THE ROLE OF SELECTED ARTHROPODS IN THE TRANSMISSION OF POULTRY CESTODIASIS

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY, UNIVERSITY OF NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY ENTOMOLOGY.

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OCTOBER, 2012
DECLARATION

I declare that the work described in this dissertation is my original work and has not been previously submitted for any degree to any university or similar institution.

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CERTIFICATION AND APPROVAL

This is to certify that Dr. Iheagwam, Chijioke Nwabueze, a postgraduate student in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka and with Registration Number PG/MSc/05/40140, has satisfactorily completed the requirements for research work for the degree of Masters of Science in Veterinary Entomology. The work embodied in this dissertation is original and has not been submitted in part or in full for any other degree of this and any other university. The dissertation has therefore been approved for the award of Master of Science in the department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.

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DEDICATION

This dissertation is dedicated to my beloved friend and wife, Blessing and to my lovely daughter, Munachimso
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ABSTRACT

Intermediate hosts are usually necessary for the transmission of cestode infections and insects which are a group of arthropods, constitute a greater population of such intermediate hosts. Many of these insects are common in the environment, in and around poultry houses as well as in poultry feed and its raw materials. Three of such insects namely *Tribolium castaneum* (Flour beetle), *Alphitobius diaperinus* (Litter beetle), and *Periplaneta americana* (American cockroach) were investigated for their possible roles as intermediate hosts in the transmission of poultry cestodiasis using the cestode model *Hymenolepis diminuta*. *Hymenolepis diminuta* eggs were harvested from the faeces of infected rats. In all the experiments, insects were starved for 5 days prior to exposure to a number of eggs incorporated in small amounts of feed to ensure consumption. In the first experiment, 3 different groups of 10 flour beetles each were exposed to eggs from fresh, 7 and 14 days old faeces to ascertain the viability of the eggs post defaecation. The second experiment assessed the vectoral potential of these insects whereas the final experiment evaluated the effect of starvation on cysticercoids establishment and development was also assessed. In all cases insects were dissected 2 weeks post infection and examined microscopically for cysticercoids. Data generated were presented as percentages and means. The results of the study showed that the eggs of *Hymenolepis diminuta* survived in faeces up till the 14th day post defaecation as observed by the presence of cysticercoids in beetles fed eggs harvested from those faeces. Sixty percent (60%) of the beetles infected with eggs from freshly voided faeces contained cysticercoids but this reduced to 38% in those fed with eggs from 14 days old faeces. The capacity to serve as an intermediate host was observed only in flour beetles in which 52.8% of all the infected beetles supported cysticercoid development. *Alphitobius*
*diaperinus* and *Periplaneta americana*, however, did not support cysticercoid development. Starvation affected cysticercoid establishment and development. Developmental alterations were observed as slight morphological variations, in the form of smaller sized cysticercoids in beetles from the starved group when compared with those in the fed group. The number of cysticercoids in starved beetles were remarkably higher (75%) than in the fed ones (35%) and the number per infected beetle ranged from 1-3. We therefore conclude that *Tribolium castaneum* can serve as a vector in the capacity of an intermediate host for poultry cestodes since they supported the development of cysticercoids which are the forms infective to the final host and are commonly found in poultry feed, feed raw material as well as in and around poultry facilities. Also, the starvation of this beetle species does not prevent the establishment of cysticercoids within them but probably promotes it, though with slight morphological variations from the normal.
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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The effects or roles of environmental factors in the sustainability of poultry production are unequivocal. However, the impact of creatures found in and around poultry houses especially in the tropics and subtropics is largely unknown. Over the years however, the presence of some of these creatures in and around poultry houses has been known. Among these include aves, reptiles, ants, beetles, weevils and bugs. Others are flies, rodents, cockroaches and endoparasites. The roles associated with most of them in the poultry houses are highly varied and characteristically permanent (Nwokoro et al., 2007). For instance, considerable losses are sustained in the production of meat and egg each year through a multitude of parasitic worms (Cavier, 1973). The major groups of parasites concerned are the gastrointestinal nematodes, cestodes and trematodes. Parasitic gastroenteritis is highly prevalent in widespread areas of Nigeria and results in enormous economic losses due to the associated morbidity, mortality and reduced productivity (Ahmed et al., 1994), and sometimes susceptibility to other microorganisms (Soulsby, 1982).

The poultry industry occupies an important position in the livestock industry of any nation and plays a vital role in national development through the generation of revenue for individuals, corporate bodies and government. Poultry is one of the most profitable agricultural business and certainly the most profitable livestock enterprise (Obioha et al., 1992).
Poultry production offers the hope in most developing countries for bridging the widening gap between demand and supply for animal protein intake of the populace (Ganiyu, 2005). Poultry, in particular domestic fowl, is a common food in the Nigerian diet. It is among the main if not the major source of animal protein, particularly in southern Nigeria (Obanu et al., 1984).

Globally, poultry is amongst the most intensively reared of all domesticated animals and generally the poultry production in Africa and other parts of Asia can be subdivided into commercialized and traditional subsectors, each having it’s own peculiarities. The commercialized subsector comprises of the distinctive units based on primary products into parent stocks, layers and broilers. It is confined mainly to the urban and peri-urban areas where the infrastructure necessary for production and market exist (Njue et al., 2001). These involve the use of improved breeds or strains selectively bred for improved meat and/or egg production. The traditional subsector also called rural or backyard production consists of local indigenous birds which have not been classified into breeds and involve many ecotypes (Njue et al., 2001). The local chicken is also called village chicken, backyard chicken or free-range chicken. They are indigenous to the particular locality where they are found. The term best describes the scavenging chickens because of the effect of the village socio-economic and biophysical environment on the production and health status of the chickens (FAO, 1996).

Returns from poultry are quick if appropriate and standard management practices are strictly adhered to. Under such situations poultry is seen to be efficient converter of feed to meat and egg within a short period.

The poultry industry provides employment both directly and indirectly, directly in poultry production enterprises and poultry product processing enterprises and indirectly in industries
which produce materials required by the industry, for instance, vaccine production, feed manufacturing and poultry equipment manufacturing. Besides, more and more people are involved in present day transportation and storage of poultry products and financing of the industry by the private sectors are also sources of employment. The industry also provides a basis for the establishment of agro based/allied industries to process by-products such as feather, offal and droppings into more manageable products such as pillows, feeds and manure respectively.

1.2 STATEMENT OF THE PROBLEM

The above merits notwithstanding, sustainable development of this sector is faced with quite a number of constraints, prominent among which are diseases especially helminthoses. The effects of helminths on their host are generally insidious, undermining host health and increasing susceptibility to secondary infections with ensuing mortality, particularly when compounded by additional stress such as poor hygiene, overcrowding, concurrent infections and malnutrition among others. Parasitized birds may be emaciated, stunted, diarrhoeic, weak or paralyzed (Hungerford, 1979; Oluyemi and Roberts, 1980; Soulsby, 1982; Gordon and Jordan, 1985). Various pathologic conditions such as severe catarrhal inflammation, ulceration and thickening of the gastrointestinal tract (Hungerford, 1979; Gordon and Jordan, 1985; Okoye and Chime, 1988) and production losses such as drop in egg production (Fabiyi, 1972; Malaki, 1976), low carcass grade or mortality especially in acute cases (Hungerford, 1979; Oluyemi and Robert, 1980) may be observed. Apart from the economic losses due to reduced production associated with helminth infections, several helminthic infections remain major public health problems (Adebisi, 2008).
Most poultry and mammalian gastrointestinal helminthoses occur through accidental ingestion of the various intermediate hosts in which the metacestodes develop. The intermediate hosts in most cases are arthropods, which birds or other mammalian hosts readily pick up while feeding, either accidentally or purposely, due to the relative abundance of these arthropods in the environment. The role of arthropods as intermediate hosts could to a large extent depend on the type of management system in use.

A great number of cestode parasites have an indirect lifecycle and therefore require suitable intermediate hosts to aid their survival and propagation. Most cestodes associated with the terrestrial food chain (cyclophyllideans), use various arthropods which serve as obligatory intermediate hosts; these are either coprophagous or scavengers in their feeding habits during their larval and adult stages. These habits enable them to take up the eggs, which might be contained in the faeces of the definitive host or a dead or decaying definitive host. This gives room for further development to a metacestode stage which is infective to a susceptible final host provided the ingested eggs contains viable onchospheres. Some of these arthropods include fleas; *Xenopsylla cheopsis, Ctenocephalides canis, Pulex irritans* (infection occurs during the larval stages), beetles; *Tenebrio molitor* (larva), *Dermestes peruvianus, Tribolium castaneum* etc. and cockroaches; *Periplaneta americana, Blatella germanica, Blaberus giganteus and Parcoblatta* species to mention a few. Some, such as the cockroaches have been suspected to serve as vectors but their precise roles in the transmission of helminth diseases has not been fully assessed (Young and Babero, 1975).

The indiscriminate disposal of both animal and human waste coupled with the cosmopolitan nature of arthropods and their diverse feeding habits predisposes these arthropods to a variety of
cestode infections to which they can serve as intermediate hosts. Poultry on the other hand, to a very large extent readily feed on arthropods and this predisposes the birds to a variety of cestode infections and likely other helminth infections that could be harboured by these arthropods, resulting in a gradual decline of the health status and productivity of affected birds and ultimately economic losses of varying magnitudes.

1.3 OBJECTIVES OF THE STUDY

This study has the following specific objectives:

1. To determine the survival of *Hymenolepis diminuta* in faecal samples and their viability in *Tribolium castaneum*.

2. To determine the vectorial potential of *T. castaneum*, *A. diaperinus* and *P. americana* in the transmission of cestodes using *Hymenolepis diminuta* as a model.

3. To assess the effect of starvation of *T. castaneum* on the establishment and development of *Hymenolepis diminuta* larval stages.
CHAPTER TWO

LITERATURE REVIEW

Arthropods continually infest livestock and these infestations can result in the transmission of disease pathogens, which in turn can cause lower body weight, reduced meat, milk and egg production, slower development and in many cases death (Seller, 1981). In addition, they cause direct injury to livestock and reduced economic returns because of severe annoyance and blood loss (Steelman, 1976).

Despite increased research in and understanding of arthropod-borne diseases and significant advances in technology aimed at disease detection, control and prevention, debilitating and often fatal diseases associated with insects and other terrestrial arthropods continue to plague people, their domestic animals, pets and wildlife. Rapid transportation of people, domestic animals and commodities, emerging or re-emerging pathogens and their vectors put nearly everyone at risk of contracting arthropod-borne diseases (Eldridge and Edman, 2004).

2.1 ARTHROPODA

The Arthropoda (Arthropod= “jointed-footed”) is an enormous phylum in the animal kingdom and contains over 80 percent of all known species of animals. It consist of invertebrates whose major characteristics are a hard chitinous exoskeleton, a segmented body and jointed limbs (Urquhart et al., 1987). This phylum is the most successful life-form on earth in terms of variety because they are able to exploit a wide range of ecological niches. They have a versatile exoskeleton; highly protective and mobile, and prevent dehydration relatively. They are also the most successful life-forms as they have highly developed sensory organs and cells that are
directly ventilated in land arthropods through direct gaseous diffusion into small circular openings called spiracles in the cuticle of the exoskeleton (tracheal system). From the spiracle, the air enters a system of branching tracheae and tracheoles which ramify through most parts of the body. In aquatic species, gaseous exchange is not via the spiracles but rather through direct integumentary/cuticular respiration (Romoser, 2004; Urquhart et al., 1987; Service, 1980). Because of the different types of foods ingested, there is great diversity in the morphology of the alimentary canal (Urquhart et al., 1987). The feeding activity of arthropods usually causes trouble of varying degrees for man and animals. According to Romoser (1996), arthropods can be recognized as being saprophagous, phytophagous, mycetophagous, carnivorous or omnivorous depending on the source of nutriment. The body cavity (coelom), which is the space between the alimentary canal and body wall, is often called a haemocele because it contains the arthropod’s blood (Haemolymph). This blood is colourless and carries out most of the functions of both blood and lymph, which are separate fluids in vertebrates. While haemolymph contains both cellular and fluid portions, as in vertebrates, it differs from vertebrate blood in that except over very short distances, it does not transport oxygen to the tissues, this is done by the tracheal system. The haemolymph has several functions which include, the transportation of molecules from digested food materials absorbed into the midgut cells and released into the haemocoel, thus serving as a reservoir for raw building materials, the transportation of metabolic waste such as CO₂, nitrogenous wastes of protein metabolism from the tissues to the malpighian tubules or other excretory, it acts as a hydraulic medium (as seen in the hydraulic effect which aids the splitting and shedding of old cuticle during moulting) and it also functions in the lubrication of the various tissues and organs within the insect (Romoser, 2004). The haemocytes which are blood cells are not concerned with respiration but are mainly phagocytic (Service, 1980).
Sexes are separate and growth is restricted to the immature stages of their life-cycle and can be achieved only by casting off the exoskeleton; a process known as moulting or ecdysis. The transformation from the egg stage through the immature stages to the final adult form is referred to as metamorphosis, a word meaning “change in form”. There are two types of metamorphosis namely; Holometabolous (complete) and Hemimetabolous (Incomplete) (Urquhart et al., 1987; Service, 1980).

Arthropods exhibit complex behaviours and evolution of social systems. It is therefore not surprising that many groups have exploited their parasitic lifestyle spending a portion of their life attached to a host (ectoparasites, like ticks and mites) while some act as intermediate host for parasitic diseases like flea, lice, flies, beetles etc. According to Romoser (2004), the phylum arthropoda is made up of four classes as follows:

- **Class crustacean**: they possess mandibles for feeding, and biramous appendages E.g. crabs, shrimps, lobsters, barnacles etc.

- **Class myriapoda**: possess mandibles for feeding, unbranched appendages on all post-headed segments. E.g. centipedes, millipedes etc.

- **Class arachnida/chelicerata**: possess chelicerae for feeding, most with unbranched appendages. E.g. spiders, ticks etc.

- **Class insecta/hexapoda**: possess mandibles, uniramous appendages E.g. beetles, ants etc.

Most of the arthropods suspected to be involved in the transmission of poultry cestodiasis fall under the class insecta. Some members of this class are holometabolous, that is, they undergo complete metamorphosis (complete lifecycle) and a good number of them are considered
harmful. For this reason, there is a tendency to be overly preoccupied with insects as pest because of the damage they do and their importance in relation to human and animal affairs.

The definition of pest can vary in detail according to the precise context in which it is considered but in the widest sense, a pest is an insect (or organism) that causes harm to humans, their livestock, crops or possessions. The key word is “harm” and is usually interpreted as “damage” which of course, can often be quantitatively measured (Hill, 1997).

The arthropod species considered in this work all qualify to be grouped as pests because they cause harm to man, his livestock, crops (on the field and during storage) and his possession.

2.2 The role of arthropods in human and animal health

Medical entomology addresses the important role played by arthropods in the dissemination of diseases in people and other vertebrate animals. Arthropods can cause diseases directly (2-component relationships) or serve as vectors or mechanical carriers of pathogenic microorganisms (3-component relationships). Arthropods serving as vectors of disease pathogens represent by far the most significant aspect of the relationships between arthropods and vertebrate animals. In many of such cases, the arthropod serves as a host, at times the primary host, for the pathogen as well. On the other hand, direct association with arthropods can cause allergic reactions, physical injury and secondary infection due to the arthropod-induced injury as well as injury resulting from severe scratching of the affected areas by the animal (Eldridge and Edman, 2004).

According to Barnett (1962), certain criteria have to be met by arthropods before they are considered as vectors of human or animal pathogens. These include:
• Demonstration that members of the suspected arthropod population commonly feed upon vertebrate hosts of the pathogen, or otherwise make effective contact with the hosts under natural conditions;

• Demonstration of a convincing biological association in time and space between the suspected vectors and clinical or subclinical infections in vertebrate hosts;

• Repeated demonstration that the suspected vectors, collected under natural conditions, harbor the identifiable infective stage of the pathogen, and;

• Demonstration of efficient transmission of the identifiable pathogen by the suspected vectors under controlled experimental condition.

2.2.1 Arthropods as direct causes of disease

Arthropods directly affect human and animal health in various ways. These include

2.2.1.1 Ectoparasitoses

These are diseases caused by various kinds of contact between arthropods and the external body surfaces of hosts. Arthropods blood-feeding, burrowing, crawling or scraping at and just beneath the skin surface cause adverse reactions, which could manifest as dermatoses, allergic reactions, reduced growth rate and feed conversion efficiency and decrease in or total loss of productivity. For instance, it is known that in cattle, the activities of ectoparasites cause weight loss and lowered milk production (Steelman, 1976) and result in costly damage to hides and skin and exposure to secondary bacterial infection which in severe cases may result in death.
2.2.1.2 Endoparasitoses

Some arthropods invade tissues or body cavities of vertebrate host. When the invasion is by diptera larvae, the condition is called myiasis. Although humans can suffer from myiasis, it is much more common in livestock and wildlife. Examples of myiasis include, tumbu fly myiasis caused by the larvae of *Cordylobia anthropophaga*, the nasal myiasis caused by the larvae (nasal bots) of *Oestrus ovis* and somatic myiasis by the larvae of *Gastrophilus* species (bot flies), though it is considered of little pathogenic significance (Eldridge and Edman, 2004; Urquhart *et al.*, 1987).

2.2.1.3 Envenomization

This is the introduction of venom or other toxins by arthropods through stinging or biting. Toxins are often proteinous substances produced by a variety of organisms and which cause poisonous reactions in other animals. Examples include, bees and the blister beetle with urticating hairs which have open tips for ejecting venoms.

2.2.1.4 Allergy

Most animals have physiological mechanisms that defend against the introduction of foreign or non-self substances. A foreign substance that results in the production of antibodies by the animal is called an antigen (mostly proteins). Antigens that produce unusually strong defensive reactions in animals are called allergens and the conditions in animals are allergies. Generally an allergy is a hypersensitivity disorder of the immune system. These hypersensitive reactions often are associated with adverse symptoms such as itching, redness, swelling and rash. Venoms of
stinging insects such as wasps and bees can also act as allergens, and can result in a particularly serious condition known as anaphylaxis (Kay, 2000).

2.2.1.5 Annoyance

Arthropods can cause considerable discomfort and annoyance to people and their animals merely by their presence and normal activities, even when they produce no serious physical harm. The larger the number of arthropods the greater the possibility of severe annoyance and this ultimately affects productivity of both humans and animals.

2.2.2 Arthropods as vectors and hosts of pathogenic microorganisms.

Arthropods serve as vectors and intermediate hosts of pathogens of both humans and other vertebrate animals. Several arthropod-borne infectious diseases affect livestock and wildlife with some being zoonoses, humans at times serve as dead-end hosts of the pathogens.

The most significant role played by arthropods in human and animal health is as vectors of pathogenic microorganisms. In epidemiology, a vector is any agent (person, animal or microorganism) that carries and transmits an infectious pathogen into another living organism (Roberts et al., 2008). An intermediate host can equally be considered a vector but it performs this function in a rather passive way as opposed to others that are actively involved in the transfer of pathogen from one host to another. Even though vertebrates can serve as vectors, invertebrate animal species make up a great percentage of vectors and in addition to hosting the development and/or reproduction of the parasite, they also serve as the vehicle for the transfer of the infectious agent between infected and non-infected vertebrates (Edman, 1988). There are instances of disease transmission involving no arthropod vector, but arthropods can serve as
intermediate hosts of a disease pathogen. Examples include the common domestic dog and cat tapeworm, *Dipylidium caninum* having the fleas (*Ctenocephalides canis* and *C. felis*) as intermediate hosts, the anoplocephalid tapeworms (*Anoplocephala* species and *Moniezia* species) having various forage mites in the soil and pasture as intermediate hosts and small aquatic arthropods which serve as intermediate hosts of other parasitic flatworms (Eldridge and Edman, 2004; Urquhart *et al*, 1987).

There are two principal ways through which arthropods transmit disease-producing organisms between animals. The first and even more important mode of transmission is known as the **biological transmission**, in this mode the vector or host species acquire the pathogen from an infected animal, usually during feeding activity. Generally, the acquired pathogen requires a certain period of time between ingestion by the vector and the subsequent transmission to a new host. During this time interval, the pathogen undergoes a vital stage of development inside the arthropod vector and becomes infective. In many cases, this period of time is also accompanied by movement of the infected vector to a new location proximate to other host animals and finally, the pathogen can then be transmitted when the vector next feeds or when it is accidentally ingested by a suitable definitive host (Reid, 1985). Often in such cases the arthropod is more than just a vector and is in fact an intermediate host playing a major role in the pathogen’s lifecycle (Hill, 1997). Biological transmission of a disease organism is a natural finely tuned affair, because specific arthropod species are usually required for successful development of a specific pathogen. In addition, geographic and environmental conditions may be quite important, and a certain “threshold population” of arthropod vectors must often exist if transmission is to be successful.
The other mode of pathogen transfer which is the simplest form of dispersal is termed **mechanical transmission**. In this case, the vector (usually a species of fly) contaminates its mouthparts or other body structures while feeding on an infected host or contaminated medium and no form of pathogen development is required prior to further transmission. The contaminated vector may subsequently visit another, uninfected host and perhaps transmit the pathogenic organism in a manner similar to use of an unsterilized or contaminated hypodermic needle (Hill, 1997). Mechanical transmission usually has to take place within a few hours of the initial attack on a diseased animal or contaminated medium because the longevity of most pathogens when exposed on arthropods is generally short. This type of transmission is often noted among individuals in a herd or flock rather than the introduction of a pathogen into disease free group of animals.

Generally, vectors involved in biological transmission are considered to be more dangerous than those in mechanical transmission because apart from being transmitters they are also reservoirs of infection.

### 2.2.3 Forms of biological transmission to vertebrates.

Biological transmission is the most common and important means of pathogen transmission by insects. According to Edman (2004), the most successful arthropod-borne parasites are those that depend on their arthropod vector or host to support part of their lifecycle. Each parasite has its own peculiarities of life-style in the insect. Some of these characteristics can be used to subdivide biological transmission into the following categories (Huff, 1931).

1. Propagative transmission
2. Cyclo-developmental transmission
3. Cyclo-propagative transmission

2.2.3.1 Propagative transmission - In this form, pathogen transmission is characterized by a period of acquisition of the pathogen by a vector (typically an insect), a latent period before the vector is able to transmit the pathogen, and retention of the pathogen by the vector for a long period because the pathogen reproduces or replicates in the vector. They do not pass through any developmental stages. Examples are seen in viruses, rickettsiae and bacteria.

2.2.3.2 Cyclo-developmental transmission - In this form of transmission, parasites undergo developmental transformation (morphological and biochemical changes) within the vector before being transferred back to another vertebrate host. There is no replication and not all ingested parasites survive to the stage infective to a vertebrate host. Thus the number of parasites leaving the vector always is less than the number entering. Examples are the filarial worms ingested by the vector with the blood meal, they undergo development to the third-stage larvae (L3) before being transferred back to another vertebrate host (Edman, 2004) as well as most helminth parasites which develop from egg to infective larval forms before they are further transferred to another host.

2.2.3.3 Cyclo-propagative transmission - In this form of transmission, parasites not only multiples in the insect but also changes its form in some manner. A large number of parasites are included in this group, such as many of the trypanosomes, leishmaniases and the malaria parasites (Lehane, 1991).

For survival and propagation of their species, parasites depend on a reliable mechanism for their movement from one host animal to another and it is conceivable that it is easier for transmission
to be achieved by ectoparasites that live on or near the surface of the host than by endoparasites that must break through exterior host defences to get inside.

### 2.2.4 Vector transmission efficiency

This refers to factors that determine a vector's competence in ensuring the success of a transmission process. Various factors either promote or reduce successful parasite transfer and therefore determine the success of a disease condition. Some factors work singly while others work in combination to determine how efficient a transfer process would be. Transfer might be an active process, involving movement and penetration by the immature stages of the parasite, or it may be entirely passive, in which case the parasite relies entirely on the host to ingest materials containing infectious forms of the parasite. Certain poorly developed parasites depend on chance for their transmission.

Some factors that can influence transmission efficiency of a parasite include,

a) The prevalence and availability of an appropriate vector or intermediate host.

b) The precarious nature of changing of host by the parasites.

c) Climatic factors (temperature, rainfall, humidity etc).

d) Level of environment cleanliness.

e) Human factors (clearing vegetation, bush burning, chemical control strategies, netting).

f) The presence of natural enemies to these parasites.

g) Whether transfer process is active or passive.

h) Frequency of transfer.
i) Duration of the parasite’s lifecycle.

j) Arthropod behaviour (defaecation behaviour of those involved with stercorarian transmission)

Parasites that depend on vectors for their transmission are often considered complex because their success and survival would depend greatly on their ability to adapt to both a vertebrate and an invertebrate host which are two very different but equally important hosts. The geographic and/or host distribution of parasites and the incidence of the parasites and the diseases they cause is determined greatly by the transmission efficiency.

2.2.5 Transmission frequency

This term describes the rate of parasite transfer by vectors and according to Edman (2004), the shorter the lifecycle of a parasite, the more frequently it must be transferred between hosts and the more efficient its transfer mechanism must be. If parasitism causes acute infection that can kill the host, rapid transfer to a new, non-infected hosts needs to take place urgently or else the parasite itself might be lost. Vector-borne parasites tend to be transmitted frequently because the life span of the vector is often short, and frequent transmission favours greater pathogenicity (Ewald, 1993, 1996).

2.2.6 Parasite enhancement of transmission

Most parasites are not passive to their transmission but often enhance the probability of their own transfer by actively manipulating the behaviour or physiology of both their vertebrate and invertebrate hosts. This increases the feeding success of vectors that attack infective hosts (Day and Edman, 1984; Rossignol et al., 1985) and also the availability of the intermediate hosts for predation by the definitive host in the case of indirect transmission, thereby increasing the
probability of successful transmission. Hosts on the other hand, through their own behavioural patterns, unknowingly encourage transmission. It is therefore clear that parasite transmission often involves a combination of parasite-induced and parasite exploited behaviour on the part of both the vertebrate and invertebrate hosts (Edman, 1988).

Parasite enhancement of their transmission can be seen in the following example:

- Blood provides the humoral defence system that protects vertebrates from infection and may also, after repeated exposure, provide some protective immunity against protozoan and helminth parasites. To limit exposure of their surface antigen to the hosts immune system, they tend to be present in blood only when the probability of transmission is high. The infective stage of many filarial worms have a circadian or seasonal patterns of appearance in host blood, which mirrors the daily and seasonal biting cycle of their mosquito vector (Sasa and Tanaka, 1972).

- Certain parasites also enhance transmission at the biochemical level, such as the malarial parasite in the salivary gland of mosquitoes which disrupts the production of apyrase, a component of the salivary secretion that aids the probing mosquito in locating a blood vessel. Lack of apyrase prolongs probing time and thereby increases the probability of transmission of the malarial parasites (Rossignol et al., 1984; Ribeiro et al., 1985; Ribeiro, 1989).

- Phlebotomine sand flies are attracted to selectively feed around the skin lesion associated with cutaneous leishmaniasis and so their chances of becoming infected with highly localized leishmanial parasites are enhanced (Coleman and Edman, 1988).

- The Leishmania parasite promotes changes in the Phlebotomine sand fly which enables it secrete various molecules to assist the establishment of the leishmanial infection in a vertebrate (Ramalho-Ortigao et al., 2010).
• Behavioural assays by Webster et al. (2000) on the predation of beetles (Tenebrio molitor) infected with tapeworms (Hymenolepis diminuta) showed that infected beetles were more often exposed (not concealed under boxes) than uninfected beetles, thereby increasing the likelihood of this intermediate host being predated upon by the definitive host, Rattus species. The behavioural alteration was induced by the parasites to aid their transmission.

• Seppala et al. (2004) showed that the parasite Diplostomum spathaceum (Trematoda) reduces the vision of its fish intermediate host. It’s metacercariae lodge themselves in the eyes of the fish and induce cataract formation, which gives them the opportunity to affect fish behaviour (escape behaviour) thereby making them more vulnerable to predation by bird hosts such as gulls and terns. This implies that the ability of D. spathaceum eye flukes to alter fish behaviour may be a parasite strategy evolved to enhance transmission

2.2.7 Mechanisms of parasite transfer by vectors.

Transmission of parasites by arthropods may be from vector to vertebrate, from one life cycle stage to another within vectors (generational) or even between vectors.

2.2.7.1 Vertical transmission (Vector to vector)- This could be transovarially from the female vector via her eggs to the next generation as seen in the transmission Babesia bovis by Boophilus microplus ticks (Howell et al., 2007) or transstadially from one immature stage to the next or to the adult stage as seen in the transmission of Borrelia burgdorferi by bird-feeding ticks (Anderson et al., 1990).

2.2.7.2 Horizontal transmission (Vector to vector)- This form of transmission can be achieved either through mating as seen in the transmission of La Crosse virus by infected male mosquitoes (which do not bloodfeed) to females or while co-feeding amongst vectors in close proximity on a
naive host with the host tissues acting as a conduit (Randolph et al., 1996, Randolph, 1998). An example is the transmission of *Borrelia burgdorferi* by ticks.

### 2.2.7.3 Horizontal transmission (Vector to vertebrate)-
This can occur through any of 6 different mechanisms:

i. **Salivarian transmission**- This is via salivary secretion and is the most common and efficient method of transmission of pathogens by arthropods. Examples are seen in the transmission of certain trypanosome parasite species (*T. brucei, T. congolense* and *T. vivax*) by Glossina species and the transmission of malaria parasites (*Plasmodium falciparum, P. vivax*) by mosquitoes.

ii. **Stercorarian transmission**- Certain parasites exit from their arthropod host via faeces and gain entrance into vertebrate hosts through skin openings or abrasions. Examples are certain trypanosome parasites involved in the ”posterior station development”, they include *T. theileri* and *T. melophagium* transmitted by tabanid flies and sheep keds respectively and *T. cruzi* the cause of Chagas’ disease in man and is transmitted in the faeces of ruduviid bugs (Urquhart et al., 1987).

iii. **Regurgitation**- Some gut-inhabiting parasites exit during regurgitation by the arthropod while attempting to feed, this is due to blockage of the alimentary tract by masses of replicating parasites, these parasites eventually gain entry into a vertebrate host through skin openings or abrasions. Examples are *Leishmania* parasites in the gut of phlebotomine sandflies and plague bacilli (*Yersinia pestis*) transmitted by fleas while regurgitating infected blood. Regurgitation is as a result of blockage of the alimentary canal by a large number of multiplying parasites, this interferes with normal feeding ability of the vector.

iv. **Assisted escape and passive transfer of pathogens**- Some parasites rely on the vertebrate host to macerate the body of the annoying arthropod vector, thereby releasing the parasites onto the
surface of the host, these find their way into host tissues via skin openings. Example is *Borrelia recurrentis* transmitted by the human body lice (Houhamdi and Raoult, 2005) and is associated with severe bacteraemia (Guo et al., 2009).

v. **Active escape and active transfer of pathogens**- Here parasites migrate to the mouthparts of the vector and escape by active process into the host via the feeding wound created by the vector. Example is seen in the transmission of *Onchocerca gutturosa* by blackflies.

vi. **Ingestion of vector**- Here parasites gain entry into vertebrate host when these hosts accidentally ingest infected arthropods or food contaminated with infected arthropods. An example is the transmission of *Dipylidium caninum* by fleas.

### 2.2.8 Barriers to transmission

Transmission involves the movement of parasites from one host to another and such event are usually associated with potential hazards. Since most parasites would survive for just a short period outside a host, there is an urgent need to ensure a quick and safe means of parasite transfer to reduce or avoid transmission losses as is seen in the use of arthropod vectors by some parasites. Using arthropod vectors does not rule out losses during transmission, rather the degree of risk depends on the efficiency of the transmission provided by the vector. It is clearly conceivable that parasites that are transmitted in vector salivary fluids or through ingestion of infected vector are not exposed to the external environment because they are directly introduced into the new host. In contrast, mechanical transmission is high-risk due to exposure of parasites to various detrimental external factors.

The major barriers parasites face during transfer between hosts include;
1. The local environmental conditions, especially desiccation, humidity and adverse temperature.

2. The resilience behaviour and immune status of the invaded hosts (Vector-borne diseases, 2002).

A very close parasite-host relationship exists within arthropod vectors and considering the absolute requirement of the arthropod for the survival of these parasites, it seems apparent that understanding those factors that contribute to successful or unsuccessful arthropod-parasite associations would contribute significantly to our understanding of the epidemiology of arthropod-borne diseases. In immunocompetent hosts, following invasion by parasites, the host’s defensive armament immediately confronts the parasites, any parasite transmission to resistant or immune hosts is wasted transmission. Parasites in order to make the best of their invasion need to develop mechanisms for evading susceptible hosts defensive responses. Several other works have shown that different forms of defence are mounted by arthropod hosts against various forms of invading parasites.

**2.2.9 Parasite maintenance and amplification**

Not all hosts that become infected are essential to perpetuating or maintaining parasites. Because some parasites are transmitted inefficiently, they frequently end up in hosts that either cannot support development and/or reproduction or that are unlikely to serve as reservoir hosts of infection for the future transmission of the parasites. Transmission to hosts that cannot serve as reservoirs of future infection is referred to as dead-end transmission and the hosts involved are called dead-end hosts. There are also dead-end arthropod hosts that become infected but cannot serve as either vectors or intermediate hosts. This is why finding a parasite in an arthropod species is not sufficient proof that it serves as a vector for that parasite (Edman, 1988). Vector or host incrimination involves several other criteria as mentioned earlier by Barnett (1962).
Parasite amplification is a period of rapid spread or transmission of infection by vectors, this occurs for limited periods when ideal environmental and biological conditions for transmission exist. These periods of accelerated transmission lead to an increased incidence of parasitism and disease and also widen the distribution of parasites into hosts and geographic regions where they normally do not occur.

2.3 Order: Coleoptera, Beetles

Beetles have hard, shell-like outer wing covers called elytra that lack venation. Metamorphosis is complete. Beetles like cockroaches, are important as intermediate hosts of parasitic worms that infect domestic animals and humans. The spirurid nematodes Gongylonema and Physocelphalus, the acanthocephalans Macracanthorhynchus and Moniliformis, and the cestodes Hymenolepis and Raillietina, all develop in beetles to the stage infective for the definitive host (Bowman et al., 2003)

2.3.1 Tribolium castaneum

Is a beetle species of the order Coleoptera and family Tenebrionidae. It is a type of darkling beetle known as flour beetle, and is a very common insect pest of stored flour and grains. It is one of the most destructive insect pests of grains and other food products stored in silos, warehouses, grocery stores, and the home. Other species under the genus Tribolium include T. confusum, T. destructor and T. madens. Tribolium species are hard to identify, and being stored grain pests, several species may occur in the same infestation.

T. castaneum is also known as red flour beetle and T. confusum as confused flour beetle. The red flour beetle is of Indo-Australian origin (Smith and Whitman, 1992) and is found in temperate
areas, but will survive the winter in protected places, especially where there is central heat (Tripathi et al., 2001). The confused flour beetle, originally of African origin, has a different distribution in that it occurs worldwide in cooler climates (Smith and Whitman, 1992).

Red and confused flour beetles attack stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, biscuits and even dried museum specimens (Via, 1999; Weston and Rattlingourd, 2000). These beetles have chewing mouthparts, but do not bite or sting. The red flour beetle may elicit an allergic response (Alanko et al., 2000), but is not known to spread disease and does not feed on or damage the structure of a home or furniture. These beetles are two of the most important pests of stored products in the home and grocery stores. They are both reddish brown in colour but distinguished primarily by the physical difference in the shape of their antennae and thorax: the red flour beetles antennae ends in a 3-segmented club (Bousquet, 1990) and the sides of the thorax are slightly curved. The confused flour beetle has no apparent club on the antennae and the sides of the thorax are more parallel (Anonymous, 1986). Additionally, red flour beetles have been known to fly short distances, while confused flour beetles do not.
Diagrams A and B above showing T. castaneum and T. Confusum respectively (Baldwin and Fasulo, 2003).

2.3.1.1 Life Cycle and Biology

Although small beetles, about 1/8 of an inch long, the adults are long-lived and may live for more than three years (Walter, 1990). These beetles can breed throughout the year in warm areas. The life cycle takes from 40 to 90 days. All forms of the life cycle may be found in infested grain products at the same time. The red and confused flour beetles live in the same environment and compete for resources (Willis and Roth, 1950; Ryan et al., 1970). Eggs, larvae, and pupae from both species are very similar and are found in similar environments (Ryan et al., 1970). The eggs
are white, microscopic and often have bits of flour stuck to their surface. The slender larvae are creamy yellow to light brown in colour.

2.3.2 *Alphitobius diaperinus*

This also belong to the order Coleoptera and family Tenebrionidae. It is a type of darkling beetle known as lesser mealworm or litter beetle. It is a cosmopolitan insect pests of broiler houses where they often occur in large numbers in the bedding litter material that is used on the floors. The floors of these houses most often consist of compacted earth and the litter bedding material is usually wood shavings. Because of the beetle’s natural association with animals, in particular birds, this species is well suited to the conditions that occur in broiler houses (Trevor, 2006). It is a general stored products pest of particular importance as a vector and competent reservoir of several poultry pathogens and parasites. It can also cause damage to poultry housing and is suspected to be a health risk to humans in close contact with larvae and adults. Adults can become a nuisance when they move en masse toward artificial lights generated by residences near fields where beetle-infested manure has been spread (Axtell, 1999).

The lesser mealworm adults are broadly oval, moderately convex, black or brownish-black and usually shiny in appearance. Colour can be variable depending on age or strain. Length is approximately 5.8 to 6.3 mm. The pronotum is twice as broad as long, slightly narrowed from base to apex, with sides feebly curved and narrowly margined. The pronotal disk is finely and sparsely punctured, with punctures much coarser laterally. The elytra have moderately impressed striae with finely punctured, feebly convex intervals. Elytral punctures are sparse and nearly as large as those of the striae. The ventral surface of the lesser mealworm is dark reddish-brown,
with the prosternal process horizontal between coxae and having a prominent apex (Dunford and Kaufman, 2006).

2.3.2.1 Life Cycle and Biology

*Alphitobius diaperinus* is usually found infesting flour, meal, and other grain products, especially in poorly maintained grain processing plants (Spilman, 1991). It has been associated with wheat, barley, rice, oatmeal, soybeans, cowpeas, and peanuts. It has also been reported from linseed, cottonseed, oilseed products, tobacco, skims, and drugs (Hosen *et al.*, 2004). Because of its tropical origin, the lesser mealworm is well suited for warm, humid conditions. It is an important inhabitant of poultry or brooder houses where it lives in poultry droppings and litter. Both adults and larvae are abundant in manure from henneries and poultry farms (Francisco and Prado, 2001).

This species has been observed in a wide variety of habitats such as bird nests, bat colonies/caves, and in rodent and other vertebrate living areas. Beetle populations in the hundreds of thousands have been found on and in caves inhabited by bats in various parts of the world, including Texas and Kenya (Falomo, 1986). Larvae are known to feed on bat guano, mould, and on sick or dead bats, chickens, and pigeons (Falomo, 1986). They also will feed on animal parts such as feathers, and other lesser mealworm individuals (cannibalism).

After mating, the female has the potential to lay over 2,000 eggs (with an average closer to 200 to 400). Adults lay their eggs in cracks and crevices in the poultry house, in manure or litter, in grain hulls, and under feed and water lines. Adults can live three to twelve months, with females continuing to produce eggs most of their life at one to five day intervals. Larvae hatch in four to seven days and complete development to the adult stage in 40 to 100 days, depending on
temperature and food quality. Mature larvae disperse, especially when crowded, to find isolated pupation sites, and often tunnel into thermal insulation materials where they construct pupal cells. The tunnels are expanded further when adult beetles eclose to leave the tunnels to find food. Both the larval and adult stages are primarily nocturnal, with greatest activity occurring at dusk. They are very active and quickly burrow into the litter when disturbed. Adults are long lived, normally persisting for more than a year, and under experimental conditions have survived for more than two years (Falomo 1986).

Although *A. diaperinus* is not considered of major economic importance to whole grains, it does occur commonly on products already damaged by other biological agents, especially moulds. However, it is of considerable importance in the poultry business as an avian disease vector, and there are human health risks associated with exposure to *A. diaperinus*.

In very developed countries, lesser mealworms can cause poultry house structural damage. When searching for suitable pupation sites, larvae will chew holes in Styrofoam, fiberglass, and polystyrene insulation panels in the walls of poultry houses. The resulting damage can cause increased heating bills and additional building repair costs when the infested area is replaced. Energy costs in beetle-damaged broiler houses are reported to be 67% higher than in houses without beetle damage (Geden and Hogsette 2001). When beetle activity is intense it promotes manure aeration and drying.

### 2.3.2.2 Veterinary and Medical Importance

Lesser mealworms are important vectors of a number of poultry pathogens and parasites, such as the viruses of leucosis or Marek's disease, Gumboro disease (Falomo, 1986), turkey coronavirus (Watson *et al.*, 2000; Calibeo, 2002), Newcastle disease, avian influenza (Hosen *et al.*, 2004);
bacteria such as *Salmonella typhimurium*, *Escherichia coli*, *Aspergillus* spp. and *Staphylococcus* spp. (De Las Casas *et al.*, 1968; Harein *et al.*, 1970; McAllister *et al.*, 1996; Chernaki-Leffer *et al.*, 2002), protozoans such as *Eimeria* spp. that cause coccidiosis (Goodwin and Waltman, 1996, Hosen *et al.*, 2004); fungi (De Las Casas *et al.*, 1972; Eugenio *et al.*, 1970); helminthes such as the nematode *Subulura brumpti* (Karunamoorthy *et al.*, 1994); and fowl cestodes (Elowni and Elbihari, 1979).

Both adults and larvae of *A. diaperinus* can cause intestinal obstruction in poultry for slaughter since these birds lack chitinasis (enzyme to digest chitin) (Elowni *et al.*, 1979). This may eventually cause microscopic lesions along the bird's intestinal wall.

Another area of concern regarding *A. diaperinus* is associated health problems in humans. Tenebrionid beetles, including *A. diaperinus*, produce highly reactive benzoquinones as defence against predation (Tschinkel, 1975). Quinones can be hazardous to human health and cause health risks when exposed to the insect for extended periods. Reported health related ailments caused by *A. diaperinus* include symptoms of asthma, headaches, dermatitis, allergic angioedema, rhinitis, erythema (reddening), and formation of papules (Falomo, 1986; Schroeckenstein *et al.*, 1988, Tseng *et al.*, 1971). Exposure to quinone vapours can also result in conjunctivitis and corneal ulceration (Falomo, 1986; Schroeckenstein *et al.*, 1988).

It may be important for individuals with known insect allergies to avoid lesser mealworms as much as possible. Wearing protective gloves and masks while working under filtered and vented conditions may be required if conducting research on *A. diaperinus*. Quinones produced by tenebrionids are also suspected carcinogens; thus, quinone producing insects also represent a
health hazard at all levels of food production and distribution (Ladisch, 1965; Phillips and Burkholder 1984).

2.4 ORDER: DICTYOPTERA (COCKROACHES)

Cockroaches are insects of the order Blattaria. There are about 4,500 species of cockroach, of which 30 species are domestic pests (Valles et al., 1999; Schall and Hamilton, 1990). The most medically important species are the American cockroach, *Periplaneta americana*, which is about 30 millimetres (1.2 in) long, the German cockroach, *Blattella germanica*, about 15 millimetres (0.59 in) long, the Asian cockroach, *Blattella asahinai*, also about 15 millimetres (0.59 in) in length, and the Oriental cockroach, *Blatta orientalis*, about 25 millimetres (0.98 in) and a few others. They are sometimes called roaches or steam-bugs and are cosmopolitan in distribution especially in the tropics and sub-tropics (Service, 1980).

Cockroaches are generally rather large insects with a broad, flattened body and a relatively small head. They are generalized insects, with few special adaptations. The mouthparts are on the underside of the head and include generalized chewing, gnawing and scraping mandibles. They have large compound eyes (each having over 2000 individual lenses), two ocelli, and long, flexible, antennae. The first pair of wings called the tegima are tough and protective, lying as a shield on top of the membranous hind wings which can be used for flying. All four wings have branching longitudinal veins, and multiple cross-veins. The legs are sturdy, with large coxae and terminate in a pair of claws. The abdomen has ten segments and several cerci (Hoell, 1998). The segmented abdomen is more or less oval in shape but is either completely or partly hidden from view, depending on the species, by the folded overlapping wings. In both sexes, a pair of prominent segmented pilose cerci arise from the last abdominal segment, but they are hidden
from view in some species by the wings. In the males a pair of styles which are unsegmented and thinner than the cerci project from the end of the abdomen between the cerci.

The life cycle is incomplete, from egg to nymph and the adult. Cockroaches are nocturnal in habit and are rarely seen during the day unless they are disturbed from their hiding places. They become very active at night, crawling over the floor, tables and other furniture to seek food. They move very quickly and can be both seen and heard scuttling along when lights are suddenly switched on. They may live for five to ten weeks without water and for many months without food (Service, 1980).

Cockroaches are readily distinguished from beetles (order Coleoptera) by having the fore wings placed over the abdomen in a scissor-like manner, in beetles the fore wings (elytra) are not crossed over but meet dorsally to form a distinct line down the centre of the abdomen. Also the elytra of beetles are generally stouter than the tegima of cockroaches (Service, 1980).

2.4.1 American cockroach

The American cockroach, Periplaneta americana (Linnaeus), is the largest of the common peridomestic cockroaches measuring on average 4 cm in length. The American cockroach is second only to the German cockroach in abundance. Forty-seven species are included in the genus Periplaneta, none of which is endemic to the U.S. (Bell and Adiyodi, 1981). The American cockroach, *P. americana*, was introduced to the United States from Africa as early as 1625 (Bell and Adiyodi, 1981), but has spread throughout the world by commerce. The cockroach often resides indoors as well as outdoors; found mainly in basements, sewers, steam tunnels, and drainage systems (Rust *et al.*, 1991). It is also readily found in commercial and large
buildings such as restaurants, grocery stores, bakeries, and where food is prepared and stored. They can develop to enormous numbers, greater than 5,000 sometimes being found in individual sewer manholes (Rust et al., 1991).

American cockroaches can also be found in moist shady areas outdoors, in yards, hollow trees, wood piles, and mulch. They are occasionally found under roof shingles and in attics. The cockroaches dwell outside but will wander indoors for food and water or during extremes in weather conditions. Areas such as trees, woodpiles, garbage facilities, and accumulations of organic debris around homes provide adequate food, water, and harbourages for peridomestic cockroaches such as the American cockroach (Hagenbuch et al., 1988). Mass migrations of the American cockroaches are common (Ebeling, 1975). They migrate by crawling or flying into structures often entering houses and apartments from sewers via the plumbing, by trees and shrubs located alongside buildings or trees with branches overhanging roofs facilitate the entry of cockroaches into the home. During the day the American cockroach, which responds negatively to light, rests in harborages close to water pipes, sinks, baths, and toilets, for example, where the microclimate is suitable for survival (Bell and Adiyodi 1981).

2.4.2 Life cycle and Biology

Females lay their eggs in a hardened, purse- shaped egg case called an ootheca which protrudes from the tip of the abdomen. After about two days, the egg cases are placed on a surface in a safe location either by simply dropping it or gluing it to a surface with a secretion from her mouth. The deposited ootheca contains water sufficient for the eggs to develop without receiving additional water from the substrate (Bell and Adiyodi, 1981). A typical egg case contains about 14 to 16 eggs. Egg cases are about 0.9 centimetres (0.35 in) long and brown. Immature
cockroaches emerge from egg cases in 6 to 8 weeks and require 6 to 12 months to mature. Adult cockroaches can live up to one year, during which females produce an average of 150 young. The females on average produce an egg case about once a month for ten months laying 16 eggs per egg case.

The adult American cockroach is reddish brown in appearance with a pale-brown or yellow band around the edge of the pronotum. The males are longer than the females because their wings extend 4 to 8 mm beyond the tip of the abdomen. Males and females have a pair of slender, segmented cerci at the tip of the abdomen. The male cockroaches have cerci with 18 to 19 segments while the female has 13 to 14 segments. The male American cockroaches have a pair of unsegmented styli between the cerci while the females do not (Barbara, 2008; Service, 1980).

It is an omnivorous, voracious and opportunistic feeder. It consumes decaying organic matter but since the cockroach is a scavenger it will eat almost anything. It prefers sweets and has been observed eating paper, boots, hair, bread, fruit, book bindings, fish, peanuts, old rice, putrid cake, the soft part on the inside of animal hides, cloth and dead insects (Bell and Adiyodi 1981).

It habitually disgorges partially digested food and deposits excreta on almost anything, including food. Cockroaches may live for five to ten weeks without water and for many months without food, but in practice this is not an important limiting factor because they very rarely occur in areas where food of some kind is not available. Nymphs may die within about seven to ten days in the absence of food (Service, 1980).
2.4.3 Medical and Economic Significance

American cockroaches can become a public health problem due to their association with human waste and disease, and their ability to move from sewers into homes and commercial establishments. The cockroach is found in caves, mines, privies, latrines, cesspools, sewers, sewage treatment plants, and dumps (Bell and Adiyodi 1981). Their presence in these habitats is of epidemiological significance. At least 22 species of pathogenic human bacteria, virus, fungi, and protozoans, as well as five species of helminthic worms, have been isolated from field collected American cockroaches (Rust et al., 1991). Cockroaches are also aesthetically displeasing because they can soil items with their excrement and regurgitation (Barbara, 2008; Service, 1980).

They are also seen dwelling in poultry facilities particularly where poultry feed is compounded and stored and at times even in the litter as a result of spilled feed and since birds occasionally eat them as part of their meal the tendency exists that infections could readily be picked up through this means. Cockroaches have been incriminated as intermediate hosts of several poultry helminths such as Gongylonema ingluvicola, Oxyspirura mansoni, Subulura brumpti and Tetrameres Americana (Mercks, 1986). They also serve as mechanical vectors of filth-borne diseases of humans. Inspection of premises where food is prepared is often a veterinary function and the presence or absence of cockroaches is an important criterion of the adequacy of food sanitation (Bowman et al., 2003).

2.5 HELMINTHS

The name helminth is derived from the Greek word helmins or helinthos, a worm, and is usually applied only to the parasitic and non-parasitic species belonging to the phyla
Nemathelminthes (roundworms and their relatives) and Platyhelminthes (flukes, tapeworms and other flatworms). The Annelida (earthworm, leeches) are fundamentally different from both the Platyhelminthes and Nemathelminthes and are not regarded as helminthes, though some (leeches) may be parasitic and others (earthworms) may serve as intermediate hosts for helminths (Soulsby, 1982). However, the host becomes infected by ingesting the substance containing the infective larvae of the parasite (Banner and Philip, 1960). However, the rate of transmission of helminth infection is influenced by density-dependent mechanism acting at various stages of the parasite life cycle. Worm fecundity, survival and establishment affect transmission dynamics and have implication for understanding the epidemiology of the infection and the impact of the control programme (Churchur et al., 2005). The epidemiology and also the transmission dynamics is also affected by the indiscriminate disposal of both animal and human waste, which in most cases are used as a source of improving soil fertility. These infected hosts faeces could serve as vital sources for helminth infections, but this would largely depend on the survivability of the eggs in faeces within the environment over time. Some wastes, particularly those of humans are sanitarily disposed of in sewage treatment plants. Some parasites, e.g., *Trichinella, Eimeria, Toxoplasma, Entamoeba*, and perhaps *Giardia* and *Schistosoma*, are eliminated by sewage treatment processes (Cram, 1943; Fox and Fitzgerald, 1976; Fitzgerald and Prakasam, 1978), but there are others that are resistant. Eggs of the enteric parasites *Ascaris, Toxocara, Toxascaris*, and *Trichuris* have been shown to pass through digesters and remain viable and capable of infection (Nolf, 1932; Fitzgerald and Ashley, 1977; Osborn and Hattingh, 1977). These parasite eggs are commonly found in sludge samples from various countries (Keller, 1951; Osborn and Hattingh, 1977).
Fitzgerald and Ashley, (1977) demonstrated that 77% of *Ascaris* eggs kept in sludge at 38°C for 25 days later embryonated and were infective. Hence, if sludge containing these pathogens are used in topical soil applications, there is a danger of transmission of parasite infection. *Ascaris* eggs have been able to survive in soil for at least 7 years and cysts of *Entamoeba*, for 8 days (Mueller, 1953; Burge, 1974; Ognov, 1975).

Parasitic helminths have the gastrointestinal tract as their predilection site, where they cause enteritis of varying degree. The susceptibility of the domesticated livestock to gastrointestinal helminth infection is highly dependent on factors such as food, feeding habit of the host, sex, lactation, stress, immune status, parturition, age, species, genetic resistance, body size, geographical range, hygiene and seasonal fluctuation in the host environment.

Helminth parasites of concern in poultry are roundworms, hairworm and tapeworm. Worms are extremely common, particularly in free-range poultry. Being a primary parasite, they drain the birds of nutrients, causing ill-thrift, a general failure to thrive, a vulnerability to other diseases, and in severe infections, death (APC, 2011). A vast number of poultry cestodes have been identified, with a wide range of intermediate hosts. These are not considered of great pathogenicity, especially in modern poultry production systems.

2.5.1 Phylum: Nemathelminthes

Though the phylum Nemathelminths has 6 classes, only one of these, the nematoda contains worms of parasitic significance. The nematodes are commonly called round worms from their appearance in cross-section (Urquhart *et al*., 1987). They maybe free-living or parasitic, are unsegmented, cylindrical and elongate in shape; tapering at both ends. The body is covered by a colourless, somewhat translucent layer, the cuticle. A few exceptions occur, for instance, the
female of *Tetramereres*, which swell up after copulation, becoming almost spherical, and those of *Simondsia*, in which the posterior part of the body also assumes a globular shape. An alimentary canal is present. With a few exceptions, the sexes are separate and the life cycle may be direct or indirect (include an intermediate host). They are the most important of helminths (Soulsby, 1982).

2.5.2 Phylum: Platyhelminthes

The phylum contains the two classes of parasitic flatworms, the Trematodes (Flukes) and Cestodes (Tapeworms). They are dorso-ventrally flattened, bilaterally symmetrical (in contrast to the radial symmetry of the nemathelminths) and are leaf- or ribbon-shaped, and almost all hermaphrodite worms with solid bodies without a body cavity, all of which, except most of the species of the class Tuberllaria are parasitic.

The organs are embedded in tissue called the parenchyma and the excretory organs are the flame cells. Respiratory and blood vascular systems are absent. They are not metamerically segmented.

Unlike nematodes, which rarely remain in the same place for long, and must move to feed, the platyhelminths have developed special organs for attachment to the host, these are suckers which may be armed with hooks. In cestodes the hooks are arranged in circlets.

The life cycle of the species of veterinary importance is always indirect, and may involve one or more intermediate hosts (Dunn, 1969).

2.5.2.1 Class:- Trematoda

These are the flukes and are usually leaf-shaped and have a blind alimentary tract and one or two
suckers for attachment to the site in the host, they are unsegmented. Some common examples include; *Fasciola* (Liver flukes), *Dicrocoelium*, *Paramphistomum* and *Schistosoma*.

### 2.5.2.2 Class:-Cestode

The most obvious gross feature of the class cestoda is the long, segmented, ribbon-like form from which the name derives (Dunn, 1969). Generally, members are referred to as tapeworms and an adult is essentially a chain (strobila) of independent, progressively maturing reproductive units with the scolex a holdfast organ on one end for attachment to the wall of the host’s intestine.

In a fully developed adult tapeworm, all stages of development are displayed in a linear array starting at the scolex and terminating at the distal end. Although from a reproductive view point a tapeworm appears to be a colony instead of an individual, all segments are served by a common osmoregulatory and nervous systems.

According to Bowman *et al*, (2003), there are no organs of prehension or digestion; all nutrients are absorbed through the tapeworms specialized integument. The body of an adult tapeworm is so flattened that for the purposes of argument it can be said to have two surfaces and two edges. This shape affords maximum surface area per unit volume, a distinct asset for an organism that absorbs all of it’s nourishment through the skin. Some tapeworms grow to considerable sizes. The strobila of *Taenia saginata*, for example, may contain as many as 2000 segments and reach a length of 3.6m (30 feet) in the human small intestine (Arundel, 1972).

There are eleven orders of cestodes but only two are of greatest interest to veterinarians; Pseudophyllidea and Cyclophyllidea.
2.5.2.2.1 Order: Pseudophyllidea.
Members of this order are associated with aquatic food chains and are represented by only two genera of importance: *Diphyllobothrium* and *Spirometra*. Both use copepods as the first intermediate host in which the oncosphere develops into a second-stage larva called a procercoid. The second intermediate host may be a fish, amphibian or reptile and supports development of the procercoid into a third-stage larva called a plerocercoid. The definitive host becomes infected when it ingests a second intermediate host containing plerocercoids (Bowman *et al.*, 2003).

2.5.2.2.2 Order: Cyclophyllidea
Members of this order are associated with terrestrial food chains and contains six families of veterinary importance: *Taeniidae, Anoplocephalidae, Dilepididae, Davaineidae, Mesocestoididae* and *Hymenolepididae*. Most cyclophyllideans require only one intermediate host. Depending on the family of tapeworm, the intermediate host may be a mammal (*Taeniidae*), mollusc (*Davaineidae*) or an arthropod (*Anoplocephalidae, Dilepididae, Hymenolepididae*).

Cyclophyllideans produce oncospheres with a protective capsule of embryonic membrane origin. The oncospheres develop into metacestodes in the body cavities or tissues of an intermediate host. Usually, the metacestode is infective for the definitive host on ingestion. The oncosphere consists of a hexacanth embryo surrounded by two embryonic membranes. The hexacanth embryo, is infective to the first intermediate host and develops in this host into a metacestode. In most cyclophyllideans of interest to us, there is only one intermediate host, and the second-stage larva is the stage infective to the definitive host in which it matures (Bowman *et al.*, 2003).
2.5.2.2.3 Morphology of Cyclophyllidea

The complete cyclophyllidean cestode consists of a ‘head’, or scolex, and a ‘body’, or strobila. The anterior part of the scolex usually bears a mobile and often retractable structure, the rostellum, which may have a number of circlets of hooks. On the sides of the scolex are four suckers, which sometimes also carry hooks. A cestode with hooks on the rostellum is said to be armed.

The segments, or proglottids, grow by being budded off from the “neck” part of the scolex, and hence the most mature ones are those furthest from the scolex, at the distal end of the worm. Clearly, no matter how successful an anthelminthic may be in eliminating lengths of strobila, unless the scolex itself is removed, complete replacement of the tapeworm will eventually occur (Dunn, 1969). Each proglottid is hermaphrodite, the reproductive organs being single or paired, with the genital pores usually opening at the lateral margins. The male cirrus and the female vagina open into a common cup-shaped, genital atrium, and there may be self-fertilization in the proglottid, or cross-fertilization between proglottids.

The cyclophyllidean cestode disseminates its eggs by the detachment of a whole gravid segment full of eggs, passed out in the faeces; it does not lay eggs in the alimentary tract usually, as is the invariable custom in the pseudophyllideans.

When it is becoming gravid, and the uterus is filling with eggs, the internal structure of the segment begins to change in preparation for the shedding of the segment. The eggs become located in various adaptations of the tissues. Sometimes they are enclosed in packets formed from the lining of the uterus, as seen in Dipylidium caninum, one of the commonest dog and cat
tapeworms, so that when the segment is outside the body these packets, and not individual eggs, are strewn about. In other cases, dilatations or pockets are formed in the uterine wall and these become isolated groups of eggs being distributed in the parenchyma of the segment; these are the par-uterine organs. Often, however, the undifferentiated uterus simply becomes a bag of eggs (Soulsby, 1982; Bowman et al., 2003).

Outside the body, some segments begin the discharge of the eggs through the genital pore, and eventually the rest are liberated by disintegration of the segment but, in others (for example, *Moniezia*, the ruminal tapeworm), there is no active expulsion, and the whole process of egg liberation depends upon disintegration.

The egg has a complex structure. Seen in the faeces, it is a small compact object with a thick shell. But what appears to be the shell is really an inner structure called the embryophore, logically for it carries the embryo inside. The embryophore consists of two stout layers, an outer vitelline and an inner chitinous layer. A third, very fine, membrane encloses the embryo itself. The embryo is called the onchosphere, for it has hooks, and because there are six of these it is often called the hexacanth embryo.

### 2.5.2.2.4 Life cycle

The cyclophyllidean life cycle is indirect. There is usually only one intermediate host.

The onchosphere cannot be released until it is in the alimentary tract of the intermediate host. Here, the embryophore is broken down by digestion, liberating the onchosphere, still in its innermost membrane, which becomes activated by the effect of pancreatic secretion and bile salts in mammalian intermediate hosts, the absence of bile salts in insect digestive systems (Rockstein, 1965) indicates a different mechanism of activation.
Some workers (Smyth and Clegg, 1959; Llewellyn, 1965) have suggested that the shell material in cyclophyllidean cestode eggs might be untanned and therefore destroyed by the intermediate host’s digestive enzymes but Lethbridge (1971) provided evidence that physical damage to the shell by the insects (intermediate host) mouth parts is obligatory for the hatching of *Hymenolepis diminuta* eggs in vivo as well as in vitro. However, when arthropods with non biting mouthparts become infected, they could act as dispersal agents.

According to Dunn (1969) and Urquhart et al. (1987), once the onchosphere is active and invasive in mammalian intermediate hosts, it attacks the intestinal mucosa, using its hooks, it tears through the mucosa to reach the blood or lymph stream or, in the case of invertebrates, the body cavity. Once in its predilection site the onchosphere loses its hooks and develops, depending on the species, into one of the following larval stages, often known as metacestodes.

i. **Cysticercus**: fluid-filled cyst containing an attached single invaginated scolex, sometimes called a protoscolex.

ii. **Coenurus**: Similar to a cysticercus, but with numerous invaginated scolices.

iii. **Strobilocercus**: The scolex is evaginated and is connected to the cyst by a chain of asexual proglottids. The latter are digested away after ingestion by the final host, leaving only the scolex.

iv. **Hydatid**: This is a large fluid-filled cyst lined with germinal epithelium from which are produced invaginated scolices which lie free or in bunches, surrounded by germinal epithelium (brood capsules). The contents of the cysts other than the fluid, i.e scolices and brood capsules, are frequently described as “hydatid sand”. Occasionally also, daughter cysts
complete with cuticle and germinal layer are formed endogenously or, if the cyst wall
ruptures, exogenously.

v. Cysticercoid: A single evaginated scolex embedded in a small solid cyst. Typically found in
very small intermediate hosts such as arthropods.

vi. Tetrathyridium: Worm-like larva with an invaginated scolex; found only in mesocestoididae.

2.6 Family: Hymenolepididae

This family contains many species that occur in birds, small mammals and man. The family is
considered of minor veterinary importance, though the genus Hymenolepis itself is widely used
as a basis for the studies on cestode physiology, biochemistry, chemotherapy and immunology
(SRG, 2010). They are sometimes associated with enteritis and intestinal obstruction in game
galliforms and in aquatic fowl (Dunn, 1969).

2.6.1 Genus: Hymenolepis

Members use a great variety of arthropods as intermediate hosts and they occur world wide. The
small intestine is its predilection site and according to Dunn (1969) species of veterinary interest
include;

- *H. carioca* in galliform domestic fowl. This is the commonest avian species; the cysticercoids
  are in beetles of many genera.
- *H. cantaniana* in galliform domestic fowl; the cysticercoids are in dung beetles.
- *H. lanceolata* in anseriform domestic fowl; the cysticercoids are in water crustaceans.
- *H. nana* in the mouse and man; the cysticercoids are in grain beetles, fleas and in the final host.
• *H. diminuta* in the rat, mouse and occasionally man; the cysticercoids are in a very wide range of arthropods and their larvae.

The largest species is *H. diminuta*, which is less than a meter in length. The majority of the avian species are only up to 8cm long, and the commonest of these, *H. carioca*, though one of the longest in the group is very slender, measuring only 700µ across at its widest segments. This species unusually in the genus, is unarmed. Slenderness of the strobila, is a useful diagnostic character for the genus.

The eggs differ in form with the species. Most are large, spherical to ovoid, with a maximum diameter of 80µ. The eggs of *H. nana* and *H. diminuta* have the characteristic thickened walls of all cestode eggs, but may easily be differentiated. Those of the yellowish brown *H. diminuta* eggs are much rounder than colourless *H. nana* eggs and are larger at 60-80µm in diameter. In *H. nana*, the eggs are oval in shape, measuring approximately 40 by 50µm and contains an onchosphere equipped with 3 pairs of embryonic hooks (i.e. a “hexacanth” larvae) and long wavy filaments (absent in *H. diminuta*) which lie in the space between (i.e. the cytoplasmic layer).
2.6.1.1 *H. diminuta*

This parasite in common with most tapeworms does require an intermediate host. Embryonated eggs pass out of the body of the definitive host in the faeces and are ingested by the insect intermediate hosts. Many insects may act as intermediate hosts for this parasite, the most common being fleas and beetles such as the flour beetle. When ingested by the intermediate host the oncosphere larvae becomes activated, breaks out of the egg shell and penetrates into the
insects body cavity where it develops into a cysticercoid larvae. For completion of the life cycle the infected intermediate host must be eaten by the definitive host. On ingestion the cysticercoid larvae becomes activated, the scolex becomes attached to the gut mucosal wall, and the parasite develops into the adult tapeworm. Interestingly, *H. nana*, is a parasite of rats and mice as well as man as the definitive host, and differs from *H. diminuta* and almost all other tapeworm in that an intermediate host is not required, although fleas and beetles may be used. The embryonated eggs are passed in the faeces where they contaminate soil. If the eggs are ingested by the definitive host the oncosphere is activated and breaks out of the egg and penetrates the gut villus. Here it develops as a cysticercoid larvae in ~ 4 days before rupturing into the gut lumen. Once rupture the scolex attaches to the gut mucosa and the parasite develops into the adult tapeworm after ~ 15 to 20 days. If the insect intermediate hosts are utilized the lifecycle is similar to that of *H. diminuta* as described above. In heavy infections eggs liberated by adult worms in the intestine may hatch there rather than passing out of the body, to give auto infections.

An interesting feature of *Hymenolepis* tapeworms is that they undergo a diurnal migration within the gut, which is associated with the feeding patterns of the host. From ~ 4pm to 4am few parasites are seen in the lower part of the small intestine, whilst from ~ 4am to 4pm many parasites are seen in the upper part of the small intestine. This was first observed in *H. diminuta* and subsequently in other species, and is indicative of a nocturnal feeding pattern by the parasite.

### 2.6.1.2 Pathology of Infection.

These parasites are not very pathogenic and usually infections are asymptomatic infections. In man infected with *H. nana* there may be a slight irritation of the gut mucosa and slight abdominal
pain, and with very heavy infections (>2000 worms) there may also be some diarrhoea. In the
bird species there may be enteritis and intestinal obstruction with some species.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of experimental arthropods

A collection of a few selected insects commonly found in poultry houses were used in this study and they included *Tribolium castaneum* (Flour beetles), *Alphitobius diaperinus* (Darkling, lesser mealworm or Litter beetles) and *Periplaneta americana* (Cockroaches). They were identified using standard entomological procedures (MAFF, 1971).

*Tribolium castaneum* (Flour beetle). Source: lemur.amu.edu.pl

Collectively, over 400 young and adult *Tribolium castaneum*, which are normally seen in poultry feed, feed spill-over from poultry houses and also in contaminated flour or flour based food substances were collected from contaminated flour, poultry feed, cleaned out litter from poultry houses and the laboratory animal house in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. Some others were also collected from raw materials used in feed production in feed mills.
The adult *Alphitobius diaperinus* were collected in large numbers (about 300) from beneath drinkers and feeders in poultry houses as well as beneath sacks containing cleaned out litter from poultry farms in Nsukka and Enugu and the laboratory animal house in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Enugu state.

As a result of the relatively large size of cockroaches, 30 *Periplaneta americana* were collected from various hiding places in poultry houses, particularly at feed storage points. Others were
collected from either drawers and cupboards or trapped in a box baited with a piece of freshly spiced boiled fish.

These arthropods were used as the culture stock from which the population used for the experiments proper were obtained.

3.2 Feeding and maintenance

The collected beetles (flour and litter beetles) were each introduced into glass petri-dishes previously lined with filter paper and were left for three (3) weeks in the laboratory to acclimatize at room temperature. They were maintained on pelleted poultry feed and oat meal. In addition, *Tribolium castaneum* also received flour and other flour based food materials like biscuits for its maintainance.

The cockroaches were kept in a large circular plastic container (254cm²) in the laboratory with a netted cover (to prevent their escape and aid aeration) for a period of 3 weeks to acclimatize. A knowledge of the fact that they are known to be omnivorous, voracious and opportunistic feeders, which feed on almost everything, prompted the idea to maintain them on various kinds of food such as bread, biscuits, cake crumbs and pelleted poultry feed as well as water.

3.3 Experimental Helminth Parasite Used

The Cestode, *Hymenolepis diminuta*, from naturally infected albino rats was used in this study and was routinely maintained in the laboratory in 6 albino rats. Eggs were recovered for use from the faeces of the rats whenever needed.
3.4 Collection of Helminth Eggs

Eggs were collected using 2 techniques: Sedimentation and salt floatation.

3.4.1 Sedimentation

This technique is a combination of washing and sieving of faeces, to remove the smallest and the largest faecal particles (Roepstorff and Nansen, 1998). It utilizes the high specific gravity of the eggs, which facilitates their sedimentation. The eggs for some experiments were collected from freshly voided fecal pellets and others from 7 day and 14 day old faecal pellets of infected rats. Following collection, the samples were preserved separately in petri-dishes. Collected fecal samples were mixed with water to soften them, crushed with spatula and washed several times to get rid of colouring matter, feed materials and other debris to obtain a cleaner and clearer fecal suspension. This was achieved by allowing the faecal suspension to stand for a while in a beaker to enable the debri to settle, the supernatant fluid was decanted and the process was repeated several times until the solution was clean and clear. The resultant solution was then vigorously stirred and poured through a coffee strainer and the retained faecal debris discarded. The filtrate which contains the eggs was then centrifuged using a bench centrifuge (Model 800D, Techmel & Techmel, Texas, USA) and the supernatant was carefully pipetted out. The eggs contained in the pellets were resuspended in normal saline to prevent the occurrence of any form of damage and were carefully preserved to be used for the infection of the experimental arthropods.

3.4.2 Salt floatation

Floatation is the most widely used technique for the concentration of parasite eggs (Roepstorff and Nansen, 1998). Here eggs were collected from freshly voided faecal pellets and from 7 and
14 day old faeces from infected rats. The faecal pellets were crushed and thoroughly mixed with floatation fluid (saturated sodium chloride solution) using a spatula. The faecal suspension was immediately poured through a coffee strainer into a large beaker and the retained faecal debris was discarded. The sieved suspension was then transferred from the beaker into test tubes which were placed in vertical positions in a test tube rack. The test tubes were topped up with the floatation fluid and left for about 5 minutes to allow eggs present to float to the surface. The eggs at the surface of the test tubes were gently poured into a beaker by carefully tipping the upper portion of the test tubes. The possible destructive or hatching effect of the salt solution on the recovered eggs was neutralized by immediately adding an excessive amount of distilled water to the egg suspension. The excess distilled water was then decanted after allowing the solution to stand for about five (5) minutes to ensure sedimentation of the recovered eggs. The above process was repeated two more times to ensure perfect dilution and neutralization of the salt solution. The diluted egg suspension was then transferred from the beaker to several test tubes and was centrifuged for five (5) minutes at 11,000rpm. Thereafter, the supernatant fluid in the test tubes was carefully removed using a pipette. The collected eggs were used for the infection of the experimental arthropods.

3.5 Preservation of eggs

Separate egg suspensions from the freshly voided, 7 and 14 day old faeces were made. The eggs were preserved by storage in the refrigerator at a temperature of 4°C until required for use.
3.6 Infection of experimental arthropods

The arthropods were infected by depositing about 3800 and 6000 *Hymenolepis* eggs on oat-meal for *T. castaneum* and *A. diaperinus* beetles respectively and about 10,000 of the same eggs on bread and biscuits for the cockroaches. They were exposed to these infected food substances after a 5 days starvation period. They completed the ingestion of the infected meal in less than 5 days after exposure.

3.7 Dissection of arthropods and recovery of cysticercoids.

At the expiration of two weeks post ingestion of eggs for each group of infected insects in the 3 experiments above, each insect was carefully dissected using the dissection procedure described by Service (1980). The insects were killed with ether and the legs and wings were pulled from the body which was placed on a clean microscope slide. A drop of normal saline was placed on the slide around the abdomen of each insect to be dissected, a greater quantity of normal saline was used for the cockroaches since they are larger insects. Dissection was achieved using a simple dissecting microscope by cutting either side of the integument of the abdomen using dissecting needles and firmly pulling away the partially severed portion of the abdomen, this was followed by gradually teasing the cut portion to liberate cysticercoids if present. Cysticercoids, where present, were recovered using a Pasteur pipette and were preserved in Phosphate Buffered Saline (PBS).
CHAPTER FOUR

SURVIVAL OF HYMENOLEPIS DIMINUTA EGGS IN FRESH AND SEVERAL DAYS OLD FAECES FROM INFECTED RATS.

4.0 INTRODUCTION

Various conditions could affect the survival of helminth eggs that are voided onto the environment. Thus, the survival of eggs in the environment is considered very important as it affects the availability, sustenance and distribution of the eggs and the chances of infection of the intermediate hosts or final hosts as the case may be. With increase in human and animal populations, the society is faced with the problem of adequate municipal waste disposal. Most animal waste disposal involves spreading the waste on pastures and farmlands as manure to aid soil fertility while most human wastes if not disposed as in the case of animals are sanitarially managed in sewage treatment plants. In the latter case, most treatment of raw sewage consists of settling and activated sludge, sometimes followed by anaerobic or aerobic digestion with the aim of concentrating the wastes into manageable solids and concomitantly, parasite eggs where present (Black et al., 1982). The presence of parasite eggs in infected animal and human wastes, the concentration of wastes into solids during treatment and the spread of waste on pasture or farmland to improve fertility will all promote the spread of these parasite eggs and therefore the diseases they cause.

Since in most cases animal wastes are used as manure, infected faecal samples could be a vital source of infection to certain arthropods that could serve as vectors and which have close association with waste disposal points due to their saprophagous or coprophagous behaviours. Of great importance in the success of transmission of parasites is the survivability of the eggs in the environment.
A knowledge of the length of time for which certain helminth eggs such as cestodes, survive in the environment becomes important as it would help in the control of such helminthosis. The present study is therefore aimed at determining parasite egg survival rate in fresh, 7 and 14 days old faeces.

4.1 MATERIALS AND METHODS

Ten (10) *T. castaneum* beetles were picked at random from the stock culture and dissected to ensure that they did not contain any cysticercoids prior to infection. This was followed by the collection of a large quantity of freshly voided faeces from infected rats, which were divided into 3 portions and kept in separate petri-dishes. The eggs in the first portion were collected as described above and were used on day 0 to infect 10 *T. castaneum* beetles randomly selected from the same stock culture as above and which had been starved for a period of 5 days prior to infection. They were kept in a petri–dish lined with filter paper. Infection was achieved by dropping approximately 3000 *H. diminuta* eggs onto oat meal which the arthropods were exposed to. The second and third portions of faeces were kept under room temperature in the laboratory animal house for a period of 7 and 14 days respectively after which the eggs within them were separately harvested and used to infect two other groups of 10 *T. castaneum* beetles each. The various groups were monitored until the infected feed were completely consumed within a period of 3 days after which they were maintained on pelletized poultry feed. For each group, dissection of individual beetles for the presence of cysticercoids followed at the expiration of two (2) weeks post ingestion of egg contaminated feed.
4.2 RESULTS

Survivability of eggs from freshly voided faeces

Following the dissection of beetles fed feed contaminated with *H. diminuta* eggs from freshly voided faeces, cysticercoids were found to be present in 60% of the infected population. All the 10 beetles infected with eggs from freshly voided faeces (day 0 post defecation) survived after two weeks prior to dissection. A range of 1-2 cysticercoids per infected beetle was obtained (Table 4.1).

Table 4.1: Determination of the survivability of *Hymenolepis diminuta* eggs in fresh and several days old faeces from infected rats.

<table>
<thead>
<tr>
<th>Age of faecal sample</th>
<th>No of beetles</th>
<th>No surviving</th>
<th>No (%) positive</th>
<th>Mean number</th>
<th>S.E M</th>
<th>Range of No of cysticercoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly voided</td>
<td>10</td>
<td>10</td>
<td>6 (60)</td>
<td>1.33</td>
<td>± 0.25</td>
<td>1-2</td>
</tr>
<tr>
<td>7 days post defecation</td>
<td>10</td>
<td>9</td>
<td>6 (67)</td>
<td>1.33</td>
<td>± 0.21</td>
<td>1-2</td>
</tr>
<tr>
<td>14 days post defecation</td>
<td>10</td>
<td>8</td>
<td>3 (38)</td>
<td>1.66</td>
<td>± 0.66</td>
<td>1-3</td>
</tr>
</tbody>
</table>

Survivability of eggs from 7 days old faeces

Of the 10 beetles infected with eggs from 7 days old faeces, 9 survived out of which 6 (67%) were positive for cysticercoids. A range of 1-2 cysticercoids per infected beetle was also recorded for this group (Table 4.1).
Survivability of eggs from 14 days old faeces

In the group infected with eggs from 14 days old faeces, 8 survived the two weeks interval for cysticercoids development following infection. Only 3 (38%) were positive for cysticercoids. Although the number of positive cases dropped for this group, it still accounted for a higher range of 1-3 cysticercoids per infected beetle (Table 4.1).

4.3 DISCUSSION

The choice of Tribolium castaneum for this experiment is as a result of the success recorded in an initial pilot study which indicated it’s suitability to serve as an intermediate host for Hymenolepis diminuta as well as the successes of Keymer and Anderson (1979) and Keymer (1981, 1982) in infecting T. confusum, a closely related beetle species to T. castaneum, with Hymenolepis diminuta while studying their infection dynamics.

From the present study, the length of time Hymenolepis diminuta eggs survive after being voided in faeces of infected rats was investigated. Survival rate was observed to decline with increase in the age of the eggs for up to 2 weeks, even though there was slight increase in the number of cysticercoids in those infected with 7 days old eggs when compared with those infected with fresh eggs before the sharp drop observed in those infected with 14 days old eggs (Table 4.1). Survival of the eggs was determined by the presence of H. diminuta cysticercoids in the haemocoel of Tribolium castaneum beetles fed such eggs during dissection. These findings are similar to those of Pawlowski and Schults (1972) and Jepsen and Roth (1949) in which the eggs of Taenia saginata survived for several weeks and even months in sewage, river and on pasture. The eggs may remain alive on pastures for at least eight weeks and on dry sunny pastures may remain alive for 14½ weeks (Seddon, 1950).
Few deaths were observed in the infected beetle populations prior to dissection, this may be explained by normal die-off kinetics due to age, as beetles of all ages were used or it could have been as a result of parasitism. According to Hurd et al., (2001), deaths probably could have been reduced or avoided completely within the duration of this experiment if infection had been carried out on beetles two days post-emergence. Both male and female *Tenebrio molitor* beetles experienced increased survivability when infected two days post-emergence. Environmental conditions such as temperature or humidity levels could equally have had their effects.

The gradual decline in the number of beetles positive for cysticercoids as the age of the faecal samples increased is as a result of a gradual drop in the survivability of the eggs with age. It is believed that for *Hymenolepis diminuta* and other closely related cestode species, the survivability could last well beyond the 2 weeks considered in this study though for infection to occur in such delayed or prolonged cases, a higher concentration of eggs would be needed to makeup for those that might have lost their viability with age. According to Lethbridge (1971), hatching of *H. diminuta* eggs is initiated when the eggshells are broken by the insect’s mouthparts and this mandibular damage is usually confined to a small area at the periphery of the egg and results in an irregular-shaped opening in the shell. This shell damage also simultaneously results in the rupturing of the underlying sub-shell membrane. It is natural to believe that to some extent, mandibular damage on some eggs could result in the destruction and therefore death of some hexacanth embryos contained within.

Although the relationship between sex and infection was not considered in the study, Shea (2010) observed that male beetles avoided infective faeces while the females showed no preference. This suggests that probably, a greater number of the infected beetles could be
females. The observation by Shea (2010) shows a foraging behaviour in beetles which is believed to be influenced by the tapeworm. Generally, male vertebrates tend to show a greater intensity and prevalence of parasites than females (Zuk and McKean, 1996; Poulin, 1996; Schalk and Forbes, 1997), while the same pattern is not commonly observed in invertebrates (Sheridan et al., 2000). However, there are some exceptions in which experimentally infected arthropods resulted in a male infection bias (Pappas et al., 1995; Gray, 1998; Wedekind and Jakobsen, 1998). This male infection bias in invertebrates may be linked to differences in infection susceptibility that are immunological (Kurtz et al., 2000; Adamo et al., 2001), differences in infection exposure that are behavioural (Reimchem and Nosil, 2001; Hecker et al., 2002), or some combination of these factors (Klein, 2000).

The maximum of 3 H. diminuta cysticercoids per infected Tribolium castaneum was quite low when compared with 161 H. nana cysticercoids per infected T. confusum, 23 H. nana cysticercoids per infected Tribolium castaneum and 36 H. nana cysticercoids per infected Galleria mellonella reported by Schiller (1959). This variation probably resulted from the mode of infection, for in this case infection was achieved by exposing insects to eggs recovered from faeces as against allowing insects to feed directly on gravid proglottids. It is also likely that differences in the species of the cestode and/or the insect hosts used may have accounted for the variations. In the present study, the low percentage of infection obtained and the small number of cysticercoids present per vector suggests that, T. castaneum is a less efficient intermediate host than T. confusum. Thus, although various arthropods might successfully serve as intermediate hosts to cestodes the efficiency of that capability could vary with species of parasite and host. Another likely factor that could have contributed in the low infection rate of T. castaneum with
*H. diminuta* is the arthropod host immune response to the invading parasite which could have resulted in fewer established cysticercoids.

The beetle which carried 3 cysticercoids surprisingly was from the group fed eggs from 14 days old faecal sample, indicating that irrespective of the age of eggs within faeces, they still survive for long periods, with the faecal sample serving as an additional protective covering over the eggs against adverse environmental conditions.

The ubiquitous nature of arthropods facilitates their contact with various helminth eggs in the environment. An almost invisible dot of faeces in the environment might contain eggs or larvae which vectors could transmit. The coprophagous behaviour of some arthropods coupled with the fact that certain helminth eggs survive over long periods of time readily predisposes some of these arthropod vectors to infections. As a result of the close association between man, his domestic animals and various arthropods, infection can readily be transmitted. The results of this study clearly indicate the need for proper enlightenment, adequate epidemiological and control programmes to check the transmission of helminth infections particularly those that require arthropod intermediate hosts to complete their lifecycle.
CHAPTER FIVE
VECTORAL POTENTIAL OF TRIBOLIUM CASTANEUM, ALPHITOBIIUS
DIAPERINUS AND PERIPLANETA AMERICANA USING HYMENOLEPIS DIMINUTA.

5.0 INTRODUCTION

Vectors as carriers and transmitters of diseases may do so in various capacities, either as primary, intermediate, paratenic, reservoir or as transport hosts. Arthropods being the most successful of all animals have been incriminated as vector in many cases of disease transmission. Insects as consumers of agricultural crops and parasites of our livestock are also humankind's number-one competitor for resources. They are found in every type of habitat and in all regions of the world. They feed on a wide variety of plant or animal materials and have been known as major causes of diseases for centuries. Without the vector, the parasite life cycle would be broken and the pathogen would die. Vectors can cause harm in different ways particularly through their biological or mechanical means of transmitting disease agents. A good number of arthropods are confirmed vectors of various disease agents which they transmit while feeding or when they are accidentally ingested by appropriate host. Accidental ingestion usually occurs due to the infestation of food products in human and animal dwellings by some of these arthropods while in search of food. Infection of some of these arthropods with parasitic worms arises through ingestion of feed contaminated with faecal samples containing helminth eggs, direct ingestion of faeces from infected hosts by coprophagous arthropods as well as through the soil in the case of soil transmitted helminths (STH). The STH are relatively common parasites in the slum and rural areas of many countries (Sornmani et al., 2004; Che Ghani et al., 1993). The high prevalence of some of these helminths is closely related to poverty, poor environmental hygiene, and impoverished health services (Montresor et al., 1998). Some of these arthropod are of major
concern in veterinary medicine due to their capacity to act as vectors of several pathogenic organisms such as protozoa, helminth parasites, enteropathogenic bacteria, and enteroviruses (Graczyk et al., 2001).

Poultry as one of man’s commercial domestic flock and his most profitable livestock enterprise is faced with the problem of helminth infections. This could, to some extent, be attributable to the predatory behaviour of birds on insects which could be vectors of some of these helminthes. Insects are found virtually everywhere including in and around poultry houses where some species are found in feed, stored grains and even in feed mills. Their search for food leads to their infestation of poultry feed, grains and the immediate poultry environ which may be open to faecal contamination by various other hosts that may be harbouring helminth parasites. The coprophagous character of some of these insects enables them to pick up infection from infected faeces which is eventually transferred to birds when they accidentally or purposely ingest these insects while feeding. Infestation by these insects rapidly increases over time if unchecked. Not only do the presence of these insects cause damage and reduce feed quality, but importantly also, is their likely potential to serve as vectors to a variety of disease agents within poultry facilities as well as those introduced from outside.

Based on the foregoing facts, this study is aimed at determining the vector potential of Tribolium castaneum, Alphitobius diaperinus and Periplaneta americana, common poultry house insects, in the transmission of poultry cestodes using Hymenolepis diminuta, as a model.
5.1 MATERIALS AND METHODS

Ten (10) each of *T. castaneum*, *A. diaperinus* and *P. americana* were randomly picked from their various stock cultures and dissected to ensure that they did not contain any cysticercoids prior to infection. This was followed by a random selection of forty (40) *T. castaneum* beetles, thirty (30) *A. diaperinus* beetles and ten (10) *Periplaneta americana* cockroaches (based on their relative abundance) from the same stock cultures for use in this study. They were kept in Petri-dishes previously lined with filter paper except for the cockroaches which were kept in a large circular plastic container as a result of their larger size. They were all starved for five (5) consecutive days prior to infection. The *Tribolium* beetles, *Alphitobius* beetles and cockroaches were infected with approximately 3800, 6000 and 10,000 *H. diminuta* eggs respectively. The infection dose variations is due to the relative size differences of the arthropods and also the quantity of infecting eggs that can be accommodated by the food medium. Infection was achieved for the beetles by placing the said quantity of eggs on oat meal to which the beetles were exposed until it was completely consumed. For the cockroaches, the eggs were placed on bread and biscuits. They were all monitored until they finished the contaminated food within 3 days and were subsequently maintained as previously mentioned above. All the beetles and cockroaches were dissected two (2) weeks after the ingestion of eggs i.e. two weeks after they finished consuming the infected food.

5.2 RESULTS

Of the three arthropod species used, only *Tribolium castaneum* showed a level of vectorial potential to *Hymenolepis diminuta* infection as shown in Table 5.1. Out of the 40 *Tribolium castaneum* beetles exposed to infection, 36 survived after 2 weeks, out of which only 19 contained varying numbers of cysticercoids within them. *Alphitobius diaperinus* and *Periplaneta*
americana did not show any vectorial potential to this parasite as they gave negative results for the presence of cysticercoids during dissection. There were slight decreases, due to death, in the number of surviving arthropods for each of the species prior to dissection. There was no difference in the number of deaths in T. castaneum and P. americana. They both lost one of every 10 while A. diaperinus lost 0.67 out of every 10. The overall number of Tribolium species containing cysticercoids accounted for 52.8% of the surviving population.

The number of cysticercoids per infected Tribolium species varied. The highest number of infected beetles was with 1 cysticercoid per beetle while the least number was with 3 cysticercoids per beetle, some others had 2 cysticercoids per beetle.

A total of 25 cysticercoids were dissected out from all the infected beetles. (PLATES 1,2,3 and 4).

**Table 5.1: Vectoral potential of Tribolium castaneum, Alphitobius diaperinus and Periplaneta americana using Hymenolepis diminuta.**

<table>
<thead>
<tr>
<th>INSECT SPECIES</th>
<th>No of insects</th>
<th>No surviving</th>
<th>No (%) POSITIVE</th>
<th>Mean no/infected insect</th>
<th>SEM ±</th>
<th>Range of cysts/infected insect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tribolium castaneum</td>
<td>40</td>
<td>36</td>
<td>19 (52.8)</td>
<td>1.32</td>
<td>± 0.13</td>
<td>1-3</td>
</tr>
<tr>
<td>Alphitobius diaperinus</td>
<td>30</td>
<td>28</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Periplaneta americana</td>
<td>10</td>
<td>9</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
5.3 DISCUSSION

Certain insects are believed to have the potential to serve as vectors to various disease agents in varying capacities. The results of the present study have shown that *T. castaneum* has a positive vector potential for *H. diminuta* as shown by the development of cysticercoids in infected beetles during dissection. *A. diaperinus* and *P. americana* did not show any positive vector potential to *Hymenolepis diminuta*. Similar vectorial potentials have been shown to exist between *T. confusum* and *H. diminuta* (Keymer, 1981, 1982; Keymer and Anderson, 1979), *T. confusum* and *H. nana*, *T. castaneum* and *H. nana*, *Tenebrio molitor* and *H. nana*, *Galleria mellonella* and *H. nana*, *Sitophylus oryzae* and *H. nana* and *Dermestid lardarius* and *H.nana* (Schiller, 1959), though with varying ranges in the number of cysticercoids per infected insect. The development of *H. diminuta* cysticercoids in *T. castaneum* beetles indicates that the physiological and biochemical environment within these insect species supported development. *Tribolium castaneum* is therefore a possible or probable vector of this cestode parasite, serving as an intermediate host. In this case, the development of cysticercoids is indicative of a cyclo-developmental mode of biological transmission. Since chickens feed on insects and *T. castaneum* often occurs in large numbers in infested poultry feed, stored grains and around the poultry house, it is therefore conceivable that birds could readily acquire helminth infections by consuming infected *T. castaneum* present in feed, grains or on their own. *Tribolium castaneum* are attracted to grains with high moisture content and their infestation of grains promotes the growth of mould (Baldwin and Fasulo, 2003). The presence of these beetles in grains reduces both the commercial and nutritional values; thus affecting the production output of birds fed on such grains and lowering the financial gains of grain producers and poultry owners.
The occurrence of a few deaths prior to dissection in all the infected insect species is believed to be age dependent. Death could also be attributed to parasitism, since parasite infections often induce a reduction in host immune response either because of a direct manipulation of the immune system by the parasite or because of energy depletion (Cornet et al., 2010).

*Alphitobius diaperinus*, a common inhabitant of poultry houses, did not support cysticeroid development. This could be as a result of an effective internal defence mechanism against these parasites. The tremendous number and diversity of species within the phylum arthropoda suggests that an equally diverse array of internal defence mechanisms likely exists within the group designed to retain the integrity of self (Christensen and Tracy, 1989). The presence of *H. diminuta* cysticeroids in *T. castaneum* as described above could be as a result of a successful evasion of the hosts immune mechanism by these parasites. This could be by avoiding immune detection and/or by actively inhibiting the immune processes.

Though *A. diaperinus* showed no vectorial potential to *H. diminuta* as shown in the study, its veterinary and medical importance should not be overlooked. Several studies have shown that *A. diaperinus* is an important vector of a number of poultry pathogens and parasites, such as the viruses, bacteria, protozoans, fungi and helminths. They are capable of maintaining and shedding some of these pathogens such as *Salmonella typhimurium* in their faeces onto the environment for extended periods of time (Kuney, 1997), thereby being potentially capable of contaminating poultry feed and litter. This suggests that direct ingestion of the beetles by birds may not be required to cause infection in poultry. Elowni and Elbihari, (1979) showed that *A. diaperinus* under natural and experimental conditions could serve as a vector to some tapeworms of chicken including *Choanotaenia infundibulum*, which usually has the housefly, *Musca domestica*, as its
intermediate host. This beetle did not support the development of the tapeworm *H. diminuta*. This indicates that differences in vectorial potential could exist between arthropods from different classes, orders, genera and even between various species within the same genus. This is probably based on diverse individual genetic makeup, which determines the ability to support the development of particular parasites.

In large numbers within the intestine of birds, litter beetle can cause intestinal obstruction because they cannot be digested (Elowni and Elbihari, 1979). Medically, symptoms of headache, asthma, rhinitis, dermatitis, erythema and papule formation (Tseng *et al.*, 1971; Falomo 1986; Schroeckenstein *et al.*, 1988,) in humans have been associated with this insect. Consequently, there is need for caution as this is a potential source of occupational hazard for poultry farm and feed mill workers.

Cockroaches are among the most notorious pests of premises, which not only contaminate food by leaving droppings that can cause food poisoning (Rueger and Olson, 1969) but also they transmit bacteria, fungi and other pathogenic microorganisms in infested areas (Czajka *et al.*, 2003; Kopanic *et al.*, 1994).

*Periplaneta americana*, a species of cockroach, from the study did not support the development of cysticercoids of *H. diminuta*. On the basis of this observation, *P. americana* cockroaches, may not serve as intermediate hosts for this tapeworm species. This finding concurs with the observations of other workers (Young and Babero, 1975; Riley and Johnson, 1919; Chandler, 1922), who were unable to infect cockroaches with *H. diminuta*. Young and Babero (1975), in a similar experiment fed many groups of four species of cockroaches with designated eggs of twenty-one species of helminths, 11 nematodes, 9 cestodes and 1 acanthocephala. The eggs of
these helminthes were recovered intact in the faeces of the cockroaches. Eggs of most of the cestodes were recovered from faeces in viable form from the cockroaches within 4 days after feeding. The viability was confirmed by re-infecting flour beetles with these recovered eggs and cysticercoids developed. This indicates that the cockroaches were unable to digest the egg-shells of the cestodes (Young and Babero, 1975). Cockroaches can therefore be said to be vectors of certain helminth infections (Ascaris suum, Ascaridia galli, Toxocara canis, T. cati, Dipylidium caninum, H. nana, Choanotaenia iola, Taenia pisiformis, H. diminuta and a few others) only as transport hosts but not as biological transmitters. It can also be assumed, that natural cross-transmission of eggs from cockroaches to various intermediate hosts such as beetle species is possible and these beetles and other insect vectors could readily serve as sources of helminth infections when predated upon by birds.

As a result of their unsanitary habits, several reports have emphasized the vectorial potential of cockroaches for parasitic diseases of both man and animals such as bacterial (Cotton et al., 2000; Leffer et al., 2010), protozoan (Graczyk et al., 2001), fungal (Salehzadeh, 2007; Fotedar and Banerjee, 1992; Thyssen et al., 2004) and helminthes (Young and Babero, 1975).
CHAPTER SIX
THE EFFECT OF STARVATION ON THE ESTABLISHMENT AND DEVELOPMENT OF CYSTICERCIOIDS IN EXPERIMENTALLY INFECTED TRIBOLIUM CASTANEUM

6.0 INTRODUCTION
When living things are deprived of food, naturally they die after a given period of time. The response to starvation could vary from one animal species to the other. The response to starvation when occurring simultaneously with parasitism could also vary. Larval development takes place in the haemocoele (body cavity) of the insect intermediate hosts, it can therefore be assumed that every nutritional need of the developing larval stage would be gotten from the haemolymph (blood), but it is desirable to know the effect of starvation on larval development. This study therefore seeks to determine the effect of starvation of T. castaneum on the establishment and development of H. diminuta cysticercoids.

6.1 MATERIALS AND METHODS
Ten (10) T. castaneum beetles were picked at random from the same stock culture used for previous experiments and dissected to ensure that they did not contain any cysticercoids prior to infection. Thereafter, forty (40) T. castaneum beetles were randomly selected from the same stock culture and these were kept in a Petri-dish lined with filter paper where they were starved for five (5) days prior to their exposure to oat meal contaminated with approximately 3000 H. diminuta eggs. They were monitored until they finished the infected food. After the exposure period, the beetles were separated into two groups of 20 individuals in each. One group was provided with maintenance food following infection while the other was kept without food for the duration of the experiment. All of the beetles were dissected two (2) weeks after the ingestion of egg contaminated feed.
6.2 RESULTS

The effect of starvation on cysticercoid establishment and development in infected T. castaneum was determined. Table 6.1 shows that following infection and prior to dissection i.e. 2 weeks for cysticercoid development, all the 20 beetles in the fed group survived but only 7 of these were positive for cysticercoids, representing 35% of the surviving population. In the unfed (starved) group, 16 beetles out of 20 survived, out of which 12 were positive for cysticercoids; representing 75% of the surviving population for this group.

Table 6.1: The effect of starvation on the establishment and development of cysticercoids in experimentally infected Tribolium castaneum.

<table>
<thead>
<tr>
<th>Feeding status</th>
<th>No of beetles</th>
<th>No surviving</th>
<th>No(%) positive</th>
<th>Range of cysticercoids</th>
<th>Mean No</th>
<th>S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>20</td>
<td>20</td>
<td>7 (35)</td>
<td>1-2</td>
<td>1.28</td>
<td>± 0.18</td>
</tr>
<tr>
<td>Unfed</td>
<td>20</td>
<td>16</td>
<td>12 (75)</td>
<td>1-3</td>
<td>1.33</td>
<td>± 0.19</td>
</tr>
</tbody>
</table>

6.3 DISCUSSION

In this study, starvation of T. castaneum affected both cysticercoid establishment and development. Developmental alterations were observed as slight morphological variations, in the form of smaller sized cysticercoids in beetles from the starved group when compared with those in the fed group. These findings are similar to those of Schiller (1959) who, though made no mention of disparities in the sizes of cysticercoids, reported a significantly high number of abnormal cysticercoids in starved beetles when compared with the non-starved ones. His study revealed the occurrence of the abnormal cysticercoids to be as a result of incomplete
development. In the present study, there was also an increase in the fragility of the cysticercoids as observed by the easy breaking off of the tail end of the cysticercoids from starved beetles during dissection. Schiller (1959) reported that starvation of the host may limit the occurrence of some specific growth factor normally present in the haemolymph in minimal amounts, so that development of the cysticercoids beyond a certain stage may be inhibited. It is likely that the limited availability of these growth factors could have resulted in the small size and fragility of cysticercoids from the starved group as observed in the study. As would naturally be expected, starvation resulted in the death of some of the beetles. Surprisingly, the beetles in the unfed group accounted for a higher number of positive cases when compared with those in the fed group and the highest number of 3 cysticercoids per beetle was also from this group, this is quite unusual as the reverse would have been expected.
CHAPTER SEVEN

CONCLUSION AND RECOMMENDATIONS

The study is a clear indication that *Hymenolepis diminuta* eggs survive beyond 2 weeks in faeces deposited by infected individuals. It also shows that *Tribolium castaneum* a common inhabitant of poultry houses has potential to serve as an intermediate host to this parasite and probably other cestode species. This poses great threat to birds. There is therefore, great need for proper enlightenment of poultry personnel on the likely detrimental effects this and other seemingly harmless insects might pose to profitable poultry business. It also highlights the need for adequate control programmes to check the transmission of helminth infections particularly those that require arthropod intermediate hosts to complete their lifecycle.

Regular deworming and treatment of infected birds should be an adopted practice and proper poultry waste disposal should also be regularly carried out. The avoidance of feeding birds with insect infested grains or the use of infested feed raw materials to compound feed would also help curb the occurrence of helminth infections in poultry birds. The restriction of free range birds coming close to intensively managed birds is equally a step in reducing the occurrence of helminth infections.

Care should be taken and the necessary zoonotic awareness created since *Hymenolepis diminuta* affects man and *Tribolium castaneum* is also commonly seen infesting cereal based food consumed by man. The inability of starvation to prevent cysticercoid establishment and development is note worthy, particularly so because man is fond of removing some of these insects from his infested grains without ensuring that they are adequately destroyed before
consumption. These undestroyed insects eventually reinfest other grain products with time while still harbouring the infection they might have acquired during a previous exposure.

Finally, even though *Alphitobius diaperinus* and *Periplaneta Americana* did not appear to support the development of *Hymenolepis diminuta*, these insects and other poultry arthropods should be adequately eradicated through a carefully planned control programme since they may serve as vectors of various other pathogenic organisms of poultry and other domestic animals in varying capacities.
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PLATE 1

CYSTICERCOID
PLATE 2

CYSTICERCOID
PLATE 3

CYSTICERC OID
PLATE 4

CYSTICERCOID