chad republics has

continually served as a transmission route. In addition, many of the rural families in this area live in close proximity to their flocks of sheep, goats and cattle and participate in taking care of them. The consumption of uninspected meat and products of animal origin is a very common practice in many rural communities in the under developed countries (Torgerson *et al*., 2003). In Kano municipal, the centralized meat processing facilities in the modern abattoir built in the 1980's have been abandoned for a small ill-equipped unsupervised abattoir.

Despite the facts that Islam forbids the rearing of dogs and 99% of the populace in Kano is Muslims, dogs are kept in some homes and by hunters and pastoralists as guards to livestock and property. Once in a year during their religious festivals, tens of thousands of sheep and cattle are slaughtered. The offal are disposed indiscriminately thus providing the stray dogs with offals from the infected intermediate hosts, man too consume such offals (Craig *et al.*, 1986), thus making the control of the disease very difficult.

The cultural level of the individuals coupled with their lower education level and lack of public enlightenment programmes on the disease has made control of the disease almost impossible. There is no consistent state policy on the prevention and control of the disease in Kano State. Meat inspection is never carried out in the abattoir, no quarantine; there is infiltration of camels from neighboring countries, lack of trained manpower. All these pose a problem in the area and there is the need to conduct proper investigation in order to know the actual status of the disease in the area.

Due to lack of facilities, diagnosis of the disease in Nigeria and Kano state in particular have been based on postmortem findings (Dada *et al*., 1979), retrospective findings (Ajogi *et al.*,1995), and serological methods (Dada and Belino, 1979) such as haemagglutination (IHA), ouchterlony's double diffusion (DD) and counter current immunoelectrophoresis (CCIE).Currently in developed countries, the diagnosis of cystic echinococcosis in intermediate hosts is based mainly on necropsy procedures (Lightowlers and Gottstein,1995). Accurate serological diagnosis of CE infection in livestock is difficult (especially when crude antigen is used) it results to serological cross reactions with several other species of taeniid cestodes including T*aenia hydatigena* and T*aenia ovis*.

1.3 Justification

Based on the reports by Dada *et al.* (1979) and Ajogi *et al.* (1995), hydatidosis is prevalent amongst domestic animals including camels a high number of which is being slaughtered in Kano State. In Nigeria, the prevalence of the disease is underreported. (Dada *et al.*, 1979) recorded 0.06% of the disease in domestic animals slaughtered in Zaria abattoir. Dada and Belino. (1979) recorded 57.5%, 22.8%, 20.9% and 16.0% of the disease in camels, goats, sheep and cattle in Sokoto abattoir, Northern Nigeria. A 2% infection rate was recorded in sheep and goats slaughtered in Ibadan abattoir. In addition to that, Ogunsan *et al.* (2000) recorded 26.2% of the disease in camels slaughtered in Sokoto abattoir. Onah *et al.* (1989) recorded 0.002% of the disease in cattle, 0.05% in goats and 0.003% in

pigs slaughtered in Anambra State. Most of these studies are based on postmortem findings from abattoir records (Anyanwale *et al.,* 1982).

However, surveys of adult tapeworm infection in dogs have shown either relatively low infection rates or high ranging from 0.01% in Kaduna State (Dada *et al.*,1979) to 4.4% recorded among rural dogs in Anambra State, Eastern Nigeria (Okolo, 1986).

On a general note, there are a few available data on the clinical effects of cystic hydatid disease in animals compared to man (Ibrahim *et al.,* 1996). This is attributed to the fact that the cysts grow slowly and animals are often slaughtered before the cysts create sufficient pressure on the tissues or organs. Camels which are one of the intermediate hosts of hydatidosis in Africa are becoming more acceptable as meat in northern Nigeria. There is the need to use more sensitive serological techniques to conduct studies in Nigeria in order to know the actual status of the disease especially in areas where camels are slaughtered. This will help to assess the high risk of infection to which the consuming public is exposed to and the zoonotic implications of the disease as it relates to camels, sheep, cattle and goats, dogs and man.

In view of the fact that the clinical signs of the disease are inapparent, reliable and sensitive serological tests using purified antigen for the diagnosis of the disease in live animals is vital for prompt control and prevention of the disease. It is with this background that the present study was undertaken.

1.4 Objectives

1. To determine the major antigenic subunits, common antigenic bands and immunodominant species specific antigenic component in the camels, sheep and goat hydatid cyst fluids.

2. To determine the prevalence of antibodies to hydatidosis in domestic animals including camels slaughtered at the Kano abattoir, Kano State.

3. To evaluate the validity of some serological techniques used for the diagnosis of the disease viz: Enzyme Linked Immunosorbent Assay (ELISA), Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)and western blotting with the aim of getting a quick means of monitoring the hydatid disease status in animals.

4. To investigate prevalence of the disease in humans using a ten- year (1993-2002) hospital records in Kano State.

1.5 Research Questions

a) What are the subunits of the cyst fluid antigen obtained from the domestic animals slaughtered in Kano abattoir?

b) Are there common antigenic bands in camel, sheep and goat?

c).Which is the immunodominant species specific antigenic component in hydatid cyst fluid of camels, sheep and goats?

d) Is there any significant difference in antibody titre between species and age of animals?

d) Is there any significant difference in antibody titre levels between males and females in each species?

e) Is there significant difference in means optimal density (OD's) between the two dilutions (1:100 and 1:200) for antigen from each species?

f) Is there any significant difference in retention time (RT) of the purified antigens among the different species?

g) Can the location of cyst influence the level of antibody titre obtained in different animals?

h) Which of the diagnostic methods is the most reliable, fast and appropriate for use in establishment of hydatidosis control scheme in Kano state?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of the disease

Echinococcosis and hydatidosis are synonyms for infection with larva (metacestode) of the dog tapeworm, *Echinococcus.* Hydatidosis was first described by Hippocrates in 460-379BC. The first book on the disease was written by Dr Abdallah Bey formally Karl Edward Hammer Schmidt and was published in 1876 (Jenkins *et al.,* 1998; Altintas, 2003). In 1773, John Hunter made an accurate description of the parasite; he noted that hydatids were uniformly round and filled with clear water in transparent bag of two coats hence the name Echinos (Greek-Hedgehog); Kokkos (Greek-berry); Granulum (Latin-small grain);Hydatids (Greek-drop of water) (Khuroo, 2002).

Hydatidosis is a public health problem in Asia, the Mediterranean, South America and Africa (Kanwar *et al.*, 1992; Ito *et al.*, 1999; Seimenis, 2003;).Immigration has increased the prevalence of the disease in Europe and North America in the recent years (Jenkins, 1998; Sotiraki *et al.*, 2003) causing substantial mortality and morbidity in humans and in livestock downgrading and condemnation of tissues for human consumption in many developing countries (Lightowlers and Gauci, 2001). There has been considerable progress in the diagnosis and surgical treatment of the disease; drug therapy and percutaneous aspiration have been successfully used to treat the disease (Deplazes and Eckert, 1996).

2.2 Echinococcus Species

Echinococcus is a small endoparasitic flatworm belonging to the Kingdom-Animalia, Subkingdom-Metazoa; Phylum-Platyhelminthes; Class-Cestoda; subclass-Eucestoda; Order-Cyclophyllidea; Family-Taenidae and Genus-*Echinococcus (*Soulsby, 1982; Thompson, 1995). Four species are commonly recognized in the genus *Echinococcus*; *Echinococcus granulosus* which causes cystic hydatid disease; *Echinococcus multilocularis* which causes alveolar hydatid disease; *Echinococcus vogeli* and *Echinococcus oligarthrus* which cause polycystic hydatid disease. The four species are distinguished by morphological and biological characteristics in both adult and larval stages and to some extent by geographical distribution, definitive

and intermediate host range and the nature and location of the hydatid cyst (Schantz *et al.,* 1995; Thompson, 1995). There are nine host strains of *E. granulosus*, which include the sheep strain, horse strain, cattle strain, camel strain, pig strain, buffalo strain, tasmanian strain, cervid strain and lion strain Eckert *et al.* (2000). *E. multilocularis* has three strains including the European strain, Alaskan strain and North American strain, while *E .vogeli* and *E. oligarthrus* have only one strain each (Jenkins and Macpherson, 2003).

Echinococcus species is a "true worm". It has no gut and all metabolic interchange take place across the syncytial outer covering, the tegument. Anteriorly, the adult possesses a specialised attachment organ (scolex) bearing two rows of hooks and four muscular suckers. The body or strobilla is segmented and consists of a number of reproductive units (proglottids). *Echinococcus* has an indirect life cycle in which the adult is hermaphroditic and the larva, that is hydatid cyst, proliferates asexually (Rausch, 1995; 2003).

Cystic echinococcosis (CE) caused by the larval stage of *E.granulosus* is the most widespread ; dogs are the usual definitive hosts while a large number of mammalian species including domestic ungulates and man is an accidental host (Torgerson and Heath, 2003). *E. multilocularis* has been identified in Tunisia, The southern part of France, Northern Italy and Turkey (Larrieu, 2001; Altintas, 2003).The ability of *E.granulosus* to adapt to a wide

variety of host species and the repeated introduction of domestic animals from endemic areas to other parts of the Mediterranean contribute to the broad distribution of the parasite (Seimenis, 2003).

2.2.1 *Life Cycle of Echinococcus species*

Echinococcus species requires two hosts for completion of its life cycle (Permin and Hansen; 1994 Rausch, 1995;). The definitive hosts which are carnivores harbor the mature tapeworm in the intestine. The dog is the urban definitive host (Craig *et al.*, 1995) while the foxes, wolves and jackals are the sylvatic definitive hosts (Heath, 1999). Metacestodes develop in the liver and viscera of the intermediate hosts which include goats, sheep, cattle, camels and pigs. Humans become accidental hosts by close contact with these domestic animals but do not participate in the full life cycle. (Gemmell *et al.*, 1987; Khuroo, 2002).The adult tapeworm is found in different parts of the small intestine of the definitive hosts. The worms may be in thousands. The eggs which are ovoid having a diameter of 30-36micromitres are shed with the faeces of the canid definitive host. These eggs contain oncospheres (fully developed larval stage). These can survive for several days outside the host and may be carried by wind and water and then are ingested by the intermediate hosts which are primarily cattle, sheep, goats, camels, other domestic animals and man (Jenkins, 1998). The oncospheres hatch in the

small intestine, become activated and penetrate the epithelial layer and migrate via blood or lymphatic vessels to the lungs, liver, brain, bones and other organs of the intermediate host with liver as the most commonly affected organ (Hamdy *et al.,* 1980; Gemmell and Robert,1995; Khuroo, 2002). In these organs, the oncospheres undergo a series of events involving degeneration, cell proliferation, differentiation and vesiculation resulting in the formation of primary vesicles (metacestode) which develop into hydatid cysts. Scoleces are formed within the cysts after a minimum of 10-12 months from the time of infection and render the cysts fertile. Ingestion of intermediate host offals containing infective protoscoleces by the definitive host completes the cycle (Rausch, 2003).

The hydatid cysts develop slowly over several months, forming an outer laminated membrane and an inner membrane called the germinal layer. From the germinal membrane, brood capsules develop, each containing one or several invaginated heads (protoscoleces) that can develop into the adult tapeworm upon ingestion by the definitive host (Soulsby, 1982). Cysts of *E. granulosus* grow throughout the life cycle of the host It may reach a volume of several litres, but unilocular. The cysts of *E. multilocularis* consist of masses of small interconnected cysts (multilocular or alveolar) that spread throughout the affected organ behaving like a slowly growing malignant tumour (Jenkins, 1998). Subspecies of *E. granulosus* differ in their

preferences for intermediate hosts. *E. granulosus granulosus* hydatids belong to the subspecies adapted to sheep and man, where as *E. granulosus equinus* is the subspecies found in horses and asses (Rausch, 2003).

2.3 Epidemiology

Cystic Echinococcosis (CE) caused by the larval stage of *E.granulosus* is the most widespread of Echinococcosis (Jenkins, 1998, Sotiraki *et al.,* 2003). In the United Kingdom, the parasite has a restricted distribution, being found mainly in the middle and southern Wales (Torgerson, 2003a; Jenkins, 1998; Khuroo, 2002). In Europe, zoonotic strains of *E. granulosus* are present except in Ireland, Iceland and Denmark (Sotiraki *et al.,* 2003) It is the most intensely endemic parasite in the Mediterranean areas and parts of Eastern Europe. Todorov and Boeva (1999) conducted an epidemiological study of human echinococcosis in Bulgaria and they discovered that it had been a public problem in the country for over 40 years; with an annual incidence of 6.5 per 100,000 populations and that the infection rate in domestic animals was very high.

Prior to the break up of the Soviet Union, cystic echinococcosis in man was at relatively low level. However, independence of the Central Asian Republics resulted in widespread structural and economic reforms, which resulted in privatization of farms, abandonment of centralized meat

processing facilities and a return to small subsistence-type of agricultural practices, and the collapse of veterinary services due to lack of government funding. This led to an epidemic of human cystic echinococcosis with an annual incidence of surgical cases reported by hospitals in excess of 4-5 times the number prior to 1991 (Torgerson *et al.*, 2003b; Willingham, 2003).In some parts of China, the parasite is regarded as an important re-emerging zoonosis (Yana Bai *et al.*, 2002).The disease has been recovered in more than 87% of inhabitants of China. In some parts of central Asia, Kazakhstan, it is regarded as the number one silent killer. A similar pattern is also emerging in other former communist countries like Bulgaria (Todorov & Boeva, 1999). It's also endemic throughout Indian subcontinent and the Middle East (Khuroo, 2002; Youngster *et al*., 2002).

In most endemic countries, there are no data regarding canine population size; national estimates of total number of dogs vary. A survey conducted on the prevalence of infection in dogs in Greece showed that there is an average dog population of 500,000-1,500,000 in Greece. The prevalence in dogs differs significantly from place to place, according to the population of dogs in the environment and the occupation of the people. A prevalence of (50.4%) was recorded in shepherd dogs, (26.9%) in sentinel dogs, (19.2%) in hunting dogs, (9.3%) in stray dogs and (0-1%) in companion (pet) dogs. (Vassalos, 1978; Tsaglas, 1985). In addition, mass

diagnostic examination was conducted on the dogs between 1985-1987, 3.3% of the 110,093dogs were infected with *E.granulosus* the infection was also reported in wolves (14.3%) (Papadopoulos, 1989).

In Africa, the parasite is widespread and is a particular problem in Northern African countries such as Tunisia, Morocco, Libya and Algeria (Willingham, 2003; Torgerson *et al.,* 2001). It is considered a serious problem of the nomadic pastoralist tribes of Eastern Africa (i.e. Kenya, Tanzania, Uganda, southern Sudan and Somalia) who have very close associations with their ruminant livestock and dogs (Chemtai *et al.,* 1981; Sotiraki *et al.,* 2003). Inspite of the availability of new tools for effective surveillance and control of the disease, it remains a serious problem of the nomadic pastoralists tribes of Eastern Africa i.e Kenya, Uganda, Tanzania, Southern Sudan and Somalia where prevalences are considered the highest in the world, and the economic and social status of the populace has made prevention and control a very difficult task (Altintas, 2003). In North America, the parasite is prevalent in Canada and Alaska (Torgerson *et al.*, 2003). In the United States of America, the parasite is sporadic with just a few foci such as certain communities in Utah and California. In South America, the parasite is extensive particularly in Argentina, Uruaguay and Penivian Andes. (Larrieu *et al.,* 2001). In the Rio Negro region of Argentina, the disease affects human and animal health with considerable economic

losses to sheep and cattle production. The social, economic and cultural habits of the rural population favour the persistence of habits that ensure the maintenance of the transmission cycle of the disease especially homeslaughter of mature sheep for human consumption and feeding of infected viscera to dogs (Kanwar *et al.*, 1992; Larrieu *et al.*, 2000; Lightowlers *et al.*, 2001;).

Karpathios *et al.* (1985) conducted a retrospective study to estimate the surgical prevalence among Greeks over 18years of age. 4,202 adults were diagnosed for hydatidosis, out of which 3,397(80.8%) underwent surgery (489 per year or 7.9 surgical cases per 100,000 inhabitants).The incidence of the disease varied between different geographical areas in the country being more prevalent in rural areas. Solitary organ involvement occurred in 95.9% of the cases and cysts were localized in the liver of 69.9% and in the lungs of 23.4% of the people.

Studies conducted in various domestic farmed animals showed significant differences in the prevalence of the disease from year to year and from one area to another (Sotiraki et *al.*, 2003). Vassalos (1978) conducted a prevalence study among domestic animals in Greece from 1973-1977 and recorded a prevalence of 53% in cattle raised outdoors, 17% in cattle raised in doors, 61% in sheep, 7.2% goats, 1.9% in pigs. More precisely, cysts were detected in 16 (80%) of sheep less than 2 years old; cyst fertility rate of (64.2%) was recorded in adult goats and the infection rate was between 1 and

22.8%. In cattle over 3 years old, the rate varied from 12.8% to 18.9% with cyst fertility of 6.6%. The prevalence rate in pigs over 4 months varied from 0.04% to 4.9%.It was also discovered that the sheep harbored more cysts and had a higher proportion of fertile and viable cysts than other intermediate hosts. This supports the primary importance of the sheep-dog life cycle pattern in the Mediterranean region and other endemic areas (Jenkins, 1998; Seimenis, 2003., Sotiraki *et al.*, 2003).

In Australia, the parasite was detected among the urban dwelling domestic dogs used for pig hunting and in wild foxes that have migrated into urban environments (Thompson and Lymbery, 1988). The distribution in rural domestic dogs has fallen steadily during the last 40years (Jenkins, 1998). Nevertheless, cases of human unilocular hydatidosis are still regularly diagnosed in the region. This is due to the high prevalence of the disease in local wild dogs and fox population in the area (Jenkins and Morris, 1991).

In most underdeveloped countries of the world particularly where sheep farming is an important agricultural industry, the disease is widespread. A substantial increase in the incidence rate of cystic echinococcosis has been reported in Kyrgyzstan, and it's neighboring Kazakhstan in central Asian republic, due to a change in agricultural practices, increased small livestock units and home slaughtering of animals (Todorov and Boeva, 1999; Torgerson *et al.*, 2001; Shaikenov *et al.*, 2003).

In a similar development, the disease has been tagged the most important helminthic disease in Turkey (Altintas, 2003). The disease was first discovered in the country in 1861. This is not unconnected with the animal husbandry which is a predominant occupation in the area and almost every farmer keeps one or two dogs, and lack of a consistent state policy on the prevention and control of the disease. There is difficulty in the prevention and treatment of the numerous dogs in the area because 99% of the populace are Muslims and once in a year, during their religious festivals they slaughter tens of thousands of sheep and cattle thus providing the stray dogs the offals from the infected intermediate hosts (Seimenis, 2003).

In Greece, the disease was discovered long before the 1960's and by 1970, the incidence in human was 51 per 100,000 inhabitants (Tsaglas,1985), the incidence rose to 10.9 per 100,000 inhabitants in 1981 (Sotiraki *et al.,* 2003).The ability of *E.granulosus* to adapt to a wide variety of host species and the repeated uncontrolled introduction of food and domestic animals from endemic areas to other parts of the world has always contributed to the broad distribution of the parasite (Seimenis, 2003).In the Mediterranean region, such conditions and activities have facilitated the spread of the disease. In addition, numerous rural families live in close proximity to their flocks of sheep, goats, cattle and camels and participate in taking care of them.

Man is normally a dead end host of this parasite. The estimated global incidence of human hydatidosis is more than 100,000 per year in endemic areas, the surgical rate is more than 10/million population per year, and the disease is common among immigrants (Janssen and Rycke, 1990).

In Belgium, there was no statistical data on the disease until 1975 when a survey was conducted and 16 patients had the disease 14 out of them were not born in Belgium but in Morocco and Italy and very low incidences were recorded in domestic animals. Due to the application of successful control programmes in some developing countries, E. *granulosus* is becoming increasingly uncommon most notably on islands such as Iceland, New Zealand, Tasmania and Southern Cyprus (Eckert *et al*., 2001).

Human alveolar echinococcosis caused by the metacestode of the fox tapeworm, *E.multilocularis* is considered the most pathogenic zoonoses in temperate and artic regions (Tamas *et al.*, 2003).It has been discovered that the parasite has a limited distribution in the northern hemisphere, within a stretching from the northern Tundra zone (Khuroo,2002). The disease has also been reported in the central parts of Europe (southern Germany, western Austria, Switzerland and Eastern France), some parts of Russia (Siberia), Eastern Azerbarjan, some parts of Turkey (Eastern Anatolia), Northern Iran, Northern Afghanistan, Northern India (Kashmir), Northwestern Canada, western Alaska and Northern Japan (Hokkaido). The annual incidence is low

(0.03 to 1.2 per 100,000 per annum) in most areas where the disease is reported (Jenkins, 1998; Willingham, 2002; Torgerson and Budke, 2003).

In the 1990's and early 2000's, the infection rate of foxes increased drastically in some areas of France and Germany (Janssen and Rycke, 1990; Eckert *et al.*, 2000). Several new endemic foci were detected in Switzerland, Germany and Austria and the parasite was reported from Netherlands, Belgium, Luxembourg, Poland, the Czech Republic Slovak Republic and Italy (Deplazes and Eckert, 2001).

High prevalence of hydatidosis occur frequently among the tribes of northern Kenya (Klungsoyr *et al.*, 1993) and Southern Ethiopia (Craig *et al.*,1986;1995).The prevalence of the disease may be up to 5.6% in some of the tribal groups in East Africa, with women accounting for the majority of the cases. This is attributed to the involvement of most of the populace in agriculture especially livestock husbandry. Klungsoyr *et al.* (1993) conducted a retrospective studies using hospital records in southern Ethiopia, they reported that 42 cases of hydatid disease were recorded between 1973 and 1981 with 25 being females, 12 males and no information on sex in five patients. Similarly, a population based study was conducted among the Hamar people of Ethiopia using a portable ultrasound scanner, a prevalence of (0.7%) was recorded, cysts were most common (4.7%) among women about 40 years old. Only one man presented a cyst, six of the seven hydatid

patients had liver enlargement of 4-5cm on palpation, the remaining one, a male (9 years old), had no evidence of liver enlargement. the prevalence of the disease increased with age. The results showed a sex-associated public health problem among the people.

Over the years, accurate estimates for hydatidosis exist for the northern part of Israel. Prevalence studies in the area showed that infection rates of (0.48%) and (0.57%) were recorded among adults and children respectively A high prevalence was also recorded in sheep and goats (5.6%) and in dogs (11.2%) belonging to the non-Jewish communities (El-on *et al.*, 1997). Until recently, in the southern part of Israel, which comprises mostly of the arid desert, the disease was considered of minor importance, due to the fact that the desert conditions are unsuitable for the development of the parasite eggs. However, during the last decade an increasing number of hydatidosis patients have been recorded among the Muslim Bedouins population (Nahmias *et al*, 1993). In 1999, a total of 125 patients were examined for cystic echinococcosis in the Soroka University Medical Center, 31(24.8%) were found to be infected of whom 22 (70.2%) were Bedouin.

In 2001, a seroprevalence study on cystic echinococcosis was again conducted among the Muslim and Jewish population in southern Israel using seroepidemiological surveys, a prevalence of 7/1026 (0.68%) was recorded in Bedouin urban group including children under the age of 10 years Among the

Jews, a seroprevalence of (0.51%) was recorded in patients over 60 years of age only. A high rate of infection (22.9%) was recorded in goats and sheep slaughtered in abattoirs of Bedouin localities. A high seroprevalence (4.44%) was recorded among the nomadic community compared to (0.68%) in the urban area. This was attributed to the flock breeding by the nomadic communities which is their traditional occupation and lifestyle of keeping dogs as an important means of protecting their livestock (Youngster *et al.*, 2002).

Human cystic echinococcosis is a serious problem in West China; because of close human relationship with the definitive and intermediate hosts. Yana Bai *et al* (2002) conducted a survey using serological method in three communities of the Tibetans in western China and recorded an overall prevalence of (13.7%) in Jijitan, (11.1%) in Quanbao and (7.6%) in Minzhong. The results revealed a significantly higher prevalence in females with a two-fold increase over the rate for males at all ages due to the closer contacts between females and dogs and the greater time spent by dogs and women in the home area. This is similar to the work conducted by Watson-Jones and Macpherson (1988) in Kenya, Youngster *et al.,* (2002) in Isra

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2.5.2 Diagnosis in Humans

The definitive diagnosis for most human cases of CE is by physical imaging methods (Ammann and Eckert, 1995; Coleman, 2000; Eckert *et al.*, 2001; Khuroo, 2002; Macpherson *et al.*, 2003) such as radiology, ultrasonography, computed topography (CT scanning) and magnetic resonance and Imaging. Detection of circulating *E. granulosus* antigens in sera is less sensitive than antibody detection which remains the method of choice (Ayadi *et al.*, 1995; Zhang *et al.*,2003).Various serological tests have been used in the diagnosis of human cases (Torgerson *et al.*,2003; Klungsoyr *et al.*,1993; Janseen *et al.*,1990). There

are considerable differences among various tests in both sensitivity and specificity (Iacona *et al*., 1980; Zhang *et al.,* 2003). Insensitive and non-specific tests including cassoni intradermal test, the complement fixation test, the indirect haemagglutination test and the latex agglutination test have been replaced by the enzyme linked immunosorbent assay (ELISA), the indirect immunolectrophoresis (IEP), and immunoblotting (IB) in routine laboratory applications (Otupiri et al., 2000;Rybicki and Purves,1996; Siles and Gottstein,2001).

Hydatid cyst fluid is the most important source of antigen used in routine serological diagnosis of hydatid disease in man and animals (Chordi and Kagan, 1965; Kittelberger *et al.*,2002). Studies have shown that antigen prepared from human hydatid cyst fluid (HCF) was found to be unsuitable for diagnosis because it contains host proteins such as IgG (Baveja *et al.*,1997). Sheep HCF obtained from fertile cyst has been used routinely to prepare and standardize antigen (Chordi & Kagan, 1965;Varela-Diaz *et al*.,1974; Ortona *et al*.,2003). Bovine HCF can be used as an alternative antigen source (Lightowlers *et al*., 1989). The camel HCF has also been used as a source of antigen in many studies (Dada *et al*., 1981; Ibrahim *et al.,* 1996; Rybicki and Purves, 1996).

Two antigenic molecules are defined i.e antigen 5 (Oriol *et al.*,1975; Lightowlers *et al*., 1984; Rogan *et al.*,1991) which is a dominant heat – labile lipoprotein antigen which forms the diagnostic 'Arc 5' in immunoeletrophoresis with sera from majority of humans infected with *E. granulosus.* The antigen has a high molecular mass (approximately 400kDa). It is composed of 57 and 67 kDa

components and under reducing conditions dissociates into 38 and 22 to 24 subunits. Lightowlers *et al.,* 1984; 1989; Hans- Peter & Bucher,1986). Studies have shown that antigen 5 is cross reactive with human antibodies to other taeniid cestodes most notably *E. Multilocularis, taenia solium* and other helminthes (McManus, 1987; Maddison *et al.,* 1989; Yarrow *et al*., 1991; Poretti *et al.,* 1999; Shepherd and).

Antigen B is a polymeric (thermostable) lipoprotein with a molecular weight of 120 kDa. It plays an important role in the biology of the parasite and its relationship with the host (Shepherd and McManus,1987). It is a highly immunogenic molecule (Oriol *et al.*,1971; Olga *et al.*,2001) a characteristic that underpins its value in serodiagnosis under reducing condition on SDS-PAGE It produces bands with molecular sizes of approximately 8 or 12, 16 and 24kDa.

2.5.3 Diagnosis in the Intermediate Hosts

In comparison with investigations in humans, relatively little research has been directed towards the development of immunodiagnostic techniques for *E. granulosus* infection in domesticated animals Lightowlers *et al*.,,1989; Zhang *et al.,* 2003; Kittelberger *et al.*,2002). Currently, the diagnosis of CE in intermediate hosts is based mainly on necropsy procedures. Accurate serological diagnosis of CE infection in livestock is difficult due to serological cross reactions with several other species of taeniid cestodes including T*aenia hydatigena and Taenia ovis (*Lightowlers and Gottstein, 1995). Furthermore, natural intermediate host animals

produce very poor antibody responses to infection compared with the relatively high levels of specific antibody seen in human infection (Lightowlers and Gottstein,1995). ELISA techniques using a variety of antigens have been applied to the diagnosis of CE (Gottstein *et al.*,1987). In experimentally infected sheep, antibodies to hydatid antigens can be detected as early as 4 years (Olga *et al.*, 2001). However, serological cross reactions with other cestodes limit the specific diagnosis of hydatid infection by ELISA with crude parasite antigens (Kanwar 1994; Janseen *et al.*, 1990., Poretti *et al.*,1999). Purification of crude antigens with antibodies from animals immunized with homologous antigen partly reduces the cross reactivity. DNA Techniques are now available that allows the identification of echinococcus species and *E. granulosus* strains by using metacestode materials from intermediate host (Craig and McManus, 2003).

2.5.4 Diagnosis in the Definitive Hosts

Accurate determination of Echinococcus species prevalence in definitive hosts (Dogs, Foxes, and Cats etc.) is essential for estimating the potential risk for humans and to undertake transmission studies (Eckert *et al* ., 2001). Diagnosis of *Echinococcus* species in the definitive hosts is complicated by the fact that eggs of species in the genera of both E*chinococcus* and *Taenia* are morphologically indistinguishable. Therefore parasitological diagnosis relies on direct detection of the tapeworms (Craig *et al.*,1995). Two major parasitological methods are used i.e. purgation with arecoline salts and examination of the small intestine at necropsy

(Deplazes and Eckert,1996;2001).Both are time consuming, laborious and require special safety precautions and not suitable for large scale epidemiological studies.

To enable diagnosis in living animals, several indirect methods have been described. Detection of serum antibodies, coproantigens and parasite DNA in faecal samples (Zhang *et al.*, 2003; Lightowlers and Gottstein., 1995; Gasser *et al.*,1990) and even these serological diagnostic techniques often revealed poor sensitivity and specificity. As many as 60% of infected dogs are negative when tested for specific IgG antibody (Jenkins and Morris, 1991).

2.6 Symptomatology

Up to 65% of the cases of cystic echinococcosis can be asymptomatic, and *E.granulosus* cysts and lesions induced by them are often incidentally detected during examinations of other diseases (Eckert *et al.,* 2001) in symptomatic cases, clinical signs are extremely. Variable in the liver echinococcosis, pain is a frequent symptom (in 77% out of 100 patients) while Jaundice (22%) is less frequent. On physical examination, an enlarged and indurated liver may be diagnosed (Eckert *et al.*, 2000).

Clinical signs of pulmonary cystic echinococcosis are chest pain, cough, fever, haemoptysis and sometimes expectoration of cyst contents (Jerray *et al.*, 1992; Zhang *et al.,* 2003). Since cysts can be localized virtually in any anatomic site, the symptoms are manifold and depend primarily on The organ involved, size

of lesions and their sites, interaction between the expanding cysts and adjacent organ structures particularly by the bile ducts and the vascular systems of the liver, complications caused by spontaneous or traumatic cyst rupture, bacterial infection of cysts and spread of protoscoleces or small vesicles and immunological reactions such as asthma anaphylaxis or membranous nephropathy (Ammann and Eckert, 1995).

2.7 Control

Hydatidosis can be controlled through preventive measures that break the cycle between the definitive and the intermediate hosts (Permin et *al.*, 1994; Craig *et al.*, 1995). The major obstacle to the control of the disease is the wide host range and lack of active surveillance for the disease in most endemic areas which prevents the medical and veterinary authorities from having detailed information on the disease. *Echinococcus granulosus* has been viewed to be highly vulnerable to the implementation of preventive measures (Schantz *et al.*, 1995). The earliest successful control programme was in Iceland initiated nearly 130 years ago when cystic hydatid disease was recognized as affecting approximately one of every six Icelanders (Torgerson *et al*., 2000) Studies on the transmission dynamics between the environment, intermediate hosts and definitive hosts are very important for the successful implementation of control programmes (Torgerson, 2003b).

Hydatidosis can be controlled by public health enlightenment or education which entails conducting a uniquely effective health campaign that can sensitize

the entire population to the disease. Subsequent measures which include educating the population and motivating them to change the practices of home slaughter of sheep and adopting proper meat inspection in the abattoir would gradually eliminate or reduce transmission. (Torgerson and Heath, 2003; Craig and McManus, 2003). Diagnostic purging of dogs with arecoline as a surveillance technique which helps in effective monitoring and identifying problem farms, quarantine of infected dogs and sheep, preventing the dogs from consuming infected viscera from sheep and other domestic ungulates and elimination of stray dogs and wild canids where they are involved in transmission also serve as effective control measures.

Studies have revealed that the most effective method of *E. granulosus* control would be to treat both the definitive and intermediate hosts at the same time (Torgerson *et al.*, 2001; 2003). Treatment options for the disease include drug therapy, surgery and percutaneous drainage (Khuroo, 2002; Zhang *et al.*, 2003). The World Health Organization (WHO) recommends medical therapy for patients with inoperative hydatid disease, multiple or disseminated cysts, incomplete surgery or relapse and for prevention of spread after rupture or cyst aspiration (Ammann and Eckert., 1995; Olga *et al.*, 2001).

Several compounds have been assessed as chemotherapeutic agents, the most promising being the benzimidazole derivatives (albendazole, mebendazole) with cure rates of up to 43%.Also praziquantel which is an isoquinolonen derivative have been found to be effective in treating dogs which do

not acquire protective immunity to infection and thus must be treated every 6 weeks to ensure break in transmission. However, in most cases, resources are not made available to sustain such control activities. Benzimidazole treatment increases 15 years survival rates by up to 80% (Khuroo, 2002). Albendazole have been found to be more effective than mebendazole due to its superior absorption and cystic penetration.

Recently, attention has been given to the potential of treating cysts in livestock with oxfendazole after it was found to be extremely effective against *Taenia solium* tissue cysts in pigs. Results of preliminary studies conducted by the African Medical Research Foundation (AMREF) in Turkana, Kenya on the efficacy of oxfendazole against hydatidosis have been found to be promising. (Craig and McManus, 2003). There is information about a local herb being used in southern Sudan to cure human hydatidosis with very good success. There are plans to evaluate the efficacy of the herb in both man and livestock in the highly endemic Turkana district of Kenya.

Research is on in China at the National Hydatid Disease Center in Urumqi Xingiang province and Lanzhou Veterinary Research Institute in Gansu province trying to develop a subcutaneous transplant form of timed-release praziquantel for dogs. The device, if placed under the dogs' skin, would ideally release a therapeutic dose of praziquanted every 6 weeks to ensure that dogs receive treatment at the necessary intervals to block transmission (McManus and Thompson., 2003).Recently a hydatidosis vaccine (EG95) for sheep and goats

based on non-living oncosphere antigens has been developed (Lightowlers *et al.*,2000). Vaccine trials in Argentina and China indicated that it induces > 95% protection against experimental challenge infection in sheep and goats with similar protection seen in naturally challenged Chinese sheep. Immunity lasts for at least one year after two immunizations and can be transferred passively to neonates (Willingham., 2003).

Successful control programmes, eventhough significant at the local level, have not markedly changed the global distribution and public health importance of the disease. In most endemic areas, effective control has not been achieved or attempted (Willingham, 2003).

2.8 Economic Impact of Hydatidosis

It is becoming increasingly important to study the economic effects of hydatidosis as such effects are vital in the evaluation of potential control programmes (Torgerson, 2003a). In 1979, WHO recommended that an economic evaluation of the effects of parasitic zoonoses should be an integral part of any control programme. It is very important to gain an understanding of the potential financial implications of cystic hydatid disease which can be used to define cost effective control programmes. The most obvious human health cost and the easiest

to define is the cost of medical and or surgical treatment of patients suffering from clinical echinococcosis. (Torgerson and Dowling, 2001).

Other potential effects on human health include human morbidity and mortality costs. Long term quality of life in patients treated for cystic echinococcosis (CE) is permanently affected. This could be due to individuals having impairment in the work place and thus having less highly paid work than would otherwise have been the case, having greater levels of absenteeism from work or possibly requiring greater levels of long-term nursing care (Domingo, 2000).The mortality rate for CE is generally reported at 1-2% (Torgerson and Budke.,2003), which is much higher than alveolar echinococcosis(AE). The total human health costs are normally less in poor developing countries (World Bank,1993) than in rich industrialized countries because hospital charges tend to be smaller, medical services less sophisticated and less well-equipped and the labour charges cheaper in poor countries (Torgerson,2003a).

The animal health costs include the cost of loss of edible offal, mainly liver, from animals with CE (Nessari and Robert, 2000). At worst such condemnations result in the total loss of that organ, although in some countries such as Uruguay, there is the possibility that condemned organs may still have a value in the pharmaceutical industry (Torgerson *et al.*,2000).

However, potentially the largest costs to animal health could be due to production loss in livestock affected by CE which can be in form of a

reduction of live weight gain, reduced yield of milk, reduction in fertility rates and reduction in the value of wool or other products. Some of these losses are estimated as 10% of agricultural loss. Many of these losses are difficult to estimate due to the fact that there have been few controlled studies. Nevertheless, data such as from Ramazanov (1982) and that reviewed by Polydorou. (1981) and Altintas. (2003), suggest that these losses are potentially very significant and should not be ignored.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out at the Kano abattoir, Kano state. Kano is an ancient city in Northern Nigeria. It is the capital of Kano state. It is one of the commercial centers in Nigeria with a lot of industries. The city is located on latitude 11^0 58 North and longitude 8^02^1 east (NPC,1991) The state has an area of 42,592 square kilometers and a population of over 12 million one third of which live within the 27 kilometers radius of Kano metropolitan. Kano state is bounded by Jigawa state to the north, Kaduna and Katsina state to the west, Yobe and Bauchi states to the east and Kaduna state to the south. The metropolitan is about 240km from the edge of the Sahara desert (NPC, 1991).

The vegetation is characteristic of the Sudan savanna zone with short grasses and medium height trees interspersed with shrubs and herbs. The dominant inhabitants of the state are the hausa/fulanis, whom are mostly muslims. Kano has an annual temperature that ranges between 31^0C to 22^0C and possesses a tropical continental climate. It has an annual average rainfall of 750 mm per annum. The earliest rainfall in Kano is in March, with August usually being the wettest month, the rainfall ending in September. The dry season starts in October and ends in April every year. Low temperature is experienced in December through February

part of the dry season due to cold, dry harmattan winds, which blow from northeast.

Kano abattoir is located in the heart of Kano metropolitan city. It is the only abattoir utilized in the city. It was constructed 40 years ago with a lot of facilities which has depreciated over the years. The abattoir is fenced; there are staff offices, three slaughter halls and inbuilt slaughter slabs. Blood, water and animal wastes pass through the narrow gutters that run in and outside the building, the floor of the abattoir is cemented.

The species of animals slaughtered in the abattoir include cattle, sheep and goats purchased from the neighboring villages like, Maigatari, Suletankarkar, Danbatta, Kazaure, Roni and Wudil. The camels are imported from the neighboring Niger and Chad republics. The goats, sheep and a fraction of cattle are slaughtered in the slaughter rooms, while the camels and some cattle are slaughtered in the open space. There is a lot of congestion in the abattoir and different animals are slaughtered in separate halls by individual butchers.

An average of 85 camels, 860 goats, 1,200 sheep and 2,500 cattle are slaughtered daily. All animals are normally registered at the gate, a receipt issued before taking them in for slaughter.

Routine meat inspection is not conducted in the abattoir; there are no veterinary and health infrastructures, no first aid facilities and no proper abattoir records. Animals hides and skin are processed in the abattoir and sold to individuals and companies. Home slaughter is very common in the area especially

during their festive periods, when animals are slaughtered indiscriminately at home.

3.1.1 The study population

The study population includes camels, goats, sheep and cattle. Permission was obtained from officials of the Kano abattoir, after they were briefed on the purpose and benefits of the research work. A total of 551 blood samples were collected from the animals viz 138 sheep, 130 goats, 147 cattle and 136 camels A total of 21 hydatid cysts were collected from eight goats, six sheep and seven camels.

A survey was carried out to detect the presence of hydatid cysts in infected animals. The random sampling method was used for the collection of samples. Numbers one to ten were written on papers and placed at the location of slaughter prior to arrival of the animals to the abattoir. Blood samples were collected from the selected animals and hydatid cysts were sought for from the same animals.

3.2 Collection of samples

The blood samples were collected from animals slaughtered in the abattoir between 6am –8am during the study period. The blood was collected in clean bijou bottles, labelled and stored in icepack. The liver, lungs, peritoneum, diaphragm, thoracic cavity of animals whose blood were collected were examined for the presence of cysts when found, the cysts were also kept in bottles and

labelled. Hydatid cyst is the larval stage of the tapeworm *Echinococcus* species which contain daughter cysts each of which contain many scoleces. Samples were transported to the laboratory immediately for analysis. Samples were collected once a week between January 12th to May 8th 2003.

3.3 Administration of questionnares

Questionnaires were administered with the aim of obtaining information on the animals and abattoir including: animal species, estimated age of the animal, presence or absence of cysts, and location of cysts. For the abattoir, presence or absence of stray dogs around the abattoir, means of disposal of offals, and general hygiene of the abattoir (Appendix I).

3.4 Laboratory analysis of the samples

Blood sample from each animal was centrifuged at 1,000 revolutions per minute (rpm) for 5 minutes then the serum was separated using a Pasteur pipette, transferred to sterile bottles, labelled and stored at 4 ^{0}C. The hydatid cysts were neatly kept in sample bottles. Both the cysts and serum samples were packed in ice pack and transported to the Chemistry Laboratory, Department of Agriculture and Food Studies, University of Plymouth, Seale Hayne campus, United Kingdom. The serum was used as a source of primary antibody while the hydatid cyst fluid was used as a source of antigen for the study.

3.4.1 Purification of Hydatid cyst fluid (HCF) antigen

In order to identify the species specific antigenic component in hydatid cyst fluid preparations, that invoke antibody responses in animals, the crude HCF obtained from the animals were purified and ran on SDS-PAGE. The hydatid cyst fluid (HCF) was processed according to the method of Oriol *et al.* (1971) reviewed by Rogan *et al.* (1991) in order to obtain a fraction enriched with the subunits of the main antigens (Lightowlers *et al.*, 1989; Siracusano *et al.*, 1991; Leggart *et al.*, 1992; Kittelberger *et al.*, 2002).The hydatid cyst fluids were aspirated from hydatid cysts of individual animals and were processed differently. Clear cyst fluids were kept frozen at – 20^0C until further use.

The cyst fluid was centrifuged at 15,000 g at 4 ^{0}C and dialyzed (3.5 kDa cut-off membrane) against 50 volumes of distilled water overnight. The dialyzed HCF was frozen and then freeze dried. The freeze dried material was redissolve in Tris buffered saline (TBS-HCl pH 7.5) Appendix (IIa) and centrifuged for 15 g at 4 ^{0}C. The resulting clear supernatant containing the concentrated HCF was carefully decanted into sterile eppendorf tubes and kept frozen at – 20^0C for further use.

Aliquots of the concentrated HCF was incubated at 100^0C in a water bath (Ibrahim *et al.*,1996; Kittelberger *et al.*,2002) and centrifuged at 15,000 g for 20 minutes at 4^0C. In order to remove any immunoglobulin (IgG) contaminants from

the host hydatid cyst fluid, the supernatant which is the concentrated HCF was passed through the gel filtration chromatography (Lightowlers *et al.*, 1984) in a G-Sepharose S-300 protein column (Pharmacia LKB, Uppsala, Sweden) at 0.5 ml/h in PBS containing 0.1% SDS. Fractions of 3 ml of sheep, goat, and camel HCF were each loaded unto the affinity column along side with the buffer i.e 3 ml of PBS pH7.4. The antigen and buffer were allowed to remain in the column overnight after which the affinity depleted antigen was collected. After each adsorbtion, the column was cleared of any remaining unbound material by washing with 2 column volumes of Phosphate Buffered Saline (PBS). Aliquots of the fractions was mixed in 1 ml of Sodium Dodecyl Sulphate (SDS) in polyacrylamide gel electrophoresis (PAGE) sample buffer (SB) containing 2-mercaptoethanol (SB/ME = 62 mM Tris- HCL pH 6.9, 5% SDS, 1% ME, 10% glycerol), (Appendix IIIe) boiled for 5 minutes and then centrifuged for 5 minutes at 5000 g. The supernatant (i.e. the antigen preparation) was then used to run ELISA to detect antibodies to hydatid cysts. SDS – PAGE was run with the aim of characterization of the major hydatid cyst fluid antigen of *E. granulosus* principally, the low molecular weight subunits of antigen B and antigen 5 (Lightowlers *et al.*, 1989). The western blotting was run to serve as a confirmatory test. That would help in comparing the validity of these serological techniques in order to suggest a quick and reliable means of diagnosis of hydatidosis in domestic animals and man.

3.5 Detection of antibodies to *Echinococcus granulosus* in hydatid cyst fluid by Enzyme Linked Immunosorbent Assay (ELISA)

The purified hydatid cyst fluid antigen from the camels, sheep and goats would be assayed in ELISA for the detection of IgG antibodies in sera obtained from slaughtered sheep, cattle camels and goats naturally infected with *E. granulosus.* ELISA procedure was carried out essentially as described by Craig *et al*., (1986). The antigen preparations were optimally diluted in 0.05M-phosphate buffer (pH 7.5) Appendix (IIb). The microtitration plates (96 well, limbro/Titerlek) were coated by adding 150 µl per well of the purified hydatid cyst fluid diluted 1/200 and 1/100 in phosphate buffer and incubated at 4 ^{0}C overnight in a moist chamber. The plates were washed five times with deionized distilled water and frozen at – 20 ^{0}C for later use.

After thawing, the plates were blocked with 0.15M phosphate buffered saline containing 0.05% Tween 20 (PBS/T20) (Appendix IIc) .And were washed three times. The control positive and negative samples (supplied by Prof. Kittelberger, University of Melbourne) were included in each plate. Serum incubations were performed by adding 100 µl of serum per well, diluted 1/25 in PBS-T and incubated at 37^{0}C for 3 hours. The plates were washed three times to remove unreacted antibodies after which the plates were incubated with 100 µl per well of monoclonal anti-goat sheep IgG (clone GT –34 sigma) diluted 1:6000 in PBS –T for sheep and goat sera sample plates, and antibovine IgG (Sigma) diluted

1:2000 in PBS-T for camels and cattle sera for I hour at room temperature. The plates were washed three times. The substrate (100 µl of alkaline phosphatase yellow NPP liquid) (Sigma) was added to each well, incubated for 30 minutes at room temperature, and the reaction was then stopped by adding 3M NaOH.

The optimal density (OD) value was measured at a wavelength of 450 nm in an automatic plate reader (Dynatech MR 5000). The ELISA cut off values for each antigen preparations from the camels, goats and sheep were set at the mean optimal density (OD) values plus two standard deviations measured in sera from animals that were not infected on the basis of abattoir inspection (Table 3.1). The sera from the animals were recorded as positive or negative by comparing the ELISA OD readings with the cut off absorbance values of the different preparations of the hydatid cyst fluid antigens. The phosphate buffered saline containing 0.05% Tween 20 was used as the wash and diluents solution in order to minimize non specific binding of conjugate to the microtitre plates or antigen. The antibody titre values of individual animals were calculated and corrected to a reference position.

Table 3.1: ELISA cut-off absorbance value at 450 nm for different hydatid cyst fluid antigens using serum from the individual animals

Antigens	**Mean**	**Standard Deviation (SD)**	**Mean + 2SD**
Cm CF	0.073	0.042	0.157
Sh CF	0.070	0.037	0.183
Gt CF	0.050	0.043	0.181

CmCF = Camel hydatid cyst fluid; ShCF= Sheep hydatid cyst fluid; GtCF = Goat hydatid cyst fluid.

3.6 Detection of subunits of major cyst fluid antigens and comparism of antigenic composition of different hydatid cysts using SDS-PAGE

3.6.1 Preparation of gels

Disposable hand gloves were worn during the experiment to prevent contamination with skin proteins. The glasses were cleaned by degreasing with ethanol. The 0.75 mm grey spacers were used to separate the glass plate from the aluminium backing. Wax paper was used to separate the plates from each other when making the gels. The glass plates were assembled to form the gel mould. The resolving gel buffer stock (Appendix IIIa) was used to prepare the (12.5%) resolving gel (Appendix IIIg). poured into the space between the glass plates leaving sufficient space at the top (2-3 cm) for the stacking gel to be polymerized later. The resolving gel was overlayed with water saturated n-butanol, allowed to polymerize for 1½ hour, after which the water saturated n-butanol was rinsed out with distilled water. The stacking gel buffer stock solution was used to rinse out the water. The equipment was inverted unto a towel to dry off excess water.

The stacking gel (Appendix IIIh) was prepared using the stacking gel buffer stock (Appendix IIIb). A dropping pipette was used to add the stacking gel to the individual wells. The combs were inserted gently and firmly making sure that all the teeth are in the plate groove. The gel was allowed to polymerize for 1 hour after which the apparatus was dismantled. A scalpel blade was used to gently separate the plates from each other. The plates were rinsed under running tap

water, gently removing excess gel from the surface of the sand - witched plates. The combs were removed and the wells were flooded with water to keep it hydrated, and were sealed with cellophane and stored in the refrigerator for further use.

3.6.2 Running the gel

Tank buffer stock solution (Appendix IIIi) was used to prepare 1/10 tank buffer freshly. The buffer was poured into the tank up to 1cm level and the gel plate (containing gels) was clamped to both sides of the cooling apparatus which was inserted into the tank. The gel template was placed on the glass to line up the wells as a guide for loading the samples. The tank buffer was poured unto the cavities between the gel and cooling apparatus. Each well was loaded with 15 µl of the sample using the fine tipped GC syringe after which the lid was placed on the tank with correct orientation of the electrodes. The apparatus was switched on and gels were run at 150 V for 1 hour and then either stained with comassie or used for western blotting.

3.6.3 Staining

The tank lid was removed and gel unclamped using a plastic spatula. The gel plate was separated from the glass. The stacking gel was chopped off and discarded. The gel was picked and placed in the staining tank containing staining

solution (Appendix IIIj). This was placed on a rocking platform for 45 minutes to ensure even staining after which it was transferred to the destaining solution (Appendix IIIk) and left for 1 hour after which it was photographed and kept in water. Protein profiles from the antigen preparations were obtained after staining the gels.

3.7 Determination of immunodominant species specific antigenic components of Echinococcus granulosus by Western blotting analysis

The protein separated was transferred from the unstained gels to the nitrocellulose membrane (Biorad Richmond, CA, USA) following the standardized procedures of Towbin *et al.* (1979). The electrophoretic transfer of antigen from polyacrylamide gels unto the membrane was carried out in a Trans - Blot cell (Biorad, Richmond, CA, USA) (Rybicki and Purves, 1996) .

The orientation of the gel was marked by cutting the corner of the gel. The sheet of nitrocellulose membrane (0.22 μm), the gel and blotting filter paper were placed in the electrode or Towbin buffer (Appendix IVa) for about 30 minutes. The transfer sandwich was arranged in the following order.

One prewetted fibre pad was placed on the greyside of the cassette (facing the positive electrode). A sheet of wetted filter paper was placed on the fibre pads. The equilibrated gel was placed on the filter paper the nitrocellulose membrane was placed on the gel and the piece of filter paper was placed on the membrane. A

glass tube was used to gently roll air bubbles out. The cassette was closed firmly and carefully, placed in the tank, which was filled with towbin buffer. A standard still bar was placed in the tank to ensure even buffer temperature and ion distribution in the tank. The tank was covered and the transfer apparatus switched on and run at 100 V and 360 mA for 1 hour under high intensity field with super cooling coil. Following transfer the effectiveness of transfer was verified by staining the membrane with the Amido black (Appendix IV h). The unstained membrane was placed on a glass plate, cut into strips, placed in labelled petridishes and blocked for 2 hours at room temperature in blocking solution (Appendix iv d) TBS (50 mM Tris-HCL, 150 mM NaCl pH 7.5) containing 5% (w/v) bovine serum albumin (BSA) (Sigma-Aldrich). These dishes were placed on an orbital shaker to gently agitate the solution. The blocking solution was poured away and TTBS was used to wash the strips 5 times for 5 minutes each with gentle agitation at room temperature. TTBS was poured away and the primary antibody solution (Appendix IV f) was added, and allowed to incubate overnight with gentle agitation. Longer incubation periods increase the sensitivity of detection. The antibody was diluted in antibody buffer (TTBS-BSA) (Appendix IVe) in a 1:25 ratio. Removal of the unbound primary antibody was done by washing the membrane in TTBS 3 times for five minutes at room temperature. The secondary antibody (Appendix IVg) i.e alkaline phosphatase conjugated donkey anti-sheep immunoglobulin G (Sigma) was diluted 1:1000 in antibody solution (TTBS-BSA)

was added to the membrane and allowed to incubate for 1 hour at room temperature.

Final washes were carried out to remove the conjugate solution, prior to colour development. The membrane was washed in TBS for 5 minutes at room temperature with gentle agitation to remove residual Tween-20 from the surface of the membrane. The colour developer containing Nitroblue tetrazolium in aqueous dimethyl formamide (DME) containing magnesium chloride and 5-Bromo-4 chloro-3-indolylphosphate in DMF (Sigma Aldrich) were prepared shortly before use. The membrane was immersed in the colour development solution, incubated at room temperature with gentle agitation until colour development was completed. The development was stopped by washing the membrane in distilled deionized water for 10 minutes with gentle agitation to remove residual colour development solution. Strips of animals that had antibodies to hydatid cyst fluid antigen had bands. Photographs were taken immediately and the strips were dried on filter paper and stored in brown envelops to protect it from light in order to minimize fading.

3.8 Validity of the diagnostic tests used

The results of ELISA were compared with that of western blotting. This was achieved by comparing the percentage positive in both tests with reference to the results obtained from animals that had hydatid cysts at slaughter. The

diagnostic sensitivities for ELISA and western blotting were calculated as number of positive sera at slaughter divided by total number of sera from infected animals. Diagnostic specificities for both tests were calculated as number of negative sera divided by total number from non-infected animals. This was done so as to determine the most sensitive, fast and accurate diagnostic method of diagnosing hydatidosis in live animals.

3.9. Collection and analysis of hospital records

Permission was sought from the Chief Medical Directors of the four hospitals via the Executive Secretary, State Ministry of Health, Kano State in order to visit and obtain hospital records on hydatidosis recorded in man. The hospitals that were visited include:

A. Mohammad Wase Specialist Hospital

B Infectious Disease Hospital zone 1

C. Murtala Mohammed Specialist Hospital and

D. Mallam Aminu Kano Teaching Hospital.

This was done by means of interviews of nurses and other paramedical personnel in the hospitals. The names of wards in each hospital were written on papers, squeezed and four were randomly picked. The staffs of those wards were interviewed. Physicians in charge of the surgery unit and the laboratories were also interviewed. Records of admissions into the hospitals from 1993 to 2002 were

also examined. Special emphasis on cases that required surgery and records of stool examinations conducted during the same period were also obtained.

3.9.1 *Statistical analysis*

Comparism of the overall prevalence between the camel, sheep, goat and cattle was calculated using the one way analysis of variance (ANOVA). Prevalence levels by age and sex were also compared. The chi-square test and calculation of odds ratio (OR) were used to find out whether there was an association between the factors (age and sex) and occurrence of antibodies to Hydatid cyst disease.

The student's t-test (paired) was used to determine the statistical significant differences between the level of infection/antigenicity at different antigen dilutions of 1:100 and 1:200**.**

CHAPTER FOUR

4.0 RESULTS

4.1 Responses from the questionnaires

A summary of the response from the questionnaires administered at the abattoir during the sample collection showed that most of the animals that were slaughtered in the abattoir especially the camels were imported from the neighboring Niger republic while the cattle, sheep and goats are mostly purchased from the neighboring villages around Kano city. The hydatid cyst varied in size and thickness of the walls or membrane. Cysts collected from older camels ranged from 5-10 cm in diameter while in younger camels, cyst were 1-4 cm in diameter .in sheep, cysts in older animals were 2-4 cm in diameter while younger ones had cysts that ranged from 0.5-1.5 cm. the younger goats had cysts with diameter of 0.5-1 cm, while in adults it ranged from 1-5 cm in diameter. Older camels had multiple cysts of 5-20 per animal while in sheep and goats; they had at least 10 cysts per animal.

A total of 551 questionnaires were completed out of which 136 were for camels, 138 sheep, 147 cattle and 130 goats. Reports also showed that most of the cattle and camels that are slaughtered in the abattoir are adults while most of the

sheep and goats were young. This was observed during the sample collection where 119 (80.9%) of the blood samples were collected from adult cattle, while 28 (19%) were from young (<12 months) cattle. In camels, out of the 136 blood samples collected, 130 (95.5%) were from adults (≥ 12 months while only 6 (4.4%) were young. In sheep, out of 138 samples.108 (78.2%) were collected from young sheep while 30 (21.7%) were from adults. In goats, out of the 130 blood samples, 116 (89.2%) were from young goats while only 14 (10.8%) were from adults (Table 4.1).

The abattoir is properly fenced and there were no stray dogs seen around but the level of hygiene and method of disposal of animal wastes is very poor. Infected offal's, which are supposed to be disposed, are rather sold to individuals for human consumption. A total of 21 hydatid cysts were collected during the study period comprising 8 from goats (Plate I), 2 from the liver and 6 in the lungs of the goats, 6 from lungs of sheep (Plate II) and 7 from lungs of camels (Plate III), and no cyst was collected from cattle during the sampling period. The camel HCF preparation was therefore used as the source of antigen in the detection of antibodies to hydatidosis in serum samples collected from the cattle. (Table 4.5).

4.2 Purification of Hydatid cyst fluid antigen

In order to remove any other immunoglobulins and contaminant from the host crude Hydatid cyst fluid, the high pressure liquid chromatography (HPLC)

was used which showed that the retention time (that is the time at which the antigen is purified) for the different antigen preparations differ from each other. The sheep had the highest retention time of 19.868 minutes 20.139 minutes followed by the camel 19.147 minutes while Goats had the least 19.037 minutes. The buffer had the lowest retention time of 18.470 minutes as compared to that of the animals. The difference in retention time (RT) of the antigen preparations from the different animals was plus or minus one unit. (Appendix v).

Table 4.1: Age distribution of antibodies to hydatid cyst fluid in domestic animals slaughtered in Kano abattoir

Animal	Age	No. w.a. (%)	No. wt.a.	Total Exam.	O.R.	C. I.	(χ^2)	P. value
Camel	Young	3 (50)	3	6	1.66	0.25-10.82	0.03	0.86
	Adult	50 (38.4)	80	130				
Sheep	Young	29 (26.8)	79	108	6.36	2.42-17.10	17.10	0.00
	Adult	21 (70.0)	09	30				
Cattle	Young	04 (14.2)	24	28	2.02	0.60-7.51	0.97	0.32
	Adult	30 (25.2)	89	119				
Goats	Young	21 (18.1)	85	116	10.12	2.54-47.42	14.61	0.00
	Adult	10 (71.4)	04	14				

No.w.a. = Number with antibodies, No.wt.a. = Number without antibodies, Total exam. = Total number examined; O.R = Odds ratio; C.I. = Confidence interval; χ^2 = Chi square

Plate I

Plate II

Plate III

4.3 Detection of prevalence of antibodies to *Echinococcus granulosus* by enzyme linked immunosorbent assay (ELISA)

The overall prevalence of antibody to hydatidosis was 180 out of 551 serum samples (32.6%). The prevalence of the antibody to hydatid cyst differed among animals at the 1:100 antigen dilutions with 38.9% in camels, 36.2% in sheep, 31.2% in cattle and 23.8% in goats (Table 1). The mean absorbance value of known positive sera out of 138 sheep serum was 0.070, standard deviation 0.037, the highest wavelength determined was 0.230 and this serum originated from one of the sheep that had a cyst in the lungs .The mean absorbance value of known positive sera out of the 130 goat serum was 0.050, standard deviation, 0.043.The highest wavelength determined among the goats was 0.200 from the serum of a goat that had cysts in the liver and lungs. In camels, the mean absorbance value of known positive sera out of the 136 serum samples was 0.073, standard deviation 0.043 the highest wavelength was 0.350, this serum originated from one of the camels that had cysts invaded in the lungs. Due to the fact that no single cyst was recovered from the cattle, the camel antigen was used in the diagnosis of antibodies to hydatidosis in cattle. Of the 147 serum samples collected from the cattle, the highest wave length value was 0.290.

The camel purified hydatid cyst fluid (HCF) was used to run the camel and cattle serum, sheep HCF was used to screen the sheep serum while goat HCF was used to screen goat serum respectively. The highest ELISA mean cut-off absorbance value (0.073) was recorded in the camel HCF antigen , followed by the sheep HCF antigen (0.070),the lowest cut off absorbance value (0.050) was recorded in the goat HCF antigen. (Table3.1).

The camel hydatid cyst fluid was found to be most reactive with antibodies in sera from camels than cattle. The camel and sheep HCF preparations were superior to goat HCF in detecting serum antibodies in the animals with natural hydatid infection confirmed at meat inspection. High antibody titres were recorded from serum samples of camels, sheep and goats that had hydatid cysts at meat inspection. Antigen dilution of 1:100 at serum dilution of 1:25 showed higher antibody titre as compared to the 1:200 antigen dilutions with the same serum dilution.

The prevalence of the antibodies to hydatid disease by age among the animals showed that in the cattle, sheep and goats, higher prevalence were recorded in older animals than the younger ones with prevalence of 25.2% in cattle, 70% recorded in sheep and 71.4% in goats while in the camels, the younger animals had the highest prevalence of 50% (Table 4.1). There was no significant difference between prevalence of *E. granulosus* antibody titre in young and adult camels and cattle, and no significant association between the age of the animals and level of *E. granulosus* antibody titre ($P>0.05$) but in goats and sheep,

significant differences in level of antibody titre to *E. granulosus* were recorded between the young and adult animals ($p<0.05$).the adult animals had higher antibody titre values than the younger animals.

The prevalence of antibody to hydatid disease by sex among the animals showed that males were more infected among the sheep, cattle and goats, the antibody titres were beyond the maximum dilution at which any other serum from infected female sheep, goat and cattle showed detectable antibody binding. In camels, higher prevalence was recorded among the females (Table 4.3). There was no statistical significant difference in prevalence of the antibody to hydatid disease between males and females in all the animals ($p>0.05$).

On a general note, there was no significant difference between the age and sex of the sheep, goat, cattle and camels and the antibody titre levels ($P>0.05$). There were no associations between the level at which young goat showed detectable antibody binding and sex, while in adult goats, odds ratio values showed associations between the age and sex of the goats and antibody titre value. In sheep, the odds ratio values showed associations between the sex of young and adult sheep and the antibody titre level.

Table 4.2**: Prevalence of antibodies to hydatidosis in domestic animals slaughtered in Kano abattoir, Kano State using ELISA**

Species	Number examined	Number with antibodies	Number without antibodies	Species specific rate (%)
Camel	136	53	83	38.9
Sheep	138	50	88	36.2
Cattle	147	34	116	31.2
Goat	130	31	99	23.8
Total	551	168	386	30.4

F=54.217**

**=highly significant at P≥0.01

Table 4.3: Sex distribution of antibodies to hydatidosis in domestic animals slaughtered in Kano abattoir, Kano State detected by ELISA (No.w.a. = Number with antibodies

Animal	Sex	No. w.a. (%)	No. wt.a.	Total Exam.	O.R.	C. I.	(χ^2)	P. value
Camel	Male	22 (48.9)	23	45	1.85	0.84-4.09	2.19	0.14
	Female	31 (34.1)	60	91				
Sheep	Male	33 (38.8)	52	85	1.23	0.57-2.69	0.16	0.70
	Female	18 (33.9)	35	53				
Cattle	Male	27 (27.0)	73	100	2.11	0.79-5.88	2.00	0.16
	Female	07 (14.9)	40	47				
Goats	Male	17 (27.4)	45	62	1.46	0.60-3.54	0.50	0.48
	Female	14 (20.6)	54	68				

No.wt.a. = Number without antibodies, Total exam. = Total number examined; O.R = Odds ratio; C.I. = Confidence interval; χ^2 = Chi square

Distribution of the disease based on antigen dilution showed an overall prevalence of 32.6% was recorded in the 1:100 antigen dilutions while a prevalence of 7.6% was recorded in the 1:200 dilution (Table 4.4). The camel had the highest prevalence (38.9%) the goat had the least (23.8%) in the 1:100. The highest prevalence (12.3%) in the 1:200 dilution was recorded in the sheep, followed by the camels (10.3%), goats (6.20%) and cattle had the least (2.04%).There was statistical significant differences between the prevalence rates recorded among all the animals in the 1:100 and 1:200 dilutions ($p < 0.05$). The antibody titre value to Hydatid cyst fluid in the 1:100 antigen dilution is greater than in the 1:200 dilution.

The overall prevalence of the antibody to Hydatid disease in different animals showed that there was a statistical significant difference between the level of prevalences recorded in camels and sheep as compared to goats ($P<0.05$) and no statistical significant difference between the prevalence rate in cattle as compared to that in goats ($P > 0.05$) (Table 4.4).

Table 4.4: Prevalence of antibodies to hydatidosis based on antigen dilutions (1:100 and 1:200) among the animals

Animal	Antigen Dilution	Number Examined	Number with antibodies	Prevalence of antibodies	P. Value
Camel	1:100	136	53	38.9	0.00
	1:200	136	14	10.2	
Sheep	1:100	138	50	36.2	0.00
	1:200	138	17	12.3	
Cattle	1:100	147	34	31.2	0.01
	1:200	147	3	2.04	
Goats	1:100	130	31	23.8	0.00
	1:200	130	8	6.15	

4.4 Detection of subunits of major cyst fluid antigens and comparism of antigenic composition of different hydatid cysts using SDS-PAGE

In order to identify immunodorminant, stage and species specific antigenic components in the hydatid cyst fluid (HCF) preparations that invoked antibody responses in the animals, the crude hydatid cyst fluid obtained from camels, sheep and goats were purified and run on Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The results showed that the HCF contained subunits of the two major antigens i.e antigen B and antigen 5 (Plate IV).

The antigen 5 contained the 38-150 kDa (Plate IV) while antigen B component contained low molecular weight subunits of 8 –24 kDa (Plate V). The camel purified HCF preparations showed prominent bands of 32, 66 to 118, 150 and 160 kDa respectively (Plate Va). Goat purified antigen preparation showed five prominent bands of 45 to 52, 66, 100 and 118Kda (Plate Vb) while the purified antigen preparation from sheep contained the 16, 24, 36 to 52, 66, 96, 118 and 150

kDa respectively (Plate Vc). Common or cross reactive antigens occur mainly between 30 and 67 kDa in electrophoresed cyst fluid of the various animals.

The dominant and most consistently demonstrable proteins in *Echinococcus granulosus* positive sera occurred as a complex in the 52-62 kDa regions and the 118-150 kDa regions in all the animals that were examined. (Plate IV) with some variability observed between the occurrence and intensity of antigenic bands of 8-38 kDa throughout the electrophoretic path (Plate V).

The antigenic profile on SDS-PAGE showed that when the same quantity of purified hydatid cyst fluid from the sheep, goat and camel were loaded in separate wells (5 µg/ml, the variation in thickness of the bands revealed that the camel antigen preparation was more concentrated followed by the sheep and goat was the least with faint bands (PlateIV).

The purified antigen from infected camels and sheep had more identical electrophoretic bands (Plate V a &c) than the infected goats (Plate Vb) .The camel hydatid cyst fluid preparation recognized higher molecular weight proteins than that of the sheep and goats. Despite the fact that the same quantity of camel, goat and sheep antigen containing 5 µg/ml of protein were loaded per well, the bands from the goat HCF were faint compared to that of sheep and camel (Plate Va ,b & c).

PLATE IV

Table 4.5: Location of hydatid cysts recovered from different animal species obtained from Kano abattoir

Animal	**Location of cysts**		**Total**
	Liver	Lungs	
Camel	0	7	7
Sheep	0	6	6
Cattle	0	0	0
Goats	2	6	8
Total	2	19	21

PLATE V

4.5 Detection of immunodominant species specific antigenic components of *Echinococcus granulosus* by Western blotting analysis

A total of 110 sera from the animals representing 19.9% of the 551 serum samples that bind at least one of the 8, 24, 30,36,38,42,45,48,92 or 118 kDa specific antigen bands served as criteria for the determination of positivity in this study (Plates VI,VII,VIII &IX).Immunoblot analysis revealed that positive serum from goats recognized the 12, 66, 75, 100 & 150 KDa (Plate VI), that of sheep recognized the 8, 24, 36,48, 66, 92, 118 and 150 kDa (Plate VII),that of camel recognized 36,52, 66, 118, 150 and 160 kDa (Plate VIII) and that of cattle recognized the 8, 48, 66,92, 118 & 150, kDa bands (Plate IX). The 8-25 kDa bands were recognized by sera from animals that had hydatid cysts but with low frequency. These bands were mostly faint in the SDS-PAGE but when transferred unto the nitrocellulose membrane they became visible.

The bands of 66, 118 and 150 kDa persisted throughout the study period among the infected animals while those of lower molecular weights including the 8, 24, and 36 kDda fluctuated. Western blot showed clear differences and degrees of similarities between different antigen preparations than the SDS – PAGE (Plates Vc and VII). This is more evident where the 8kDa band in sheep HCF was revealed by the western blot (Plate VII) and not by SDS-PAGE and considerably produce weaker or barely visible bands in the goat antigen preparation (Plates Vb & VI).

PLATE VI

PLATE VII

PLATE VIII

PLATE IX

4.6 Validity of the diagnostic tests

The overall prevalence of antibodies to hydatidosis using ELISA was (32.6%) recognized a wide range of serum with maximum antibody titre values which classify them as being positive. Western blotting only recognized 110 (19.9%)of the sera as being positive based on the recognition of at least one of the 8, 24, 36, 38, 48, 92 or 118 kDa specific antigen bands by the sera of camels,sheep,goats and cattle and their non recognition of other antigens. Diagnostic sensitivity of ELISA and Western blotting were 90.5% and 80.9% while the specificities were 67.3% and 83.2% for ELISA and Western blotting. Western blotting was more and specific while ELISA was more sensitive in the diagnosis of hydatidosis.

4.7 Analysis of hospital records

Using available data in the parasitology laboratories in the chosen hospitals, the status and pattern of infection in the area over a ten-year (1993-2002) were determined. There was no proper documentation of the cases. The results obtained showed that there were no records of *Echinococcus granulosus* eggs recovered in the faecal samples of individuals that attended the hospital within 10 years. Most of the intestinal parasites frequently encountered in the faecal matter as recorded over a ten-year period were hookworm (41.63%), *Ascaris* (27.25%), *Taenia* species (13.76%), *Entamoeba histolytica* (8.17%),

Schistosoma mansoni (5.77%), *Trichuris trichuira* (2.19%), *Entamoeba coli* (0.66%), *Hymenolepis nana* (0.57%) (Table 4.6).

The data obtained showed that hookworm was encountered more than other parasites, followed by *Ascaris*, *Taenia* species and *E.histolytica*. Highest number of intestinal parasites were encountered in 2002(15.54%) followed by 2001(14.46%), 2000 (10.72%) and the least was recorded in 1993(7.23%).

In the surgery departments of Murtala Mohammed specialist, Nassarawa and Infectious disease hospital, the heads of department reported that no single case of Hydatidosis has been operated since they came into office and there are no available records of surgery on the disease conducted in the past. The only available record of surgery being conducted was in Mallam Aminu Kano hospital in 1999 where a 30 year old male patient had a unilocular cyst in the lung which was discovered accidentally during surgery.

Table 4.6: Ten -year hospital records of intestinal parasites and Bacteria obtained from four hospitals in Kano municipal, Kano State (1993-2002)

Parasite	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**Total**	**Percentage**
E. granulosus	0	0	0	0	0	0	0	0	0	0	0	0.00
Ascaris	2580	1805	2360	1288	2281	2793	2121	2788	4006	3146	25168	27.25
S. mansoni	502	489	971	477	462	487	516	441	463	518	5326	5.77
Taenia spp.	584	1659	1706	1203	759	932	1073	1556	1877	1365	12714	13.76
Hookworm	2745	2932	3762	4062	3980	1479	2736	3977	5130	7652	38455	41.63
T. trichiura	38	47	25	61	858	85	91	64	700	52	2021	2.19
E. histolytica	165	205	382	439	1080	1153	535	960	1087	1539	7545	8.17
H. nana	24	44	51	72	70	64	77	32	48	49	531	0.57
E. coli	39	84	45	60	70	86	63	82	45	38	612	0.66
Total	6677	7265	9302	7662	9560	7079	7212	9900	13356	14359	92372	-
Percentage	7.23	7.86	10.07	8.29	10.35	7.66	7.81	10.72	14.46	15.54	-	-

4.8 Data analysis

A summary of the results of the statistical analysis to show the associations between the various epidemiological variables and the disease are presented in tables 3 & 4. The responses were categorized into factor positive and factor negative, the number of animals that have the antibody to the disease and those that do not have antibody to the disease. Odds ratio value greater than unit one which indicates associations between each of the factors and prevalence of the antibody to the disease was obtained for the distribution of the disease by sex and age.

The one way Analysis of variance (ANOVA) showed that the F calculated (54.217) was greater than F tabulated at $P \geq 0.01$ for antigen preparation obtained from the sheep, goats and camels. This showed a significant difference in level of *E.granulosus* antibody titre in sera obtained from the different animals.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Responses from the questionnaires

The official figures of the number of animals slaughtered annually in Kano State during the study period came to an average of 912,500 cattle, 438,000 sheep, 313,900 goats and 31,025 camels. This greatly underestimates the actual numbers as this exclude large numbers slaughtered privately in homes and especially during festive activities in the municipal and the surrounding villages.

The people of Kano state are predominantly Muslims and their religious beliefs consider the dog as an unclean animal. Dogs are rarely allowed inside Muslim homes and humans have little contact with dogs. In this study, dogs were not seen around the abattoir, its been reported that in the villages, the pastoralists keep dogs as guards this therefore enhances transmission of the disease before animals are brought to Kano for slaughter. Whether the animals were sent directly to the abattoir for slaughter or reared for several months in Kano city is not known due to the absence of accurate records. There is lack of routine meat inspection at the abattoir, proper monitoring of movement of people and livestock from endemic countries across the borders. This situation is further complicated by the large scale and uncontrolled movement of food animals between most of the northern states and some of the states and the neighbouring countries like Niger,

and Chad Republic where a high prevalence of hydatidosis have been recorded (Develoux *et al*., 1985).

In addition, the traditional collective slaughtering of animals during religious festivities of the Muslims may account for a significant high number of animals being slaughtered indiscriminately at home which exposes stray dogs and man to the disease by feeding on infected offals that are not properly disposed (Dada *et al*.,1981; Anyanwale *et al* .,1982).

5.2 Purification of Hydatid cyst fluid

The High pressure liquid chromatography (HPLC) showed that the retention or elution time (the period when the antigen is purified and other materials such as solutes are completely eluted) for the different antigen preparations were closely related with a difference of plus or minus one minute which is negligible. This is an indication that the antigen preparations from the goat, sheep and camel have similar components which are separated according to differences in size. The character of the column also showed similarities in the antigen preparations and the slight differences in the retention time of the sheep and camel HCF is an indication that the sheep puried HCF has components that the camel and goat HCF lack.(Lightowlers *et al.,* 1984).

The antigenic components responsible for the non- specific binding of normal sheep, camel and goat immunoglobulin were removed by passage through the affinity column .This reduced but did not eliminate the non-specific binding of

immunoglobulins. The affinity depletion or purification of the crude Hydatid cyst fluid antigen resulted in improved differentiation in ELISA between sera from infected and uninfected sheep, goats, cattle and camels.

5.3 Detection of prevalence of antibodies to *Echinococcus granulosus* by Enzyme linked Immunosorbent Assay (ELISA)

The application of serodiagnostic techniques for epidemiological studies of hydatidosis provides useful information on prevalence rates and may detect asymptomatic cyst carriers and contributes to planning and assessment of effective control programmes (Varela-Diaz *et al.*, 1983). In this study, crude hydatid cyst fluids obtained from the camels, sheep and goats were purified and tested using the ELISA for their reactivity with Immunoglobulin G (IgG) antibodies in sera collected during routine slaughter from these animals in order to identify their suitability to detect infected animals.

The high mean absorbance value recorded in the camel and sheep HCF as compared to the goat HCF was due to the fact that the camel and sheep HCF had a high level of antigenicity as compared to that of the goat (Lightowlers, 1990; Lightowlers & Gottstein, 1995). The prevalence rate (38.9%) recorded among camels is in accordance with the (55.5%, 17.12% and 26.2%) obtained in various abattoirs in Nigeria by Dada and Belino. (1978) in Kano, Ajogi *et al.* (1995) in Sokoto, and Ogunsan *et al.* (2000) in Sokoto by use of simple serodiagnostic methods like the countercurrent immunoelectrophoresis, double diffusion and

indirect haemagglutination tests in the diagnosis of hydatidosis in camels. This result may be attributed to the fact that camels often grow to maturity before they are being slaughtered. This enables the hydatid cysts to become fully developed and fertile (Pandey *et al.*,1986; Gusbi *et al.*,1990) The camel cysts has been found to be rich in antigen B and other cyst fluid antigens and therefore provides an important source of *E. granulosus* antigens especially in areas where camel Hydatidosis is prevalent (Lightowlers *et al*.,1989;Lightowlers and Gottstein,1995; Khuroo, 2002; Craig and McManus,2003).The high prevalence of infection (38.9%) in camels In comparison with prevalence values in sheep (36.2%),cattle (31.2%) and goats(23.8%),indicate the suitability of camels and as susceptible intermediate hosts of *E.granulosus* in Nigeria.

Higher prevalence levels were recorded among sheep than goats in this study.The level of antigenicity among the infected sheep was higher than that of the infected goats. This might be due to the fact that sheep hydatid cyst has been found to be highly fertile thereby contain higher concentration of antigen than those of other animals. (Rickard and Lightowlers 1986; Lightowlers, 1990; Kanwar *et al.,* 1992; Ibrahim *et al.,* 1996).

The lowest prevalence recorded among the goats as compared to other animal species might be as a result of higher number of goats that were slaughtered were young and the antigen level was still very low. In the case of the sheep, older animals are slaughtered as the Muslims keep them for slaughter during their annual religious festival.

The overall prevalence of the antibody to hydatidosis recorded among various animals in this study might be attributed to the fact that natural intermediate host animals produce very poor antibody responses to infection Craig *et al.,* (1995) as compared with the relatively high levels of specific antibody in humans (Lightowlers and Gottstein, 1995;Craig and McManus,2003; Kittelberger *et al.,* 2002). This must have contributed to the significant difference recorded in the prevalence levels in the 1:100 and 1:200 antigen dilutions. (Rogan *et al.*, 1991).

Despite the fact that no cyst was recovered from all the cattle during the study period, there were cattle sera which elicit *E. granulosus* antibodies responses. This may be due to the fact that at meat inspection, if cysts are small, well encapsulated or calcified, in active and localized in sites where they do not induce significant pathology, the further course of infection may remain asymptomatic permanently or for a certain period of months or years (Ammann and Eckert, 1995; Macpherson *et al*., 2003; Macpherson and Milher, 2003).

Higher prevalence recorded in animals than the younger ones might be attributed to the fact that in older animals the cysts must have developed to a matured stage before the animal were slaughtered unlike in younger ones that were being slaughtered before the cysts become fully matured since they grow slowly (1 cm/year) . The clinical signs of the disease do not develop until when the cyst is localized in the viscera when the cysts are about 8-10cm in diameter. This study agrees with work conducted by Shaikenov *et al.,* (2003) in Kazakhstan; Ibrahim &

Craig, (1998) in Libya where cysts were found in animals of all age groups, but animals of 1-2 years of age had infertile cysts, while older animals between 2½-3 years accounted for 10% of the fertile cysts recovered.

The present study shows high level of antigenicity in camels was due to the fact that camels are unlikely to be slaughtered before the age of 6-10 years and therefore the risk of them acquiring infection becomes relatively high. In addition, the number of cysts per infected camel was also higher in adult camels ranging from 5-25 cysts. Younger camels had less than 5 cysts per animal. This coincides with the works of Ibrahim and Craig. (1998) and Ibrahim *et al.* (2002).The age related difference in prevalence of infection, number and sizes of cysts in camels might be because older camels have a greater chance of ingesting large number of *E.granulosus* eggs Craig *et al.* (1995) and the cyst being likely to increase in size in this long lived host. In young sheep and goats, the sizes of the cysts ranged from 0.5-1.5 cm, while in older sheep and goats the sizes range from 2.5 to 5 cm. This is due to the small size of these animals as compared to the camel.

Viable fertile or non- fertile cysts with healthy germinal layers contain antigens suitable for diagnostic tests this has a great influence on the level of antigenicity in the hydatid cysts of the animals. This explains why higher antibody titers were recorded among the older animals than younger ones and no significant associations between antibody titer level with age and sex of the animals. The slight variation obtained in cattle and goats where females were more predisposed to the disease than males was due to the small sample sizes of the females.

In this study, most of the cysts were collected from the lungs this might be the cause of the low titre values and weak bands obtained in the antigen/sera of some infected young animals. Studies have shown that absence or reduced antibody responses could be associated with the site of hydatid cyst involvement and variation in immunological response in the animal which may or may not lead to production of sustained levels of specific circulating antibody (Williams *et al*., 1971; Todorov *et al.,* 1979; Lightowlers *et al.,* 1989; Leggart *et al.,* 1992). Pulmonary hydatidosis has been consistently reported to result in lower specific antibody responses than hepatic cases.

It has been discovered that in camels organ localization of *E. granulosus* is similar to that in cattle, as the majority of the hydatid cysts are found in the lungs. It has been reported that 68%-86% of camels infected with larval *E. granulosus* harboured cysts in the lungs where as cysts in the liver are much less frequent about 20-32% (Hamdy *et al.,* 1980).

The serologic response to infection among all the animals was weak because the animals were naturally infected and could be associated with the fact that specific immune responses might occur following infection but antibody production is often transient (Lightowlers,1990; Lightowlers *et al*., 2000). False positive reactions may occur in animals with other helminthic infections, cancers and chronic immune disorders.Negetive test results does not rule out hydatidosis because some cyst carriers do not have detectable antibodies (Lightowlers *et al*.,1989). The findings that sex-specific infection rates in camels,cattle, sheep and

goats are higher in males than females is because of the small sample size of the females which makes them seem more exposed than the males. In previous studies Ibrahim *et al*. (1996) have shown that there is no association between sex of the animal and infection. This finding may be due to the fact that more infected males were encountered during the study period in case of the camels,cattle, sheep and goats.

Camel, which is considered to be an important intermediate host for the local maintenance of the life cycle of hydatidosis in Africa (Macpherson *et al.*, 1986; Schantz *et al.*, 1995) are imported to Nigeria via the Northern parts from neighboring Niger and Chad republic they are being accepted as food and so serve as an important means of spreading the disease to other animals. The poor hygienic conditions in and around the abattoir and the definitive host being kept as guard dog by most pastoralists create the favorable conditions for the spread of the disease.

5.4 Detection of subunits of major cyst fluid antigens and immunodominant species specific antigenic components of *Echinococcus granulosus* by Western blotting analysis

Records have shown that this study is the first to be conducted in this area using the SDS-PAGE and western blotting as diagnostic techniques. The purified hydatid cyst fluid was used as a source of antigen in order to evaluate the specificity and its application in order to analyze *Echinococcus* proteins to know which of the antigens invoke antibody response in the animals and to identify the

species specific antigens which might be used for immunodiagnosis of the disease in live animals.

Reports from previous studies conducted on hydatidosis in animals slaughtered in major abattoirs in Nigeria were very few and often based on parasitological or retrospective abattoir records (Dada and Belino, 1978; Anyanwale *et al.,* 1982; Onah *et al.,* 1989; Ajogi *et al.,* 1995; Garba and Maigandi, 1995; Ogunsan, 2000). Studies on the general distribution of the disease in Nigeria is very scarce, (Willingham, 2003; Schantz *et al.*, 1995) making it difficult to compare our findings with existing data.

The protein bands of 32-150 kDa were revealed by SDS-PAGE in antigen preparations of sheep, goats and camels, while in the 8-24 kDa faint bands were seen in this molecular weight range. While immunoblotting picked the same bands sharply indicating that SDS-PAGE is not as specific as western blotting in comparing HCF from different intermediate hosts. In western blotting, clear differences and degree of similarities between the hydatid cysts fluid from the animals was revealed (Janseen *et al.,* 1990).

The results of these findings are in accordance with work done by Janseen *et al.* (1992); Kanwar *et al.*(1992); Sbihi *et al.* (1996); Olga *et al,* (2001); Ibrahim *et al.* (2002) and Kittelberger *et al.* (2002) which showed that the protein bands of 8-24 kDa are derived from antigen B while those of 36 –150 kDa from antigen 5.

Oriol *et al.* (1971), Shepherd and McManus (1987) and Kanwar *et al.* (1992) reported that western blotting revealed the presence of 8, 24, 66 and

118Kda bands which is of high specificity in diagnosis of hydatidosis while Verastegui *et al.* (1992) showed the presence of these bands in 12% of infected humans with cysticercosis.

The relatively faint bands of the 8-24 kDa obtained in this study might be attributed to the location where the cysts were collected Khuroo, (2002). Our data showed that polypeptides of 8, 24, 36, 52, 66 and 118 KDa were recognized in western immunoblot assay of the sera of hydatid positive animals. This agrees with the work of Shepherd and McManus. (1987); Kanwar *et al.* (1992) and Kittelberger *et al.* (2002) who made similar representations with a slight difference between molecular weights of their results and ours. This might be due to the method of processing the hydatid cyst fluid before electrophoresis, which can alter the polarity, size, configuration and optimal concentration of antigenic molecules (Towbin *et al*. (1979); Ibrahim *et al.* (1996); Olga *et al.* (2001) and Lightowlers *et al.* (2000).

This result also showed that the dorminant and most consistently demonstrable protein occurred as a complex in the 52-66 Kda region and 118-150Kda region in the antigen preparations (Plate1b) this coincides with work conducted by Craig *et al.* (1981); Gasser *et al.* (1981); (1988); Kittelberger *et al.* (2002) who discovered that these cyst fluid components react specifically with sera from animals with hydatidosis. The sensitivity of the 8-24Kda bands in our western blotting however proved less than that described by Sbihi *et al*. (1990); Kittelberger *et al.* (2002). In our case, such discrepancies might be explained by

the presence of antigens of intermediate molecular weights and probably the location of the cysts in the animal which greatly influences sensitivity, Zarzosa *et al.* (1999) or this protein might have migrated with the 12-16 kDa which are multimers of 8KDa protein. (Siracusano *et al.,* 1991; Verastegui *et al.,*1992).

The variability observed between the occurrence and intensity of antigenic bands may be attributed to poor antibody response to infection in most natural intermediate hosts especially in goats which had the faint bands in most instances Ibrahim *et al.* (1996) and Jenkins and Macpherson. (2003); and strain variation Rickard and Lightowlers. (1990) absence or reduced antibody responses is associated with the site of hydatid cyst involvement. this has been reported from previous work that pulmonary hydatidosis consistently results in lower specific antibody response than hepatic cases (Craig *et al.,*1986),The fewer bands obtained in this study is attributed to the antigen purification method used which removed some impurities hence reducing the chances of having serological cross- reactions between *E. granulosus* specific bands and those of other cestodes which in most cases prohibit the specific diagnosis of hydatid infection when crude hydatid cyst fluids are used as a source of antigen (Oriol *et al.,* 1971; Olga *et al.,* 2001).

The physical condition of the hydatid cysts might also be an important criterion for determining the level of prevalence as calcified cysts and cysts with intact (hyaline membranes) regardless of the site might result in low seroreactivity probably by lack of release of adequate antigen resulting in antibody stimulation(Olga *et al.,* 2001).in this study, all the cysts from the camels, sheep

and goats had thin membranes and posses protoscolex which is an indication of fertility of the cyst (Craig *et al.,* 1981;Janseen *et al*.,1990).

The most frequent cross reactions in the western blots of Hydatidosis are mainly due to *Taenia solium* and *Taenia hydatigena* infection Schantz and Kagan. (1980); Lightowlers. (1990). Antigen 5 produces more cross-reactions than antigen B which is less immunogenic but more specific to *E granulosus i*nfection. These cross-reactions are due to the presence of a common epitope (phospholylcholine in all the cestodes species (Ito *et al.,* 1999).

The ability of the western blot analysis to recognize bands that are faint in SDS- PAGE shows the high resolving power of the technique for being able to detect and characterize a multitude of proteins especially those that are of low abundance; Gel electrophoresis is limited in purpose, owing to the fact that the separated proteins in the gel matrix are often difficult to access with molecular probes until when transferred to an adsorbent membrane which gives a clearer resolution. (Towbin *et al.,* 1979).

5.5 Validity of the diagnostic tests

The result of the study clearly indicated that despite the fact that ELISA revealed higher prevalence among all the animals, there are some limitations .Western blot analysis showed that antibody titers that were positive in ELISA failed to recognize *E.granulosus* bands. This might be due to cross reactions

between hydatid antigen and antigen from other helminth parasite or with non specific host proteins in hydatid fluids.

Therefore, western blotting using purified hydatid cyst fluid as a source of antigen has been found to be more specific and relevant though very expensive method for the diagnosis of hydatidosis in live animals. this is due to its ability to detect lower levels of prevalence. Results obtained by other authors have recorded variable specificity and sensitivity of western blotting which in most cases depends on the antigen and technical modalities used. (Zarzosa *et al*.,1999).At present, the best way of conducting serological diagnosis is by using a combination of tests. Enzyme immunoassays or indirect haemagglutination is used to screen all specimens and a positive reaction is confirmed by western blotting or gel diffusion assay.

5.5 Relationship between results of hospital records and the results obtained from the survey

The results of the present study was compared with that obtained from a 10 year retrospective study using hospital records from four hospitals in Kano municipal. It was observed that the formol -ether concentration technique and the direct wet smear are the methods used in diagnosis of intestinal parasites in all the hospital laboratories that were visited. These simple, inaccurate methods cannot distinguish eggs of *E. granulosus* from other taeniid species due to similarities in structure of eggs of cestodes Lightowler. (1990). In some cases, eggs of

E.granulosus could have been be mistaken for that of *Taenia* species this could be one of the reasons why no single case of *E. granulosus* egg was encountered in the hospitals while in this study, sensitive techniques were used and positive cases were encountered in animals and hydatidosis is zoonoses In view of this, it has become necessary to introduce the use of specific and accurate serologic techniques in the diagnosis of hydatid disease in live animals and man because such facilities are not available in the hospitals.

There are factors which must be taken into account when determining the most useful and appropriate test. Practical considerations such as availability of equipments for conducting the tests, conditions under which tests are to be performed and skills of the operators have an overriding consideration in determining the test of choice. The results obtained in the hospitals is attributed to lack of adequate manpower, facilities for conducting serologic diagnosis and where available, the lack of constant supply of light which affects diagnosis. In addition, there are no trained personels that can conduct such tests in the hospitals and government may not be able to release the huge amount of money to purchase facilities and reagents needed for the tests.

Summary and Conclusion

The epidemiological studies of hydatidosis among domestic animals slaughtered in Kano abattoir using serological techniques have shown that the

disease is endemic in the area. The overall prevalence of the disease was higher in camels followed by sheep, cattle and goats. Higher prevalence of antibody to hydatid disease was recorded among older animals than younger ones. The sex of the animal had no effect on the level of antigenicity in the animals. Most of the sheep and goats that were slaughtered were young as compared to the cattle and camels in which older animals were mostly slaughtered.

The ELISA and western blot analysis using purified antigen has proved to be useful in the diagnosis of the disease by giving a promising result. ELISA has the advantage of being fast, sensitive and cheaper while western blotting is more specific, time consuming, more expensive but serve as a confirmatory test. The purification of the cyst fluid reduces the impurities in the antigen.

SDS-PAGE and Western blot analysis revealed that the dominant and most consistently demonstrable proteins occurs as a complex in the 52-66 kDa region and 118-150 kDa region of the antigen preparation from all the animals. The *E. granulosus* specific antigen bands includes the 8, 24, 30, 36, 38, 42, 48, 52, 66, 92 or 118 kDa proteins

In many developing countries of Africa and Asia, hydatidosis causes serious human sufferings and considerable losses in agricultural and human productivity thus posing a significant hindrance to development in the endemic countries. Livestock will continue to play a major role in developing countries as a source of high quality protein, source of wealth and as a supplement to the diets and income of poor families. The uncontrolled commerce and exchange of animals

and animal products, consumption of uninspected meat and products of animal origin is a very common practice in our communities which serves as a common source of transmission of the disease in the community. The traditional collective slaughtering of animals combined with religious or local festivities constitutes ideal conditions for the spread of the infection to dogs and man.

Kano abattoir is very close to human settlements it is the only major abattoir utilized by the densely populated city. The abattoir lack appropriate facilities. The poor hygienic conditions in and around the abattoir creates the favourable conditions for the spread of the disease. Studies on the incidence of the disease are underreported in Nigeria. Since the disease is known to pose a serious constraints to human, agricultural, economic development, food security and safety, human health and well being, and environmental sustainability, much attention is needed to inform the public on the risk of infection, through education and community participation, small scale programmes which could be implemented locally, inexpensively and target specific groups such as consumers, farmers, those that keep dogs as guards or pets, abattoir workers and retailers in order to create awareness on the impact of the disease in man, domestic animals and dogs.

There is the need to conduct more comprehensive surveys using systemic serological techniques inorder to determine accurately the status of the disease in dogs and food animals, and thus to assess the risks to human health in northern Nigeria. Abattoir data are important, particularly in the surveillance of hydatid

control programmes but it can be very difficult to identify specifically small lesions in the lungs and or liver of young animals without additional histological examination therefore a specific serological test would be useful.

The study, being the first of its kind in Kano, has the limitation of not being able to compare it at a close range with the few previous studies that has been conducted in the area which were based mainly on collection of abattoir records and the use of less sensitive serologic techniques. Application of ELISA and western blotting for the detection of *E. granulosus* antibodies in sheep, goat, camel and cattle has been hampered by lack of facilities and adequate standard laboratory facilities in our educational and health sectors.

Recommendations

Serology and imaging techniques have been found to be the best means of studying the actual status of hydatidosis the control of which needs the collaborative work of the veterinarians, in conjunction with the ministry of Agriculture in order to ensure that proper meat inspection are conducted in the abattoirs. Proper disposal or handling of offal, proper handling of dogs and educating the public on the epizootic implications of the disease in man and domestic animals is also recommended.

The findings of this study also suggests that further studies on the use of serological techniques in diagnosis of hydatidosis should be conducted in order to know the actual status of the disease in live animals in Nigeria.

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APPENDIX I

Questionnaire on the Prevalence of Hydatidosis in Domestic Animals Slaughtered in Kano Abattoir, Kano State-NIGERIA

1. Information about the animal

   a. Animal species: Bovine/Ovine/Caprine/Camelidae

   b. Estimated age of Animal: Adult (≥ 1 year old) /Young < 1 year old.

   c. Sex: Male/Female.

   d. Presence of cyst: (Yes/No)

   e. Location of cyst (Liver/Lungs/ others.

2. Information on the Abattoir

   a. Fenced (yes/No)

   b. Presence of stray dogs around abattoir (Yes/No)

   c. Means of disposal of offals.

   d. General hygiene of the abattoir (good/fair/poor.

APPENDIX II: ELISA working solutions

a. TBS - Tris Buffered saline pH7.5

Tris buffered saline (TBS) 50 mM Tris-HCL

150 mM NaCl, 0.02% sodium azide PH 7.5

Weigh 6.05 g Tris in 1 litre of TBS.

Weight 8.76 g NaCl and add to the TBS

Dissolve 6.05 g tris (50mM) and 8.76 g of NaCl (150 mM)

In 800 ml of sterile water, adjust to pH 7.5 with conc. HCl; make up to 1 litre using sterile water.

b. 0.05M phosphate buffer (pH 7.5)

i.e 15M phosphate buffered saline containing 0.05% Tween 20,

APPENDIX III: Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) working solutions

a. Resolving gel buffer stock

3M Tris HCl (pH 8.8)

36.3g Tris dissolved in water

Add HCl to adjust pH

Make up solution to 100ml.

Keep in the fridge.

b. Stacking gel buffer stock

0.5M Tris-HCl pH 6.8.

Dissolve 6g of Tris in distilled water.

Add HCl to adjust pH

Make up to 100mls,

Store in fridge.

c. Acrylamide stock solution (30%)

30g Acryl amide

Dissolve in about 60ml of distilled water

Weigh 0.8g of Bis-acrylamide add in the same beaker allow to dissolve

Filter the solution to 100ml with distilled water,

Store in fridge.

d. 10% Sodium dodecyl sulphate (SDS)

Weigh 10 g of SDS; dissolve in 60 ml of distilled water make up solution to 100 ml.

e. Sample buffer

1% mecaptoethanol, 62 mM Tris-HCl, pH 6.9 5% SDS, 10% glycerol.

To prepare 250 ml of SB/ME,

Weigh 1.878 g of tris in 200 mls of ddH2O

Adjust to pH 6.9, add 5% SDS=12.5g SDS,

Add 1 % mecaptoethanol=2.5 ml

Add 10% glycerol =25ml glycerol.

f. Ammonium per sulphate (APS) 1.5%

Dissolve 0.15g Ammonium per sulphate in 10mls of distilled water. Make fresh everyday.

g. Resolving gel (12.5%)

12.5ml Acryl amide stock

19.75ml distilled water

3.75ml resolving gel buffer

0.3ml 10% SDS

1.5ml APS

15µl TEMED

h. Stacking gel

2.5ml Acryl amide

11.3ml distilled water

5ml stacking gel buffer

0.2ml 10% SDS

15 µl TEMED

1mls APS

i. Tank buffer stock solution (1 * 10 Tank buffer)

30.3g Tris

144g glycine

10g SDS

Dissolve in distilled water and make up to 1 litre.

Store in fridge.

To prepare Tank buffer dilution should be 1:10.

j. Stain solution (0.1% Comassie blue)

2 g Comassie blue G 90 (CI 42655)

500 ml Iso Propanol

100 ml Acetic acid

Dissolve stain in distilled water, add acetic acid and make up to 2 litres with Distilled water.

k. Destain solution.

200ml Iso Propanol

200ml acetic acid

Mix and make up to 2 L with distilled water.

APPENDIX IV: Western blotting working solutions

a. **Electrode or Towbin buffer**. (25Mm Tris, 192mM glycine 20% methanol pH 8.3)

30% Tris

14.4 g Glycine

200ml methanol

Make up to 1 litre with double deionised water (dd H2O).

b. Working solution (TBS) .

Tris- buffered saline (20mM, 500mM NaCl pH 7.5)

Take 100ml of stock TBS (Biorad)

900 ml distilled deionized H2O =1:10 TBS working solution.

c. Wash solution (TTBS)

Tween Tris buffered saline (20mM Tris, 500Mm NaCl, 0.05% Tween 20, pH 7.5.

350W Tween 20 in

700ml working TBS solution.

d. Blocking solution (TBS - BSA)

(TBS) 50Mm – HCl, 150Mm NaCl, pH 7.5, 3% w/v Bovine serum Albumin (BSA).

Weigh 2.8g of BSA

Dissolve in 100ml of TBS.

e. Antibody buffer (TTBS with 2% BSA)

To prepare 200ml

Take 3.84g BSA,

Dissolve in 200ml TTBS.

f. Primary antibody solution

Primary antibody i.e. animal serum diluted in Tris buffered saline –

containing

Bovine serum albumin in a 1: 25.

g.. Secondary antibody solution

Secondary antibody of choice diluted in TBS-BSA in a ratio of 1: 1000

e g 100µl: 100ml.

h. Amido black stain

0.1% Amido black – 0.25 g Amido black

25% IsoPropanol – 62.5 ml

10% Acetic acid -25 ml

Make up to 250 ml with distilled water.

i. Amido black destain

25% Isopropanol – 62.5 ml

10% acetic acid – 25ml

Make up to 250 ml.

APPENDIX 5: High Pressure Liquid Chromatography (HPLC) chart showing the Retention Time (RT) of the Sample Buffer, Sheep, Camel and Goat purified Hydatid Cyst Fluid (HCF); (Mw Ca = 264,000).

