SARCOCYSTIS INFECTION IN CATTLE AND PIGS AND ITS PUBLIC HEALTH IMPLICATIONS IN ZARIA, NIGERIA

BY

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FEBRUARY, 2012

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MSc./VET-MED/00332/08-09

A THESIS SUBMITTED TO THE SCHOOL OF POST-GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

FEBRUARY, 2012

DECLARATION

I declare that the work in the thesis titled: '*Sarcocystis* Infection in Cattle and Pigs and its Public Health Implications in Zaria, Nigeria', has been performed by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Prof. I. Ajogi, Prof. J. U. Umoh and Prof. Idris A. Lawal.

The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis titled "*SARCOCYSTIS* INFECTION IN CATTLE AND PIGS AND ITS PUBLIC HEALTH IMPLICATIONS IN ZARIA, NIGERIA" meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENTS

I thank God Almighty for his protection, guidance and providence during the period of my study. My sincere thanks go to my supervisors Professors I. Ajogi, Idris A. Lawal and J. U. Umoh for their immeasurable advice and patience during the course of my study. I also thank my beloved parents for their love, understanding, moral and financial support. I could not have done this without them. They shall live long to see their great grand and grand children, Amen. I also thank Professor C.A. Kudi, Dr M. Bisallah and Dr O.O. Okubanjo for their professional guidance during the study. I wish to appreciate the Heads of Department and all the academic and technical staff of Veterinary Public Health and Preventive Medicine and Veterinary Parasitology and Entomology for their immense support and patience which contributed to the success of this work. I also thank Dr J.O.S. Asekome, Dr A.S. Oloruko-Oba and the medical director of the university health services main campus Samaru Zaria for their co-operation during the study. My immense gratitude also goes to the industrial training students from the Departments of Microbiology and Biological Science, Ahmadu Bello University Zaria whose contributions I cannot quantify. I also thank my course mates and friends for their encouragement and love.

ABSTRACT

Sarcocystis infection is a parasitic zoonosis which causes acute and fatal clinical diseases in food animals and musculoskeletal pain, diarrhoea and cardiomyopathy in humans. Infected beef and pork are capable of causing systemic sarcocystosis in man; hence it is imperative to determine the prevalence of the infection. This study was designed to determine the prevalence of *Sarcocystis* infection in slaughtered cattle and pigs, dogs and humans in Zaria and to identify risk factors associated with the infection. A cross sectional study was designed in which 200 dog faecal samples were collected from Ahmadu Bello University Veterinary Teaching Hospital and dog slaughter areas. Faecal samples were collected from 390 patients from randomly selected hospitals and volunteers and information on the type of food/meat eaten, source of water, and preparation of meat/food and access to pets were obtained. Sucrose floatation technique was used to analyse faecal samples. Tissues samples (oesophagus and diaphragm) were collected from 200 cattle and 100 pigs from slaughter houses. These were analysed by pepsin-hydrochloric acid digestion and stained with Giemsa. Histological sections of tissues were stained using Haematoxylin and Eosin (H&E). Measurement of sporocyst and sarcocyst were performed for identification of species. One (0.5 %) dog was positive for sporocysts and no human sample was positive. Faecal samples from cattle and pigs were not positive. Eighty-five (42.5 %) cattle and 60 (60.0 %) pigs were positive for sarcocyst by tissue digestion. Tissue digestion was more efficient in detecting sarcocysts/bradyzoites than histology. In cattle, sarcocysts were found more in the oesophagus than the diaphragm ($p < 0.05$) while in pigs, there was no significant difference in the level of infectivity between oesophagus and diaphragm ($p > 0.05$). Age, sex and breed did not significantly influence the prevalence of *Sarcocystis* infection ($p > 0.05$). Leucocytic infiltrations were observed in oesophageal tissues of

cattle and pigs which were mostly lymphocytes and few eosinophils. *Sarcocystis cruzi* (99.0 %) and *S. hominis* (4.0 %) were identified in cattle while *S. meischeriana* (25.0 %) and possibly *S. porcifelis* (85.0 %) were identified in pigs. Species identified in dogs could be *S. tenella*, *S. capracanis* or *S. bertrami* or a mixture of the species. Questionnaire analysis shows that none of the respondents was aware of *Sarcocystis* infection. About 92.2 % reported boiling and frying meat before consumption. Sixty-one percent reported consuming suya, meat pie, sausages and burger occasionally while 22.2 % do so regularly. Also, 98.7 % consume vegetables which were mostly washed twice (49.8 %) with water and salt (64.8 %) and added directly to food to cook. Eight percent reported feeding raw meat to their pets and 62.2 % reported having wells which were covered, cemented and above ground level. This study has established the prevalence of *Sarcocystis* infection in cattle, pigs and dogs in the study area. Identified species were of public health importance. Adequate heat treatment of meat and none feeding of raw meat to pets is advocated.

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CHAPTER 1

INTRODUCTION

1.1 DEFINITION OF THE PROBLEM

Sarcocystosis is a parasitic zoonosis caused by species of *Sarcocystis*; an intracellular protozoan parasite in the phylum Apicomplexa and family Sarcocystidae. The infection is characterised by cyst formation in muscular tissues (muscular sarcocyctosis) in the intermediate host or colonization of the lamina propria of the intestines (intestinal Sarcocystosis) in the definitive host. *Sarcocystis* has a requisite two-host life cycle based on a prey-predator (intermediate-definitive) host relationship (Fayer, 2004).

Asexual stages develop in intermediate hosts after they ingest the oocyst stage from definitive-host faeces and terminate with the formation of intramuscular cysts (sarcocysts). Sarcocysts in meat eaten by a definitive host initiate sexual stages in the intestine that terminate in oocysts excreted in the faeces. Most *Sarcocystis* species infect specific hosts or closely related host species (Fayer, 2004). Unlike other species of Coccidia, *Sarcocystis* is shed in the faeces in the infective form. That is, the oocyst does not depend on weather conditions to sporulate and hence is infective (Dubey, 1976).

Sarcocystis spp form cysts in various intermediate hosts such as cattle, pigs, horses, sheep, goats, birds, rodents, camelids, wildlife, reptiles and including humans (Dubey *et al*., 1989). The cysts vary in size from a few micrometers to several centimeters, depending on the host and species (Merck, 2005). Although, species of *Sarcocystis*

have some degree of host specificity, hosts can harbour more than one species (Dubey *et al*., 1989).

Sarcocystis infection (sarcocystosis) in cattle can cause abortion, reduce milk yield, neurologic signs, loss of weight, and even death (fatal cases) depending on the specie and number of sporocysts ingested (Dubey *et al*., 1989). In pigs, weight loss, purpura of the skin, muscle tremors, disseminated intravascular coagulaopathy (DIC), abortion and death has being documented (Barrows *et al*., 1982a; Caspari, 2011).

In the definitive hosts, *Sarcocystis* generally does not cause illness. Humans can serve both as intermediate and definitive host (Fayer, 2004). In humans, eating raw or undercooked meat containing mature sarcocysts has resulted in humans acquiring intestinal Sarcocystosis with infected individuals exhibiting diarrhoea, bloat, dyspnea, tarchycardia, nausea and loss of appetite (Fayer, 2004). Currently, Sarcocystosis is being considered as one of the opportunistic infections exacerbated in Acquired Immune Deficiency Syndrome (AIDS) patients (Velasquez *et al*., 2008).

1.2 STATEMENT OF RESEARCH PROBLEM

In Nigeria, many families practice backyard animal farming as a means of livelihood and income support. Cattle rearing provides major source of income and food security to rural and urban dwellers in the Northern Guinea Savannah owing to their efficiency in converting poor quality feed into desirable food product. Also, pig husbandry is gaining popularity amongst urban dwellers and as an alternative source of protein; people patronize this meat product as it is known to be a delicacy. *Sarcocystis* has been

shown to be a zoonotic parasite when humans consume infected meat and accidental ingestion of oocyst/sporocysts from infected animals.

The presence of pet animals which are the definitive hosts of the parasite in the community ensures continuous cycling of the infection between the intermediate hosts (livestock animals) and definitive hosts (dogs and cats); as dogs are used as security tools in cattle and pig farms. More so, meat from many birds, reptiles and species of wild mammals that may harbour sarcocysts is eaten in various parts of the country with unknown consequences. Locally known predators such as dogs, cats and snakes could excrete infectious sporocysts that may find their way through contaminated water or food, eventually infecting man. Therefore, there remain many potential but unknown sources of human intestinal sarcocystosis (Fayer, 2004).

Sarcocystosis has been reported to be of world wide occurrence. However, not all species are highly prevalent (Collery and Weaver, 1981; Meads, 1976). Where data are available, the economic loss in livestock industry is enormous. This is mainly due to condemnation or down-grading of meat containing grossly visible sarcocysts (Fayer, 1976). In the absence of effective meat inspection, consumers of beef and pork are likely to be infected with sarcocysts.

1.3 JUSTIFICATION

Nigeria is presently going through a period in which considerable emphasis is placed on food production embracing both crop and livestock production to feed her teeming population. This goal can only be attained if only healthy animals are produced, thereby reducing losses caused by diseases and death. In many countries Sarcocystosis in

animals has been shown to cause acute and even fatal clinical diseases in cattle and pigs (Dubey *et al*., 1989; Caspari, 2011). In humans, muscular sarcocystosis has being reported to cause musculoskeletal pain, fever, rash, cardiomyopathy, bronchospasm and subcutaneous swellings (Fayer, 2004).

Available information on *Sarcocystis* infection of livestock animals in Nigeria dates back to late 1980s and late 1990s by the work of Aganga *et al* (1988), Kudi (1989) and Kudi *et al* (1991). Adejinmi and Osayomi (2010) reported a prevalence rate of 12.0% *Sarcocystis* infection in dogs in Ibadan but did not determine its prevalence or occurrence in livestock animals.

The current status of *Sarcocystis* infection in cattle and pigs in Nigeria is unknown. As these are food product for man and are capable of causing systemic sarcocystosis if infected meat is consumed, there is need to know if the parasite exists in our environment and to what extent and severity.

1.4 AIM

To determine the prevalence of *Sarcocystis* infection in slaughtered cattle and pigs, in dogs and humans and to identify risk factors associated with the infection in Zaria.

1.5 SPECIFIC OBJECTIVES

The specific objectives were to:

1. detect macroscopic and/or microscopic cysts of *Sarcocystis* in the oesophagus and diaphragm of slaughtered cattle and pigs in Zaria.
2. detect the presence of sporocysts and/or oocysts of *Sarcocystis* in the faeces of dogs and man in Zaria.
3. identify the species of the parasite through measurement of oocyst/sporocyst and sarcocysts.
4. identify the risk factors associated with the infection in the study area.
5. assess the awareness of individuals in the study area to *Sarcocystis* infection.

1.6 RESEARCH QUESTIONS

1. Do slaughtered cattle and pigs in Zaria harbour *Sarcocystis* species?
2. Do dogs and humans in Zaria harbour *Sarcocystis* species?
3. Is there an association between age and *Sarcocystis* infection in cattle, pigs and dogs?
4. Is there an association between sex and *Sarcocystis* infection in cattle, pigs and dogs?
5. Is there an association between breed and *Sarcocystis* infection in cattle, pigs and dogs?

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORY

Sarcocystis was first reported in 1843 by Miescher as white threadlike cysts in striated muscles of a house mouse, without a scientific name. For the following 20 years, the parasite was simply referred to as Meischer's tubules. Kuhn (1865) found similar structure found in pig muscle and named it *Synchytrium mieschерianum.* Lankester (1882) introduced the name *Sarcocystis* (sarco = muscle) because the former genus as occupied. Labbe (1899) then, changed the name *Synchytrium mieschерianum* to *Sarcocystis meischeriana*. Thus, the parasite *S. meischeriana* (Kunh, 1865) Labbe 1899 became the type species of the genus.

Between 1885 and 1972, numerous species of *Sarcocystis* species were named based on finding of sarcocysts in the host. At that time, validation of the identity of the different species was not possible because the life cycle of *Sarcocystis* was unknown not until 1972 (Dubey *et al*., 1989). During much of this time, scientists debated whether *Sarcocystis* species were protozoa or fungi.

The possibility that *Sarcocystis* were fungi arose because only the sarcocyst stage was known and, when sarcocysts and their contents were placed in various culture media, hyphae and mycelia (now recognized to be a result of contamination) were sometimes found several days later (Fayer, 2004). It was not until 1967, 124 years after the first report of *Sarcocystis*, that the spindle- or crescent-shaped bodies (bradyzoites) in the sarcocysts were studied by electron microscopy and organelles were observed like

those seen in other apicomplexan protozoa such as *Toxoplasma* and *Eimeria* (Senaud, 1967).

The life cycle and all other stages remained unknown until 1970, when bradyzoites from sarcocysts in bird muscles were inoculated into cultured mammalian cells and underwent development into sexual stages and oocysts (Fayer, 1970; Fayer, 1972). Heydorn *et al* (1975) first provided evidence that were three species of *Sarcocystis* in cattle with sarcocyst in cattle and sexual stages in man, dog and cat respectively; there were two species with sarcocyst in sheep and sexual stages in dog and cat. The sarcocysts of the species were structurally different from one another.

Previously, any sarcocyst in cattle was considered one species; *S. fusiformis*, and it was also thought to parasitise water buffalo (Dubey *et al*., 1989). Transmission studies involving *S. fusiformis* provided further clarification of the biology of this once enigmatic group of protozoan parasites. After sarcocysts were fed to different potential definitive hosts—dogs, cats, and humans— *S. fusiformis* was found to encompass three species, and the new species names *S. bovicanis (*old name*: S. cruzi)*, *S. bovifelis (*old name*: S. hirsuta)* and *S. bovihominis (*old name*: S. hominis)* (named for the intermediate and definitive hosts) were proposed (Heydorn and Rommel, 1972; Rommel and Heydorn, 1972; Rommel *et al*., 1972).

These collective findings provide the current basis for understanding the sources of infectious organisms, the transmission dynamics, the criteria for identifying and naming species of *Sarcocystis*, and the biology critical to prevention and treatment strategies.

2.2 TAXONOMY

Before the life cycle of *Sarcocystis* was discovered by Fayer (1972), numerous species were named based upon the findings of sarcocysts in the host. Equipped with knowledge of the life cycle of *Sarcocystis*, various authorities searched the literature for clues that earlier investigators recognized structural differences among sarcocysts and named them accordingly. For example Moule (1888) had named *Sarcocystis* hirsuta for a sarcocyst with hairy projections he observed in cattle. Hasselmann (1926) had given the name *Sarcocystis* cruzi for a sarcocyst without hairy projections from the heart of cattle. Levine (1977) renamed *Isospora hominis* as *Sarcocystis hominis*; another parasite found in the faeces of man, based on the observation that it was found to initiate the development of another sarcocyst in man. Because the original descriptions were inadequate and no type specimens were available, it was impossible to confirm that originally named species were identical to those species wherein the life cycles were documented (Levine, 1977).

Due to difficulties in making positive identification, Heydorn *et al*. (1975) proposed new names for the three species in cattle thus; *Sarcocystis bovicanis*; *Sarcocystis bovifelis* and *Sarcocystis bovihominis* and two species in sheep, *Sarcocystis ovifelis* and *Sarcocystis ovicanis*. This was done by combining the names of the intermediate and definitive hosts.

Levine (1977) after an extensive search of the literature reported that the old names, *S. cruzi*, *S. hirsuta* and *S. hominis* for Sarcocysts in cattle, and *S. tenella* and *S. gigantea* for *Sarcocystis* in sheep, were valid and should not be replaced by the new names, following the International Code of Zoological Nomenclature (ICZN). In this write up,

taxonomical review by Levine (1977) and Levine and Tadros (1980), and supported by the ICZN are used. *Sarcocystis* species are coccidian parasites and belong to:-

Phylum: Apicomplexa

Class: Sporozoasida

Subclass: Coccidiasina

Order: Eucoccidiorida

Suborder: Eimeriorina

Family: Sarcocystidae

Subfamily: Sarcocystinae

Genus: *Sarcocystis*; (Lankester, 1882)

The relative value of different criteria that might be used to validate *Sarcocystis* species based on research findings of various investigators are the sarcocysts, schizonts, oocysts and sporocysts, host-specificity and isoenzymes (Dubey *et al*., 1989).

The shape and size of the sarcocysts vary with its age, type of host cell parasitized and the techniques used for its identification. For example, sarcocysts of the same species in cardiac muscles and in the central nervous system are always smaller than those in skeletal muscles (Dubey, 1982b; Dubey *et al*., 1984). The size and shape of sarcocyst will also vary depending on the type of fixation (they are smaller in fixed tissues than in live specimens) and also vary with the type of fixative used. As they are often located in contractile muscles, the sarcocyst size will vary depending on whether the host cell was relaxed or contracted at the time of fixation (Dubey *et al*., 1989).

The structure of the bradyzoites also varies. While bradyzoites of some *Sarcocystis* species are densely packed within the sarcocyst, others are sparely found. Such conditions may affect the shape and size of the sarcocyst (Dubey *et al*., 1989). Measurements of length and width of the bradyzoites is also used for species identification, but unless the size differences of bradyzoites are obvious, this criterion should not be used for identification (Dubey *et al*., 1989).

The structure of metrocytes contained in the sarcocyst is not useful criterion for speciation because they are often irregularly shaped and their size is highly variable depending on the stage of division. Also, the structure of organelles such as mitochondria, rhoptries and micronemes is not a good taxonomic criterion (Dubey *et al*., 1989) whereas the structure of sarcocyst wall is a useful criterion for speciation. The structure of the sarcocyst all seems to indicate a phylogenetic relationship. For example, while Type 1 sarcocysts were found in small closely related mammals and Type 15 only in closely related cervids, Type 14 were found only in goats and sheep (Dubey *et al*., 1989).

The structure of sarcocyst wall is used with caution as a taxonomic criterion because of the following (Dubey *et al*., 1989);

i. The structure varies with fixative and the degenerative stage of the host cell. For example, the smooth thin wall of *S. cruzi* may appear thick and hirsute if not properly fixed or as a result of fixation with different fixatives.

ii. In some species, villar protrusions from the sarcocyst wall are embedded deep in the surrounding myocyte and thus may be impossible to detect with the light

microscope e.g. a hirsute sarcocyst wall could appear smooth. Therefore, the ultra structure of the sarcocyst wall is essential for describing a new species of *Sarcocystis*.

iii. The shape and size of the villar protrusions on the sarcocyst wall vary in live and fixed preparations. Therefore, undue reliance should not be placed on their size.

iv. The structure of the sarcocyst may vary with age. Therefore, it is important to be cautious when studying specimens from naturally infected animals because there is no reliable method of determining the age of the sarcocyst (Dubey *et al*., 1989).

The structure of the schizont is of limited taxonomic value because it varies greatly depending on the developmental cycle and the host cell parasitized (Dubey *et al*., 1989). The structure of the oocyst and sporocysts traditionally used to determine species of other coccidian is of little or no taxonomic value in *Sarcocystis* for the following reasons:

i. Except for minor variations is sizes, all *Sarcocystis* sporocysts and oocysts are structurally similar.

ii. Numerous species of *Sarcocystis* in a given host overlap in dimensions (Dubey *et al*., 1989).

iii. Species of *Sarcocystis* are generally more host specific for their intermediate hosts than for their definitive hosts. However, none of the species transmissible via dogs are transmissible by cats and vice versa (Dubey *et al*., 1989).

Determination of electrophoretic patterns of isoenzymes is useful in distinguishing species of *Sarcocystis* (Ford *et al*., 1987), but the zoites must be obtained from experimentally infected hosts (Dubey *et al*., 1989).

2.3 LIFE CYCLE, DEVELOPMENT AND MORPHOLOGICAL STAGES OF SARCOCYSTOSIS

Sarcocystis species are intracellular protozoan parasites with a requite two-host life cycle based on a prey-predator (intermediate-definitive) host relationship. The life cycle is characterized by alternation of asexual and sexual generation and can be divided into three distinct phases: sporogony, schizogony and gametogony (Figure 2.1). Sporogony involves the formation of sporozoites which might initiate infection in the intermediate host. Schizogony is the asexual reproduction by multiple fissions while gametogony is the sexual reproduction which is the fertilization of female gametes which takes place in the definitive host (Fayer, 1972; Dubey, 1976).

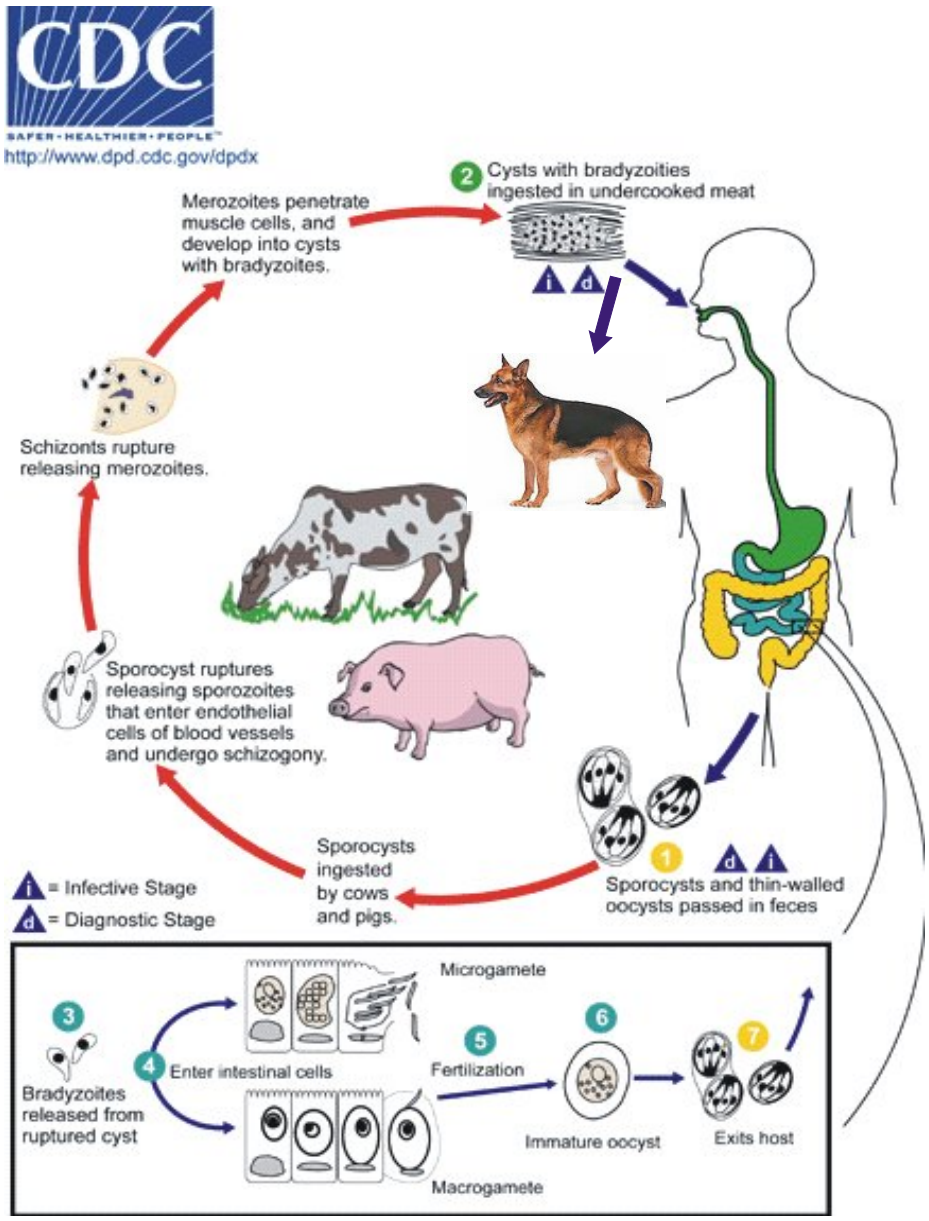


Figure 2.1: Life cycle of *Sarcocystis* species (Source: Modified from CDC, 2009)

2.3.1 Stages in the Intermediate (Prey) Host

After oocysts or free sporocysts from the definitive host are ingested by a susceptible intermediate host, they pass to the small intestine. The plates forming the sporocyst walls separate, releasing the four sporozoites held inside. Motile sporozoites migrate through the gut epithelium, eventually entering endothelial cells in small arteries throughout the body. Here they undergo the first of four asexual generations (called schizogony or merogony), producing numerous merozoites (cells morphologically similar to sporozoites and bradyzoites) about 15 to 16 days after ingestion of sporocysts (Fayer, 2004).

Second generation occur in the endothelium as early as 15 days post inoculation in the capillaries, small arteries and virtually though out the body. The schizonts were most numerous in the glomeruli of the kidney. The shape and size of schizonts vary considerably. Schizonts in the skeletal muscles are longer than those in other tissues (Dubey *et al*., 1989). Both first and second generation schizonts are located within the cell cytoplasm and are not surrounded by a parasitophorus vacuole (Dubey *et al*., 1989).

Merozoites released from these second-generation schizonts enter the blood stream and circulate extracellularly or within mononuclear cells. They multiply in the blood stream by dividing into two progeny. These have been found six weeks after inoculation. The number of such generations in blood stream is unknown. A third generation of merogony within the blood has been described (Fayer and Johnson, 1974; Speer and Dubey, 1981; Dubey *et al*., 1989).

Merozoites released from the second-generation schizonts that develop in the blood vessels penetrate muscle and nerve cells and cyst formation begins with the development of a parasitophorous vacuole (Mehlhorn and Heydorn, 1978). The boarder of this vacuole is always a single unit membrane. This membrane soon becomes strengthened by an underlying layer of osmiophilic material. The complex formed becomes the primary cyst wall and has a thickness range of about 20-100mm (Mehlhorn *et al*., 1976; Mehlhorn and Heydorn, 1977).

Merozoites enter muscle cells, round up to form metrocytes (mother cells), and initiate sarcocyst (Greek: sarkos = flesh, kystis = bladder) formation. Sarcocysts begin as unicellular bodies containing a single metrocyte. Through repeated asexual multiplication, numerous metrocytes accumulate and the sarcocyst increases in size. As sarcocysts mature, the small, rounded, noninfectious metrocytes give rise to infectious, crescent-shaped bodies called bradyzoites (Greek: brady = slow, zoite = small animal) (Fayer, 2004).

Maturation varies with each species and takes 2 months or more until bradyzoites form and sarcocysts become infectious for the definitive host (Fayer, 2004). Sarcocysts can persist for months or years. Mature sarcocysts of each species vary in size from microscopic to macroscopic, vary in length and circumference, and develop structurally distinct sarcocyst walls that vary in thickness and organization of villar protrusions, but all contain numerous bradyzoites.

At least seven structurally distinct wall patterns have been found by electron microscopy of specimens isolated from humans. By light microscopy, often the most

one can distinguish is whether the wall is thick or thin (Fayer, 2004). Sarcocysts are found in virtually all striated muscles of the body including the tongue, esophagus, and diaphragm, as well as cardiac muscle and, to a lesser extent, smooth muscle. Sarcocysts have also been found in small numbers in neural tissue such as spinal cord and brain and Purkinje fibers of the heart (Fayer, 2004).

2.3.2 Stages in the Definitive (Predator) Host

After sarcocysts are eaten by a susceptible definitive host and the wall is mechanically ruptured or digested, bradyzoites become motile, leave the sarcocyst, and enter cells of the intestinal lamina propria. Each intracellular bradyzoite develops into a male (micro) or female (macro) gamont. Within 6 hours of ingesting infected tissues, gamonts are found within parasitophorus vacuole in goblet cells near the tip of the villi. The ratio of macrogamonts to microgamonts is approximately 95:5 (Dubey *et al*., 1989).

Microgamonts are ovoid to elongate containing one to several nuclei. They undergo nuclear division with each resulting nucleus situated near the gamont surface (Fayer and Thompson, 1975). A single microgamete develops immediately above each nucleus. Each consists of a nucleus and two flagella. Macrogamonts are ovoid to round and contain a single large nucleus. They do not undergo nuclear division as the macrogamonts develop into macrogametes. Microgametes liberated from the microgamont actively moves up to the periphery of the macrogamont. After fertilization, a wall develops around the zygote and oocyst is formed (Sheffield and Fayer, 1980).

Oocyst sporulates in the lamina propria. Initially, the sporont is granular, basophilic and filled the young oocysts. During the intial stages of sporont condensation, a clear cap-like structure is seen (Dubey *et al*., 1982b). On further condensation, a similar clear area appears at the opposite end. At this stage, the oocyst contains one large nucleus with one or two nucleoli and several Periodic Acid Shiff (PAS)-positive granules (Dubey *et al*., 1989).

As sporulation progresses, the nucleus becomes elongate and divides into two nuclei, one at each pole of the sporont. A second transverse division occur giving rise to two sporoblasts which later becomes the sporocysts. Four sporozoites are formed in each sporocyst by a third nuclear division. Because sporulation is asynchronous, unsporulated and sporulated oocysts are found simultaneously (Dubey *et al*., 1989).

Oocysts pass into the intestinal lumen and then pass from the body in the faeces. Intact oocysts are usually observed only in the first few days of patency and appear as two adjacent sporocysts with the oocyst wall barely visible, if visible at all. The thin oocyst wall often breaks, releasing individual sporocysts, often the only stage observed in faeces. Sporulated oocysts are generally colourless, thin walled (<1 μm) and contain two elongate sporocysts. An oocyst residium and a micropyle are absent. Each sporocyst contains four elongated sporozoites and a granular sporocyst residium which may be compact or dispersed (Dubey *et al*., 1989).

2.4 ULTRASTRUCTURAL CHARACTERISTICS OF THE DEVELOPMENTAL STAGES OF *SARCOCYSTIS* SP.

2.4.1 Sarcocysts

This is an important developmental stage of *Sarcocystis* because of its taxonomic aid. Each *Sarcocystis* species produces a cyst wall that has unique ultrastructural features which can be used to distinguish it from other species within the same intermediate host. Sarcocysts development begins when a merozoite enters a myocyte or nerve cell. The merozoite resides in a parasitophorous vacuole. The vacuole is surrounded by a parasitophorous vacuolar membrane. This membrane later develops into a primary cyst wall (Dubey *et al*., 1989).

The primary cyst wall consists of an underlying electron-dense layer. Many of the organelles of the apical complex such as micronemes, conoid, polar and apical rings disappear as a merozoite transforms into a metrocyte, while making ribosomes, endoplasmic reticulum and mitochondria become more abundant and the nucleus larger. A granular layer develops early immediately beneath the primary cyst wall. The structure and thickness of the cysts wall varies among and within species of *Sarcocystis*. This is true especially as the cyst matures (Dubey *et al*., 1989).

The wall of the sarcocyst may be smooth, striated or hirsute when viewed microscopically (Dubey *et al*., 1989). Sometimes it may possess complex branched protrusions (Gjerde, 1985a; 1985b). The protrusions may contain minute granules, electron dense bodies, microfilaments, microtubules and small vesicles. As sarcocysts develop, the contents of the villar protrusions also change which probably reflects a

change in the metabolic rate within the sarcocysts. In mature sarcocysts, the protrusions contain granules and clusters of vesicles (Dubey *et al*., 1989).

2.4.2 Metrocytes

They are ovoid, rapidly multiplying forms which have an electron-lucent cytoplasmic matrix. It has numerous ribosomes, several mitochondria, one or more micropores, and pellicle consisting of two or three membranes, subpellicular microtubules, endoplasmic reticulum, one or two golgi complexes, a few amylopectin granules, centrioles, electron dense bodies, an occasional lipid body and a diffuse nucleus (Dubey *et al*., 1989).

Metrocytes may also contain anlagen of two progeny formed by endodyogeny. They may arise from metrocytes or from differentiating merozoites. Metrocytes in early stages of endodygeny may also contain remnants of the conoid, micronemes and rhoptries. Early sarcocysts contain only metrocytes whereas relatively few metrocytes are contained in intermediate and mature sarcocysts (Dubey *et al*., 1989).

2.4.3 Bradyzoites

Bradyzoites has a pellicle, apical rings, microtubules, micropore, rhoptries and micronomes, conoid, golgi complex, endoplasmic reticulum, ribosomes, mitochondria, multivesicular bodies and inclusion bodies (Dubey *et al*., 1989).

The pellicle consists of three membranes; an outer plasmalemma and an inner double membrane complex. The plasmalemma is a continuous unit that completely encloses the whole parasite, whereas the inner pellicular membranes are interrupted at the anterior and posterior ends and at micropores (Dubey *et al*., 1989). The plasmalemma is

separated from the inner membranes by an electro-lucent space, 15 to 20 nm thick. The inner pellicullar membrane complex is similar to tight intercellular junctions in higher mammals and is composed of 11 rows of contiguous plaques that are arranged in loosely pattern. The plaques converge at the posterior end to form the posterior pore. At the anterior tip, the plasmalemma contains a rosette of intramembranous particles (Dubey *et al*., 1989). The rosette of intramembranous particles may be involved in rhoptry secretion during host cell penetration or serve as a receptor-processor system which enables the parasite to recognize and penetrate host cells (Porchet and Torpier, 1977).

The two apical rings (also called preconoidal or conoid rings) are situated at the anterior tip immediately beneath the plasmalemma and above the conoid. The function of these apical rings is still not known (Dubey *et al*., 1989).

The polar rings have been studied less extensively in *Sarcocystis* species than in other coccidian (Dubey *et al*., 1989). A single polar ring appears to be present in *Sarcocystis tenella* bradyzoites (Porchet-Hennere, 1975) whereas other species have two polar rings (Dubey *et al*., 1989). The first polar ring appears as an electron-dense thickening at the anterior end of the anterior cape of the inner membrane complex and serves to anchor the subpellicular microtubules in those bradyzoites with a single polar ring (Porchet-Hennere, 1975). In those species with two polar rings, the second ring is located immediately beneath and slightly posterior to the first polar ring and serves as an anchoring point for the subpellicular microtubules (Dubey *et al*., 1989).

Microtubules are found in every stage of *Sarcocystis* species (Dubey *et al*., 1989). Cytoplasmic microtubules are few in number as compared to other microtubules of specialized structures such as subpellicular microtubules, conoid, centrioles, flagellar axoneme and the mitotic spindle apparatus. Microtubules are the basic building blocks of flagellar axonemes (found only in microgametes), centrioles, basal bodies, mitotic spindles, and the conoid (Dubey *et al*., 1989).

Subpellicular microtubules probably provide structural integrity to the overall shape of bradyzoites and may be involved in motility, transport of cytoplasmic components near the margin of the zoite, or serve to anchor protein molecules in the inner membrane complex (Dubey *et al*., 1989).

Rhoptries and micronemes are electron-dense, membrane-bound, elongate structures occupying the anterior half of the zoite. They constitute a single functional unit involved in secreting substances to aid penetration of cells. They may also serve to secrete proteins that become antigenic or are inserted into the zoite plasmalemma just above the conoid (Dubey *et al*., 1989).

Micropores are also present in all stages of *Sarcocystis* species except for microgametes. They consist of an invagination of the parasite plasmalemma into the parasite cytoplasma. The invaginated membrane is encircled on the cytoplasmic side by an electron-dense membranous collar which the inner membrane interrupts and is juxtapoxed at a 90^{o} angle to it. Although most micropores appear to be inactive, some are observed actively ingesting particulate matter observed in developing or multiplying stages such as metrocytes (Dubey *et al*., 1989).

The conoid is a hollow, conically-shaped organelle located at the anterior tip of sporozoites, bradyzoites and merozoites that consists of microtubular elements. The conoid is believed to be in involved in penetration of host cells because when it is protruded, the anterior tip becomes shaped like a long, narrow style that would facilitate active penetration into the cell (Dubey *et al*., 1989). Within the core of the conoid are the ducts of rhoptries and one or two eccentrically located microtubules that extend for a short distance into the zoite cytoplasm (Porchet-Hennere, 1975).

Golgi apparatus is present but in reduced numbers in bradyzoites of most *Sarcocystis* species. It is more developed in those stages that are metabolically active such as metrocytes and schizonts. Endoplasmic reticulum is somewhat reduced in bradyzoites; both smooth and rough endoplasmic reticula are present. Bradyzoites have an abundant of free ribosomes which can be found from the conoid to the posterior tip. Golgi complex, endoplasmic reticulum, mitochondria and ribosomes are present and have functions just like in the typical cell (Dubey *et al*., 1989).

Inclusion bodies such as amlopectin, lipid and electron-dense bodies, are present free in the cytoplasm. Lipid and especially amylopectin represent energy reserves. Although electron-dense bodies are seen in nearly all stages, their function is still not known. They probably serve as a site for storage or energy reserves, protein, lipid and perhaps enzymes (Dubey *et al*., 1989).

2.4.4 Oocyst

Unsporulated oocyst consists of a sporont surrounded by an oocyst wall. The oocyst wall has an electron-dense, finely granular outer layer and an inner layer of one to four

membranes (Mehlhorn and Heydorn, 1979). On the outer surface of the oocyst wall are electron dense projections. The sporont is limited by a plasmalemma and a single inner membrane. It contains a nucleus, endoplasmic reticulum, mitochondria, ribosomes, golgi complex, amylopectin bodies, micropores and an occasional electron-dense body (Dubey *et al*., 1989).

Sporulated oocysts are generally colourless, thick walled and contain two elongated sporocysts. Each sporocyst contains four elongated sporozoites and a granular sporozoite residium which may be compact or dispersed (Dubey *et al*., 1989).

2.4.5 Sporocyst

The sporocyst wall is composed of a thin continuous outer layer and a thick inner layer which consists of four plates joined at sutures, similar to those in related coccidian (Speer *et al*., 1973; Box *et al*., 1980). The inner layer has alternating bands of electron dense and electron-lucent materials and is perpendicular to the surface of the sporocyst (Dubey *et al*., 1989). A lip-like thickening (150-180 nm) is located at the margin of each plate. A thin strip of electron-dense material is interposed between the lip-like thickenings of two apposing plates.

During excystation, bile salts and or trypsin act upon sutures, causing the plates to separate from the interposed strip. This allows the sporocyst to collapse, releasing the sporozoites randomly (Dubey *et al*., 1989). Each sporocyst contains four sporozoites.

2.4.6 Sporozoites

Sporozoites are banana-shaped and have all the ultrastructural features described earlier for bradyzoites such as conoid, apical rings, micronemes, rhoptries, micropore, mucleus, mitochondria, golgi complex, endoplasmic reticulum, ribosomes, amylopectin bodies, pellicle, and subpellicular microtubules (Dubey *et al*., 1989). They differ however, from bradyzoites as well as merozoites by possessing a crystalloid body and structures similar to rhoptries and micronemes in the posterior one half of the sporozoite (Strohlein and Prestwood, 1986).

The crystalloid body consists of electron-dense and electron-luscent granules and usually located in the posterior one half of the sporozoite. This body is probably analogous to the homogenous refractile bodies of *Eimeria* species in which the body is believed to represent an energy or amino acid reserve (Dubey *et al*., 1989).

2.5 EPIDEMIOLOGY

Sarcocystis infection (sarcocystosis) is common in many species of animals globally (Dubey *et al*., 1989; Roberts and Janovy, 2006). More than 50% of adult swine, cattle and sheep probably are infected with *Sarcocystis* spp (Dubey, 1977). Dogs and cats serve as definitive hosts for a variety of *Sarcocystis* species. They shed the sporocysts in their faeccs over a period of many months after infection. The oocyst or sporocysts are known to be resistant to freezing and other harsh weather conditions (Cawthorn *et al*., 1984).

Flies may acts as transport host (Markus, 1980). The discovery of a generalized infection in dogs indicates that carnivores may develop infections typical of those

found in intermediate hosts (Dubey and Speer, 1991). Unlike other species of Coccidia, *Sarcocystis* is shed in faeces in the infective form. That is, the oocysts are sporulated when passed in the faeces; no dependence on weather conditions to sporulate (Dubey *et al*., 1989, Urquhart *et al*., 2003; Fayer, 2004).

Not all species of *Sarcocystis* are highly prevalent. Most species of *Sarcocystis* transmissible via cats have been found less frequently than those transmissible via canids (Dubey *et al*., 1989). This is because cats are poor producers of *Sarcocystis* sporocysts. Another reason may be that *Sarcocystis* of feline transmitted species require several months or years to become infective. Also, another reason may be that some host species are inherently more susceptible to infection with some agents than are others (Dubey *et al*., 1989).

From the high prevalence of symptomless infections observed in slaughtered food animals, it is clear that where dogs or cats are kept in close association with farm animals or their feed, transmission is likely to occur (Urquhuart *et al*., 2003). Acute outbreaks of the infection are probably most likely when livestock which have been reared without dog contact are subsequently exposed to large numbers of the oocysts/sporocysts from dog faeces (Urquhuart *et al*., 2003).

Aganga *et al* (1988) reported 3.04% occurrence rate of *Sarcocystis ovicanis* in Zaria. Kudi (1989) reported prevalence rates of 1.8%, 3.0% and 4.8% of *Sarcocystis* infection in Kaduna, Plateau and Bauchi states respectively, with an overall prevalence rate of 2.5%. He also reported 0% prevalence in cattle and human. In 2010, Adejinmi and Osayomi reported 12 % prevalence of *Sarcocystis* infection in dogs in Ibadan.

Several studies on *Sarcocystis* infection has being conducted in other countries. Based on examination of tissues from abattoirs, a high percentage of cattle worldwide are infected with sarcocysts, with those of *S. cruzi* (infectious from cattle to canines) being the most prevalent and easiest to identify histologically (Van Knapen *et al*., 1987). Of 238 cattle carcasses examined in Madhya Pradesh, India, over 80% contained sarcocysts (Jain and Shah, 1987). Of these, 186, 31, and 29 were identified as *S. cruzi*, *S. hirsuta*, and *S. hominis*, respectively.

The prevalence of *Sarcocystis* in Japanese and imported beef was reported, but the species were not identified (Ona and Ohsumi, 1999). In Brazil, all 50 samples of raw beef prepared as kibbe in 25 Arabian restaurants in Sao Paulo contained sarcocysts (Pena *et al*., 2001). *Sarcocystis hominis* has not been detected in the United States, whereas up to 63% of cattle in Germany have been reported to be infected (Fayer, 2004).

A prevalence rate of 6.25% *Sarcocystis* cysts were detected in meat supplied for hamburger in Iran (Khaniki and Kia, 2006). The overall prevalence of *Sarcocystis* in pigs appears low, at 3 to 36% worldwide (Fayer, 2004). *S. suihominis* was more prevalent in Germany than Austria, but little information is available from other countries. *S. suihominis* and *S. hominis* have been found in slaughtered pigs and cattle raised in Japan (Saito *et al*., 1998; 1999). Caspari *et al* (2011) made the first report of naturally acquired clinical sarcocystosis in a pig breeding stock.

Herbivores serve as intermediate hosts for *Sarcocystis* spp where it encysts in the skeletal and smooth muscles leading to formation of sarcocysts. Studies have reported

100% prevalence in adult cattle in the United States (Dubey *et al*., 1989) and 100% prevalence in pigs in Thailand (Nateeworanart *et al*., 2004).

The conditions existing that permit such usually high prevalence rate are:

i. A host may harbour many several species of *Sarcocystis*. For example, pig and cattle may become infected with as many as three species each respectively.

ii. Many definitive hosts are involved in transmission. For example, cattle sarcocystosis is transmitted via felids, canids, and primates. *Sarcocystis* cruzi, the most common species in cattle world-wide, is transmissible via dogs, coyotes, foxes, wolves and raccoons (Dubey *et al*., 1989).

iii. *Sarcocystis* oocysts and sporocysts develop in the lamina propria and are discharged over period of many months.

iv. Sporocysts or oocysts remain viable for many months in the environment. They may be further spread or protected by invertebrates (Markus, 1980).

v. Large number of sporocysts may be shed by the definitive hosts.

vi. There is little or no immunity to re-shedding of sporocysts, therefore, each meal of infected meat can initiate a new round of production of sporocysts (Fayer, 1977).

vii. Oocysts or sporocysts are resistant to freezing, disinfectants and other harsh weather conditions (Cawthorn *et al*., 1984).

viii. Unlike many other species of coccidian, *Sarcocystis* is passed in faeces in the infective form and is not dependent on weather conditions for maturation and infectivity.

In humans, Sarcocystosis manifest in two forms; intestinal and muscular Sarcocystosis (Fayer, 2004). Few large scale population surveys have been conducted for sarcocystosis in humans. Prevalence data for *Sarcocystis* infection primarily reflect case reports and findings of physicians, public health workers and researchers with specific interest (Fayer, 2004). Consequently, many infections go unreported.

Sarcocystosis has been reported to affect a wide range of humans from a 26-day-old infant to a 75 year old man (Lele *et al*., 1986). Intestinal sarcocystosis in humans was found more frequently in Europe than other continents (Dubey *et al*., 1989). Of fecal specimens examined from children in Poland and Germany, 10.4 and 7.3% were found positive, respectively (Fayer, 2004). Of 1,228 apprentices from the Hanoi-Haiphong area of Viet Nam who worked in Central Slovakia in 1987 to 1989, 14 (1.1%) were positive (Straka *et al*., 1991).

After raw beef containing *S. hominis* was prepared as kibbe and fed to seven human volunteers, six excreted sporocysts and two developed diarrhea (Pena *et al*., 2001). After eating raw beef, a patient in Spain with abdominal discomfort and loose stools was diagnosed with *S. hominis* oocysts in his feces (Clavel *et al*., 2001). In Tibet, *Sarcocystis* was detected in 42.9% of beef specimens examined from the marketplace, and *S. hominis* and *S. suihominis* were found in stools from 21.8 and 0 to 7% of 926 persons, respectively (Yu, 1991).

Muscular sarcocystosis has been found in persons living in tropical or subtropical environments. Of approximately 46 cases reported by 1990 (Dubey *et al*., 1989) most were from tropical or subtropical countries in Asia and Southeast Asia. An additional

46 cases, based on histologic findings, include 1 from China; 2 from Malaysians of Indian origin; 2 others of undetermined origin; 4 each from Africa, Europe, and the United States; 5 from Central and South America; 11 from India; and 13 from Southeast Asia (Fayer, 2004). Sarcocysts of *Sarcocystis* has being diagnosed from biopsies of skeletal muscle in humans (Mehrotra *et al*., 1996). An outbreak involving 7 of 15 military personnel in Malaysia is the largest cluster case on record (Arness *et al*., 1999).

A seroepidemiological survey in West Malaysia found that 19.7% of 243 persons had antibodies to *Sarcocystis* (Thomas and Dissanaike, 1978). Titers were highest among the Orang Aslis (aboriginals) followed by Malays, indians, and Chinese, possibly reflecting food habits and environmental sanitation levels (Fayer, 2004). Recent research works has reported systemic sarcocystosis in an acquired immune deficiency syndrome patient (Velasquez *et al*., 2008).

In Nigeria, research on human sarcocystosis is not common. Kudi (1989) reported a 0% prevalence of human intestinal sarcocystosis in Kaduna, plateau and Bauchi states. The report seems to be the only work on human sarcocystosis in Nigeria.

2.6 TRANSMISSION

2.6.1 Transmission in Animals

Intestinal infections of the definitive hosts are acquired by the eating of raw or undercooked meat containing mature cysts with infective bradyzoites (Dubey *et al*., 1989; Fayer, 2004; Smith, 2004). The bradyzoites are liberated from the cysts by enzymatic action in the digestive system. Upon reaching the lamina propria of the small

intestine, the zoites transform into male and female gametes and the microgamete fertilizes the macrogamete, giving rise to the zygote. Sporulated oocysts and liberated sporocysts are later shed in the feces. Dog and man acts as a definitive host.

Ingestion of sporocysts and oocysts in food or water is virtually the only significant mode of transmission to intermediate hosts (Dubey *et al*., 1989). Transplacental infection has rarely been documented in cattle and sheep in nature (Dubey *et al*., 1989). The possibility of transmission via milk colostrums has been postulated, however, evidence of transmission was not provided (Fayer *et al*., 1982). Muscular infections which are rare in humans are acquired when the human acts as an accidental intermediate host by ingesting sporocysts or oocysts in contaminated food or water, or by close contact with contaminated soil. The sporozoites excyst from the ingested sporocysts and begin asexual reproduction, usually in the vascular endothelial cells. These zoites give rise to schizonts, then merozoites. The merozoites encyst in muscular tissue and continue maturation within the cyst. Because humans have no natural predator, they are dead-end hosts for this parasite (Smith, 2004).

2.6.2 Transmission from Animals to Humans

Eating raw or undercooked beef and pork containing mature sarcocysts of *S. hominis* and *S. suihominis* respectively, has resulted in humans acquiring intestinal sarcocystosis (Fayer 2004). Based on histologic examination of intestinal lesions from persons in Thailand having eaten undercooked meat from *Bos indicus* cattle (Bunyaratvej *et al*., 1982) and possibly other animals (unpublished data), there could be other species of *Sarcocystis* from which humans acquire intestinal sarcocystosis. Meat from many reptiles, birds, and species of wild mammals that harbor sarcocysts is eaten in various

parts of the world with unknown consequences. Therefore, there remain many potential but unknown sources of human intestinal Sarcocystosis (Fayer, 2004).

Sarcocystis causing muscular infection has been found in fewer than 100 humans. In such cases, humans harbor the sarcocyst stage and therefore are the intermediate host. Because there is no known predatory or scavenging cycle in nature in which human tissues are eaten regularly by carnivores, humans most probably become infected by eating food or drinking water contaminated with faeces from a predator of nonhuman primates involving unknown species of *Sarcocystis* (Fayer, 2004). Similar conclusions were reached in reviews of human cases in which sarcocysts were found in muscle tissues (Beaver *et al*., 1979; Pathanathan and Kan, 1981).

Locally known predators such as cats, dogs, and pythons (Kan, 1985) could excrete infectious sporocysts that find their way through contaminated food or water, eventually infecting humans (Fayer 2004). In tropical areas where most human cases have been reported and nonhuman primates are present, 79 (21%) of 375 wild-caught monkeys examined, comprising 14 species, had sarcocysts whereas none of 369 laboratory-born monkeys had sarcocysts (Karr and Wong, 1975).

2.7 PATHOLOGY AND PATHOGENESIS OF *SARCOCYSTIS* INFECTION

2.7.1 Pathology of *Sarcocystis* Infection

2.7.1.1 Pathology in the Intermediate Hosts

Not all species of *Sarcocystis* are pathogenic for intermediate hosts. Generally, *Sarcocystis* species transmitted by canids are more pathogenic than those transmitted by other definitive hosts (Dubey *et al*., 1989).The severity of clinical Sarcocystosis is

dependent on the dose ingested. Cows are as susceptible to a specific dose as calves. Also, pregnancy, lactation, poor nutrition, weather, or other stresses may influence the severity of clinical Sarcocystosis. The weight of the host seems not to be relevant to resistance or susceptibility of clinical disease (Dubey *et al*., 1989).

Under experimental conditions, cattle usually do not develop acute sarcocystosis unless 200,000 or more sporocysts have been ingested at a given time. Beginning the fourth week after inoculation (i.e. incubation period usually one month), signs such as anorexia, diarrhoea, emaciation, weakness, muscle twitching, prostration and sometimes death may occur. Pregnant animals may undergo a premature parturition, abortion, or produce stillbirth feotus (Fayer *et al*., 1976). Clinical laboratory findings indicate anaemia, tissue damage and clothing dysfunction in animals with moderate to severe infections (Dubey *et al*., 1989).

Serum bilirubin, lactic dehydrogenase, alanine aminotransferase, sorbitol dehydrogenase and creatinine phosphokinase are elevated for brief periods during the anaemic phase (Frelier and Lewis, 1984; Mahaffey *et al*., 1986) .Blood urea nitrogen becomes elevated approaching terminal sarcocystosis (Dubey *et al*., 1982).

As infection becomes chronic, growth is adversely affected, animals become hyperexcitable, they hypersalivate and they lose hair, especially on the neck, rump and tail tip (Fayer and Dubey, 1986). Some become emaciated while some eventually develop central nervous system (CNS) signs including recumbency opisthotonous, nystagmus, cycling gait and occasionally death (Dubey *et al*., 1989). With few exceptions, clinical signs similar to those seen in *S. cruzi* infected cattle have been seen

in goats (Dubey *et al*., 1981; Heydorn and Unterholzner, 1983), sheep (Dubey, 1988) and pigs (Barrows *et al*., 1982a; Schnieder and Rommel, 1983) infected with high doses of *S. capracanis*, *S. tenella* and *S. miescheriana* respectively. Neural signs are more prominent in sheep and goat than in cattle (Dubey *et al*., 1989).

Grossly, oedema and focal necrosis in gut associated lymph-nodes are the first to be seen (Dubey *et al*., 1989). Haemorrhages later develop on the serous surface of viscera, in cardiac and skeletal muscles and in the sclera of the eyes. Skeletal muscles mottled or striped with pale areas, interspersed with dark haemorrhagic areas are characteristic of acute sarcocystosis (Johnson *et al*., 1975; Dubey *et al*., 1982). Haemorrhages vary from petochiae to ecchymoses several centimeters in fiameter. Following acute infection, body fat becomes scanty and gelatinous. Body cavities contain straw-coloured fluid, and organs become icteric. In chronically affected animals, the most notable lesion is serous atrophy of fat especially pericardial and perirenal fat, with white flecks of mineralization (Dubey *et al*., 1989).

2.7.1.2 Pathology in the Definitive Host

Generally, *Sarcocystis* does not cause illness in the definitive hosts. Tissues infected with numerous species of *Sarcocystis* fed to dogs, cats, coyotes, foxes and raccoons did not result in illness, although sporocysts were being shed. However, a few dogs vomited or became anorexic for a day or two. This was attributed to probably a change in diet from the laboratory chow to raw meat (Dubey *et al*., 1989).

Sarcocysts have been found occasionally in muscles of dogs, domestic east, lions, raccoons, foxes and other carnivores (Kirkpatrick *et al*; 1986; Everitt *et al*., 1987;

Kirkpatrick *et al*; 1987). Of these sarcocysts; the life cycle of only one *Sarcocystis* species in the fox, *Sarcocystis corsaci* is known. It is unusual because the fox acts both as the intermediate and definitive host (Pak, 1979).

Species that cause sarcocystosis in humans include *Sarcocystis hominis*, *Sarcocystis suihominis,* and other unidentified species (Frenkel, 1997; Fayer, 2004). Human acquire intestinal sarcocystosis with species *Sarcocystis hominis* and *Sarcocystis suihominis* after eating raw or undercooked beef and pork respectively containing mature sarcocysts (Frenkel, 1997; Fayer, 2004). Human volunteers in Germany who ate raw beef containing *S. hominis* became infected and shed oocysts in their feces (Rommel and Heydorn, 1972; Aryeetey and Piekarski, 1976). One person became ill. Signs that appeared 3 to 6 hours after eating the beef included nausea, stomach ache, and diarrhea; these were transient and lasted about 36 hours.

Volunteers in China consumed 1,567 to 14,740 sarcocysts of *S. hominis* from experimentally infected buffalo meat (Chen *et al*., 1999). They had abdominal pain, distension, watery diarrhea, and eosinophilia starting 1 week and ending 4 weeks after ingesting the sarcocysts and were spontaneously cured without treatment. Six persons in Thailand who reportedly ate spiced raw beef from zebu developed segmental necrotizing enteritis requiring surgical intervention (Bunyaratvej *et al*., 1982). Histology of intestine samples from these patients revealed sexual stages attributed to *Sarcocystis* and gram-positive bacilli.

Volunteers in Germany who ate raw pork containing *S. suihominis* became infected, shed oocysts, and had dramatic symptoms 6 to 48 hours later, including bloat, nausea,

loss of appetite, stomach ache, vomiting, diarrhea, difficulty in breathing, and rapid pulse (Rommel and Heydorn, 1972, Heydorn, 1977a). Volunteers who ate well-cooked meat from the same pigs remained asymptomatic (Heydorn, 1977a).

In another study involving 17 volunteers at the University of Bonn (Germany), 14 persons ate raw pork from a pig that was experimentally infected with *S. suihominis* and killed 175 days later (Kimmig *et al*., 1979). During the first 2 days after the volunteers had eaten the infected meat, they presented with the same symptoms as volunteers in the earlier study. Symptoms appeared to be related to the quantity of meat consumed, but individual reactions varied considerably (Fayer, 2004).

Muscular sarcocystosis is due to group of *Sarcocystis* species forming sarcocysts in the skeletal muscles of human beings. They were mostly incidental findings (Jeffery, 1974). Judging from the published reports, sarcocysts in humans are rare; only 46 documented cases were reported as of 1990 (Dubey *et al*., 1989). Four cases of *Sarcocystis* infection from biopsies of the skeletal muscle were also reported in 1996 (Mehrotra *et al*., 1996). Symptoms of muscular sarcocystosis in man include fever, musculoskeletal pain, rash, cardiomyopathy, bronchospasm and subcutaneous swelling. Chronic myositis, and eosinophilia was also reported in patients with muscular sarcocystosis (Van den Enden *et al*., 1995). Recent studies have shown sarcocystosis as an opportunistic infection in HIV-infected patients (Velasquez *et al*., 2008).

2.7.2 Pathogenesis of *Sarcocystis* Infection

Schizonts cause necrosis of cells and tissues depending on the species of *Sarcocystis*, location and multiplication potential (Dubey *et al*., 1982; Dubey *et al*., 1989). However,

localized tissue necrosis does not appear extensive enough to cause the severe illness or death seen in large animals (cattle, sheep and goats and pigs). The perivascular mononuclear cell infiltration seen in experimental infection seen around *Sarcocystis cruzi* suggests a host reaction to antigens, liberated from sporozoites or immature schizonts or the expression of parasite antigens by host cells (Dubey *et al*., 1989).

An intense inflammatory reaction is usually observed about the time when the second-generation schizonts mature and rupture. The myositis, during the penetration of myocytes by merozoites, may be related to products liberated from merozoites or myocytes (Dubey *et al*., 1982). The intense mononuclear cell infiltrations in the kidney, liver, lungs and other organs are probably stimulated by similar parasite antigens (Dubey *et al*., 1982). Ascites and edema in tissues are probably related to hypoproteinemia and vasculitis (Dubey *et al*., 1982; Smith *et al*., 1987).

Fever is probably related to the release or pyrogens from mature rupturing schizonts directly on the hypothalamus or indirectly by stimulating the release of prostaglandins (Dubey *et al*., 1989). The peaks of fever coincide with maturation of schizonts and release of merozoites into the bloodstream (Fayer and Dubey, 1986).

Anaemia is the most evident clinical finding of acute sarcocystosis in cattle, pigs, sheep and goats but the mechanism is unknown (Prasse and Fayer, 1981; Mahaffey *et al*., 1986; Gajadhar *et al*, 1987). Although the anaemia is regenerative, few or no reticulocytes are found. The anaemia is normocytic, normochromic and primarily haemolytic (Dubey *et al*., 1989). Many red blood cells are removed from circulation and sequestered in the spleen via immunologic mechanisms. It is also possible that

some unknown toxic factors or metabolites released from schizonts or infected host cells contribute further to the anaemia (Dubey *et al*., 1989).

Abortion and fetal death can result when animals become infected with pathogenic species of *Sarcocystis* during pregnancy. Most cattle, pigs, sheep and goats that developed clinical sarcocystosis from experimental infections induced in mid to late gestation aborted (Erber *et al*., 1978; Leek and Fayer, 1978; Dubey, 1981) whereas most infected animals without signs of infection carried their fetus to term (Dubey, 1981; Munday, 1981).

Research has shown that in natural infections, parasites, lesions, or both are found in the fetuses (Dubey and Bergeron, 1982; Hong *et al*., 1982; Jerrett *et al*., 1984). In experimentally infected host species lesions were not found in fetal tissues with the exception of one lamb (Dubey *et al*., 1989). *Sarcocystis* species were found in only 2 of 18 bovine fetuses (Barnett *et al*., 1977) and 4 schizonts were found in fetal membrane of 3 sheep (Leek and Fayer, 1978). In contrast, parasite and lesions were found in maternal placentomes of cattle, sheep and goats (Barnett *et al*., 1977). Thus, results from these experimentally infected animals indicate that although *Sarcocystis* is present in the maternal placenta, it rarely infects the fetus or fetal membranes. Unlike experimental infections, parasites as well as lesions were also found in the placentas in several natural infections (Corner *et al*., 1963). Furthermore, some naturally infected cows have no clinical signs of infection, although organisms were detected in the fetus or placenta (Dubey and Bergeron, 1982).

In naturally infected bovine fetuses, parasites were found in virtually all organs but were most often found in the brain. Immature and mature schizonts and free merozoites were found, usually within endothelial cells of capillaries, but occasionally free in the lumen of a vessel or in neural tissus (Kunde *et al*., 1980; Hong *et al*., 1982; Jerrett *et al*., 1984).

Brain lesions include nonsuppurative encephalitis or meningitis, with small foci of gial cells surrounding a central necrotic area throughout the gray and white mater of the cerebrum, cerebellum or brainstem, with some perivascular monomuclear cell infiltration and occasional microthrombi in vessels in reaction foci. Lesions in other organs include nonsuppurative myocarditis, pneumonitis, hepatitis and renal glomerulus accompanied by focal necrosis and haemorrhaage (Dubey *et al*., 1989).

Eosinophilic myositis (EM) is a specific inflammatory condition of striated muscles principally due to accumulation of eosinophils. It has been found mainly in cattle (Rimaila-Parnanen and Nikander, 1980; Jensen *et al*., 1986; Gajadhar *et al*., 1987), occasionally in sheep (Jensen *et al*., 1986) and rarely in pigs and horses (Dubey *et al*., 1989). The condition is generally detected at the time the surface of the carcass is inspected or when it is cut into prime cuts or into quarters (Imes and Migaki, 1967).

The etiology of EM remains uncertain. Because of sarcocysts are found in the same location as EM, *Sarcocystis* has been traditionally considered to be the cause of EM (Dubey *et al*., 1989). The *Sarcocystis*-induced etiology of EM was supported by the

finding of *Sarcocystis*-specific-IgE antibody in the sera of eight cases of eosionphilic myocarditis but not in two *Sarcocystis*-free calves (Granstorm *et al*., 1989).

Little is known of the pathogenesis of chronic sarcocystosis. Although sarcocysts in muscles or in the central nervous system are well adapted, usually without any host reactions, some sarcocysts probably rupture from time to time releasing toxic products (Dubey *et al*., 1989). Sarcotoxin which is an aqueous extract of bradyzoites is toxic when inoculated into rabbits (Hiepe *et al*., 1981; Tadros and Laarman, 1982). However, it is not clear how or if sarcotoxin might be released from intact sarcocysts and if released, what role it plays in chronic sarcocystosis. It's been postulated that substances released from *Sarcocystis* might stimulate tumour necrosis factor (TNF) (Fayer and Prasse, 1981). TNF is known to be associated with wasting disease.

2.8 IMMUNITY

Sarcocystis species are immunogenic in intermediate host. Available information on cellular and humoral immune responses is only from responses directed against antigen derived from bradyzoites (Dubey *et al*., 1989).

2.8.1 Humoral Response

Cattle and pigs inoculated with *Sarcocystis* sporocysts, developed immunoglobulin G (IgG) antibodies starting three to five weeks after inoculation (Fayer and Lunde, 1977; 0'Donoghue and Weyreter, 1983; Gasbarre *et al*., 1984; 0'Donoghue and Weyreter, 1984). These antibodies were detected by indirect haemagglutination (IHA), enzyme

linked immunosorbent assay (ELISA) or dot ELISA, indirect fluorescent antibody (IFA) or complement fixation tests (Cerva and Cerna, 1982; Gasbarrre *et al*., 1984; Tenter, 1988).

Immunoglobulin M (IgM) antibodies appear earlier than immunoglobulin G (IgG) but IgM are short lived and disappear by the time the sarcocyst matures (Dubey *et al*., 1989). Immunoglobulin G (IgG) antibody concentration in serum peaked during the early period of sarcocysts formation. This persisted at a relatively high concentration during the chronic phase of the infection. The onset and persistence of *Sarcocystis* antibodies varied with the species of the host, species of the parasite and source of antigen. Although *Sarcocystis* species share antigens within themselves, the antibody titre were higher using antigen from homologous species (Gasbarre *et al*., 1984).

2.8.2 Cellular Response

Immune cells are mobilized during *Sarcocystis* infection as might be expected of an intracellular parasite. Immune cells such as lymphocytes and macrophages are mobilized and infiltrate visceral and muscular tissue (Dubey *et al*., 1982). This mononuclear cell infiltration begins during the third week of infection. It may last for several months long after the parasite is no longer demonstrable in visceral tissues (Dubey *et al*., 1989).

Also, lymphocytes from peripheral circulation show a blastogenic response when stimulated with antigen-specific *Sarcocystis* species. Whether these cellular events participate in the recovery of the host from the disease has not been well established (Dubey *et al*., 1989). The passive transfer of resistance via cells or antibodies has not

been reported. In certain animals, sarcocystosis may depress their immune status (Gasbarre at al., 1984). The cellular response seen in immune animals that survive lethal challenge indicates cell-mediated immunity against *Sarcocystis* (Dubey *et al*., 1989). Cytotoxic antibodies or metabolites are known to destroy second generation extracellular merozoites (Speer and Dubey, 1981).

2.8.3 Protective Immunity and Vaccination

Cattle and pigs inoculated orally with a dose of sporocysts that resulted in subclinical infection were protected against a challenge dose that would have been lethal. This is protective immunity persisted for 80 days but not 120 days in pigs (Weyreter *et al*., 1984) and at least 252 days in cattle (Fayer and Dubey, 1984). The size of the immunizing dose is important.

Pigs dosed with 100 or more sporocysts of *S. miescheriana* were protected; those dosed with 10 sporocysts were not protected. The degree of protection was better with 1000 sporocysts than with 100 sporocysts (Zielasko *et al*., 1981). The protective immunity is induced only with homologous *Sarcocystis* species (Fayer and Dubey, 1984).

The stage of the parastite responsible for protective immunity is not known but it is likely to be either the sporozoite of first generation schizonts (Dubey *et al*., 1989). Vaccination of pigs with live, killed or fractions of *S. miescheriana* bradyzoites induced antibody production but provided no protection (O'Donoghue *et al*., 1985). The administration of colostrums from infected animals has no protective response in cattle and pig. These animals remain susceptible to clinical sarcocystosis from birth and throughout life, despite repeated natural infection (Fayer *et al*., 1976; Munday, 1979).

The persistence of live sarcocysts is probably not essential for the maintenance of protective immunity (Dubey, 1983a).

2.9 SARCOCYSTOSIS IN INTERMEDIATE HOSTS

2.9.1 Sarcocystosis in Cattle

There are three species of *Sarcocystis* in cattle. These are *Sarcocystis cruzi*, *Sarcocystis hirsuta* and *Sarcocystis hominis* with canids, felids and primates respectively as definitive hosts.

2.9.1.1 Sarcocystis cruzi (Hasselmann, 1926)

Synonym*: S. bovicanis*

The distribution is worldwide. Intermediate host include cattle (*Bos Taurus*) and bison (*Bison bison*) while the definitive hosts include dog (*Canis familaris*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), raccoon (Procyon lotor) and wolf (*Canis lupus*) (Dubey *et al*., 1989).

Sarcocysts are microscopic and are usually less than 500 µm in length. They are formed in virtually in all striated muscles, purkinje fibers of the heart and in the central nervous system (Dubey, 1982a). The sarcocyst wall is thin (less than 1 µm) and its surface is covered with long narrow ribbon-like protrusions (Pacheco *et al*., 1978; Dubey, 1982a). The protrusions are up to 3.5 µm long and 0.3 µm wide at the base (Fujino *et al*., 1982).

The sarcocyst wall has numerous small invaginations when viewed from the surface (Fujino *et al*., 1982). Following ingestion of sporulated *Sarcocystis* oocyst by cattle, two or more generations of schizonts occur in the vascular endothelia cells,

metrocytes are found in the striated muscle about one month after infection and fully formed cysts are evident within $1^{1/2}$ to 3 months (Fayer and Johnson, 1974).

Sarcocystis cruzi is the most pathogenic species seen in cattle (Fayer *et al*., 1982; Nakamura *et al*., 1982). It can cause anorexia, pyrexia, anaemia, loss of weight, hair loss, weakness, muscle twitching, prostration, abortion, reduced milk yield, hyper salivation, neurologic signs and death depending on the isolate and the number of sporocysts ingested (Dubey *et al*., 1989). Cattle fed 200,000 sporocysts become clinically ill, some die of acute Sarcocystosis while survivors do not grow to their full potential (Fayer and Dubey, 1986).

Cases of natural clinical Sarcocystosis in cattle due to *S. cruzi* have been reported in Canada (Meads, 1976), England (Lainson, 1972; Clegg *et al*., 1978), Ireland (Collery and Weavers, 1981), Australia (Carrigan, 1986), Norway (Landsverk, 1979) and U.S.A (Giles *et al*., 1980; Forcvt *et al*., 1986). Abortion and placentitis has been reported in pregnant cows naturally infected with *Sarcocystis* (Dubey and Bergeron, 1982).

Sporadic cases of bovine abortion and neonatal mortality have being reported in New Zealand (Vickers and Brooks, 1983), Australia (Carrigan, 1986), Canada (Corner *et al*., 1963) and United States (Dubey and Bergeron, 1982) in which fetal lesion and or protozoa were found. Fetal encephalitis, myocarditis and hepatitis were often associated with the outbreaks (Dubey *et al*., 1989).

Fayer and Dubey (1984) in an experiment showed it is possible to establish a protective immunity. Results from the experiment showed that calves infected with 50,000 or

100,000 *S. cruzi* sporocysts were protected from illness and death that would have resulted from challenge infections with large numbers of S. cruzi sporocysts 70 to 252 days later. Second generation schizonts were reduced in calves treated prophylactically with amprolium from 21 to 35 days after infection (Fayer and Dubey, 1984).

2.9.1.2 Sarcocystis hirusta (Moule, 1888)

Synonym- *Sarcocystis bovifelis*

The definitive host for this specie is the cat (*Felis catus*) and its distribution is probably worldwide (Dubey *et al*., 1989).

In experimentally infected cattle, the sarcocyst are as large as 800 µm long and 80 µm wide (Dubey, 1982b) and as long as 8 mm and 1 mm wide in naturally infected cattle (Bottner *et al*., 1987a). The sarcocyst wall may be as thick as 7 µm and it appears radially straited or hirsute (Dubey, 1982b). The villous protrusions contain numerous microtubules and are up to 70 µm long and 1.5 µm wide. First generation schizonts have been found only in small arteries of the intestine and mesentery and the second generation have been found only in capillaries of the heart and skeletal muscles (Dubey *et al*., 1989).

Sarcocystis hirsuta is mildly pathogenic (Dubey, 1983a). Dubey (1983a) reported in an experiment that calves fed 100,000 or more sporocysts became febrile, had diarrhoea and were mildly anaemic, none died. At necropsy, haemorrhagic lesions were not seen.

2.9.1.3 Sarcocystis hominis (Dubey, 1976)

Synonym- *Sarcocystis bovihominis*

The definitive host is man (homosapiens), rhesus monkey (*Macaca mulatta*) baboon (*Papio cynocephalus*) and possibly chimpanzee (*Pantro glodyte*s) (Dubey *et al*., 1989). The sarcocyst wall is up to 6 µm thick and appears radially striated because of numerous villar protrusions (Mehlhorn *et al*., 1975a). Bradyziotes are 7 to 9 µm long and are arranged in packets (Mehlhorn *et al*., 1975b). *Sarcocystis hominis* is mildly pathogenic (Dubey, *et al*., 1988).

Sarcocystis cruzi is the most prevalent species and it is easily recognized in histologic sections, whereas *S. hirsuta* is difficult to distinguish from *S. hominis* microscopically. However the sarcocyst wall of *S. hirsuta* and *S. hominis* can be differentiated ultra structurally (Dubey, *et al*., 1989).

2.9.2 Sarcocystosis in Pigs (*Sus scrofa*)

There are three reported species in pigs: *S. miescheriana, S. suihominis* and *S. porcifelis*.

2.9.2.1 Sarcocystis miescheriana (Kuhn, 1865) Labbe, 1899

Synonym – *S. suicanis* (Erber, 1977).

This is the type species of the genus. The distribution is probably worldwide. Definitive hosts include dog (*Canis familiaris*), raccoon (*Procyon lotor*), wolf (*Canis lupus*), red fox (*Vulpes vulpes*) and jackal (*Canis aureus*) (Dubey *et al*., 1989).

Sarcocysts are as large as 1500µm long and 200µm wide and are found in skeletal and cardiac muscles (Erber, 1977). The sarcocyst wall is 3 to 6 µm thick and appears radially striated. The villar protrusions on the sarcocyst wall are up to 5 µm long and 1.3µm wide (Erber, 1977; Mehlhorn and Heydorn, 1978), unusually large bradyzoites, up to 20µm long, are present within mature sarcocysts.

There are two known generations of schizogony which develop much more rapidly than those of cattle and sheep. First- and second-generation schizonts mature within 13 days post inoculation and immature sarcocysts are found as early as 27 days post inoculation (Heydorn *et al*., 1981a; Barrow *et al*., 1982b).

Sarcocystis miescheriana can cause weight loss and purpura of the skin, especially of the ears and buttocks, dyspnea, muscle tremors, abortion, and death, depending on the number of sporocysts ingested (Zielasko *et al*., 1981; Barrow *et al*., 1982a). Ingestion of less than 1 million sporocysts generally results in subclinical infection. Stress of pregnancy may modify the severity of infection. Of 5 pregnant sows experimentally infected with 50,000 sporocysts each, all became ill: 2 aborted 12 to 14 days post inoculation, 1 died, and 2 became moribund and were euthanatized (Erber *et al*., 1978). A similar number of sporocysts given to finishing pigs caused no clinical sign but resulted in weight gains that were 11 to 27% less than uninfected controls (Boch *et al*., 1980). Administration of 25,000 or 15,000 sporocysts neither reduced weight gains nor produced clinical signs.

Pigs immunized once with 1000 to 50,000 *S. miescheriana* sporocysts become refractive to lethal challenge with large numbers of *S. miescheriana* sporocysts (Weber

et al., 1983; Schneider *et al*., 1984; Rommel and Schneider, 1985). Pigs given multiple low doses of sporocysts (trickle infections) also developed protection (Schnieder and Rommel, 1983). The protective effect resulting from administration of *S. miescheriana* sporocysts was not protective against a challenge infection with *S. suihominis* sporocysts (Erber and Giesel, 1979).

2.9.2.2 Sarcocystis suihominis (Heydorn, 1977a)

The definitive host for this specie is Man (Homo sapiens) and nonhuman primates (*Macaca mulatta*, *Macaca irus*, *Pan troglodytes*, and *Papio cynocephalus*) (Dubey *et al*., 1989).

Sarcocysts are up to 1500 µm long (Erber, 1977). The sarcocyst wall is 4 to 9 µm thick (Erber, 1977) and appears hirsute, with villar protrusions up to 13 µm long (Mehlhorn and Heydorn, 1977). The bradyzoites are approximately 15 µm long. The two generations of schizogony develop at about the same time as and are structurally similar to those of *S. miescheriana* (Heydorn and Ipezynski, 1978; Heydorn and Mehlhorn, 1978). Immature sarcocysts were first seen 27 days post inoculation, and bradyzoites were seen 56 days post inoculation (Mehlhorn and Heydorn, 1977).

S. suihominis is pathogenic for pigs. Pigs fed 50,000 or more sporocysts became ill, and half of those fed 1 million sporocysts died (Heydorn, 1977b). Clinical signs in pigs were similar to those in pigs infected with *S. miescheriana* (Heydorn, 1977b).

2.9.2.3 Sarcocystis porcifelis (Dubey, 1976)

The validity of this species is uncertain because it has been reported only once and therefore lacks confirmation. This specie was named (Dubey, 1976) for the parasite described from the U.SS.R. (Golubkov *et al*., 1974). *Sarcocystis-infected* oesophagi from pigs were fed to four cats. The cats passed sporulated oocysts 5 to 10 days after ingesting infected swine tissues. The sporocysts were 13.5 x 7.6 µm and each contained four sporozoites measuring 9.5 x 3.8 µm.

The sporocysts from cat feces were fed to eight littermate pigs. All pigs became ill and one died 89 days post inoculation. Sarcocysts were found in skeletal muscles and the heart. Other pigs were killed 3.5 months post inoculation, and sarcocysts were found in them (Golubkov *et al*., 1974). The structure of the sarcocysts was not described.

2.10 DIAGNOSIS

Diagnosis of acute sarcocystosis is difficult to diagnose because the disease is generalised in nature with no specific signs. It has no specific signs and finding of parasites in tissues of acutely infected intermediate host is not predictable. Although, there was a recent report of acute fatal sarcocystosis in a pig breeding herd (Caspari *et al*., 2011). Diagnosis of sarcocystosis is therefore base on the elimination of other causative agents, a good epidemiologic evaluation of the herd and its relationship to other animals (especially dogs) and clinical signs.

A presumptive diagnosis of the disease can be made by:

i. Clinical findings: These include anaemia, anorexia, pyrexia, lymphadenothy, excessive salivation, placentitis, abortion in third trimester and loss of body hair especially at the tip of the tail.

ii. Clinical pathology: Findings include elevation of serum biliruin, lactic acid dehydrogenase (LDH), creatinine phosphokinase (CPK), blood urea nitrogen (BUN) with lower packed cell volume (Dubey *et al*., 1989).

iii. Serology: A number of serological tests have been developed for determination of antibody to *Sarcocystis* infection. These tests include: Agar-gel diffusion tests, haemagglutination inhibition test (HI), Indirect fluorescent antibody test (IFA) and Enzyme Linked Immunosorbent Assay (ELISA) (Dubey *et al*., 1989). At present, there is lack of standardization of these tests because antigens are obtained from sarcocysts in muscles of experiementally infected animals (Dubey *et al*., 1989). Antigens consist of lysate of bradyzoites (Lunde and Fayer, 1977) and variations occur in preparation methods. This results in antigens varying greatly from one batch to another.

iv. Biopsies: Finding of vascular schizonts in biopsies of muscles and lumphnodes, histology and post-mortem is another way of diagnosis.

The diagnosis of *Sarcocystis* induced abortion presents additional problems because the parasite is not consistently found in fetal tissues. Sarcocystosis produces gliosis and placental necrosis, therefore, brain and placenta should be examined. Within the placental cotyledons, schizonts have been seen in both the lamina propria and submucosa. The chances of diagnosis are improved if numerous fetal tissues are examined (Dubey *et al*., 1989).

2.11 TREATMENT

Several drugs routinely used as anticoccidials in poultry have been found experimentally, to be effective against sarcocystosis in cattle and pigs when administered for a month starting at or before inoculation with *Sarcocystis* species (Fayer and Johnson, 1975; Heydorn *et al*., 1981b).

Amprolium (100 mg/kg weight) administered from 0 to 30 days post inoculation prevented acute disease or death in cattle infected with *S. cruzi* (Dubey *et al*., 1989). Therapeutic treatment of cattle and pig has been ineffective (Dubey *et al*., 1989; Caspari, 2011). Most anticoccidials affect *Sarcocystis* schizonts (Dubey *et al*., 1989). Of particular interest is the activity of sulfaquinoxalin plus pyrimethamine against immature and mature sarcocysts because no other drug is known to be effective against sarcocysts (Rommel *et al*., 1981; Rommel, 1983).

There is no known prophylaxis or therapeutic treatment for intestinal sarcocystosis in humans. Infections are self-limiting, of short duration, and often asymptomatic. The efficacy of co-trimoxazole (Croft, 1994) or furazolidone (Mensa *et al*., 1999) remains to be demonstrated.

For six persons in Thailand with segmental necrotizing enteritis associated with sexual stages of *Sarcocystis* and gram-positive bacilli, surgical resection of the small intestine was followed by antibiotic treatment (Bunyaratvej, *et al*., 1982). This extremely aggressive course of treatment has not been applied in other cases. Neither prophylaxis nor therapeutic treatment for myositis, vasculitis, or related lesions in humans has been approved. The usefulness of pyrimethamine or other drugs known to be effective

against related protozoa such as *Toxoplasma* is also unknown. Because of the paucity of reported treatment cases and the lack of any controlled studies, there is no basis for evaluation, and therefore no course of treatment can be recommended as superior to any other at this time (Fayer, 2004).

2.12 CONTROL

There is no vaccine to protect livestock against clinical sarcocystosis. However, experimental studies indicate that cattle, sheep, goats and pigs can be immunized by low dosages of live sporocysts. Thus, there is hope of developing a vaccine for sarcocystosis in the future.

For the present, prevention is the only practical method of control. Shedding of *Sarcocystis* in faeces of definitive host is the key factor to the spread of *Sarcocystis* infection. Therefore to interrupt this cycle, the following methods are adopted (Dubey *et al*., 1989).

i. Carnivores should be excluded from animal houses and from feed, water, and bedding for livestock.

ii. Uncooked meat or offal should never be fed to carnivores.

iii. Dead livestock should be buried or incinerated. This is particularly important to prevent leaving dead animals in the field where carnivores can feed on them.

iv. The prophylactic use of anticoccidials may be another practical method of controlling sarcocystosis in livestock.

For humans, intestinal sarcocystosis can be prevented by thoroughly cooking or freezing meat to kill bradyzoites in the sarcocysts. Sarcocysts in pig muscles were rendered noninfectious for puppies after cooking meat at 60^{o}C, 70^{o}C, and 100°C for 20,

15, and 5 minutes, respectively (Saleque *et al*., 1990). Freezing at -4 and -20°C for 48 and 24 hours respectively, also rendered bradyzoites in pork noninfectious (Saleque *et al*., 1990).

To prevent humans from becoming infected as intermediate hosts, ingestion of sporocysts must be prevented. The most likely source of sporocysts is water contaminated with feces from a carnivore or omnivore or foods washed or irrigated with contaminated water. Where contaminated drinking water is suspected, boiling is the best method to ensure disinfection. Where contaminated foods are suspected they should be thoroughly washed or cooked before being eaten (Fayer, 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The study area covered Zaria town of Kaduna state. Zaria is one of the major cities in Kaduna state in North west geo-political zone of Nigeria. Zaria is located within the northern sudan savanna zone on a plateau at a height of 2,200 feet above sea level. It lies between latitude 7^{o} and 11^{o} North, and longitude 7^{o} 44′ East. It is characterized by cold humid wet season and cold or hot dry seasons. The average rainfall ranges from 1000 to 1250 mm and the average temperature ranges from 17^{o} C to 33^{o} C. The occupation of Zaria inhabitants is primarily agriculture. Staples are guinea corn and millet; cash crops include cotton, groundnut and tobacco (BBC news, 2002; Zaria, 2010). The major abattoir is located in Zango but there are also slaughter slabs in Sabon gari and Agoro. Pig slaughter areas are located in Basawa, Samaru and Sabon gari.

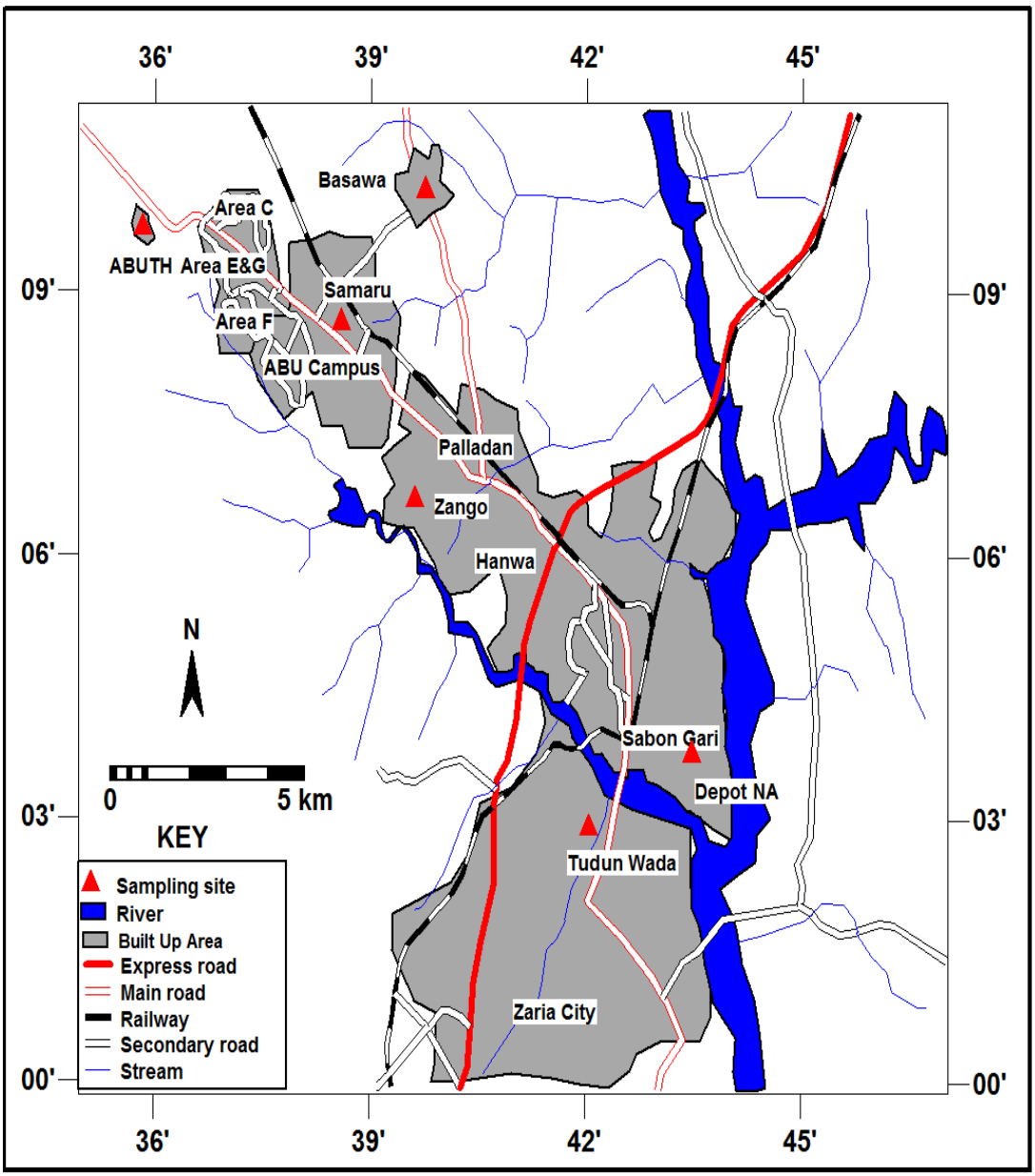


FIG. 3.1 : STUDY AREA SHOWING SAMPLING SITES Source: Modified from Google Earth, 2011

3.2 SAMPLE SIZE DETERMINATION

The sample size was calculated using the formula described by Thrusfield (1997).

$$n = \frac{1.96^2 \, P_{exp} \, (1 - P_{exp})}{d^2}$$

Where:

n = sample size

d = desired absolute precision of 5% (0.05)

P_{exp} = expected prevalence (Human = 0%, dog = 1.8%, cattle = 0%; Kudi (1989))

For human, 50% prevalence was assumed. Therefore, $n = \frac{1.96^2 \times 0.50 \times 0.50}{0.05^2}$

$= 384.16$

A total of 390 samples were collected from human.

For dogs, $n = \frac{1.96^2 \times 0.018 \times 0.982}{0.05^2}$

= 27.16; a total of 200 samples were collected from dogs.

For cattle, 50% prevalence was assumed. Therefore, $n = \frac{1.96^2 \times 0.50 \times 0.50}{0.05^2}$

$= 384.16$

A total of 400 tissues samples were collected from 200 cattle.

Two hundered tissue samples were collected from 100 pigs.The pig population and the number of slaughtered pigs per week in Zaria were considered before arriving at this figure.

In addition to the tissue samples collected from cattle and pig, faecal samples were also collected; 200 and 100 faecal samples respectively.

A total of 1,490 samples were collected for processing of which 890 and 600 were faecal and tissue samples respectively.

3.3 SAMPLING PROCEDURE

A cross sectional study was performed to determine the prevalence of *Sarcocystis* infection in dogs, humans, cattle and pigs in Zaria. Two hundred faecal samples from dogs were collected from the Ahmadu Bello University Veterinary Teaching Hospital (ABUVTH) and dog slaughter areas. Samples were collected three days in a week and at an interval of two weeks and all samples obtained from ABUVTH and slaughter houses in a sampling day were included for processing.

A total of 390 faecal samples were collected from patients in hospitals and volunteers in the study area. Hospitals in the study area were approached for their consent for the inclusion of their hospitals in the study. Out of the 6 hospitals visited, 4 gave their permission and co-operation. The hospitals that co-operated included Jamaa hospital, University Health Services main campus Samaru, MIBA memorial hospital Sabon gari, Ahmadu Bello University Teaching Hospital (ABUTH). Samples were collected from patients using a systematic random sampling method. The first patient and patients at

an interval of 3 counts were selected for inclusion in the study. Volunteers included hospital staff, family members and inhabitants of Zaria who were interested to be screened for *Sarcocystis* and other helminth parasites. Volunteers were randomly selected for inclusion in the study. Sampling was done thrice in a week at an interval of 2 weeks for a period of 20 weeks.

A total of 400 tissue samples (oesophagus and diaphragm) and 200 faecal samples were collected from 200 slaughtered cattle from Zango abattoir and Agoro slaughter area in Tudun Wada using a systematic random sampling method. Samples were collected from the first animal and at an interval of 2 animals. Sampling was done thrice in a week at an interval of 2 weeks for a period of 20 weeks.

A total of 200 tissue samples (oesophagus and diaphragm) and 100 faecal samples were collected from 100 slaughtered pigs based on the location of the slaughter houses (Samaru and Sabon gari) and the number of pigs slaughtered per location. Convenience sampling method was employed in this regard. Sampling was done thrice in a week and at an interval of 2 weeks for a period of 20 weeks.

3.4 SAMPLE COLLECTION

3.4.1 Faecal Samples

3.4.1.1 Dogs

Samples were collected directly from the rectum using the index finger into labeled clean polyethylene bags from dogs brought to the hospital. For slaughtered dogs, samples were collected by expressing the content of the rectum into labeled clean

polyethylene bags. Age, sex and breed of the dogs were recorded as stated in the hospital records while those of slaughtered dogs were determined.

3.4.1.2 Cattle and Pigs

Faecal samples were also obtained from the slaughtered cattle and pigs. Samples were collected by expressing the content of the rectum into labeled clean polyethylene bags. Information on the age, sex and breed were noted. The age of the animals were estimated using the dentition (except for pigs which could not be traced), sex was determined by examining the sex organs and breed by observing the skin colour.

3.4.1.3 Humans

Ethical clearance was obtained from the various hospitals before the collection of faecal sample commenced. Samples were collected from randomly selected patients and patients sent for laboratory examination in the hospitals. Clean sample bottles were given to patients who submitted their samples to the laboratory units of the hospitals. Age, sex and location of the patients were recorded as stated in the hospital records.

All samples were transported to the laboratory in a cool box and processed immediately or kept at 4^{o}C (for not more than 3 days) until processed.

3.4.2 Tissue Samples

Samples of oesophagus (about 15 cm) and diaphragm muscle (25 g) were collected from slaughtered cattle and pigs in Zaria. Two hundred each and 100 each of oesophagus and diaphragm muscle were obtained from slaughtered cattle and pigs respectively. The samples were placed each into a labeled polyethylene bag and

transported to the laboratory in a cool box containing ice pack. The samples were processed immediately or kept at -20^{o}C and processed within 7 days of collection.

3.5 EXAMINATION OF SAMPLES

3.5.1 Analysis of Faecal Samples

In the present study, the sucrose floatation technique for the detection of worm ova and protozoan sporocysts and oocysts was used in the detection of *Sarcocystis* sporocysts/oocyst (Dubey, 1989; Kudi, 1989). Each sample (5 g) was mixed with water (1 ml) and emulsified into a thick paste in a beaker using an applicator stick. The paste was then suspended in 10 volumes of Sheather's sugar solution specific gravity of 1.31 (150% w/v of sugar in distilled water, with 4.5 g phenol added as preservative). The suspension was filtered through cotton gauge to remove large particles while the filtrate was centrifuged at 400 g for 10 minutes. With a Pasteur pipette and a rubber bulb, two drops were taken from the top of the solution onto a clean glass slide and then covered with a cover slip. The slide was examined at x 400 magnification using an optical microscope. Patients were said to be positive if the faecal samples contained oocyst/sporocysts of *Sarcocystis*

3.5.1.1 Identification of Sporocysts/oocyst of Sarcocystis

The sporocysts were identified as spherical ova containing granular sporocyst residium and 4 sporozoites which are banana shaped. The oocyst contains 2 sporocysts.

3.5.2 Analysis of Tissue Samples

Tissues samples collected were grossly examined in the abattoir for the presence of macroscopic cysts. The method used for the detection of cysts in the laboratory was by

tissue digestion using artificial gastric juice (1% HCl-Pepsin solution) as described by Dubey *et al* (1989) and modified by Hamidinejat *et al* (2010). Twelve point five grams each of the tissue were minced, placed into a stomacher bag containing 5 mL of normal saline and homogenized using a stomacher. The homogenate were suspendended in 20 mL of the digestion medium and digested for 1 hour at 37^{o}C in a water bath. The digest was sieved and the filtrate centrifuged at 1500 g for 5 minutes. The supernatant was gently decanted and the sediment resuspended in a small volume of saline. Two drops of the sediment were transferred onto a glass slide, smeared and stained with giemsa stain and examined using optical microscope at x 400 magnification for the presence of bradyzoites (Plates II and III). All tissues samples positive by tissue digestion were submitted for histology. Also, photomicrographs of typical sarcocyst or bradyzoites were taken using a digital camera and a digital microscope camera (Tucsen®; 640 x 480 pixel resolutions). An animal was said to be positive for *Sarcocystis* infection if either the oesophagus or diaphragm contained sarcocysts/bradyzoites in the tissue processed by tissue digestion and/or histology.

3.5.2.1 Giemsa Staining of Tissue Sediment

The tissue sediment were smeared on a clean glass slide, allowed to dry in air and fixed in methanol for 3 minutes. The slides were transferred to a staining jar filled with 1:10 dilution of Giemsa stain (10 mL stock giemsa stain plus 90 mL of buffer solution pH 7.0). The slides were stained for 20 minutes; the stain was poured off and rinsed several times with distilled water. The slides were left to dry on the staining rack and examined at X 1000 magnification with oil immersion using an optical microscope (Ochei and Kolhatkar, 2000).

3.5.2.2 Identification of Bradyzoites at Tissue Digestion

Bradyzoites were identified as banana shaped structures which stained bluish with pinkish/light nuclei while the muscular tissue stained pinkish/purplish.

3.5.2.3 Processing and Staining of Tissue Sections for Histology

All tissues samples positive by tissue digestion were submitted for histology. The tissues were preserved in 10% formal saline prior to sectioning. The tissues were processed in Shandon Southern® Automatic tissue processor and sections of 5 µ thick were made using Leitz Wetzlar® base sledge microtome. Tissue sections were stained using Haematoxylin and Eosin (H&E) stain as described by Bancroft and Cook (1984). The sections were placed in water and in haematoxylin for 10 minutes and washed in tap water. The sections were placed in 1% acid alcohol for a minute and washed in tap water. "The blue" sections were placed in Scott's water and washed in tap water. The sections were then placed in eosin for 2 minutes and washed in tap water. The sections were dehydrated through grades of alcohol (70%, 90% and 100%). The sections were cleared with xylene and mounted using DPX mountant and examined at X 400 magnification using an optical microscope.

3.5.2.4 Identification of Sarcocyst in Histological Sections

Sarcocysts were identified as abnormal mass embedded in myocytes containing small rounded or banana shaped bradyzoites enclosed by a well defined membrane. The cysts stained bluish while the myocytes stained pinkish.

3.6 MEASUREMENT OF OOCYST/SPOROCYST AND SARCOCYSTS

Micrometry was done in the Helminthology Laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Oocyst/sporocysts present in positive faecal samples and sarcocysts were measured with an ocular micrometer using a calibrated microscope. The length and width of these and thickness of the sarcocyst wall was recorded and compared with those described previously by Dubey *et al* (1989). A calibrated eye piece was inserted into the micrometer; the slide containing the oocyst/sarcocyst was placed on the stage of the micrometer and focused while viewing from the eye piece. The oocyst/sarcocyst was moved towards the calibration with the help of the adjustment knob of the microscope until the calibration is placed side by side with the oocyst/sarcocyst. The zero point was placed at the beginning of the oocyst/sarcocyst and measurement was taken from one end of the oocyst/sarcocyst to the other.

3.7 LEVEL OF AWARENESS AND RISK FACTORS

In order to ascertain the awareness and risk factors associated with *Sarcocystis* infection, non-structured questionnaires were administered to individuals in the study area. Information concerning type of food/meat consumed, preparation of meat before consumption, ownership and or access to pets and source of drinking water. Knowledge of the infection was also enquired.

3.8 CLASSIFICATION OF VARIABLES

Sarcocystis infection was classified into positive and negative. Sex was classified into male and females. For cattle, age was classified into 5-8 ½ years and greater than 8½ years of age while breed was classified into white Fulani and Gudali. Muscular tissue

was classified into oesophagus and diaphragm. Laboratory method was classified into tissue digestion and histology. Species were classified into cattle, pigs, dogs and humans. Awareness of *Sarcocystis* infection was classified into aware and not aware.

3.9 STATISTICAL ANALYSIS

Data collated at the end of the study were analyzed using Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc. Chicago, IL, USA). Statistical methods employed included descriptive statistics employing frequencies and percentages. Chi-square test of association was used to establish association between the infection status and variables such as age, sex and breed. Mann-Whitney test was used to compare the level of infectivity of tissues between oesophagus and diaphragm. P values less than 0.05 were considered significant. Frequency of responses to risk factors listed in the questionnaires was determined.

CHAPTER 4

RESULTS

4.1 PARASITE DETECTION FROM FAECES OF DOGS AND HUMANS

Of a total of 200 dog samples examined, 1 (0.5 %) was parasitologically positive for *Sarcocystis* species. Also, from a total of 390 human feacal samples, none was positive for *Sarcocystis* species (Table 4.1, Plate I). Prevalence based on sex and age of dogs was not done as only one dog was positive. The only positive dog was an exotic breed, male and 10 months old.

Other findings indicated that of the 200 dogs sampled, 19 (9.5 %) were positive for *Isospora* oocysts, 14 (7.0 %) *Taenia* eggs, 24 (12.0 %) Hookworm eggs, 1 (0.5 %) *Trichuris* eggs, 1 (0.5 %) *Strongyloides* eggs, 1 (0.5 %) *Balantidium* sp, 17 (8.5 %) p *Toxocara* eggs, 8 (4.0 %) *Dipylidium* eggs, 1 (0.5 %) *Spirocerca* sp and 1 (0.5 %) mites eggs (Table 4.2).

Of the 390 feacal samples from humans, prevalence rates were established as follows; 5 (1.3 %) *Isospora* oocyst, 1 (0.3 %) *Taenia* eggs, 35 (9.0 %) Hookworm eggs, 1(0.3 %) *Trichuris* eggs, 1 (0.3 %) *Strongyloides* eggs, 49 (1.0 %) *Balantidium* sp, 17 (8.5 %) *Toxocara* eggs, 3 (0.8 %) *Entamoeba* cysts, 3 (0.8 %) *Enterobius* eggs, 2 (0.5 %) *Hymenolepis* eggs and 1 (0.3 %) *Ascaris* eggs (Table 4.2).

Table 4.1: Prevalence of *Sarcocystis* (sporocysts/sarcocysts) in definitive and intermediate hosts investigated in Zaria.

Host	Animal sp.	Total No. sampled	No. positive	Prevalence (%)
Definitive host	Dog	200	1	0.5
	Human	390	0	0
Intermediate host	Cattle	200	85	42.5
	Pig	100	60	60.0

Table 4.2: Prevalence of other protozoan and helminth ova seen in dogs and humans faeces in Zaria.

Parasite	Dog (n = 200)	Human (n = 390)
Isospora oocyst	19 (9.5)	5 (1.3)
Taenia ova	14 (7.0)	1 (0.3)
Hookworm ova	24 (12.0)	35 (9.0)
Trichuris sp	1 (0.5)	1 (0.3)
Strongyloides sp	1 (0.5)	1 (0.3)
Balantidium sp	1 (0.5)	4 (1.0)
Toxocara sp	17 (8.5)	0 (0)
Dipylidium caninium	8 (4.0)	0 (0)
Mites egg	1 (0.5)	0 (0)
Spirocerca sp	1 (0.5)	0 (0)
Entamoeba sp	0 (0)	3 (0.8)
Enterobius vermicularis	0 (0)	3 (0.8)
Hymenolepis sp	0 (0)	2 (0.5)
Ascaris sp	0 (0)	1 (0.3)

*No. in parenthesis indicates percentage positive

4.2 PREVALENCE OF *SARCOCYSTIS* IN TISSUES OF CATTLE AND PIGS

Of the 200 cattle sampled, 85 (42.5 %) cattle were positive for sarcocyst by tissue digestion (Table 4.1). Of the 85, 75 (88.2 %) and 56 (65.9 %) had sarcocysts in the oesophagus and diaphragm respectively (Plates IV, V, VI and VII). Of the 75 oesophagus samples positive by tissue digestion, 56 (74.7 %) were positive by histology while of the 56 diaphragm positive samples, 39 (69.9 %) were positive by histology. The cysts were found more in the oesophagus than in the diaphragm ($p < 0.05$) (Table 4.3). There was no significant association between sex and *Sarcocystis* infection ($p > 0.05$) (Table 4.4), age and *Sarcocystis* infection ($p > 0.05$) (Table 4.5), and breed and *Sarcocystis* infection ($p > 0.05$) (Table 4.6) in the animals.

Of the 100 pigs sampled, 60 (60.0 %) pigs were positive for sarcocysts by tissue digestion (Table 4.1). Of the 60, 52 (86.7 %) and 47 (78.3 %) had sarcocysts in the oesophagus and diaphragm respectively (Plates VIII, IX, X and XI). Histological examination showed 43 (82.7 %) to be positive of the 52 oesophagus samples positive by tissue digestion while 34 (72.3 %) to be positive of the 47 diaphrgam positive samples. There was no significant difference in the level of infectivity between oesophagus and diaphragm ($p > 0.05$) (Table 4.3). There was no significant association between sex and *Sarcocystis* infection in pigs ($p > 0.05$) (Table 4.7).

4.3 PARASITE DETECTION FROM FAECES OF CATTLE AND PIGS

Faecal samples from cattle and pigs were not positive for *Sarcocystis* species. However, of the 200 cattle samples, 71 (35.3 %) were positive for Coccidia oocyst, 41 (20.5 %) *Strongyle* type eggs, 8 (4.0 %) *Strongyloide*s sp, 2 (1.0 %) *Taenia* ova and 2 (1.0 %) *Buxtonella* sp (Table 4.8).

Also of the 100 pig samples, 53 (53.0 %) were positive for Coccidia oocyst, 52 (52.0 %) *Strongyle* type eggs, 5 (5.0 %) *Strongyloide*s sp, 2 (2.0 %) *Taenia* ova, 18 (18.0 %) *Ascaris* sp, 2 (2.0 %) *Metastrongylus* sp, 5 (5.0 %) *Stephanurus* sp, 4 (4.0 %) *Trichuris* sp and 1 (1.0 %) *Toxocara* sp (Table 4.8).

.4.4 IDENTIFICATION OF SPOROCYST OF *SARCOCYSTIS*

The average measurement of sporocysts seen in dog was 15.05 ± 0.60 µm in length and 9.03 ± 0.00 µm in width (Table 4.9).

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Table 4.3: Detection rates of *Sarcocystis* species in tissues of cattle and pigs in Zaria based on 2 methods

Animal sp.	No. positive	Oesophagus			Diaphragm		
		No. of samples positive (%)	D^+ (%)	H^+ (%)	No. of samples positive (%)	D+ (%)	H+ (%)
*Cattle	85 (out of 200)	75 (88.2)	75 (100.0)	56 (74.7)	56 (65.9)	56 (100.0)	39 (69.6)
**Pig	60 (out of 100)	52 (86.7)	52 (100.0)	43 (82.7)	47 (78.3)	47 (100.0)	34 (72.3)

No. in parenthesis indicates percentage positive
D^+ = Number positive in pepsin digestion method, H^+ = Number positive in histology
* (Mean rank; Diaphragm = 191.0, Oesophagus = 210.0, $p < 0.05$)
** (Mean rank; Diaphragm = 98.0, Oesophagus = 103.0, $p > 0.05$)

Table 4.4: Sex specific prevalence of *Sarcocystis* species in tissues (oesophagus and diaphragm) of cattle in Zaria

Sex	Total sampled	No. positive	Sex specific rate (%)	P value
Male	65	31	47.7	0.303
Female	135	54	40.0	
Total	**200**	**85**	**42.5**	

($\chi^2 = 1.062$, df = 1) $\alpha = 0.05$

Table 4.5: Age specific prevalence of *Sarcocystis* species in tissues (oesophagus and diaphragm) of cattle in Zaria

Age group	Total sampled	No. positive	Age specific rate (%)	P value
5- 8½ years	131	60	45.8	0.193
> 8½ years	69	25	36.2	
Total	**200**	**85**	**42.5**	

($\chi^2 = 1.694$, df = 1) $\alpha = 0.05$

Table 4.6: Breed specific prevalence of *Sarcocystis* species in tissues (oesophagus and diaphragm) of cattle in Zaria

Breed	Total sampled	No. positive	Breed specific rate (%)	P value
White Fulani	180	78	43.3	0.474
Gudali	20	7	35.0	
Total	**200**	**85**	**42.5**	

($\chi^2 = 0.512$, df = 1) $\alpha = 0.05$

Table 4.7: Sex specific prevalence of *Sarcocystis* species in tissues (oesophagus and diaphragm) of pigs in Zaria

Sex	Total sampled	No. positive	Sex specific rate (%)	P value
Male	51	28	54.9	0.288
Female	49	32	65.3	
Total	**100**	**60**	**60**	

($\chi^2 = 1.127$, df = 1) $\alpha = 0.05$

Table 4.8: Prevalence of other protozoan and helminth ova seen in cattle and pigs faeces in Zaria

Parasite	Cattle (n = 200)	Pigs (n = 100)
Coccidia oocyst	71 (35.5)	53 (53.0)
Strongyle type egg	41 (20.5)	52 (52.0)
Strongyloides sp	8 (4.0)	5 (5.0)
Taenia sp	2 (1.0)	2 (2.0)
Buxtonella sp	2 (1.0)	0 (0)
Metastrongylus sp	0 (0)	2 (2.0)
Ascaris sp	0 (0)	18 (18.0)
Stephanurus sp	0 (0)	5 (5.0)
Trichuris sp	0 (0)	4 (4.0)
Toxocara sp	0 (0)	1 (1.0)

*No. in parenthesis indicates percentage positive

Table 4.9: Sporocyst size and species of *Sarcocystis* from faecal sample of dog

Animal Specie	**Total Sampled**	**No. Positive**	**Average sporocyst size (L by W in µm)**	**Tentative *Sarcocystis* sp**	***Reference Size**
Dog	200	1	15.04 (± 0.60) by 9.03 (± 0.0)	*S. tenella* *S. capracanis* *S. bertrami*	15 by 10 µm

* Dubey, 1977; Dubey *et al*., 1989;

4.5 IDENTIFICATION OF SARCOCYSTS

The measurement of sarcocysts seen in cattle ranged from 228.8 µm to 1215.5 µm in length and 46.93 µm to 114.40 µm in width. The sarcocysts were microscopic in nature and 99.0 % had a thin wall (< 1.81 µm), while 4.0 % had sarcocysts which were thick walled (3.61 to 7.22 µm) (Table 4.10).

In pig, the measurement of sarcocysts ranged from 205.77 µm to 1444.2 µm in length and 36.1 to 129.96 µm in width. The sarcocysts were also microscopic in nature, 85.0 % and 25.0 % had thin (< 3.61 µm) and thick (3.61-7.22 µm) walled sarcocysts respectively. Majority of the sarcocysts (85.0%) had sarcocysts which were between 104.69 to 205.77 µm in length and 36.1 to 122.74 µm in width (Table 4.10).

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4.6 TISSUE INFILTRATION

Leucocytic infiltration was observed in 3 and 4 oesophageal tissue of cattle and pigs respectively. They were mostly lymphocytes and few eosinophils (Plates XII, XIII and XIV).

Table 4.10: Morphology of sarcocysts and species of *Sarcocystis* from tissues of cattle and pigs in Zaria.

Animal species	No. Positive	Sarcocyst size (range in µm)	Cyst wall thickness (range in µm)	Nature of cyst	Tissue reaction	**Species of *Sarcocystis*
Cattle	85 (out of 200)	L 228.8 - 1215.5	< 1.81	Microscopic	Leucocytic infiltration	*S. cruzi*
		W 46.93 - 114.40	3.61-7.22	Microscopic		*S. hominis*
Pig	60 (out of 100)	L 205.77-1444.2	< 3.61	Microscopic	Leucocytic infiltration	Possibly *S. porcifelis*
		W 36.1-129.96	3.61-7.22	Microscopic		*S. meischeriana*

*L = length, W = width, **Reference size: Dubey *et al*., 1989



Plate I: Photomicrograph of *Sarcocystis tenella* /*S. capracanis*/*S. bertrami* seen in dog faeces (Light microscopy x 400 magnification) (A) Sporocyst (B) Sporocyst residium (C & D) Sporozoites.

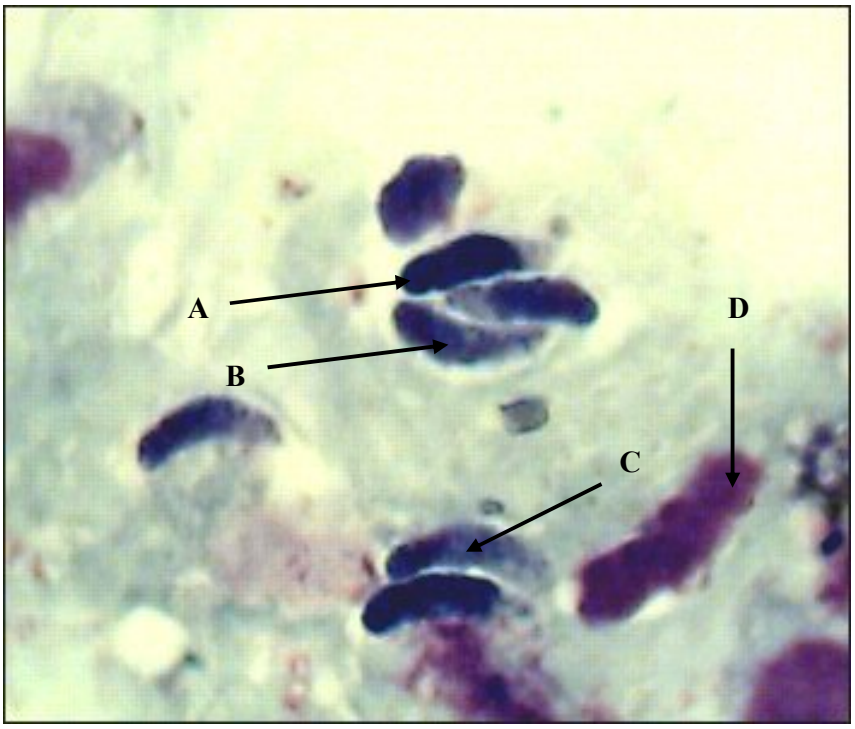


Plate II: Photomicrograph of bradyzoites released from sarcocysts in the oesophagus of pig during tissue digestion stained with Giemsa (Light microscopy x 400 magnification using digital microscope camera) (A, B, C) Bradyzoites (D) Muscle tissue

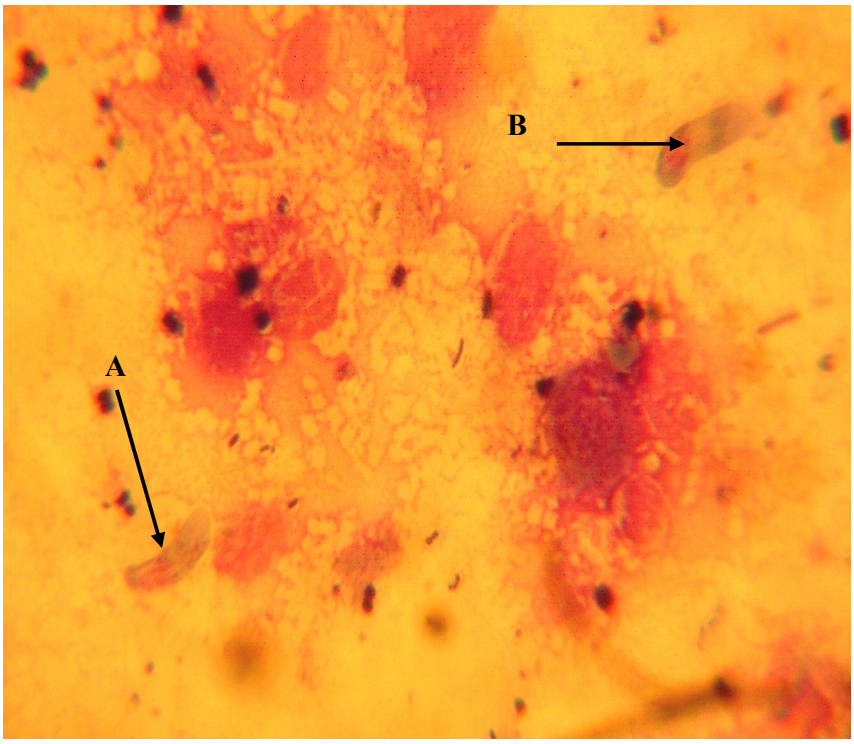


Plate III: Photomicrograph of bradyzoites released from sarcocysts in the oesophagus of cattle during tissue digestion stained with Giemsa (Light microscopy x 400 magnification). (A&B – Bradyzoites)

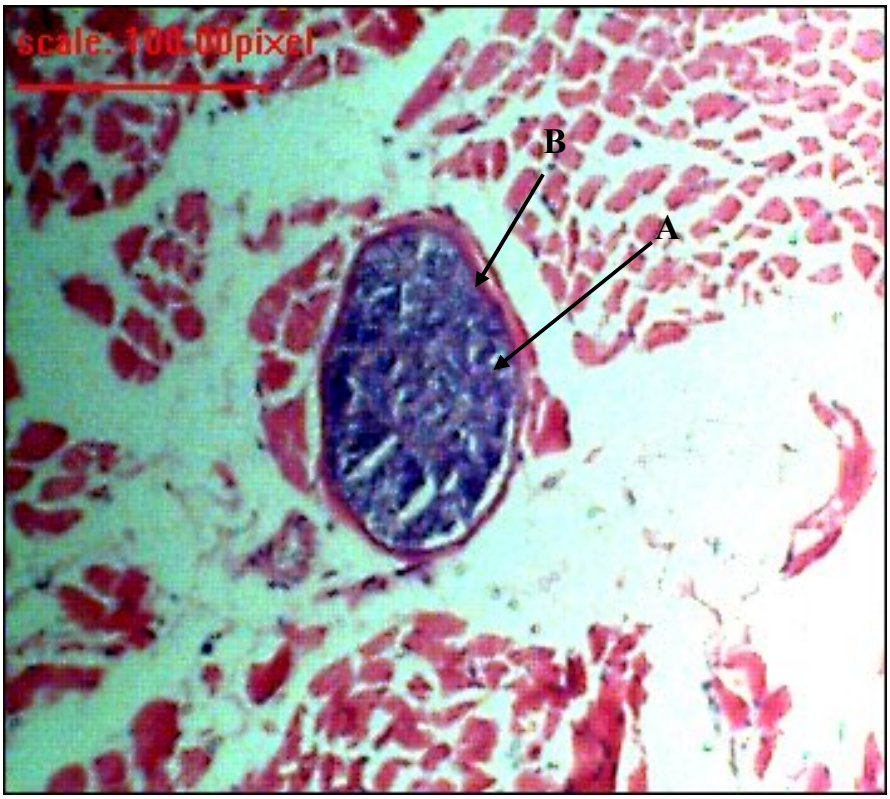


Plate IV: Photomicrograph of *Sarcocystis cruzi* in the oesophagus of cattle with H&E (Light microscopy x 400 magnification using digital microscope camera) (A) Sarcocyst (B) Thin cyst wall.

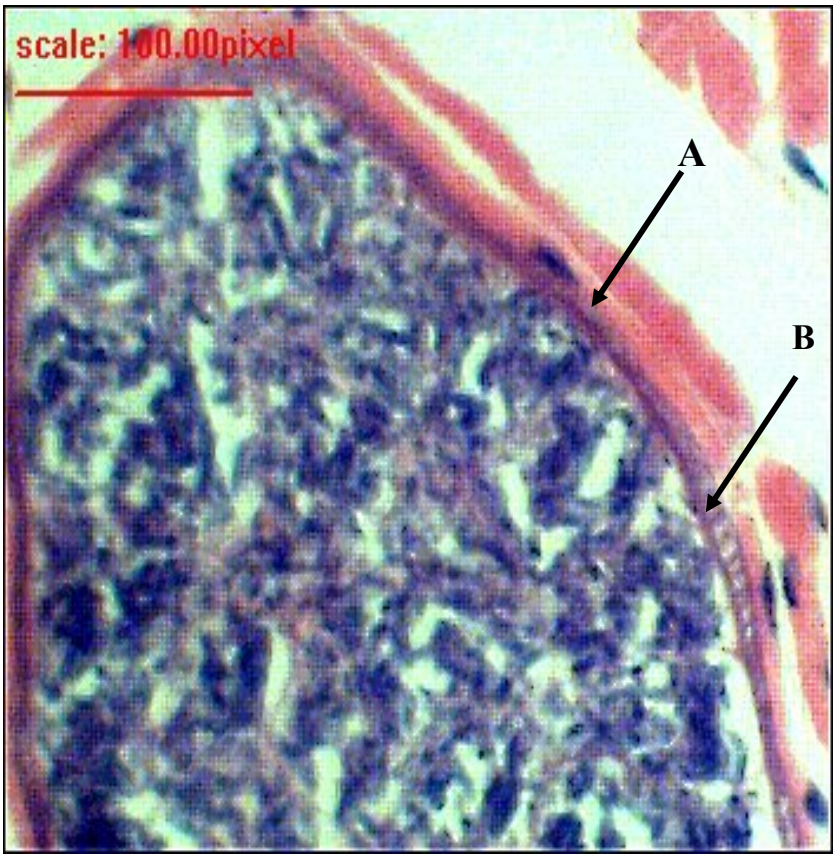


Plate V: Photomicrograph of *Sarcocystis hominis* in the oesophagus of cattle stained with H&E (Light microscopy x 400 magnification using digital microscope camera) (A) Skeletal muscle cell (B) Thick cyst wall showing villous protrusions.



Plate VI: Photomicrograph of *Sarcocystis hominis* in the diaphragm of cattle stained with H&E (Light microscopy x 400 magnification using digital microscope camera) (Arrow showing sarcocyst)

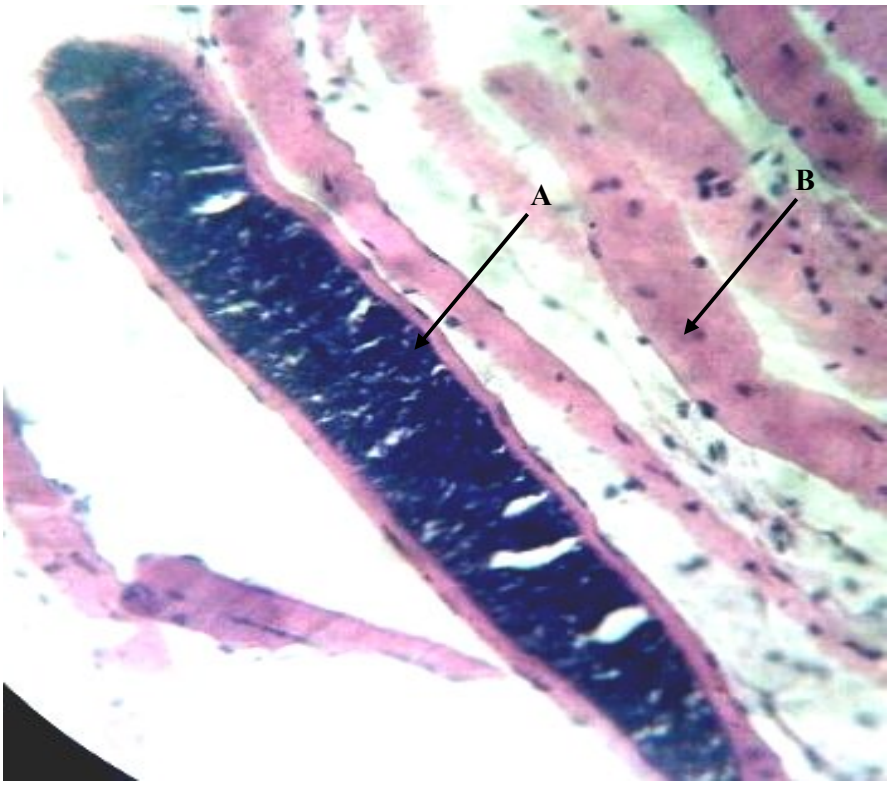


Plate VII: Photomicrograph of *Sarcocystis hominis* in the diaphragm of cattle stained with H&E (Light microscopy x 400 magnification) (A) Sarcocyst; long (B) Skeletal muscle.

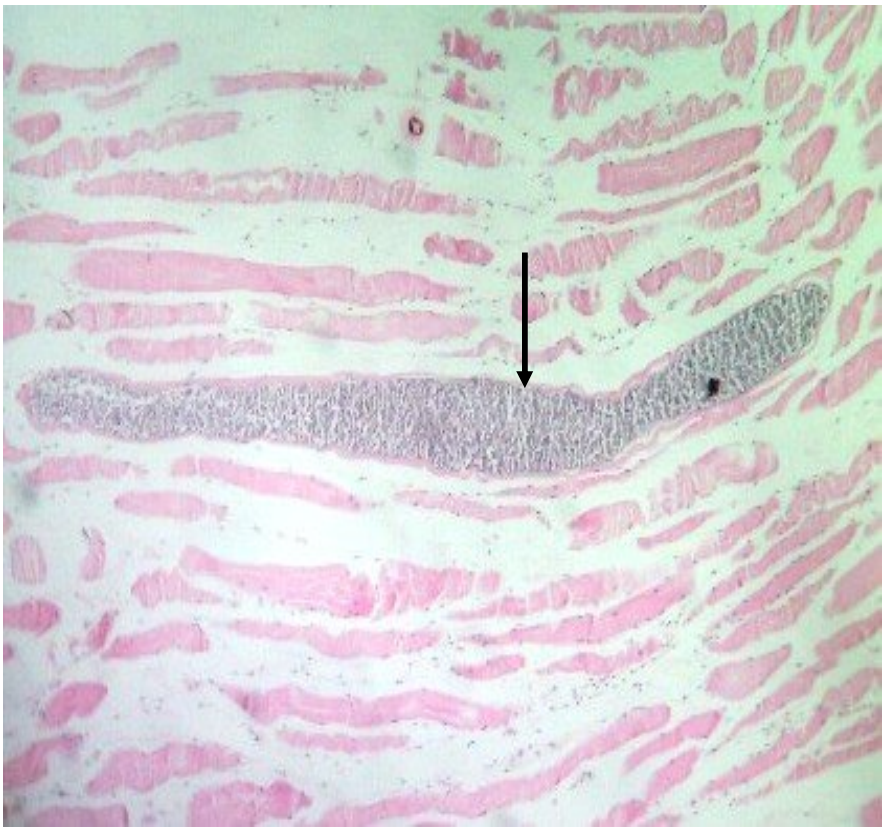


Plate VIII: Photomicrograph of *Sarcocystis meischeriana* in the diaphragm of pig stained with H&E (Light microscopy x 100 magnification) (Arrow showing sarcocyst; long).

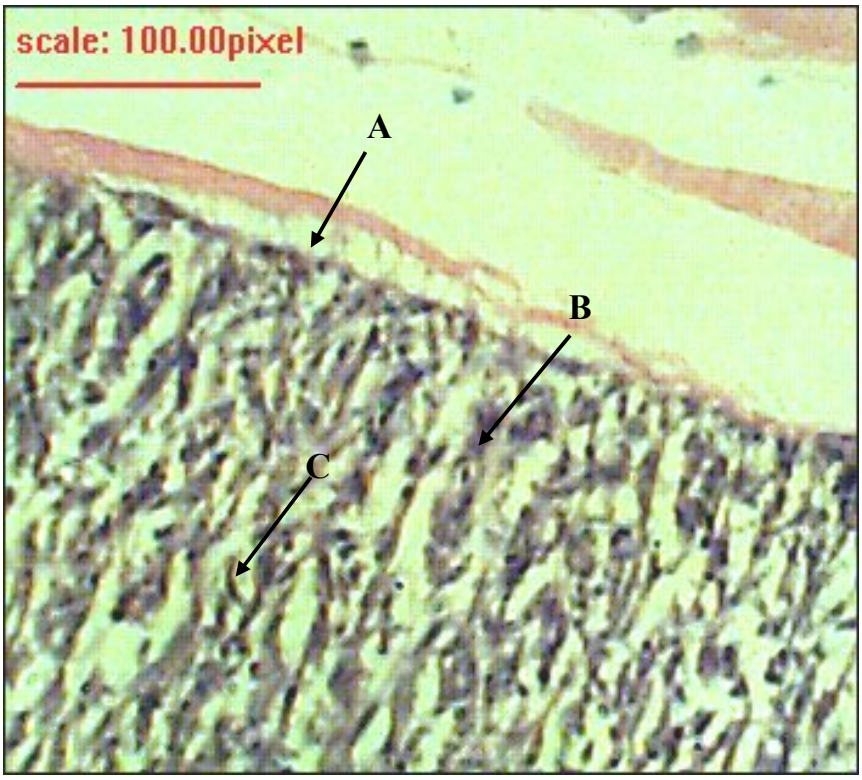


Plate IX: Photomicrograph of *Sarcocystis meischeriana* in the diaphragm of pig stained with H&E (Light microscopy x 400 magnification using digital microscope camera) (A) Thick cyst wall showing villous protrusions (B) Sarcocyst containing numerous bradyzoites. (C) Banana shaped bradyzoites with a dark nucleus.



Plate X: Photomicrograph of *Sarcocystis meischeriana* in the diaphragm of pig stained with H&E (Light microscopy x 400 magnification using digital microscope camera) (Arrows showing banana shaped bradyzoites with a dark nucleus).

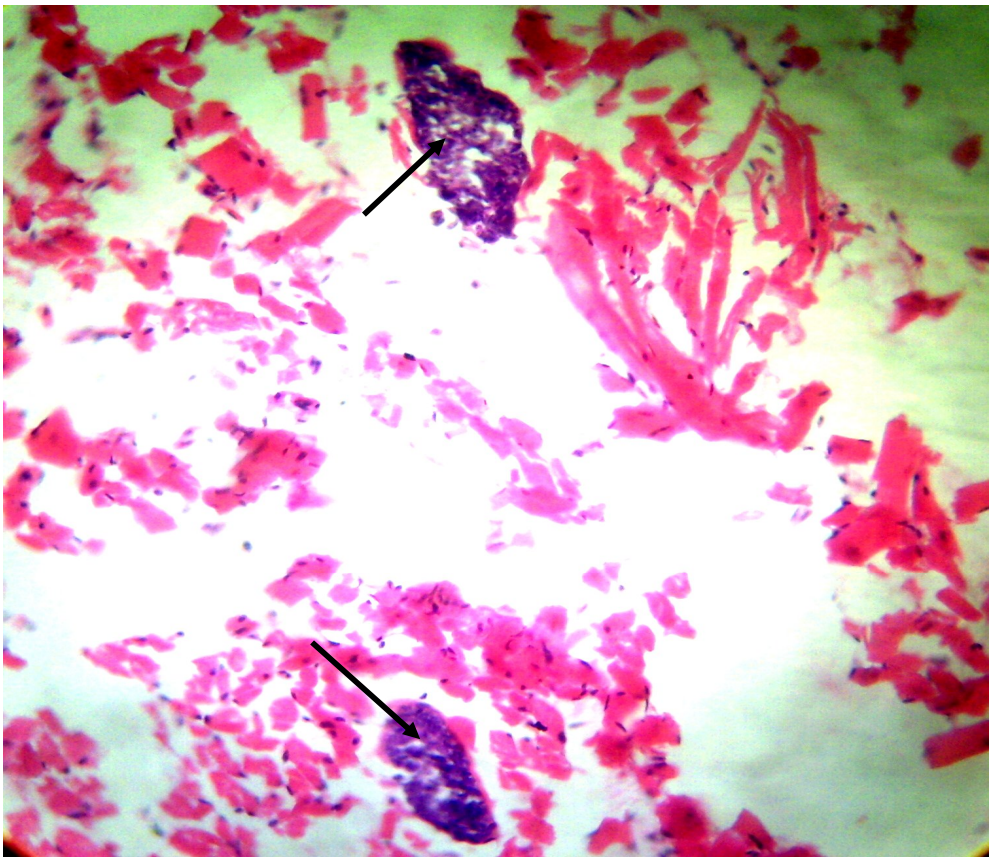


Plate XI: Photomicrograph of possibly *Sarcocystis porcifelis* in the diaphragm of pig stained with H&E (Light microscopy x 400 magnification using digital microscope camera) (Arrows showing sarcocysts).

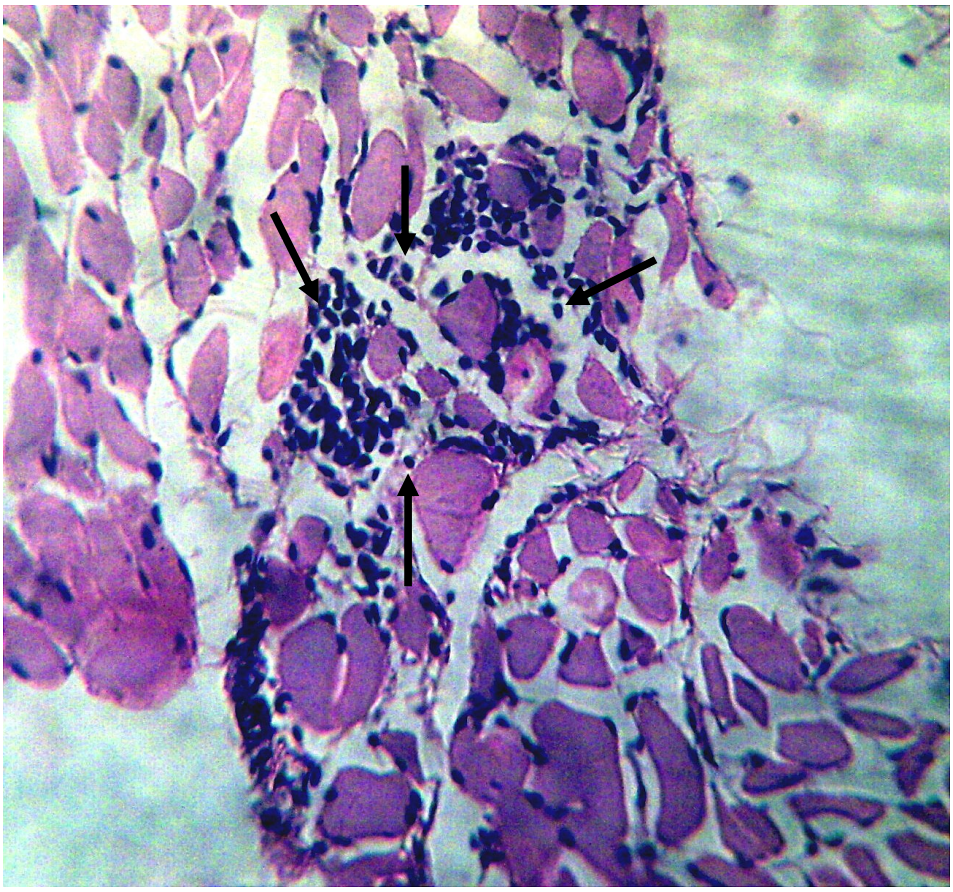


Plate XII: Photomicrograph of leucocytic infiltration in the oesophagus of cattle stained with H&E (Light microscopy x 400 magnification using digital microscope camera) (Arrows showing lymphocytes)

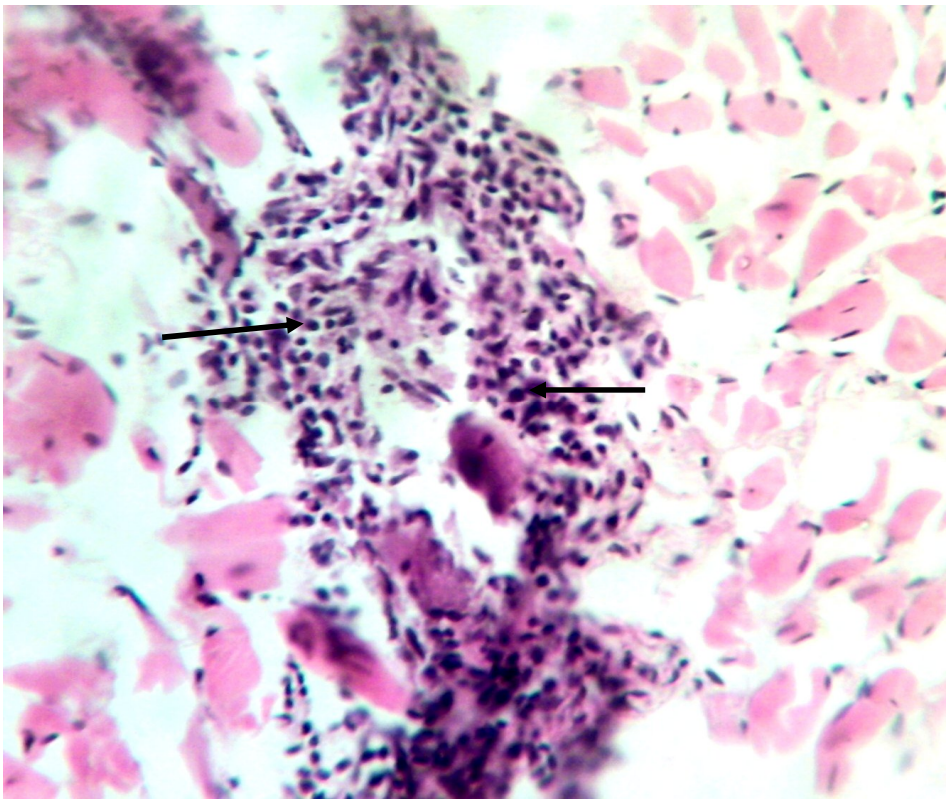


Plate XIII: Photomicrograph of leucocytic infiltration in the oesophagus of pig stained with H&E (Light microscopy x 400 magnification using digital microscope camera) (Arrows showing lymphocytes).



Plate XV: Photomicrograph of leucocytic infiltration in the oesophagus of pig stained with H&E (Light microscopy x 1000 magnification using digital microscope camera) (Arrows showing eosinophils)

4.7 RISKS FACTORS AND AWARENESS OF *SARCOCYSTIS* INFECTION

Filled questionnaires from 230 respondents were analysed for risk factors and awareness of *Sarcocystis* infection. None of the respondents were aware of the infection or disease.

Demographic information of respondents indicated that female constituted 64.3 % of the respondents while male were 35.7 %, mostly with the age range of 21-30 years (30.9 %), and the least being above 50 years (9.6 %) (Table 4.11). Housewives and students predominated the respondents while secondary level of education was mostly attained by respondents.

Ninety-nine percent of the respondents reported eating meat, which were beef, mutton, chevon, chicken and fish (Figure 4.1). Food were consumed by respondents in various forms; cooked (91.7 %), fried (0.9 %), fruits (1.4 %), and snacks (6.0 %) (Figure 4.2). Seventy point four percent of the respondents reported eating suya, 19.9 %, 25.3 % and 51.8 % reported eating beef burger, sausages and meat pie respectively (Figure 4.3). Also, 60.9 % reported eating either of the fast food meat products once a week and 22.2% reported consuming the food products always (Figure 4.4).

Ninety-nine percent of the respondents reported consuming vegetables which are mostly washed twice (50.5 %) (Figure 4.5) with water and salt (72.0 %) (Figure 4.6) and added directly to food to cook (94.0 %) (Figure 4.7).

Although 71.9 % of the respondents reported having no pets, 54.0 % reported having access to pets which were mostly dogs (Table 4.12). Hundred percent of the respondents who owned pets reported feeding them with household food while 8.2 % reported feeding raw meat to their pets (Figure 4.8).

Thirty-one percent, 12.6 %, 20.4 %, 16.5 %, 1.7 % and 3.5 % of the respondents reported drinking water from tap, well, borehole, sachet water, bottled water and water from any source respectively (Figure 4.9). Also, 25.6 % of respondents who had wells in their houses reported drinking water from it (Figure 4.10). One point nine percent of the respondents reported having wells which were not covered, not cemented and at ground level and 62.2 % reported having wells which were covered, cemented and above ground level. About 0.6% reported having wells which were covered, not cemented and at ground level (Table 4.13).

Table 4.11: Sex and age groups of respondents to *Sarcocystis* infection in Zaria

		Sex		
		Male (%)	Female (%)	Total (%)
Age group	2- 20 yrs	18 (43.9)	23 (56.1)	41 (100.0)
	21-30 yrs	17 (23.9)	54 (76.1)	71 (100.0)
	31-40 yrs	22 (37.3)	37 (62.7)	59 (100.0)
	41-50 yrs	18 (48.6)	19 (51.4)	37 (100.0)
	> 50 yrs	7 (31.8)	15 (68.2)	22 (100.0)
	Total (%)	82 (35.7)	148 (64.3)	230 (100.0)

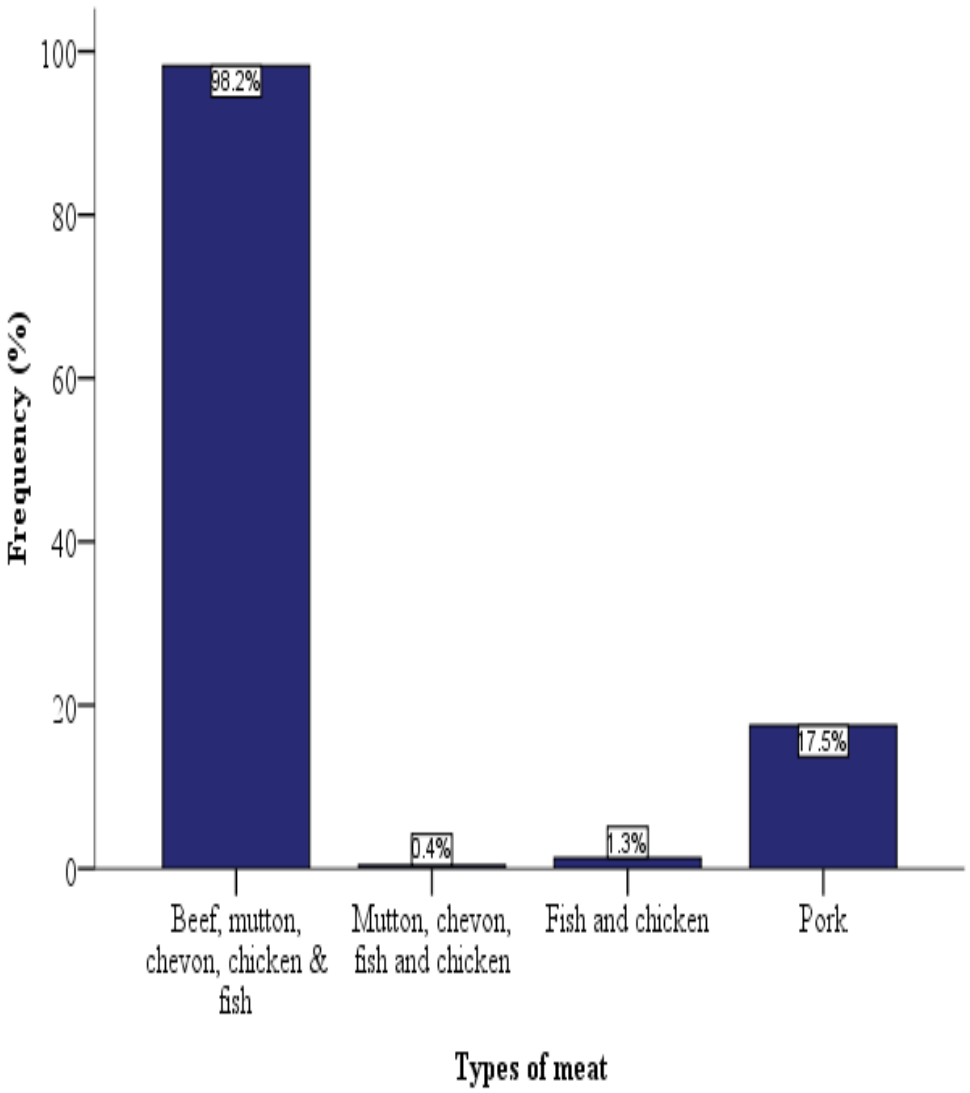


Figure 4.1: Types of meat consumed by the respondents in the study area.

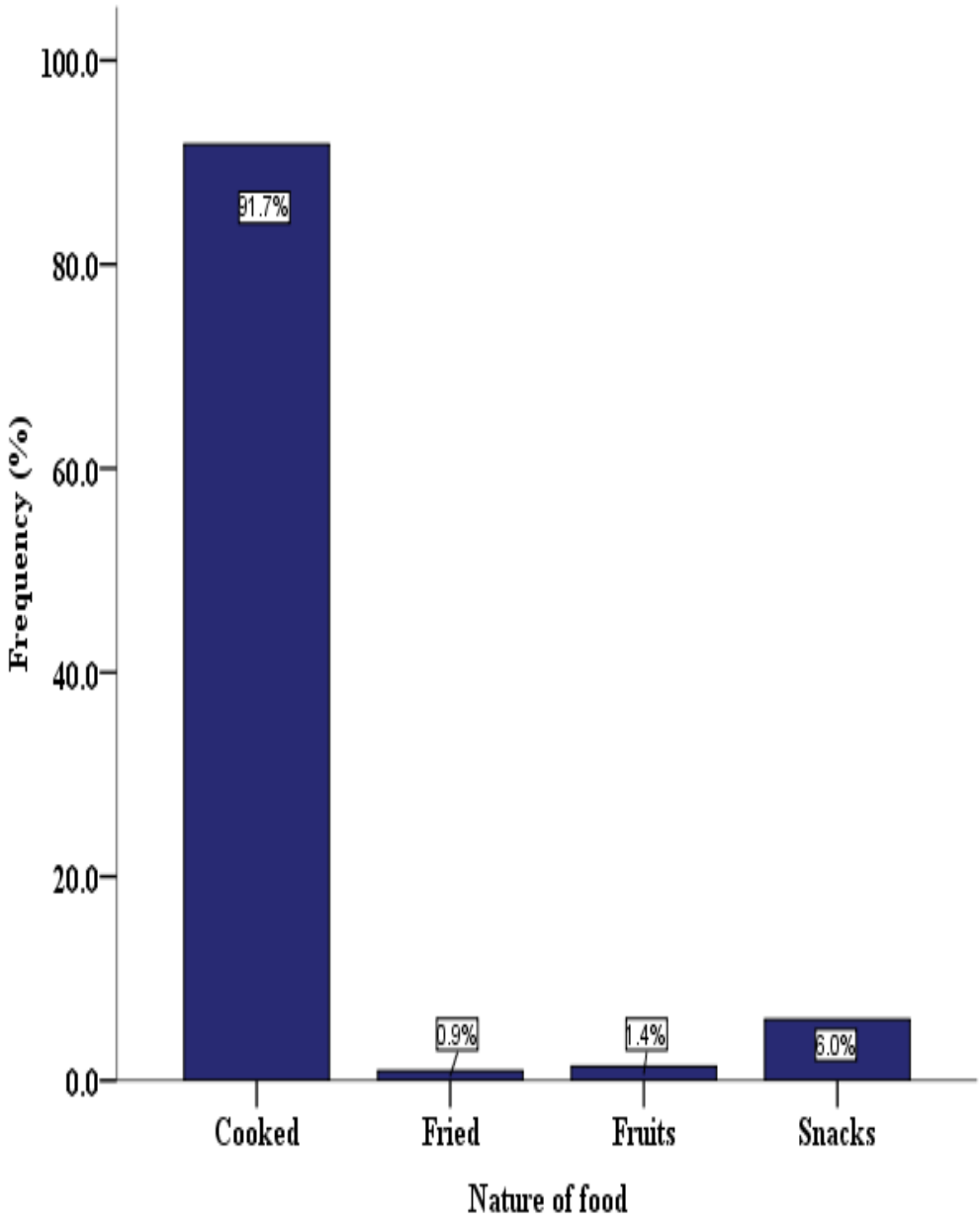


Figure 4.2: Nature of food frequently consumed by respondents in the study area

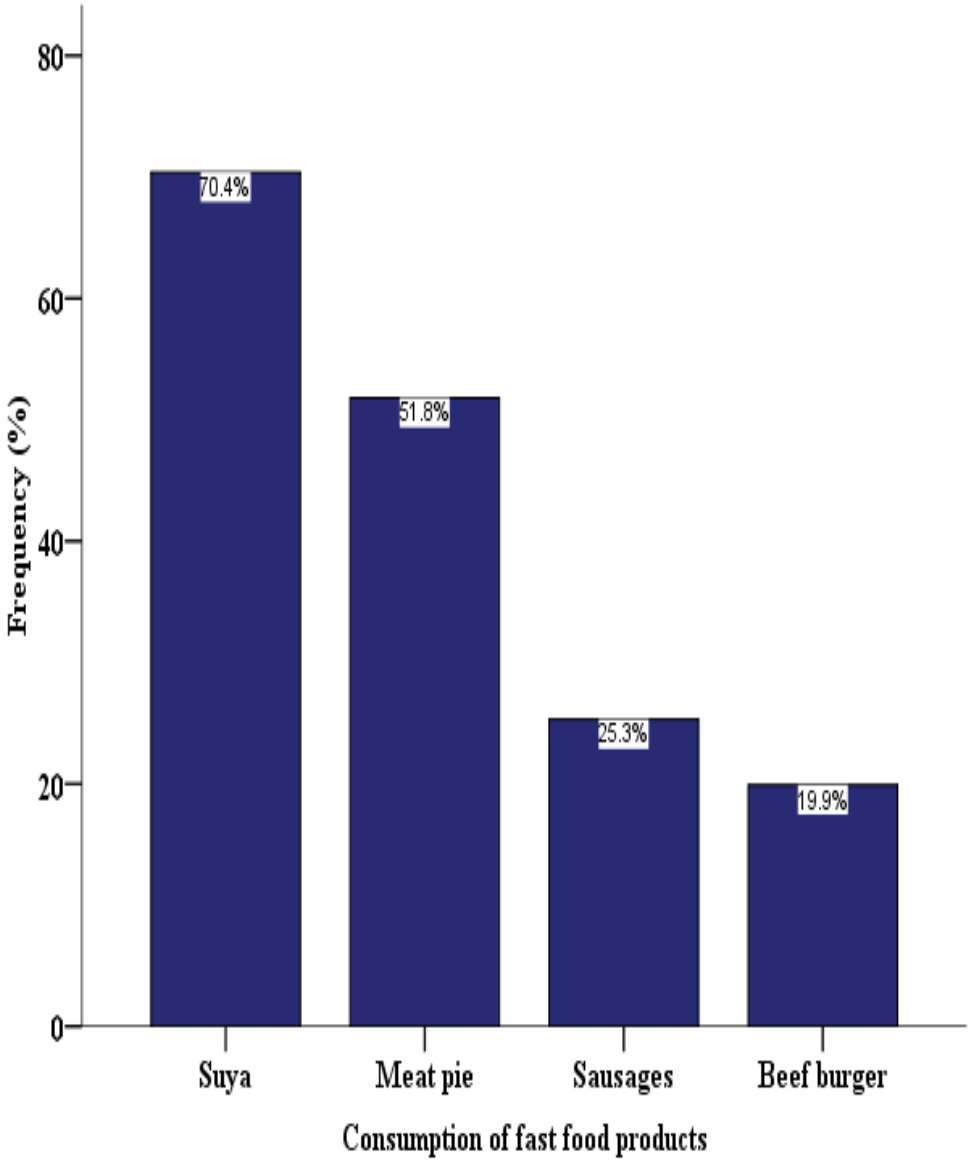


Figure 4.3: Fast food products consumed by respondents in the study area

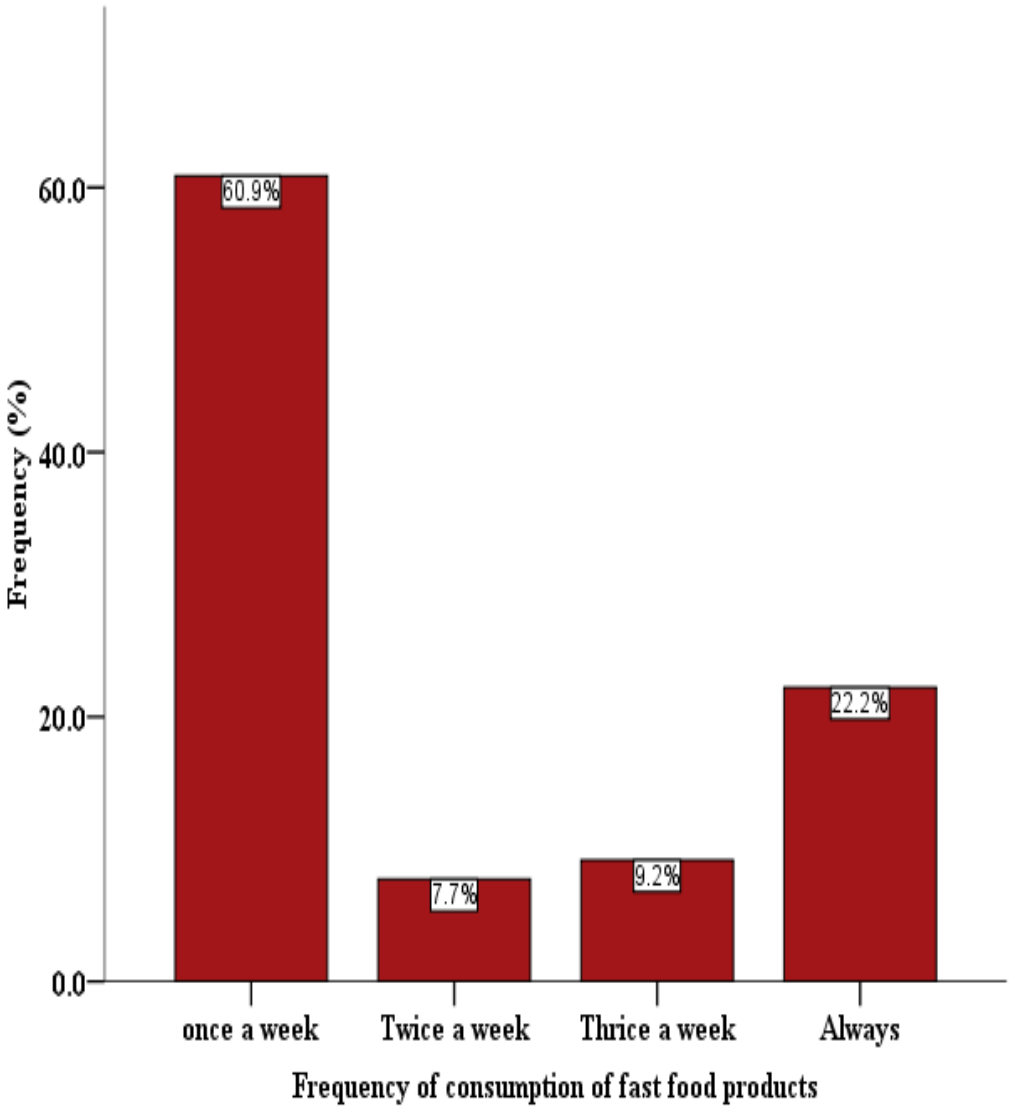


Figure 4.4: Frequency of consumption of fast food meat products by respondents in the study area.

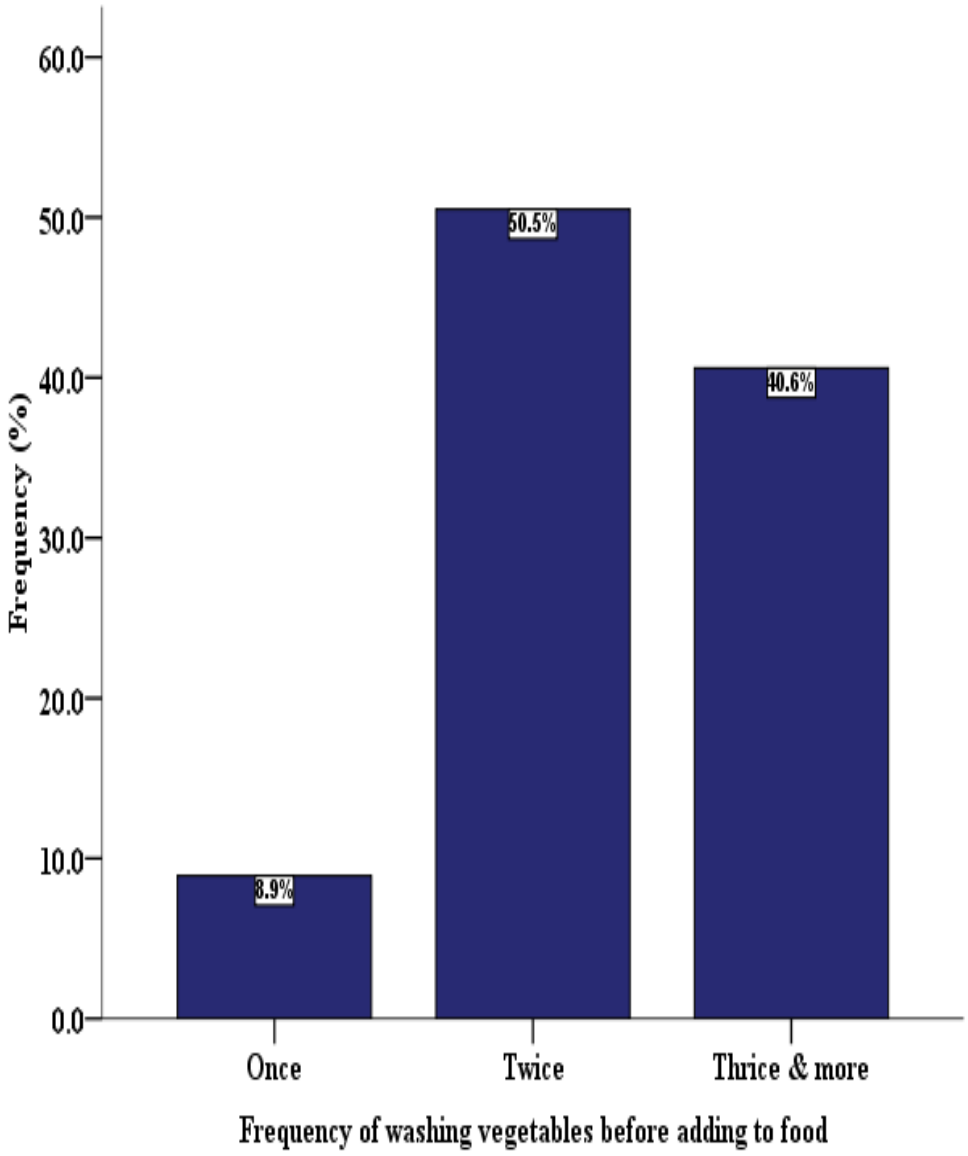


Figure 4.5: Frequency of washing vegetables before cooking by the respondents in the study area

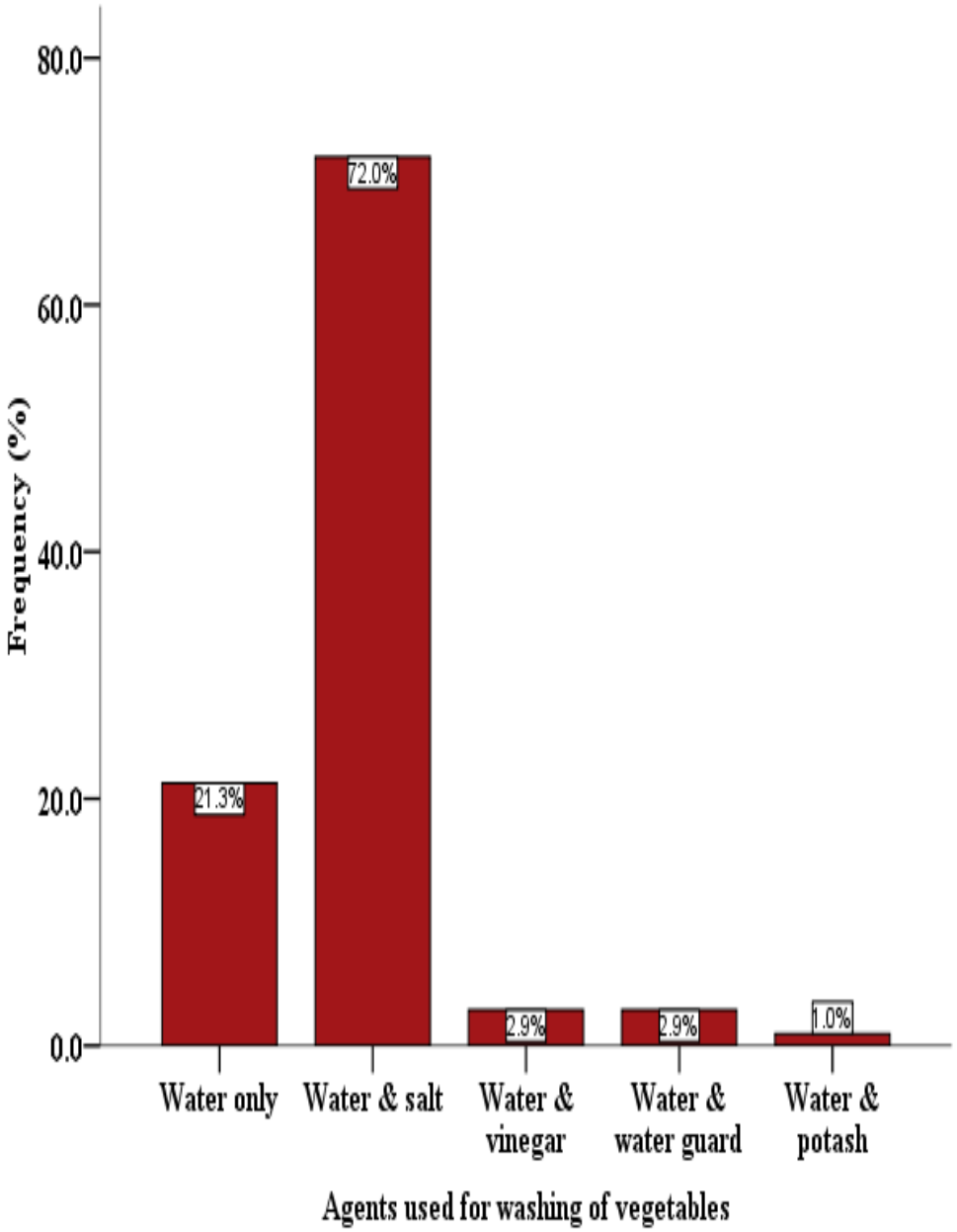


Figure 4.6: Agents used for washing of vegetables by respondents in the study area

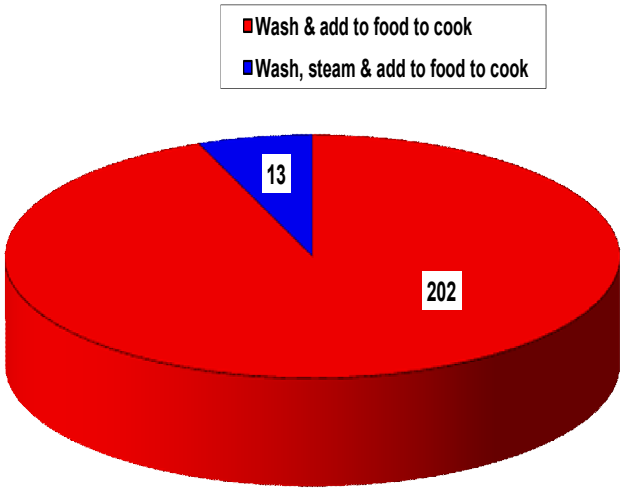


Figure 4.7: Preparation of vegetables by respondents in the study area

Table 4.12: Responses to ownership and access to dogs and cats by respondents in the study area

		Ownership of dogs/cats		
		No (%)	Yes (%)	Total (%)
Access to dogs/cats	No (%)	102 (98.1)	2 (1.9)	104 (100.0)
	Yes (%)	62 (50.0)	62 (50.0)	124 (100.0)
	Total (%)	164 (71.9)	64 (28.1)	228 (100.0)

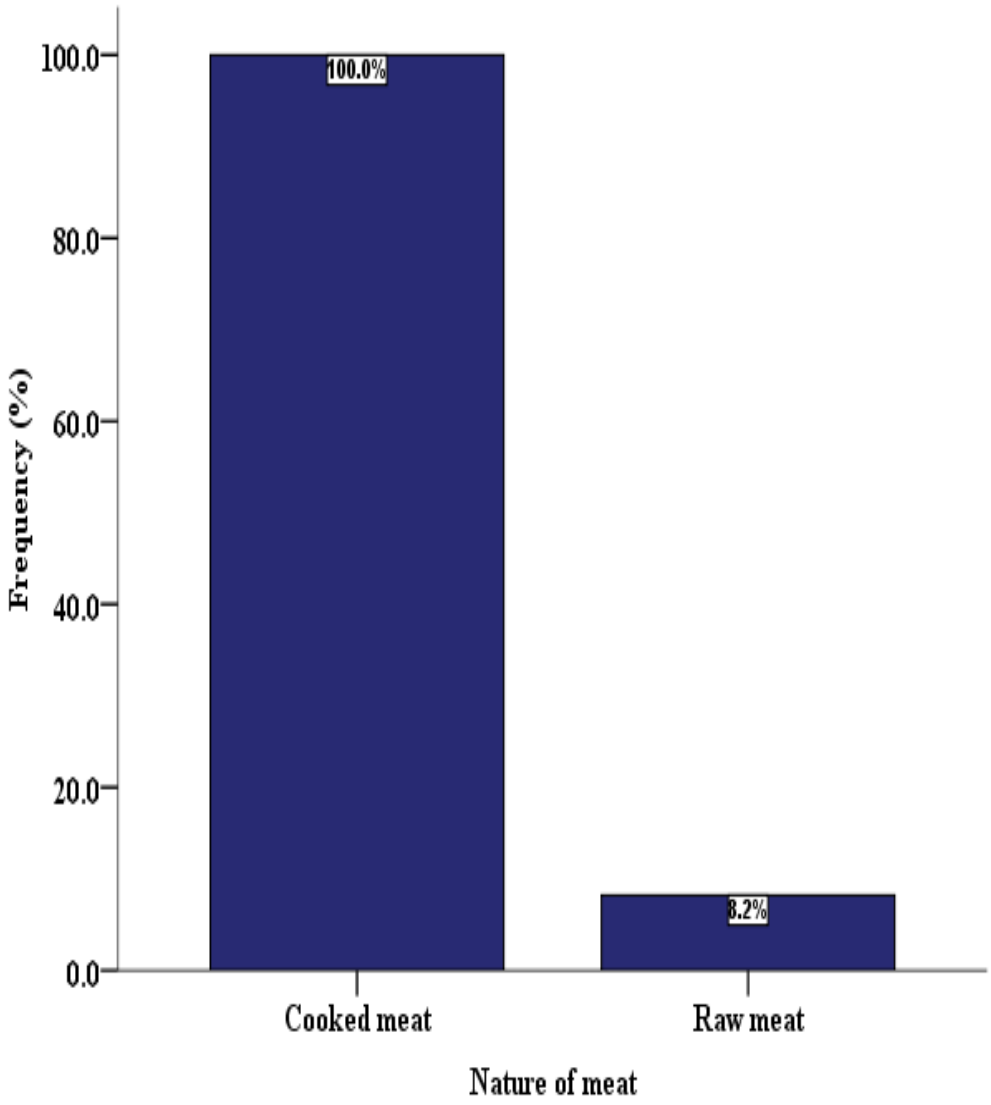


Figure 4.8: Nature of meat fed to pets by respondents in the study area

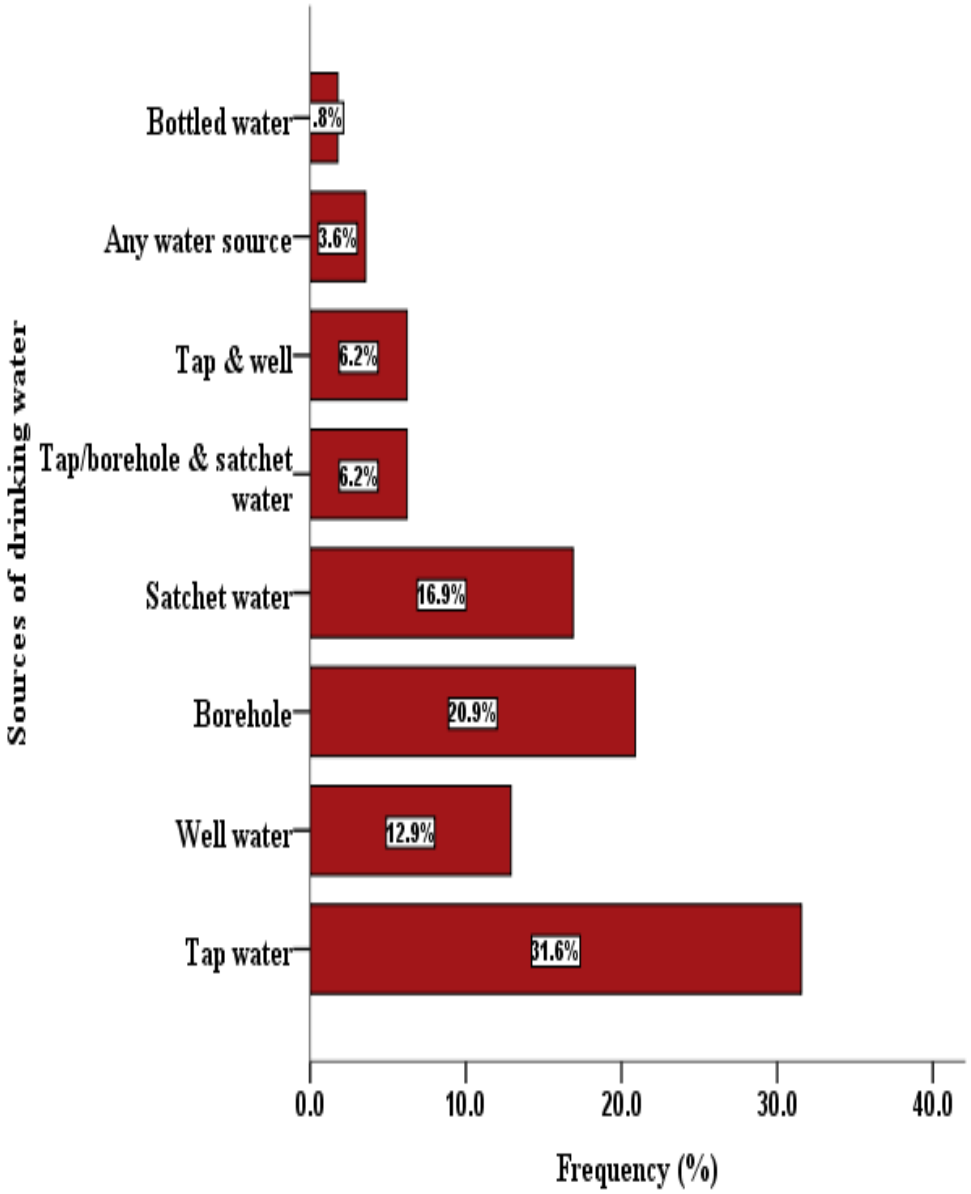


Figure 4.9: Sources of drinking water as reported by respondents in the study area

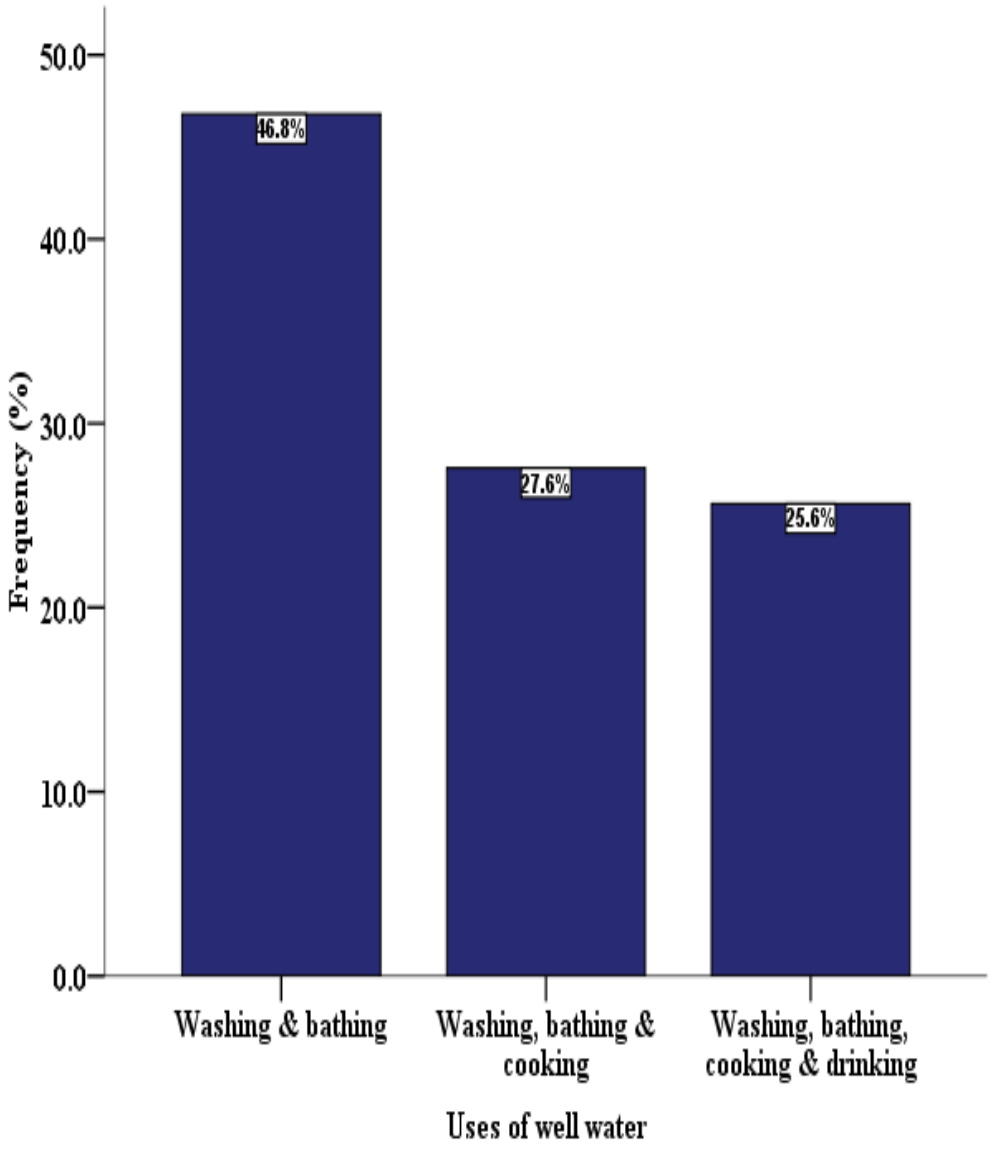


Figure 4.10: Uses of well water by respondents in the study area

Table 4.13: Descriptions of wells present in houses of respondents in Zaria

Description of well	Frequency	Percent (%)
Covered, cemented & above ground surface	97	62.2
Cemented, not covered & above the ground	27	17.3
Covered, not cemented & above ground surface	15	9.6
Not cemented, not covered & above ground surface	9	5.8
Not covered, not cemented & ground level	3	1.9
Covered, not cemented & ground level	1	0.6
Cemented, covered & ground level	4	2.6
Total	**156**	**100.0**

CHAPTER 5

DISCUSSION

Sarcocystis infection is common in many species of animal world wide (Dubey *et al*., 1989). Carnivores ingest the intramuscular cysts from herbivores and presumably from other animals too and eventually shed sporulated tetrazoic sporocysts in their faeces. In this study, a low prevalence of *Sarcocystis* species was recorded in dogs. This could be due to many reasons. It could be that the parasite has not completed the developmental stages for the oocysts/sporocysts to be visualized in the faeces since samples were collected just once from the subjects. Depending on the specie, it takes 8 to 33 days for complete developmental stages in the definitive host to shed oocyst/sporocysts in the faeces (Dubey et al., 1989).

Low prevalence of the parasite in the faeces could be as a result of pet care practised by clients who bring their dogs to the hospitals for health care services. These include deworming and other prophylactic treatment and measures. Oxytetracycline has being demonstrated to prevent death in sheep with acute sarcocystosis (Heydorn *et al*., 1981b). It is a common drug used in prophylaxis and therapeutic treatment of dogs for various diseases. Exposure to this drug in the hospitals might have affected the parasite, although *Sarcocystis* generally does not cause illness in dogs (Dubey *et al*., 1989). Furthermore, faecal samples were mostly collected from household dogs brought to the Ahmadu Bello Veterinary Teaching hospital (ABUVTH).

Although farm dogs play an important role in the establishment of sarcocystosis in cattle and pig farms, they were however not investigated in this study. The result of this

study agrees with the findings of Kudi (1989) who reported 1.8% prevalence rate in dogs in Kaduna state, but is in contrast to the findings of Adejinmi and Osayomi (2010) who recorded a prevalence rate of 12.0% in household dogs in Ibadan.

Based on the sporocyst measurement (15.05 ± 0.60 by 9.03 ± 0.00 μm), one may suggest that the *Sarcocystis* species seen in the dog faeces could either be *S. tenella, S. capracanis* or *S. equicanis* or a mixture of the three species. This is because the sporocyst size of these species is the same (the sporocysts overlap in size). Sporocysts of different *Sarcocystis* species overlap in size and shape, the species cannot therefore be distinguished solely by microscopy. Molecular biological methods involving the polymerase chain reaction (PCR) amplification of species-specific rRNA may be used for this purpose (Swierczynski and Milanesi, 2010).

Sarcocystis tenella, S. capracanis and *S. bertrami* infect sheep, goat and horse respectively. This is indicative that *Sarcocystis* present within the study area affects various animals (including food animals). In sheep, *S. tenella* has being shown to depress wool growth and cause abortion while *S. capracanis* is the most pathogenic specie of *Sarcocystis* in goats. It causes irritability, abortion and death in goats at low doses (Dubey *et al*., 1989). Kudi (1989) identified sporocysts of *Sarcocystis* seen in dog faeces in his study as *S. tenella, S. capracanis* and *S. cruzi.*

In humans, 0% prevalence rate of *Sarcocystis* infection was recorded. This could be as a result of the nature of food preparation in some parts of Africa where food is cooked up to an hour. This is inferred from the respondents who said they cook meat for more than 30 minutes. This cooking duration is enough to kill the sarcocysts if present.

Sarcocysts in pig muscles were rendered non-infectious for puppies after cooking meat at 60^{o}C, 70^{o}C and 100^{o}C for 20, 15 and 5 minutes respectively (Saleque *et al*., 1990).
It could also be that the parasite has not completed the developmental stages for the oocysts/sporocysts to be visualized in the faeces since samples were collected just once from the patients. Sporocysts of *S. hominis* and *S. suihominis* are shed in faeces 14 to 18 days and 11 to 13 days respectively, after ingestion of infected beef and pork respectively (Lund, 2000). Zero prevalence of *Sarcocystis* species in humans recorded in this study could be actual absence of the parasite in humans. The result of this study agrees with the study of Kudi (1989) who also recorded 0% prevalence rate of *Sarcocystis* species in humans.

The present research efforts represents to the best of my knowledge, the second attempt to ascertain the status of human *Sarcocystis* infection in Nigeria. Based on limited surveys (Dubey *et al*., 1989), intestinal sarcocystosis in humans was found more frequently in Europe than other continents (Dubey *et al*., 1989). Findings from other studies around the world reported an occurrence rate of 21.8 % and 0 to 7 % of 926 persons of *S. hominis* and *S. suihominis* respectively in Tibet (Yu. 1991). In 2001, a patient in Spain after eating raw beef had abdominal discomfort and loose stool, and was diagnosed with *S. hominis* oocyst (Cavel *et al*., 2001).

Intermediate hosts such as cattle and pigs become infected with *Sarcocystis* when oocyst/sporocysts of the parasite are ingested while grazing and scavenging respectively. In cattle, the high prevalence (42.5 %) of *Sarcocystis* observed in cattle in this study is in contrast to 0 % prevalence rate reported by Kudi (1989). The increased detection of the parasite or prevalence could be as a result of modification in the

technique of Kudi (1989). The method was modified by mincing and homogenizing the tissues before digestion and staining of tissue sediments with Giemsa stain. These procedures probably enhanced detection of bradyzoites in tissue smears. Also, the source of animals could have contributed to the high prevalence recorded. Presently in Nigeria, most cattle are purchased from neighbouring countries. It could be possible that they acquire the infection from these sources and are transported into the country until slaughtered. Researchers have shown that sarcocyts can persist for months or years in the tissues of intermediate hosts (Fayer, 2004).

In Nigeria, cattle farms /nomadic cattle rearers use dogs as security tools (dog shepherds) to protect their animals from danger. For nomads, they graze their cattle over various pastures moving from one location to another. The presence of dogs and other definitive hosts (including non human primates) in the grazing pastures of the animals ensures shedding of the infective oocysts into the environment, which inturn infects the animals. Moreso, Sporocysts or oocysts of *Sarcocystis* remain viable for many months in the environment and they may be further spread or protected by invertebrates (Dubey *et al*., 1989).

The sarcocysts observed are suggestive of *S. cruzi* and *S. hominis*. *Sarcocystis cruzi* because of its microscopic nature, thin cyst wall and having sarcocysts less than 500 µm in length. Some of the sarcocysts were up to 1215.5 µm in length with thick cyst wall and were microscopic in nature, suggestive of *S. hominis.* In histologic sections, *S. hominis* is difficult to distinguish from *S. hirsuta*. However, the sarcocyst walls of *S. hominis* and *S. hirsuta* can be differentiated ultrastructurally (Dubey *et al*., 1989). More so, the sarcocysts of *S. hominis* is microscopic in nature whereas *S. hirsuta is*

macroscopic at meat inspection (Rommel, 1985). No macroscopic cyst was observed in cattle in this study. Based on the above reasons, one can conclude that the species observed are most probably *S. cruzi* and *S. hominis*.

The observation or finding of *S. hominis* in cattle poses a public health risk because *S. hominis* is pathogenic in humans (Dubey *et al*., 1989). *Sarcocystis cruzi* is the most pathogenic species in cattle where it causes abortion, weight loss, neurologic sign, fever and death (Dubey *et al*., 1989). The result obtained in this study is similar to those recorded in a study by Savini *et al* (1992) which recorded a 52.0 % prevalence rate in Australia. Da Silva (2002) reported 100.0 % prevalence rate in Rio Grande do Sul state while Hamidinejat *et al* (2010) reported 100.0 % prevalence rate in Ahvaz Khouzestan, south west of Iran.

Previous studies have shown that sarcocysts are most commonly seen in the oesophagus than any other parts of the body in cattle (Bottner *et al*., 1987b; Vercruysse *et al*., 1989; Savini *et al*., 1992). The result of this study agrees with these findings. In the present study, age, sex and breed were not significantly associated with *Sarcocystis* infection in cattle. Savini *et al* (1992) reported that *Sarcocystis* infection in cattle in Western Australia was influenced by age, sex, environmental and management factors. In that study, cattle included in the study aged less than 1½ years to greater than 4 years of age. *Sarcocystis* infection was found to significantly drop in cattle greater than 4 years of age. In the present study, the age range of cattle sampled was from 5 to 11½ years (older cattle). This factor could have masked the effect of age on *Sarcocystis* infection as calves were not sampled. A lower prevalence was observed in the older cattle when compared to cattle between 5 to 8½ years, although not significant. Host

immune response to the infection might have being responsible for the fall in prevalence in the older cattle. Broken and degenerating sarcocysts surrounded by host inflammatory reactions in muscle tissue of infected pigs and goats have been reported (Barrows *et al*., 1982b; Dubey, 1983b). The rupture of the sarcocysts, which may occur spontaneously or be caused by a host-immune reaction, is more likely to occur in old cysts found in older animals and may cause progressive reduction in cyst number over time (Barrows *et al*., 1982b; Dubey, 1983b).

The finding of the present study is in contrast to the findings of Savini *et al* (1992) who reported significant effect of sex on *Sarcocystis* infection in cattle. Nonetheless, it is worthwhile to note that despite fewer bulls were sampled when compared to cows, almost half of the bulls were infected in contrast to infected cows. Breed was also found not to influence *Sarcocystis* infection in cattle. It could be indicative that perhaps the 2 breeds of cattle investigated had equal chance of being infected by *Sarcocystis* species. The absence of *Sarcocystis* species in the faecal samples of cattle; an intermediate host, is in conformity with the known life cycle of the parasite where intermediate hosts only harbour the muscular stage and definitive hosts harbour the intestinal stage of the parasite.

In pigs, a high prevalence rate (60.0 %) of *Sarcocystis* species was recorded. This study represents the first research efforts to the best of my knowledge on sarcocystosis in pigs in Nigeria. Pigs are food animals which are reared and consumed majorly by Christians resident in the study area. Apart from being food animal, pig husbandry is being practiced as a source of income for some families in form of backyard farming. The high prevalence of *Sarcocystis* infection recorded could be as a result of the

management system practiced by pig owners where they are allowed to roam about and scavenge for food. In the process, they pick up the oocyst/sporocysts of *Sarcocystis* shed by different definitive hosts such as dog, cat, humans and non-human primates, thus resulting to sarcocyst formation in the musculature of the pigs (Dubey *et al*., 1989).

In Ghana, a prevalence rate of 28.3 % of *Sarcocystis* species in pigs was reported (Permin *et al*., 1999). Other reports include 18.0 % prevalence rate in Iowa, 27.0 % in the Philippines, 43.0 % in Spain, 57.0 % in Uruguay and 68.0 % in India (Titi Tudorancea Bullentin, 2010).

The sarcocysts observed in pigs are suggestive of *S. meischeriana* because they were microscopic in nature with thick cyst wall and up to 1400 µm in length. Also, most of the sarcocysts seen were thin walled with a range of 104.69 to 205.77 µm in length which could be possibly *S. porcifelis.* There are three recognized species of *Sarcocystis* in pigs; *S. meischeriana*, *S. suihominis* and *S. porcifelis*. They are all pathogenic in pigs. *S. porcifelis* was reported but the structure of the sarcocyst was not described (Dubey *et al*., 1989). The sarcocyst of *S. suihominis* is thick walled but macroscopic in nature (Fayer, 2004). No macroscopic cyst was observed in pigs in this study. Based on the above reasons, one can conclude that the species observed in pigs in this study are most probably *S. meischeriana and* possibly *S. porcifelis*.

S. meischeriana has being shown to cause fever, weight loss, purpura of the skin, muscle tremors, abortion and death in infected pigs (Rommel, 1985; Casparii, 2011).

Sporocysts of *S. porcifelis* were fed to pigs and the pigs became ill and one died 89 days post inoculation (Dubey *et al*., 1989).

Findings from this study showed that there was no significant difference in the level of infectivity between oesophagus and diaphragm in pigs in contrast to cattle. Perhaps it could be as a result of the degree of infection recorded which is higher in pigs than in cattle, evident by the scavenging and rooting behaviour of pigs. Pigs can eat anything ranging from faeces (from any source), carcasses, waste products and from gutters too. Consequently, they can pick all sorts of parasites and microorganisms resulting to infection which could be either sub-clinical or clinical.

In the present study, sex was found not to influence *Sarcocystis* infection in pigs. Both male and female pigs are allowed to roam about and scavenge for food by pig farm owners who practice extensive system of management. This gives equal chance to both sexes of picking up parasites. This system of management is practiced by farm owners in the study area. The absence of *Sarcocystis* species in the faecal samples of pigs; an intermediate host, is in conformity with the known life cycle of the parasite where intermediate hosts only harbour the muscular stage and definitive hosts harbour the intestinal stage of the parasite.

Tissue digestion was found to be more efficient in detecting sarcocysts/bradyzoites in tissues than histology. Digestion of host tissues is the most sensitive method to detect light *Sarcocystis* infection because several hundreds or thousand of bradyzoites are released from sarcocysts as the host tissue and sarcocysts are digested making detection of the parasites easier (Dubey *et al*., 1989). In histology, only thin sections (5 micron)

are taken at a time which may not contain any cyst (Dubey *et al*., 1989). This finding agrees with the findings of Kudi (1989) and Hamidinejat *et al* (2010).

The presence of lymphocytes in some of the tissues is indicative of the chronic stage of the infection. Little is known of the pathogenesis of chronic sarcocystosis. Some sarcocysts probably rupture from time to time releasing toxic product; sarcotoxin (Dubey *et al*., 1989). Also, it has been postulated that substances released from *Sarcocystis* might stimulate tumour necrosis factor (TNF) (Fayer and Prasse, 1981) which is known to be associated with wasting disease. Presence of eosinophils is indicative of the parasitic nature of the organism, as the body reacts due to the presence of a parasite.

Findings of this study showed that management of the risk factors by the respondents did not enhance the transmission of the parasite even if it is present. All the respondents claimed to cook their meat for more than 30 minutes; a duration that is sufficient enough to render the bradyzoites non-infectious. Sarcocysts in pig muscles were rendered non-infectious for puppies after cooking meat at 60^{o}C, 70^{o}C and 100^{o}C for 20, 15 and 5 minutes respectively (Saleque *et al*., 1990).

A larger proportion of the respondents consume processed meat which included suya (smoked meat), meat pie, beef burger and sausages which may reduce the risk of infection as compared to the consumption of raw meat. In contrast, the consumption of these products regularly as reported by a proportion of the respondents could present a risk for infection, if infected meat is used in the preparation of theses products and if the heat treatment is not sufficient enough to destroy the bradyzoites. Khaniki and Kia

(2006) reported a prevalence rate of 6.25 % (5 out of 80 samples) of *Sarcocystis* in hamburgers samples collected from retail stores in Garmsar; Iran.

Water contamination with sporocysts of *Sarcocystis* from a carnivore or omnivore or foods washed or irrigated with contaminated water is the most likely source of sporocysts for human infection (Fayer, 2004). A larger proportion of the respondents consume vegetables which were mostly washed twice with water and salt. Other used vinegar, water guard and potash. These agents could be sufficient enough to destroy any parasite ova or oocyst present in the vegetables.

Almost half of the respondents had access to pets directly or indirectly while a smaller proportion (8.2 %) fed raw meat to their pets. This poses a great risk for *Sarcocystis* infection for both food animals and man in the environment. Consumption of raw meat by dogs results in the shedding of the oocyst/sporocysts of the parasite into the environment and this might have been the source of infection for the positive case recoreded in dogs. Locally known predators such as dogs, cats, snakes could excrete infectious sporocysts that find their way through contaminated water and food eventually infecting humans (Kan, 1985; Fayer, 2004).

A proportion of the respondents drink well water and had wells which were not cemented, not covered and at ground level.Wells which are either at ground level, not cemented or not covered, could possibly be contaminated with sporocysts of the parasite from local predators, which eventually infects man when such water is consumed without adequate heat treatment.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Prevalence studies for *Sarcocystis* species was carried out in Zaria, Kaduna state.

Of a total of 200 dogs and 200 humans, none of the human patients sampled were shedding sporocysts at the time of examination, while 1(0.5 %) dog was positive for *Sarcocystis* species. Faecal samples of cattle and pigs were not positive for *Sarcocystis* species

Prevalence studies of 200 cattle and 100 pigs showed that 85 (42.5 %) cattle and 60 (60.0 %) pigs were positive for sarcocysts. Age, sex and breed did not significantly influence the prevalence of *Sarcocystis* infection.

Tissue digestion was found to be more efficient in detecting sarcocysts/bradyzoites in tissues than histology. Sarcocysts were found more in the oesophagus than in the diaphragm in cattle while in pigs, there was no significant difference in the level of infectivity between oesophagus and diaphragm. Leucocytic infiltrations were observed in the oesophagus of cattle and pigs which were mostly lymphocytes and few eosinophils.

Attempts were made to identify the species of *Sarcocystis* seen through sporocyst size measurement, sarcocyst size measurement, nature of sarcocyst and cyst wall thickness. The species that were identified through sarcocyst measurement were *S. cruzi* and *S. hominis* in cattle, and *S. meischeriana* and possibly *S. porcifelis* in pigs. *S. tenella, S.*

capracanis or *S. bertrami* or a mixture of the three species were identified through sporocyst measurement.

Assessment of risk factors and awareness of *Sarcocystis* infection among respondents showed that none of the respondents were aware of the infection and there was a reduced risk for infection in humans. Although, potential risks were identified by means of regular consumption of fast food meat products, feeding of raw meat to pets, access to pets and possession of wells which were either not covered, not cemented and at ground level.

6.2 CONCLUSIONS

This study has established in the study area the prevalence of *Sarcocystis* infection in cattle and pigs using tissue digestion method and histology, and in dogs using sucrose floatation technique. The identified species were of veterinary and public health importance and age, sex and breed were not determinants of the infection. Residents in the study area were unaware of the infection and their food habits might have contributed to the zero prevalence recorded. The relatively high prevalence of *Sarcocystis* in muscle of cattle and pig indicates that the parasite is readily available and suggests the shedding of oocysts or sporocysts by definitive hosts into the environment, which contaminates the pastures for cattle. Pigs are exposed to all kinds of parasites and microorganisms because of their scavenging and rooting behaviour.

6.3 RECOMMENDATIONS

1. Adequate heat treatment of meat before consumption is advocated.
2. Feeding of raw meat to pets should be discouraged.
3. Drinking water should be safe and potable.
4. Farm dogs should receive adequate medical attention via deworming and other prophylactic treatment.
5. Abattoirs should be fenced to prevent the entrance of stray dogs and other carnivores.

Limitations of this study include financial, time, material and other logistical constraints. However, I learnt a lot from the research and hope it serves as a challenge for other researchers in Nigeria with common interest to carry on with further studies on the parasite, with this research findings serving as additional information (update) to the existing information.

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APPENDIX I

QUESTIONAIRE ON FACTORS INFLUENCING THE DISTRIBUTION OF *SARCOCYSTIS* SPECIES IN ZARIA, KADUNA STATE.

No……. **Category**.....

Area……**Other information**……..

Note: All information provided is strictly confidential and are for the purposes of this research study.

1. Sex (a) Male (b) Female
2. Age……
3. Occupation……
4. Level of education ……
5. Where do you live? .....
6. Do your meals include meat? ……
7. If yes, what kind of meat……
8. How do you prepare your meat before eating?

……

9. For how long do you prepare your meat? …….
10. Do you eat dog meat? ……...
11. Do you eat pork (pig meat)? ……
12. What do you often eat? …….....
13. Do you eat
    a) Suya Yes\ No
    b) Burger Yes\ No
    c) Sausages Yes\ No
    d) Meat pie Yes\ No

14. How often do you eat suya\burger\sausages\meat pie? ……

15. Does your meal involve vegetables? ……

16. How do you prepare your vegetables before eating? .....

17. What do you wash your vegetables with? …….

18. How many times do you wash your vegetables before cooking? ……

19. Do you have pets?……

20. Do you have access to pets? ……

21. What kind of pet? .....

22. Are your pets restricted? .....

23. What do you feed your pets with? ……

24. Do you feed raw meat to your pet(s)? .....

25. If yes, what type of meat? …….

26. What is the source of your drinking water? ……..

27. Do you have a well in your home? ……..

28. What do you use your well water for……

29. Describe your well

……..……

30. Test result……..……

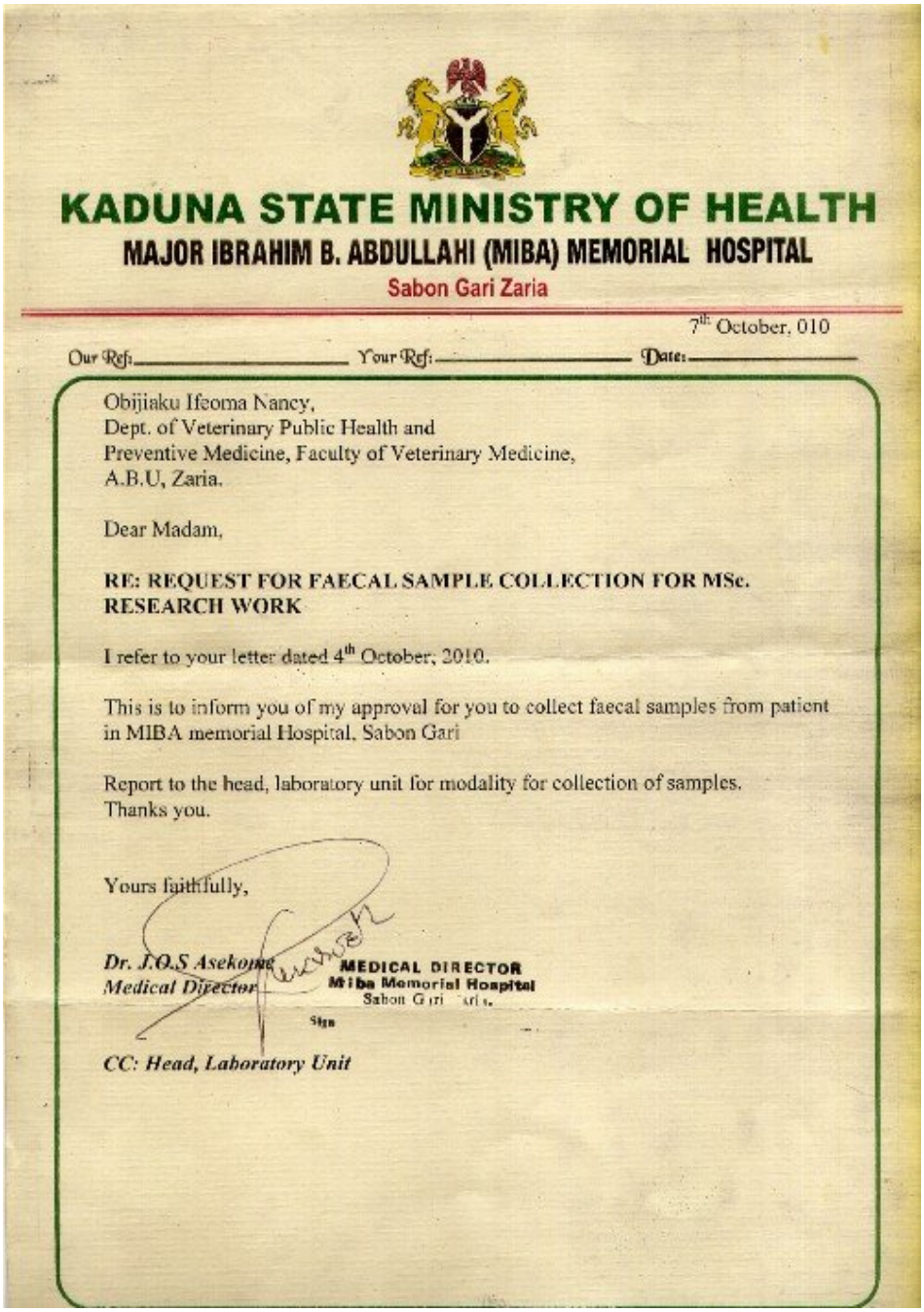
31. Have you heard about the infection Sarcocystosis? ……

32. What do you know about the infection? .....

…….

Thank you.

APPENDIX II



KADUNA STATE MINISTRY OF HEALTH

MAJOR IBRAHIM B. ABDULLAHI (MIBA) MEMORIAL HOSPITAL

Sabon Gari Zaria

7th October, 010

Our Ref:_____ Your Ref:_____ Date:_____

Obijiaku Ifeoma Nancy,
Dept. of Veterinary Public Health and
Preventive Medicine, Faculty of Veterinary Medicine,
A.B.U, Zaria.

Dear Madam,

RE: REQUEST FOR FAECAL SAMPLE COLLECTION FOR MSc. RESEARCH WORK

I refer to your letter dated 4th October, 2010.

This is to inform you of my approval for you to collect faecal samples from patient in MIBA memorial Hospital, Sabon Gari

Report to the head, laboratory unit for modality for collection of samples.
Thanks you.

Yours faithfully,

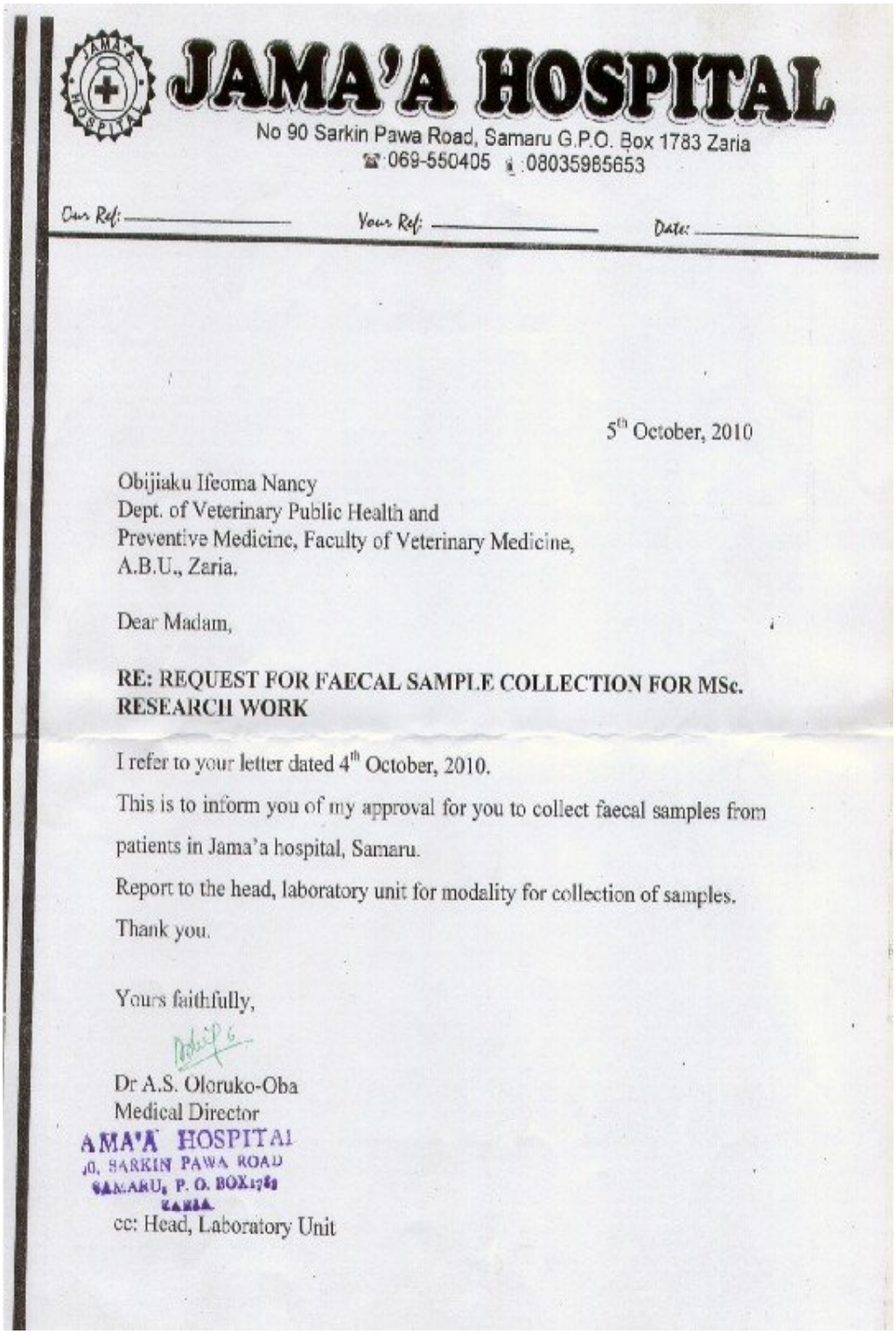
Dr. J.O.S Asekome
Medical Director

MEDICAL DIRECTOR
Miba Memorial Hospital
Sabon Gari Zaria.

Sign

CC: Head, Laboratory Unit

APPENDIX III



JAMA'A HOSPITAL

No 90 Sarkin Pawa Road, Samaru G.P.O. Box 1783 Zaria
☎ 069-550405 ; 08035985653

Our Ref: _____ Your Ref: _____ Date: _____

5th October, 2010

Obijiaku Ifeoma Nancy
Dept. of Veterinary Public Health and
Preventive Medicine, Faculty of Veterinary Medicine,
A.B.U., Zaria.

Dear Madam,

RE: REQUEST FOR FAECAL SAMPLE COLLECTION FOR MSc. RESEARCH WORK

I refer to your letter dated 4th October, 2010.

This is to inform you of my approval for you to collect faecal samples from patients in Jama'a hospital, Samaru.

Report to the head, laboratory unit for modality for collection of samples.

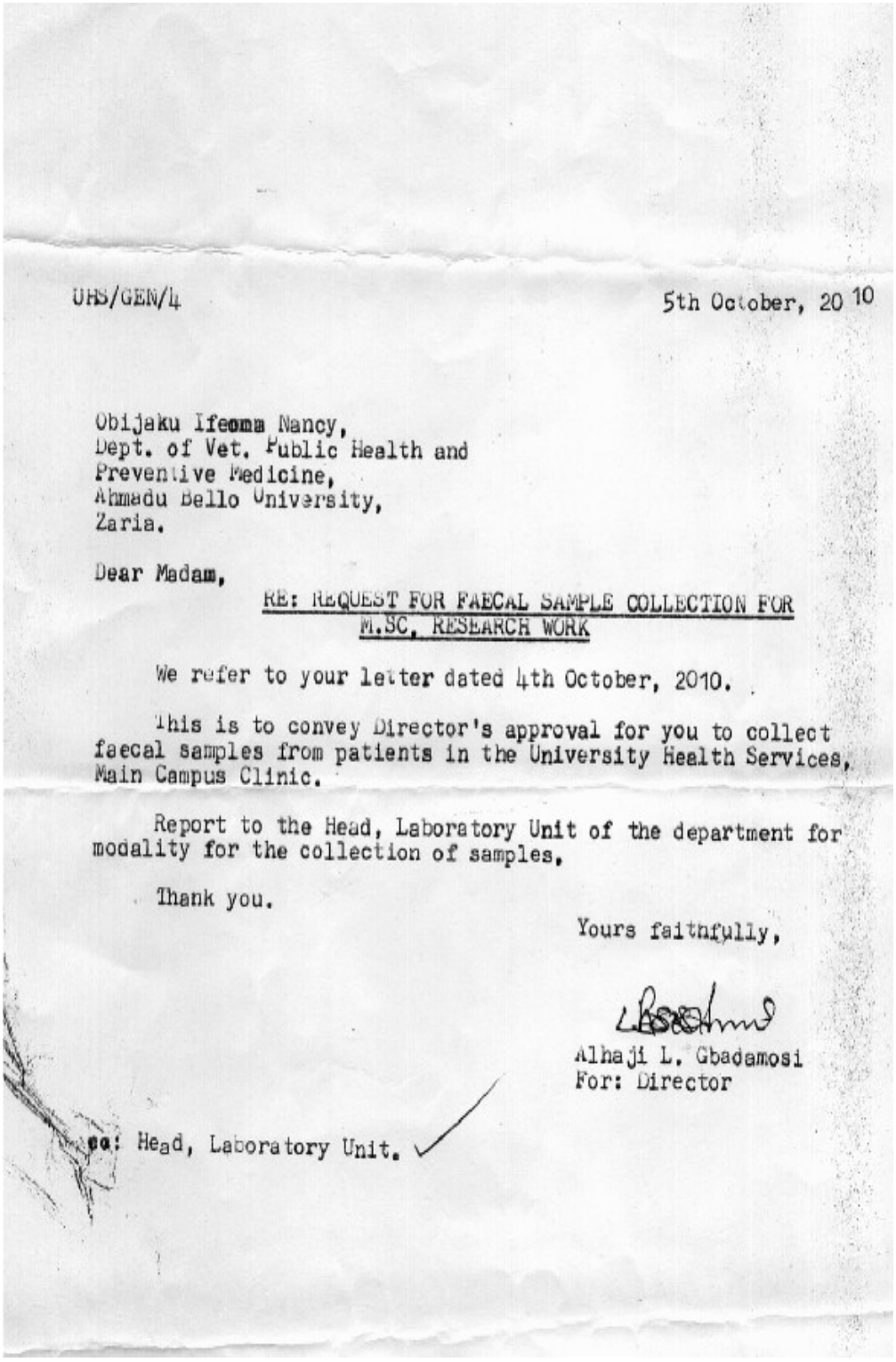
Thank you.

Yours faithfully,

Dr A.S. Oloruko-Oba
Medical Director
JAMA'A HOSPITAL
NO. SARKIN PAWA ROAD
SAMARU, P. O. BOX 1783
ZARIA

cc: Head, Laboratory Unit

APPENDIX IV



UHS/GEN/4

5th October, 2010

Obijaku Ifeoma Nancy,
Dept. of Vet. Public Health and
Preventive Medicine,
Ahmadu Bello University,
Zaria.

Dear Madam,

RE: REQUEST FOR FAECAL SAMPLE COLLECTION FOR M.SC. RESEARCH WORK

We refer to your letter dated 4th October, 2010.

This is to convey Director's approval for you to collect faecal samples from patients in the University Health Services, Main Campus Clinic.

Report to the Head, Laboratory Unit of the department for modality for the collection of samples.

Thank you.

Yours faithfully,

Alhaji L. Gbadamosi
For: Director

cc: Head, Laboratory Unit.