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PREVALENCE AND GENETIC CHARACTERISATION OF NEMATODES IN RURAL POULTRY IN THE NORTHERN REGION, GHANA

ANANE, ABRAHAM

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FACULTY OF BIOSCIENCES

DEPARTMENT OF BIOTECHNOLOGY

PREVALENCE AND GENETIC CHARACTERISATION OF NEMATODES IN RURAL POULTRY IN THE NORTHERN REGION, GHANA

UNIVERSITY FOR DEVELOPMENT STUDIES

BY

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(BSc. BIOTECHNOLOGY AND MOLECULAR BIOLOGY)

(UDS/MBT/0006/18)

DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY, FACULTY OF BIOSCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN BIOTECHNOLOGY

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DECLARATION

I, Abraham Anane, declare that this dissertation has not been presented in any previous institution for a degree other than University for Development Studies and that it is my original work conducted under the supervision of Dr. Francis Addy and Dr. Osman Dufailu. All assistance towards the production of this work and all the references contained herein have been duly acknowledged.

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Candidate's Signature……. Date. ……

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Co-Supervisor's Signature……..... Date. …….....

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Head of Department's Signature…… Date. …….

Dr. Opoku Nelson

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DEDICATION

This study is dedicated to my family and friends.

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LIST OF ACRONYMS/ABBREVIATION/SYMBOLS

%	–	Percentage
˚C	–	Degree Celsius
w/v	–	Weight per volume
IgG	–	Immunoglobin
µL	–	Microlitre
BLAST	–	Basic Local Alignment Search Tool
bp	–	Base pair
cox1	–	Cytochrome c oxidase subunit 1
CTAB	–	Cetyl trimethylammonium bromide
DNA	–	Deoxyribonucleic Acid
DnaSP	–	DNA Sequence Polymorphism
DNTPs	–	deoxyribonucleotide triphosphates
EDTA	–	Ethylenediaminetetraacetic acid
Kg	–	kilo gram
g	–	gram
h	–	hour
HCl	–	Hydrochloric acid
ITS	–	Internal transcribed spacer
KCl	–	Potassium Chloride
M	–	Molar
MEGA	–	Molecular Evolutionary Genetics Analysis
mins	–	Minutes
ml	–	mililiter
mm	–	milimeter

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mM	–	millimolar
NaCl	–	Sodium Chloride
NCBI	–	National center for Biotechnology Information
NH_4Cl	–	Ammonium Chloride
PCR	–	Polymerase Chain Reaction
PVP	–	Polyvinylpyrrolidone
RPM	–	Revolution per minutes
s	–	seconds
TBE	–	Tris-Borate EDTA
U	–	Units
UV	–	ultra-violet
PUFA	–	Polyunsaturated Fatty Acid
FASDEP	–	Food and Agricultural Sector Development Policy
MOFA	–	Ministry of Food and Agriculture
FAO	–	Food and Agriculture Organisation
AMOVA	–	Analysis of Molecular Variance

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ABSTRACT

Rural poultry production in Ghana is predominantly done under the extensive system. Extensive system of production exposes birds to parasites infection mostly nematode species. The prevalence of nematodes in rural chicken from Kumbugu, Savelugu and Tolon Districts in the Northern Region of Ghana was investigated and the genetics of *Ascaridia galli* characterised. A total of 86 village chickens were dissected and screened for parasitic nematodes and the DNA of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene (475 bp) of 20 *A. galli* isolates were sequenced. The nematode species *Ascaridia galli* and *Heterakis* spp. were found at the following prevalence; *A. galli* in Savelugu District was 9/25 (36.0 %), Tolon and Kumbungu Districts recorded 14/25 (56.0 %) and 9/36 (25.0 %), respectively. *Heterakis* spp. prevalence rate in Savelugu District was 7/25 (28.0 %), Tolon and Kumbugu Districts also recorded 5/25 (20.0 %) and 6/36 (16.67 %), respectively Chi-square test showed a significant association of *A. galli* prevalence to the Districts of origin of birds (x^2 = 6.0907, p < 0.048). From 20 DNA analysed (Savelugu n=7, Tolon n=7 and Kumbungu n=6), two haplotypes were seen, namely, GHA1 and GHA2 with a single nucleotide variable site. Haplotype GHA1 was found to have wide distribution globally compared with GenBank deposits, whereas GHA2 appeared novel in the present study. These DNA sequences presented rather very low haplotype diversity of 0.100, but having negative Tajima's D and Fu's Fs indices which indicate population expansion. An AMOVA revealed that *A. galli* populations in the three Districts not to have differentiated from each other. The data generated by the present study revealed the importance of *Ascaridia galli* and *Heterakis* spp. infection in rural chicken in Ghana and pave way for further epidemiological study and population genetics characterisation of avian nematodes.

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CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Local chickens also called village chickens (*Gallus gallus domesticus*), are domesticated birds kept in rural areas for the purposes of obtaining meat, eggs, farm manure, sometimes feathers, and as a means of livelihood to the owners (Pym et al., 2006; Wong et al., 2017). They also play an important role in the cultural lives of rural communities such as their use in traditional marriage and festivities (Dinka et al., 2010; Bettridge et al., 2018).

There has been an increase in poultry production throughout the world over the past years (Mottet & Tempio, 2017). However, it has been estimated that 80 % of poultry production in Africa is kept under free range or extensive system (Mattiello et al., 2018). In Ghana, it is estimated there are over 25 million free range or village chickens, which most of them are found in the northern part of the country (Livestock Planning and Information Unit, 2006; Osei-Amponsah et al., 2015). Predators and gastrointestinal parasites have been the major cause of low productivity and poor health to chickens (Ola-Fadunsin et al., 2019). Poor health associated with catarrh, diarrhea, intestinal obstruction, loss of appetite, anaemia, weakness, paralysis, poor feathering in birds and death (Eshetu et al., 2001;Jegede et al., 2016). Helminth parasites are commonly found in poultry of which nematodes are the most important group of helminth parasites interms of both number of species and the extent of damage they cause to the birds (Tesfaheywet et al., 2012; Ola-Fadunsin et al., 2019).

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Ascaridia galli is the most common and important intestinal roundworm of chickens and other domestic and wild birds that occurs worldwide (Martis et al., 2017). According to Poulsen et al. (2000) and Tay et al. (2017), *Ascaridia galli* prevalent rate in Ghana varying from 24 to 54.3 % in village chickens. Ascaridosis caused by *A. galli* has been associated with low productivity in poultry (Dahl et al., 2002; Prastowo et al., 2017). Chicken nematode infection is frequently controlled by application of anthelmintic drugs such as flubendazole and benzimidazole (Bistoletti et al., 2011; Mackenzie & Geary, 2011). Other control strategies are improved housing systems, proper management and hygiene practices.

Availability and use of molecular approaches to studying population genetics structure and taxonomy of animals is becoming widespread in recent years (Sadaow et al., 2018; Xie et al., 2020). Molecular markers such as internal transcribe spacer genome (ITS) and mitochondria DNA (mtDNA) have been used to study the relationship between *A. galli* infection in chicken population (Bazh, 2013; Li et al., 2013; Malatji et al., 2016). Sequences of mitochondria DNA (mtDNA) have been confirmed to be a valuable and dependable genetic marker set as a result of its maternal inheritance, fast mutation rate and comparatively conserved genome (Blouin, 2002). This has aided the study of genetic diversity in some parasites like nematodes, cestodes and trematodes by various authors (Dai et al., 2012; Malatji et al., 2016; Addy et al., 2018). In Ghana however, there is limited information on molecular characterization of *A. galli* in village chicken. This present study employed molecular approach to determine sequences variability in mitochondria DNA (mtDNA) cytochrome c oxidase subunit 1

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(*cox1*) gene, among *A. galli* isolates from three Districts in northern Ghana. Phylogenetic inference of *A. galli* from this study and other countries was also made.

1.2. Problem Statement and Justification

In Ghana, chickens are commonly kept under free range or extensive system (Blackie, 2014). This system of production exposes chickens to a wide range of parasitic helminth infection which is indicated to be a major problem in poultry production in Ghana and other countries (Poulsen et al., 2000; Luka & Ndams, 2010). Parasitic infections result in reduction in productivity and sometimes increased mortality rate. *Ascaridia galli* and *Heterakis* spp are the most commonly nematode species found in chicken, and inhibit the growth of birds and egg production and at times result in high mortality in chicks which may lead to economic loss to the farmers (Soulsby, 1968; Amundson et al., 2016).

Many studies have reported the prevalence of *A. galli* and *Heterakis* spp. infection in free-range or village chickens in parts of Ghana (Poulsen et al., 2000; Tay et al., 2017; Asumang et al., 2019). To add up to knowledge, this study investigated the prevalence of these parasites in rural chickens in the Northern Region. However, molecular information on nematode species (*A. galli*) in Ghana is lacking. To fill in the knowledge gap, this study employed molecular approaches to determine the genetic diversity of *A. galli* in rural chicken in the Northern Region, Ghana. This will help the understanding of genetic differentiation and evolutionary relationship of the parasite and also improve on the control strategies. This will also pave way for future epidemiological study and population genetics characterization of the nematodes in Ghana.

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1.3. Objectives

i. To determine the prevalence of gastrointestinal nematodes infection in rural chickens in the Northern Region, Ghana

ii. To determine the genetic diversity in *Ascaridia galli* population in the Northern Region, Ghana.

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CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Poultry Production in Ghana

Poultry that have been produced and consumed in Ghana for many generations include chickens, turkeys, guinea fowls and ducks (Balma & Richard, 2016). In Ghana, meat from livestock and poultry contributes 40 % of the national supply of animal protein with the rest coming from fish (Aboe et al., 2006). Poultry production could be categorised into two main systems; intensive production system (indoors) and the extensive production system (free-range or outdoor). Semi-intensive production system is a mixture of the intensive and extensive systems. According to several reports, the extensive system of production (the low-input system) is the most common system in Ghana, which serves an important role in farmers' livelihood (Hagan et al., 2013; Blackie, 2014; Osei-Amponsah et al., 2015). The village chickens are kept in rural and peri-urban areas throughout the country (Williams, 1990; Dankwa et al., 2000).

In Ghana, village chicken accounts for 60 – 80 % of the national poultry population, similar to other African countries (Food and Agriculture Sector Development Policy, 2002; Gyening, 2006; Banson et al., 2015). According to Osei-Amponsah et al. (2015), it was estimated thast the rural poultry population in Ghana was around 12 million in the early 2000s, but increased to about 20 million in 2005 (Aning, 2006). However, it is currently estimated that, there are over 25 million free range poultry in Ghana (Osei-Amponsah et al., 2015). Nearly, all households in rural areas in the country keep local chickens and, in some areas, they keep guinea fowls, turkeys and ducks (Aning, 2006). According

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the data from Livestock Planning and Information Unit, (2006), Upper West (92.3%), Upper East (99.7%), and Northern (100%) Regions have the highest concentration of village chickens, local guinea fowls, ducks, turkeys and pigeons as compared to exotic breeds. Rural chicken production system in Africa and their contribution to the livelihood of the farmers have been stressed in various reports (Muchadeyi et al., 2004; Blackie, 2014; Avornyo et al., 2016; Birteeb & Boakye, 2020).

The estimated consumption per capita of poultry products in Ghana have increased from 4 to 6 kg between 2010 and 2012, with further increase to 6.8 kg as projected in 2013 (Banson et al., 2015). In general, Ghanaians prefer the meat of local chicken to imported chicken because of the quality (safety), taste, patriotism and tenderness of the local chicken meat (Hagan et al., 2013; Kwakwa, 2013). Even though livestock and poultry only contribute 7 % of agricultural Gross Domestic Product but their contribution to livelihoods and food security is huge (FASDEP, 2002). Poultry production plays a key role in Ghana's agriculture by contributing greatly towards food supplies, providing manure to maintain soil fertility as well as income provision, particularly to the farmers in the northern part of the nation (Quaye et al., 2010; FAO, 2014). In the 1960s, Ghana government once cited poultry production as the greatest potential to resolve the acute insufficiency in animal protein supply and job development (Aning, 2006).

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2.2 Role of Poultry in Human Nutrition

Research have proved that chicken egg plays a role as a carrier of some essential nutrients (González-Esquerra & Leeson, 2001; Suchý et al., 2014;d Kralik & Kralik, 2017). High quality protein as well as other important minerals, vitamins and vital fatty acids are obtained from poultry products (Barroeta, 2007). In sub-Saharan Africa (SSA), only 8 % of dietary energy is derived from animal protein, compared to an average of 17 % in all developing countries, and 28 % in China (Farrel, 2008). According to Sparks (2006), a typical egg will contribute approximately 3 – 4 % of the daily average energy intake of an adult, approximately 6.5 g of protein, 15 % of vitamin B6, 10 – 20 % of folate, and a similar percentage of the total saturated and polyunsaturated fatty acid (PUFA). Various reports have shown higher levels of vitamins A, E and B12 (20-30 %) were given by eggs to enhance human nutrition (Leeson & Caston, 2003; Kovacs-Nolan et al., 2005; Karsten et al., 2010; Schiavone & Barroeta, 2011; Lesnierowski & Stangierski, 2018). A study conducted in Australia by Meyer and colleagues (2003) reported that, eggs ranked third largest source of omega-3 polyunsaturated fatty acids after meat and seafood. A research by McNaughton & Marks (2002), also conducted in Australia earlier revealed that, eggs are also rich in selenium (9.0-41.4 IgG/100 g) only after seafood and meat.

Low dietary energy and protein are associated with diseases such as kwashiorkor and marasmus (Pitt et al., 2016; Henrique de S B Xavier et al., 2017; Gonzales et al., 2020).

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2.3. Characteristic Features of Village Chicken

village chickens are also called local, extensive, tradition, native, backyard, scavenging, free-range or indigenous chicken at different areas across the world (Permin et al., 2002; Dana et al., 2010; Conan et al., 2012; Padhi, 2016). These birds are left to search for their feed in the day time around the farm and in the fields after harvesting (Maphosa et al., 2004; Mcainsh et al., 2004; Asresie, 2015). Most of these chickens are left to roost in the bushes and on trees (Maphosa et al., 2004; Gunya et al., 2020). Village chickens are sometimes supplied with feed supplements that may include yellow corn, kitchen waste, broken grains, corn bran, sunflower cake or wheat (Nyoni & Masika, 2012; Gunya et al., 2020). This method of production system is characterised by low productivity due to the poor management system (Sonaiya, 2007; Yegani & Korver, 2008; Janczak & Riber, 2015; Haile & Biratu, 2017; Milkias, 2018). Low productivity is expressed in terms of late sexual maturity, reduced egg production, reduced size of egg, slow growth rate and survivability of young birds. There is generally lack of genetic improvement of chicken kept under extensive production system, and the poor management of such birds makes them to be frequent exposed to diseases and predation (Williams 1990; Mtileni et al., 2012; Blackie, 2014; Mutua, 2018).

Chickens that are exposed to severe environmental conditions in the local production system has an adverse effect on them. As a result of the environmental factors, selective role (natural selective) is observed in village chicken population mostly eliminating those that cannot utilize poor quality feed and susceptible to diseases (Abubakar et al., 2007; Mtileni et al., 2009; Mtileni et al., 2016). Khobondo et al. (2015) and Marwa et al. (2016) noted that, chickens that are well

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adapted to the harsh environmental conditions are also able to utilize available feed and resit infections. Hassan et al. (2013) reported that, the constant infection burden on rural chickens is as a result variable ages and mixed flock where there is the likelihood of disease transfer from one animal species to the other. Many authors have associated the low production of village chickens to high parasitic infections and ranked highest amongst the factors that impends this production system (Komba et al., 2014; Lawal et al., 2016; Raza et al., 2016). This coupled with the minimal input received by resource-limited farmers, make the experience of high mortality rate quite common (Gelli et al., 2017; Aslam et al., 2020)

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2.4. The Role Played by Village Chicken in the Rural Areas.

Village poultry are used mostly for domestic consumption and plays an important role in traditional ceremonies such as festival and (Kryger et al., 2010; Masole et al., 2015; Wong et al., 2017; Alders et al., 2018; Bettridge et al., 2018)). Village chicken again, plays a role in food security and provides man with animal protein and can provide additional cash income when sold (Dessie & Ogle, 2001; Guèye, 2005;Aklilu et al., 2007; Gelli et al., 2017). Cockerels were also said to be used as alarm clocks to tell the time in some rural areas Evans & Marler (1991), while Muchadeyi et al. (2004), stated that village chickens, provide manure for use in vegetable crop production. Again, village chickens are used to control weeds thus, grazing of young grass and other vegetation (Barua & Yoshimura, 1997).

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2.5. Constraints of Village Chicken Production System

Village chicken farming is faced by factors such as harsh climatic condition, uncontrolled breeding, disease, management practice, exposure to predators and thieves, poor nutrition and inadequate quality feeds, lack of locally adapted and well performing breeds (Mapiye & Sibanda, 2005; Mtileni et al., 2009; Haoua et al., 2015; Zemelak et al., 2016; Alders et al., 2018). These village chickens are allowed to roam and scavenge for their feed to meet their nutritional requirement (Henning et al., 2007; Mengesha et al., 2011; Olaniyan & Camara, 2018) although sometimes, homestead or owners provide feed supplement but not the nutritional requirement of the chickens (Nyoni & Masika, 2012). These supplementary feeds are usually thrown on the ground which further exposes the chickens to parasitic infections in the course of feeding. Zemelak et al. (2016), revealed that diseases were one of the major challenges for village chicken production. In their study, it was also revealed that 56-70 % of the farms they visited were affected by either one of these diseases at least once, thus; either coccidiosis, newcastle or ascariasis.

As a factor, parasitism is rampant in comparison with other factors that affection this type of production system due to poor sanitary and weak biosecurity (Abdelqader et al., 2007; Ovwigho et al., 2009; Okeno et al., 2012; Malatji et al., 2016). Gastrointestinal parasites in poultry have been studied in many countries across the globe. *Ascaridia galli*, *Capillaria* spp. and *Heterakis gallinarum* have been revealed by many researchers as the most frequent nematode species encountered in chicken (Permin et al., 1999; Ferdushy et al., 2016; Wuthijaree et al., 2017; Lozano et al., 2019). *Ascaridia galli* infections have been associated with obstruction of the gastrointestinal tract, in so doing causes mortality as well

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as reduction in egg production and growth performance in chickens (Poulsen et al., 2000; Kaufmann et al., 2011; Sharma et al., 2019). Secondary infection could occur as a result of parasitic infection in local chickens and transmit diseases like cestodosis, ascariodiosis and histomoiasis (Eshetu et al., 2001; Kaufmann & Gauly, 2009) as well as avian malaria (Juhl & Permin, 2002). *Ascaridia galli* can also play a role in transmitting bacterial infection like *Salmonella* ((Eigaard et al., 2006). Since village chicken is a source of livelihood to many farmers in the northern region of Ghana, this may cause economic hardship to families.

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2.6. Global Distribution of Nematode Infections in Village Chicken

Domestic chicken feeds on a variety of food substances ranging from grains to insects. Chickens harbour infectious parasites in the course of feeding, and thus predispose them to parasitic infection mainly gastrointestinal parasites (Mungube et al., 2008; Daş et al., 2012; Dar & Tanveer, 2013; Ben Slimane, 2016). Parasitic helminth diseases play a role on health and welfare of poultry production in the world, affecting productivity in the poultry industry. According to Lozano et al. (2019) helminth and protozoa from the genus *Eimeria*, are the most frequent gastrointestinal parasitic infections that occur in village chicken. Besides, occurrence of mixed infections has also been reported in village chickens. Occurrence of *Eimeria* spp. and helminths, nematodes and cestodes and mixed infection of two or more parasites species have been reported (Luka & Ndams, 2010; Dar & Tanveer, 2013; Tomza-Marciniak et al., 2014; Ferdushy et al., 2016).

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Amongst the helminth group, nematodes species (*Ascaridia galli, Capillaria spp.* and *Heterakis gallinarum*) are also the most highly prevalent parasites in village chicken (Permin et al., 2002; Lozano et al., 2019). The rate of infection varies with geographical area, sex, age, management practice and season (Permin & Hansen, 1998; Bachaya et al., 2015; Lozano et al., 2019). These nematodes are widely distributed in diverse regions globally, and have been researched by several authors

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2.6.1. Occurrence of Nematode Infection in Village Chicken in Africa

In Ghana, there have been several reports on roundworm worm infection, with recent study by Tay et al. (2017) in the Kumasi-Metropolis, Ashanti region. Form their results, it was discovered that nematode which include *Ascaridia galli* and *Heterakis gallinarum* were highly prevalent. With overall incidence rate of 54.3 % and 51.4 %, respectively. The overall incidence rate of gastrointestinal parasites was 91.4 % in village chicken. Earlier studies on the prevalence of roundworm of poultry in Ghana have been reported by numerous authors (Poulsen et al., 2000, Ayeh-Kumi et al., 2016, Asumang et al., 2019). Across the African region, Malatji et al. (2016), reported roundworm infection in local chicken from two province in South Africa. *Ascaridia galli, Heterakis gallinarum* and *Capillaria* species were found to infects rural chickens. Moreover, several reports on nematode infection in local poultry have reported across the continent (Table 2.1).

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Table 2. 1. Prevalence (%) of *Ascaridia galli* and *Heterakis* spp. reported in African regions.

Region	***Ascaridia galli* (%)**	***Heterakis* spp. (%)**	**Host Animal**	**Reference**
West Africa				
Ghana	24.0 – 54.3	16 – 51.4	Chicken	Poulsen et al. (2000), Ayeh-Kumi et al. (2016) Tay et al. (2017), Asumang et al. (2019)
Nigeria	6.0 – 22.3	0.6 – 10.2	Chicken	Ngongeh et al. (2014), Ola-Fadunsin et al. (2019)
East Africa				
Kenya	1.41 – 33.3	0.3 – 22.8	Chicken	Mungube et al. (2008), Kaingu et al. (2010)
Ethiopia	55.3	32.6	Chicken	Ashenafi & Eshetu (2004)
Central Africa Cameroon	14.3 – 51.6	9.8 – 59.3	Chicken	Mpoame & Agbede (1995), Wozerou (2014)

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Table 2.1. Con't

Region	*Ascaridia galli* (%)	*Heterakis* spp. (%)	Host Animal	Reference
North Africa				
Morocco	9.0	10.0	Chicken	Hassouni & Belghyti (2006)
Tunisia	53.33	100.0	Chicken	Ben Slimane (2016)
Egypt	8.9	15.38	Chicken	El-Dakhly et al. (2019)
South Africa				
South Africa	14.28 – 18.77	15.56 – 25.75	Chicken	Mwale & Masika, (2011), Malatji et al. (2016),
Botswana	84.6	84.6	Chicken	Mushi et al. (2000)

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2.6.2. Occurrence of Nematode Infection in Village Chicken in Europe

Kaufman et al. (2011), investigated the prevalence of nematodes infection in rural chickens in Germany. Their investigation revealed that, *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria* spp. were occurring with a very high prevalence rate of 88 %, 98 % and 75.3 %, respectively. An epidemiological survey on the prevalence of parasitic helminth infections in indigenous layers, conducted in eight different European countries by Thapa et al. (2015) revealed

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A. galli to have an overall prevalence rate of 69.5 % and being the highest amongst other parasites (Table 2.2).

Table 2. 2: Prevalence (%) of *Ascaridia galli* and *Heterakis* spp. reported in Europe.

Country	***Ascaridia galli* (%)**	***Heterakis* spp. (%)**	**Host Animal**	**Reference**
Germany	88.0 – 89.5	87.4 – 98.0	Chicken	Kaufman et al. (2011), Thapa et al. (2015
Austria	60.66	46.0	Chicken	Thapa et al. (2015)
Belgium	54.3	77.3		
Denmark	76.6	1.6		
Italy	50.5	0.0		
Netherlands	96.7	100		
Sweden	72.6	0.0		
United Kingdom	73.3	0.0		

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2.6.3. Occurrence of Nematode Infection in Village Chicken in Asia

As nematodes infections have also been reported to occur in the gastrointestinal tract of village chickens in Asia (Table 2.3). Javaregowda et al. (2016), reported helminth infections in backyard chickens in India. Nematode group *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria* spp. were highly prevalent. Mixed infections were also observed from their investigation. The overall prevalence rate of parasitic helminth was 72.0 %. Several on occurrence of nematodes in rural chickens have report by different authors across the region in Malaysia (Rahman et al., 2009) and in Pakistan (Khan et al., 2016).

Table 2. 3: Prevalence (%) of *Ascaridia galli* and *Heterakis* spp. reported in Asia.

Country	***Ascaridia galli* (%)**	***Heterakis* spp. (%)**	**Host Animal**	**Reference**
India	62.3	22.6	Chicken	Javaregowda et al. (2016)
Malaysia	53.33	93.33	Chicken	Rahman et al. (2009)
Pakistan	27.7	38.89	Chicken	Khan et al. (2016)

2.6.4. Occurrence of Nematode Infection in Village Chicken in North and South America

A recent study in Brazil and Columbia, have reported the outbreak of nematode infections in the region. In São Paulo State, Brazil, da Silva et al. (2016), reported

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intestinal parasitic disease on poultry with different management system. According their report *A. galli* and *Heterakis gallinarum* were found to occur frequently in the chickens that were investigated. Other nematodes were also present in some of the birds. Similarly, other investigations have been conducted in Colombia and Mexico have all reported the occurrence of nematodes in chickens and their presence were associated with management system, preventive measures and sanitary condition (Hoyos et al., 2015; Cervantes-Rivera et al., 2016) (Table 2.4).

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Table 2. 4: Prevalence (%) of *Ascaridia galli* and *Heterakis* spp. reported in North and South America.

Country	***Ascaridia galli* (%)**	***Heterakis* spp. (%)**	**Host Animal**	**Reference**
Brazil	71.0 – 100	76.0 – 100	Chicken	da Silva et al. (2016)
Colombia	31.25	21.87	Chicken	Hoyos et al. (2015)
Mexico	62.7	30.2	Chicken	Cervantes-Rivera et al. (2016)

2.7. Nematodes Found in Poultry and Their Characteristics

The most common species of helminths in poultry are nematodes (Molla et al., 2012; Wongrak et al., 2014). Nematodes typically have a long, thin, and thread-

like body (*'nema'* means 'thread' in Greek), but their body is not segmented like that of earthworms (Figure 2.1) (Yamaguti, 1954; Koppenhöfer, 2000; Kiontke & Fitch, 2013; Iqbal & Jones, 2016). Their body plan is a tube-like structure within a tube thus, the intestine and gonad are enclosed within the body wall by its longitudinal dorsal and ventral muscles, epidermis and a cuticle (Fagerholm, 1991; Kiontke & Fitch, 2013). The two 'tubular glands' (a dorsal and a ventral) are attached to the anterior end of the esophagus, apparently acting as a salivary gland, with its opening between the buccal capsule's basal ring and the esophagus anterior end (Yamaguti,1954).

Between the body wall, a pressurized fluid-filled space within the internal and the external tubes that acts as a hydrostatic skeleton (Maggenti & Nickle, 1991; Nicholas & Hodgkin, 2004). This organization allows nematodes to move stylishly in sinusoidal waves while lying on one side (Burr & Robinson, 2004). This primary body organization put a powerful restriction on evolutionary change in nematodes. Since nematodes actually depends on their solid body wall and pressurized body cavity for protection because they have no appendages such as legs or wings have ever evolved (Moore, 2001; Burr & Robinson, 2004).

Roundworms that infect poultry have been classified into two part; roundworm of the digestive tract and roundworms in other organs and tissues.

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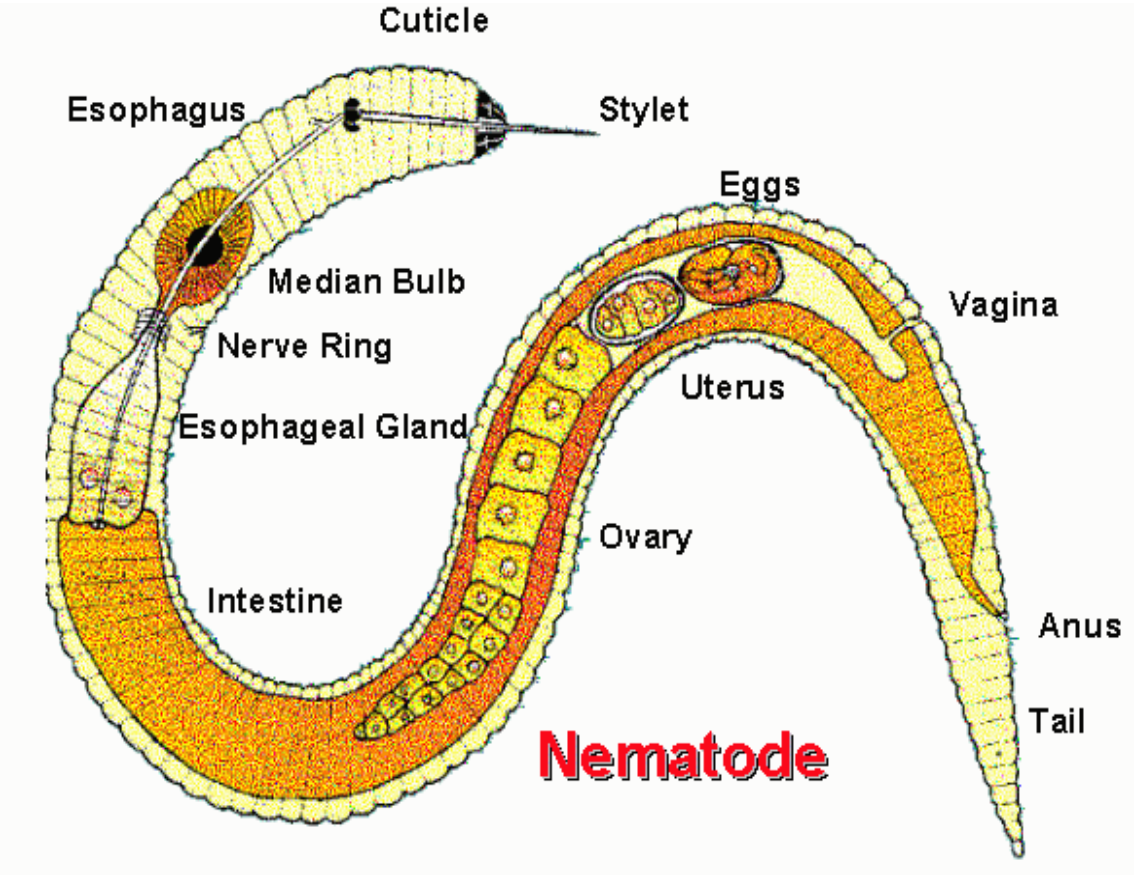

Figure 2. 1: A typical structure of a nematode that infect poultry (Source: pinterest.com, Accessed on 20/11/2020)

2.8. Roundworms That Infect Parenteral Regions of Poultry

2.8.1. *Oxyspirura mansoni*

This nematode commonly known as eyeworm (Ransom, 1904) has been reported to occur in chickens, turkeys, guineafowl and peafowl in the subtropical and tropical zones. The predilection site of this parasite is the nictitating membrane in the conjunctival sacs (Vellayan et al., 2012; Bruno et al., 2015).

Morphologically, the female adult worm measures 12 – 19 mm in length and that of the male is 10 – 16 mm. This parasite is slender and have a smooth cuticle (Permin & Hansen, 1998) (Figure 2.2).

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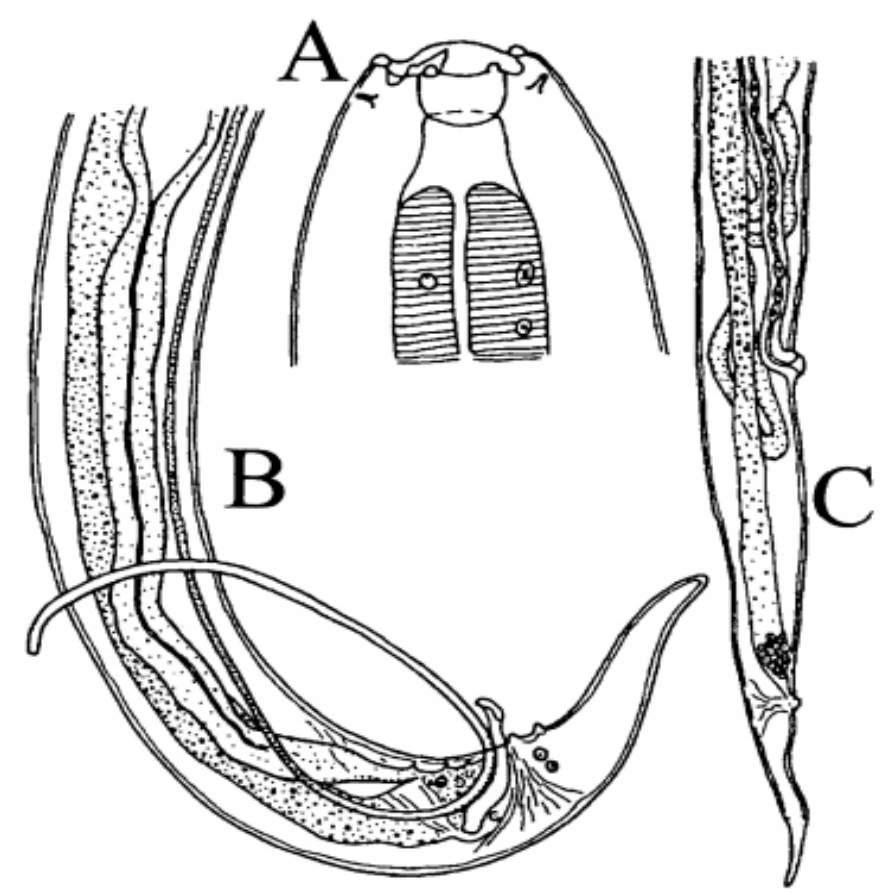

Figure 2. 2: Morphological structure of *Oxyspirura mansoni* (A) the head (B) Male tail (C) Female tail (Source: Permin & Hansen, 1998)

The worm experiences an indirect life cycle with cockroaches serving as the intermediate host for this parasite (Dunham et al., 2016; Kalyanasundaram et al., 2019). After the ingestion of the intermediate host by birds, the larvae are passed through the oesophagus, pharynx and lacrimal duct then finally settle on the eye (Ransom, 1904).

Some of the clinical signs includes scratching of the eyes of infected animals since the eyes become irritated, ophthalmitis inflamed and watery eyes (Villarreal

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et al., 2012; Dunham et al., 2017). Most chicken get blinded sight as heavy infection occur.

2.8.2. *Syngamus trachea*

Another name for this parasite is Gapeworm (Long,1957; Holand et al., 2014; Gethings, 2018). There have been several reports of this parasite to occur in both domesticated and wild birds globally. This worm infects the respiratory tract of birds. As the name indicates, this worm can be located in the trachea or in the lungs of the host (Fernando & Barta, 2008; de Paula et al., 2018).

They are red in colour because they feed on blood, and the male and female have a permanent copulation which makes them look like a "Y" shape (Figure 2.3) (Fernando & Barta, 2008; Martin et al., 2020). The female measures 5 – 20 mm and that of the male measures from 2 – 6 mm. The eggs of *S. trachea* measure 53 – 82 µm (Permin & Hansen, 1998).

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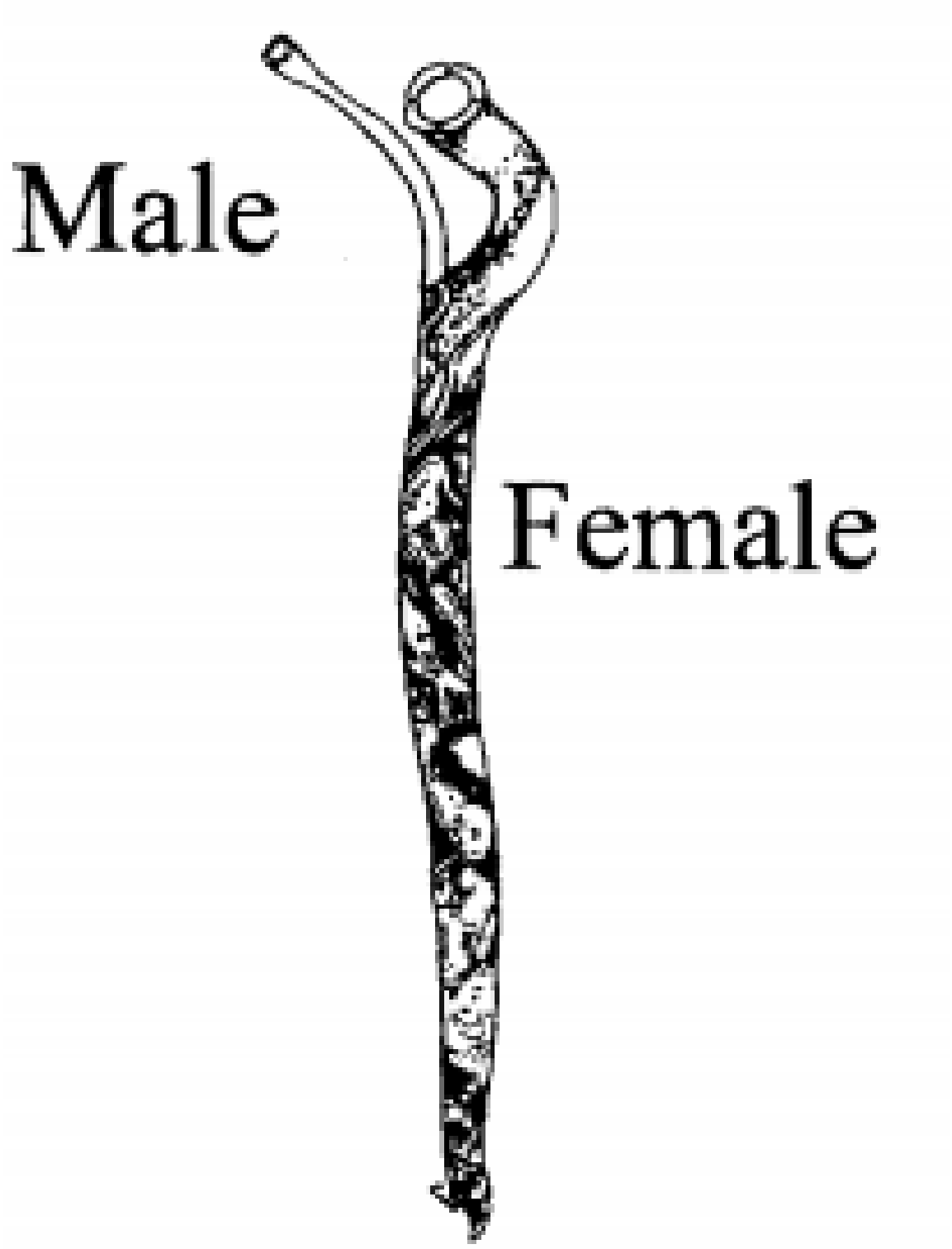

Figure 2. 3: *Syngamus trachea* morphology; male and female in permanent copulation (Source: Permin & Hansen, 1998).

The life cycle is either direct or indirect (Figure 2.4), earthworms, snails, flies or other arthropods serves as the intermediate hosts for *S. trachea* (Ripple, 1941; Davenport & Cairns, 1962; de Paula et al., 2018). After the ingestion of the intermediate host, larvae passed via the intestinal wall and they are carried by blood to the trachea or lung of birds and develop into adult worm (Fernando & Barta, 2008). According to Permin & Hansen, (1998), young animals are highly vulnerable to this infection but with turkeys, they are affected at any age.

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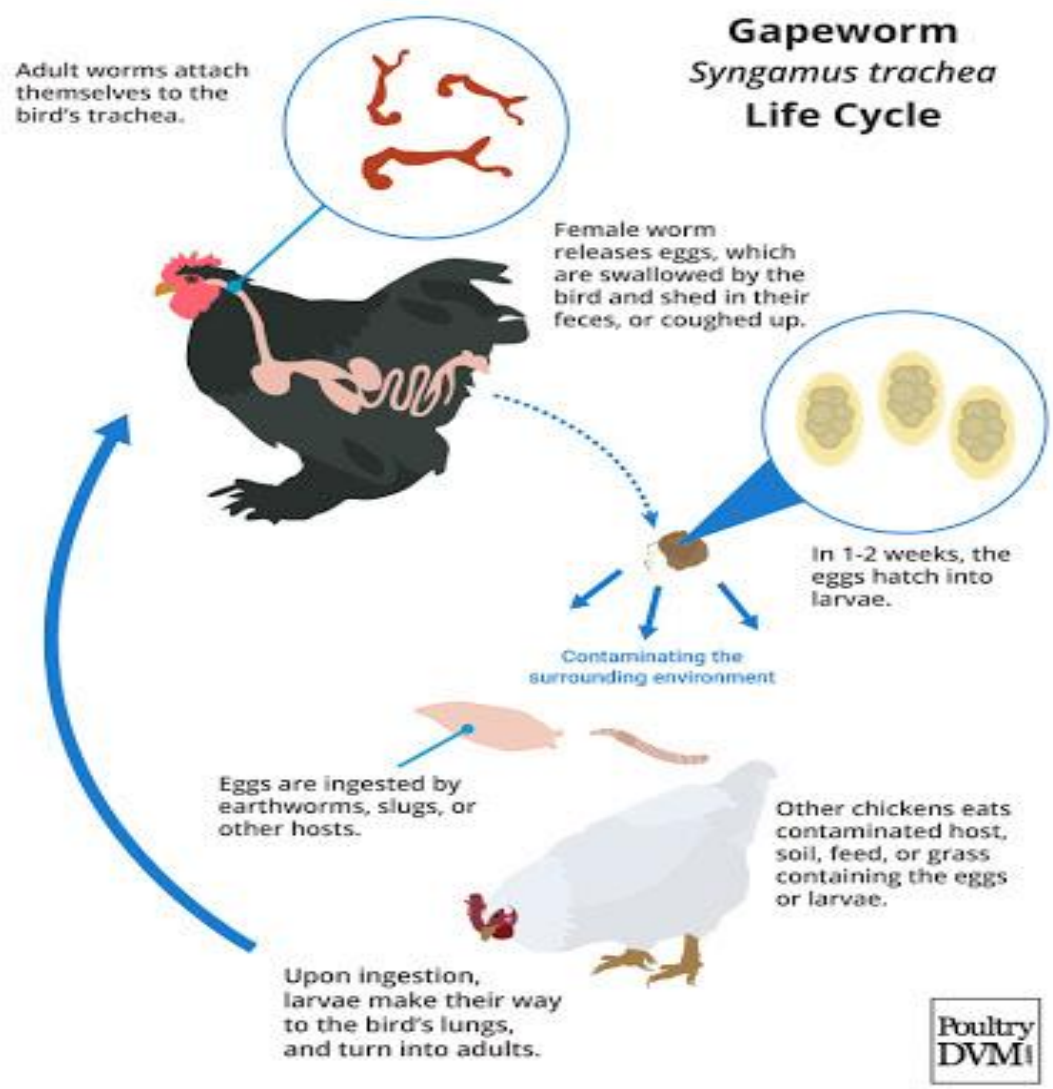

Figure 2. 4: Life cycle of *Syngamus trachea* (Source: Poultry DVM. Accessed on 20/11/2020).

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Syngamus trachea may cause respiration discomfort in birds that is; dyspnoea and asphyxia or chocking, shaking of head and coughing may occur as a result of accumulation of the worm in the trachea (Davenport & Cairns, 1962; Fernando & Barta, 2008; Gethings et al., 2016). Anaemia, emaciation and weakness as well as death are also experienced in infected animals (Ola-Fadunsin et al., 2019).

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2.9. Nematode That Infect the Digestive Tract of Poultry

2.9.1. *Gongylonema ingluvicola*

Gongylonema is a genus of roundworm parasites that have domesticated poultry as their final host. In North America, Africa, Asia and Australia, *Gongylonema ingluvicola* (Ransom, 1904) have been reported in all kinds of poultry (Permin & Hansen, 1998; Waruiru et al., 2018). The adult worm is found in the crop, oesophagus and occasionally in the proventriculus (Waruiru et al., 2018). The length of the female worm and male worm measure from 32 – 50 mm and 17 – 55 mm, respectively. At the anterior part of the body, they possess a varying number of a typical ring or oval thickenings on the cuticle called cuticular plagues (Permin & Hansen, 1998) (Figure 2.5).

The life cycle of *G. ingluvicola* is indirect. Beetles (*Copris minutus*) and cockroaches (*Blatella germanica*) act as the intermediate (Mukaratirwa et al., 2010). The larvae develop within 30 days and become contagious in the intermediate host. Fowl gets infected by consuming the intermediate host that contains stages of the infective (L3) phase (Permin & Hansen, 1998).

The worm can cause local lesions that look like a perforation in the crop which are the only damage associated with this worm. The worms and their burrows

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appear in the crop wall appear as white curved tracks, and can be confused with *Capillaria* spp. except when microscopically examined (Yazwinski & Tucker, 2008).

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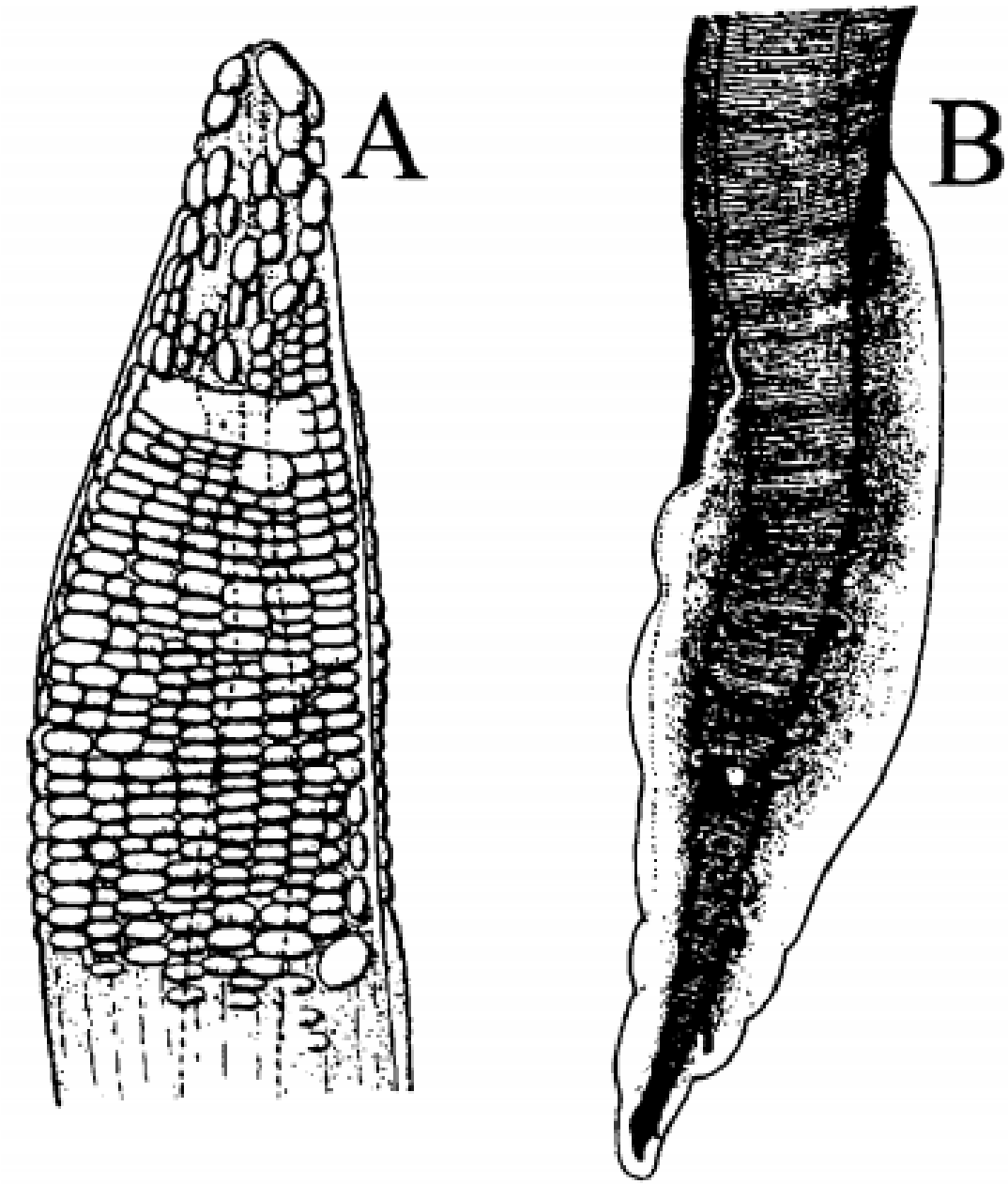

Figure 2. 5: Morphology of *Gongylonema ingluvicola* (A) Head and (B) Tail of male (Source; Permin & Hansen, 1998)

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2.9.2. *Tetrameres* spp.

Tetrameres americana is a common parasite in North America and Africa, that affect domestic and wild birds (Kellogg & Prestwood, 1968; Ewing et al., 1967). The species *T. fissispina* is a cosmopolitan parasite that infect chicken, duck, turkey, guinea fowl, and other wild birds. The worms are not very common like other gastrointestinal nematodes species of poultry, but in endemic areas, prevalence may be higher (Papazahariadou et al., 2008; Kamil et al., 2011).

Saif et al. (2008) described the morphological (Figure 2.6) characteristics of *Tetrameres* spp. as: presence of dimorphism and red in colour. In their body organization the female is globular in shape with four longitudinal furrows, long ovaries and uterus. The mouth is surrounded by three lips-like structure and with an opening buccal cavity. Their body cavity is filled with numerous coils. The egg of *Tetrameres* spp. is embryonated and measure 42–50 × 24 µm when laid. The length and width of the female and male measures 3.5 – 4.5 mm and 3 mm, and 5 – 5.5 mm and, 116 – 133 µm, respectively. The male has double of posteriorly directed spines that extends throughout the entire length of the body. Also, presence of cervical papillae and long tail at the anterior end with two unequal spicules.

Pernim & Hansen (1998), explained *Tetrameres* spp. life cycle as: the intermediate hosts being grasshoppers and cockroaches. Eggs are consumed by the intermediate host when passed with faeces. The eggs are hatched after some few days and larvae is released and form part of the tissue of the body cavity on the intermediate hosts, here, the infective phase (L3) of the larvae is complete. In the course of scavenging, the birds get infected after ingesting the intermediate

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host. They mature into adult worm and migrate to the proventriculus soon after ingestion and attaches themselves in the glands. Right after copulation the male leaves the glands and die.

Animals infected show clinical signs such as; weight loss and become anaemic. In a case of heavy infection, the proventriculus gets thickened and edematous also causes partial lumen obstruction (Fink et al., 2005; Biu & Haddabi, 2006).

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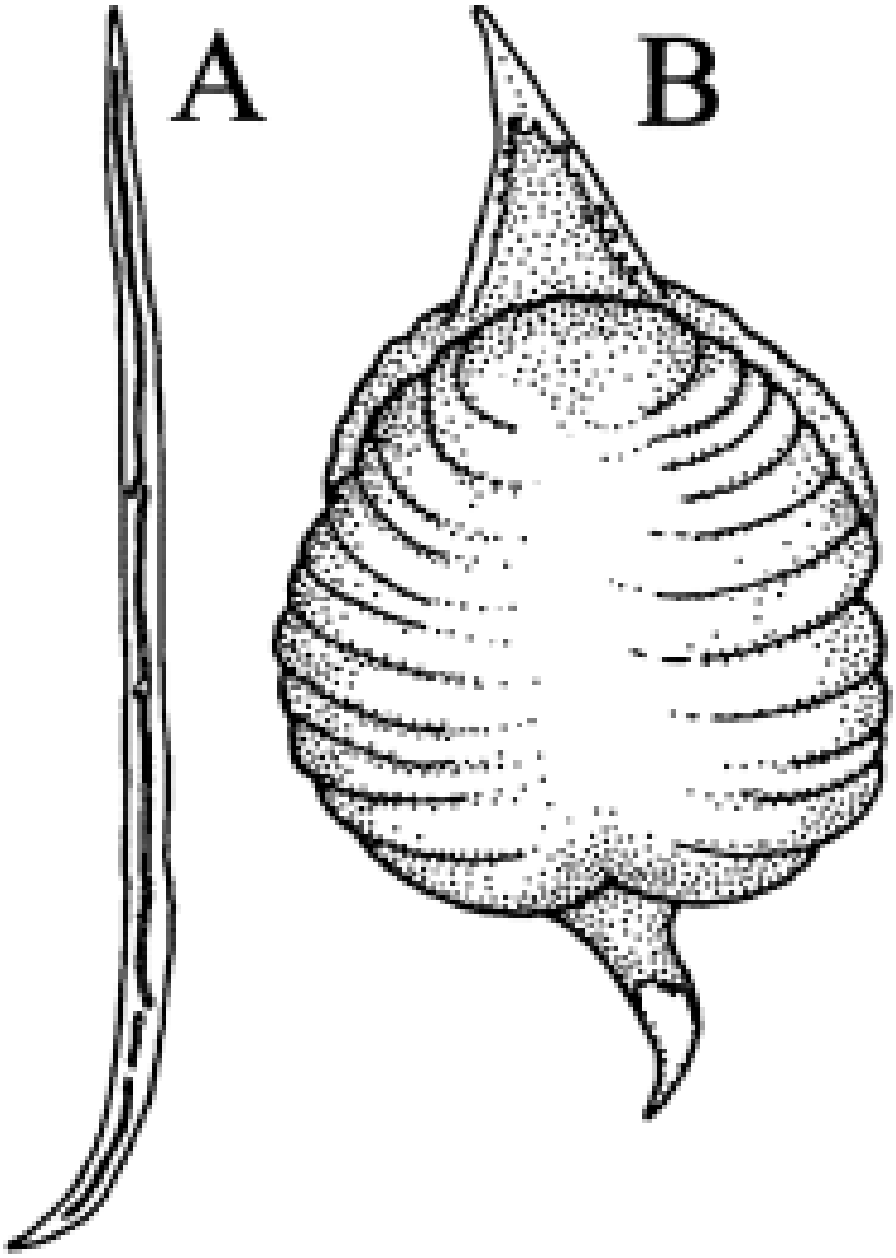

Figure 2. 6: Morphology of *Tetrameres americana* (A) Male and (B) Female

(Source: Permin & Hansen, 1998)

2.9.3. *Dispharynx nasuta*

Dispharynx nasuta occurs in North and South America, Africa and Asia with domestic and wild poultry as their final hosts. They are mostly located in the oesophagus and proventriculus, rarely in the intestine (Rickard, 1985; Sreenivasa Murthy & Panda, 2016).

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Yazwinski & Tucker (2008) described the morphological characteristics of *D. nasuta* (Figure 2.7) The adult male and female measures 7 – 8.3 mm and 9 – 10.2 mm long, respectively. At the anterior end of their body, there are four distinctive cuticular rings that recurve yet, do not fuse or anastomose. Also, they have a wavy structure to the anterior part. The egg of this worm is embryonated and measure about 33 – 40 × 18 – 25 µm.

According to different authors, the life cycle is indirect. Pillbugs (*Armandillidium vulgare*) and sowbug (*Porcellio scaber*) are the intermediate host. Embryonated eggs are ingested by the intermediate host, the eggs then hatch releasing the larvae after some days. The larvae form part of the isopod's body cavity. In the proventriculus, the adult worms mature when consumed by the bird (Cram, 1928; Hwang et al., 1961; Carreno, 2008; Erez et al., 2017).

Permin & Hansen, (1998), stated that, clinical signs such as anaemia and emaciation are observed in bird with heavy infection. Whilst in light infection inflammation is seen in the mucosa.

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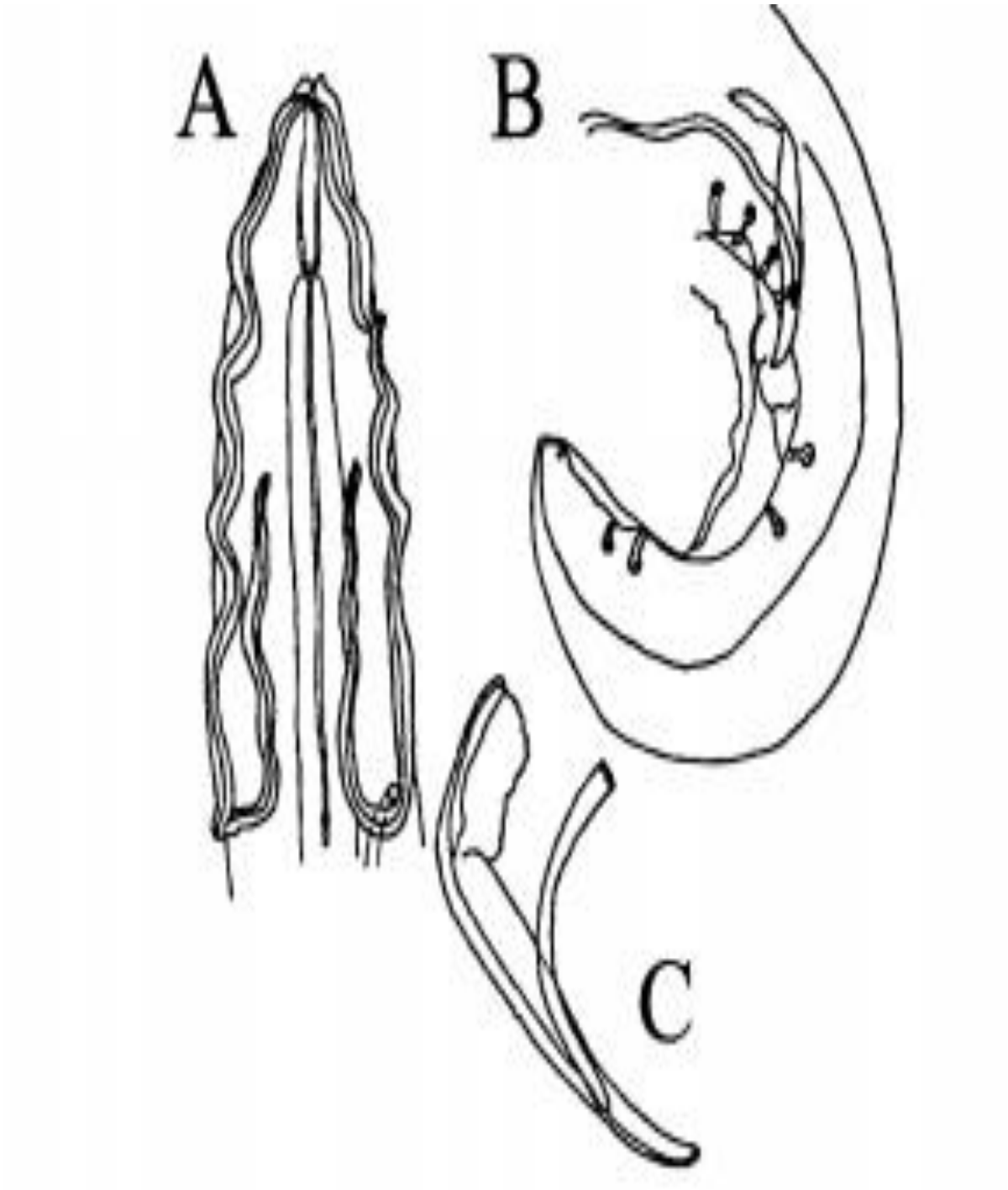

Figure 2. 7: Morphology of *Dispharynx nasuta* (A) anterior end of male (B) Male posterior end (C) Male spicules (Permin & Hansen, 1998)

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2.9.4. *Acuaria hamulosa*

Acuaria is a genus of roundworm parasites that infects various domestic birds (chickens, turkeys, pigeons, guinea fowls, etc.) (Salam et al., 2009; Tanveer et al., 2015). The worm is a cosmopolitan but not common as other nematodes species in poultry. In an endemic zone, the prevalence rate can be as high as 50 %. It is located under the keratinized layer of the gizzard, the adult worms are trapped in nodules or abscesses (Salam et al., 2009; Ben Slimane, 2016).

Morphological features of this worm were described by Permin & Hansen, (1998) (Figure 2.8) as follows; the adult female and male measure 16 – 29 mm and 10 – 14 mm long, respectively. The presence of four long cuticular cordons on their body gives their distinguished characteristics. The cordons are irregular and wavy, covering roughly two-thirds down the body. On the male body, there is a long and slender spicule on the left and right side and measures about 1.63 – 1.8 mm, respectively.

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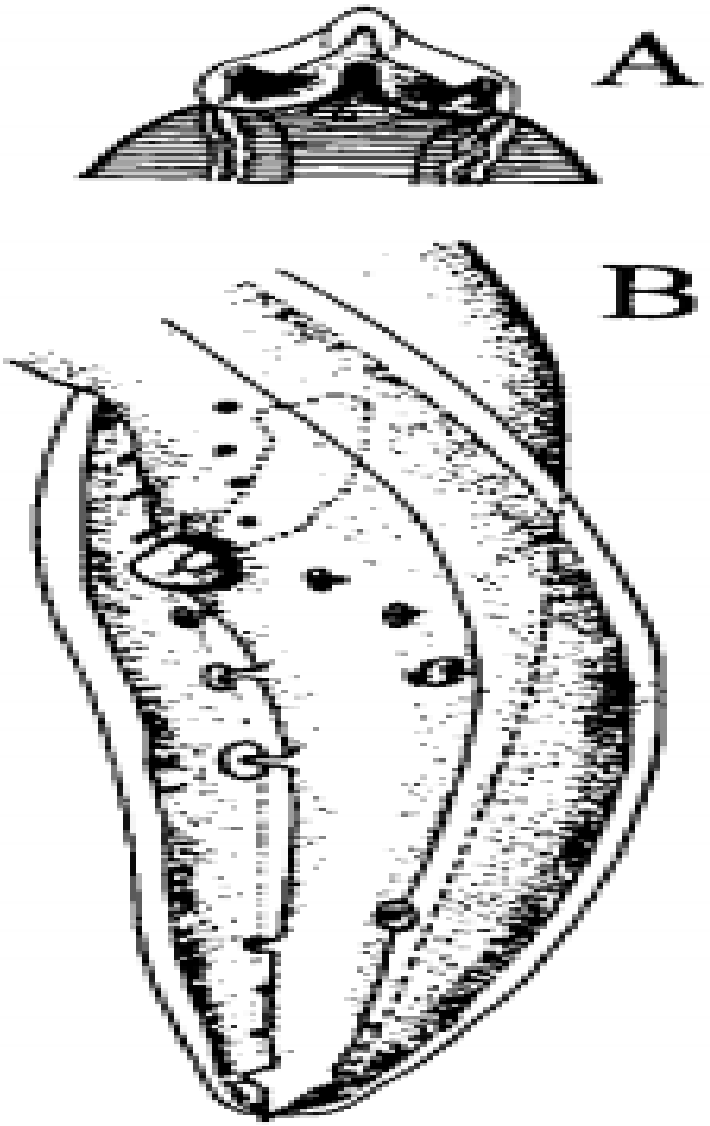

Figure 2. 8: Morphology of *Acuaria hamulosa* (A) Head and (B) Tail (Source: Permin & Hansen, 1998)

The life cycle of this parasite is indirect (Figure. 2.9) (Belete, 2016). (a) *Melanoplus* spp., *Oxya nitidula,* or *Spathosternum prasiniferum* are species of grasshoppers, various beetles, sand-hoppers, and (b) weevils, which serves as the intermediate host. Eggs passed out with faeces and ingested by the intermediate host. After three weeks in the intermediate host, the eggs are hatched mature into

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an infectious stage (larva). When the intermediate host is consumed by the final hosts thus; domestic birds, they get infected (Saif et al., 2008; Belete, 2016).

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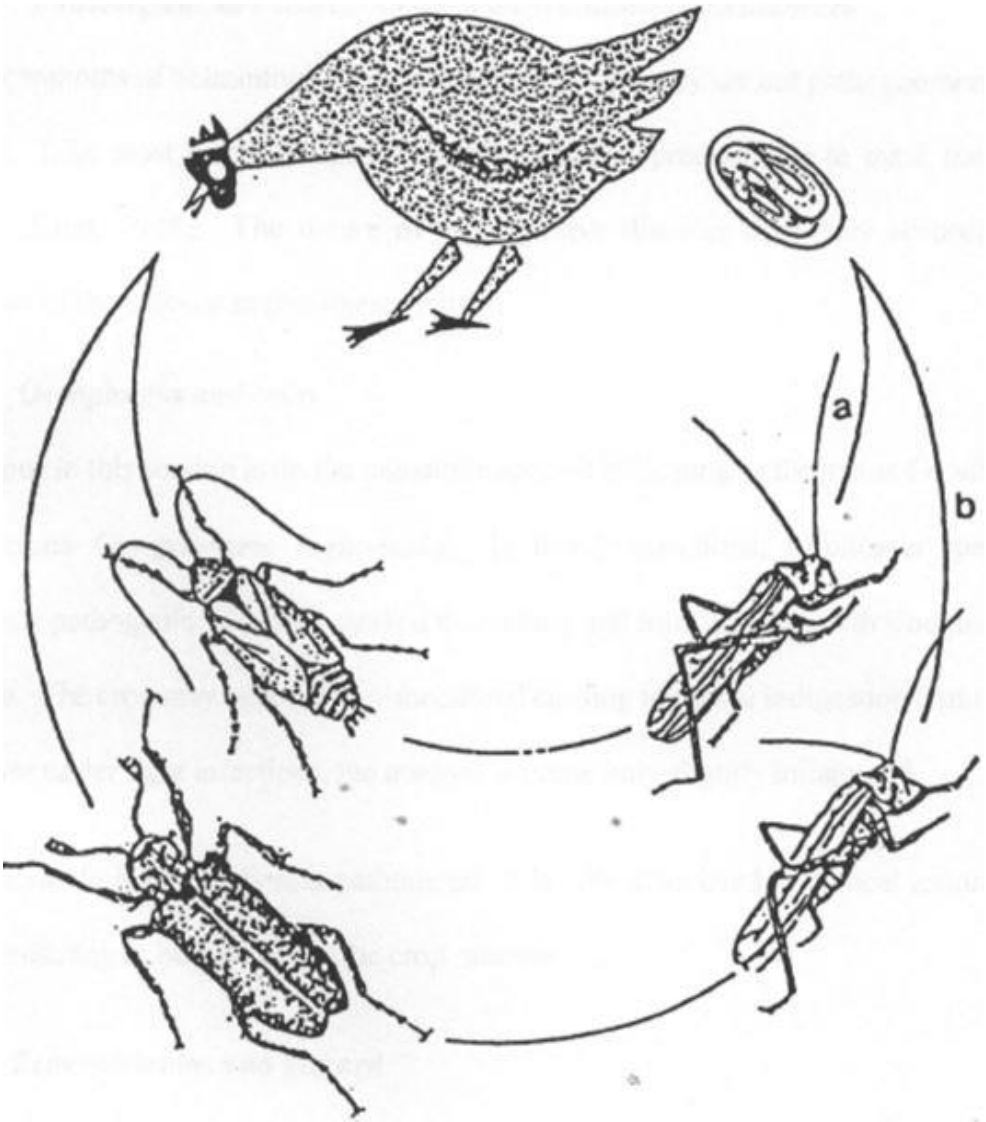

Figure 2. 9: An indirect life cycle of *Acuaria hamulosa* (Source: Belete, 2016).

Heavy infections are responsible for droopiness, fatigue, anaemia and emaciation. Sometimes the gizzard rapture due the damage they cause. In serious infections,

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the keratinized layer can even damage or even necrotic in severe cases (Permin & Hansen, 1998; Salam et al., 2009; (Kaufmann, et al., 2011).

2.9.5. *Amidostomum* spp.

Amidostomum spp. also referred to as gizzard worm, ventricular nematodiasis, amidostomiasis, epomidiostomiasis, is a parasite found worldwide in wild and domestic pigeons, ducks, geese but not very common in chicken. The predilection site of the worm is the proventriculus or in the oesophagus under the keratinized layer of the gizzard and feeds on blood (Islam et al., 1988; Fedynich & Thomas, 2008).

Morphologically, the adult worm is reddish in colour and slender (Permin & Hansen, 1998). The male worm measures 10 – 17 mm in length and 250 – 350 µm wide. The male has two spicules of the same length. Also, the female worm measure 12 – 24 mm long and 200 – 400 µm wide, with the thickest point around the vulva. Their eggs are embryonated when laid and size of the egg measure about 100 × 50 µm (Permin & Hansen, 1998) (Figure 2.10).

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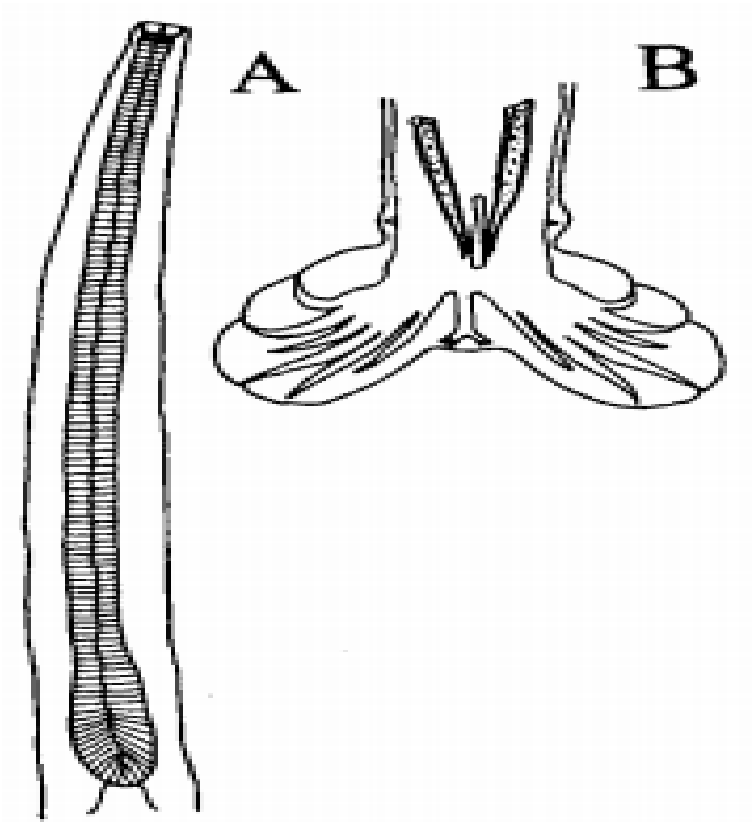

Figure 2. 10: Morphology of *Amidostomum anseris* (A) Head (B) Tail

The life cycle of *Amidostomum* spp is direct, with a 14 to 25 day prepatent period (Borgsteede et al., 2006). The eggs are deposited into the environment with faeces, and hatch to release the larvae and develop into infective stage after some days. Development may occur within or outside the egg. Susceptible animals become infected through contaminated food or water by ingestion or drinking (McDougald**,** 2020).

Appetite loss, emaciation, weakness and anaemia are clinical signs showed by birds as a result of *Amidostomum* spp infection (Johnston, 2011). The birds are likely to experience diarrhoea and die if stressed. The worm is highly pathogenic and young animals are easily susceptible whilst older birds are carriers of this infection. Severe inflammation, necrosis and haemorrhages are seen as they are

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caused by the adult parasites. Since the worm feeds on blood, in heavy infection severe anaemia is also experienced in birds (Permin, 1999; Tsiouris et al., 2019).

2.9.6. *Capillaria* spp.

Another name for *Capillaria* nematodes is hairworms, threadworm due to their extreme thinness in size (Yabsley, 2008; Park & Shin, 2010), and they are divided into two groups that is; those which burrow into the upper digestive tract (oesophagus or crop and those which burrows into the lower digestive tract epithelium (small intestine and rarely the ceca) **.** Six *Capillaria* spp. have been reported. Out of the six species reported, two of the species have been reported to occur in the upper digestive tract epithelium (oesophagus or crop) and the other four species reported to occur in the lower digestive tract epithelium (small intestine and rarely the ceca). *Capillaria nnulate* (Moin, 1958) and *C. contorta* (Creplin, 1839) are located in the oesophagus. Those reported to be found in the small intestine are; *C. caudinflata* (Molin, 1858), *C. bursata* (Freitas & Almeida, 1934) and *C. obsignata* (Madsen, 1945). Species *C. anartis* (Schrank, 1790) only occur in the ceca (Soulsby, 1968; Witter & Schat, 2003; Park & Shin, 2010).

Worms that belong to *Capillaria* spp. are hair-like, making them hard to identify in the intestinal content (Park & Shin, 2010). *Capillaria annulata* male measure 15 – 25 mm whilst the female measure 37 – 80 mm long. Typically, their eggs have a bipolar plugs and measure about 60 × 25 µm. *Capillaria contorta* males are of the same size as *C. annulata* but the females are only 27 – 38 mm long which makes them shorter. The *C. contorta* eggs measures about 60 × 25 µm *Capillaria caudinflata*, *C. bursata*, *C. obsignata*, and *C. anatis* measures only 6

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– 35 mm long because they are smaller. Their egg measures 45 x 25 µm (Permin & Hansen, 1998; Saif et al., 2008) (Figure 2.11).

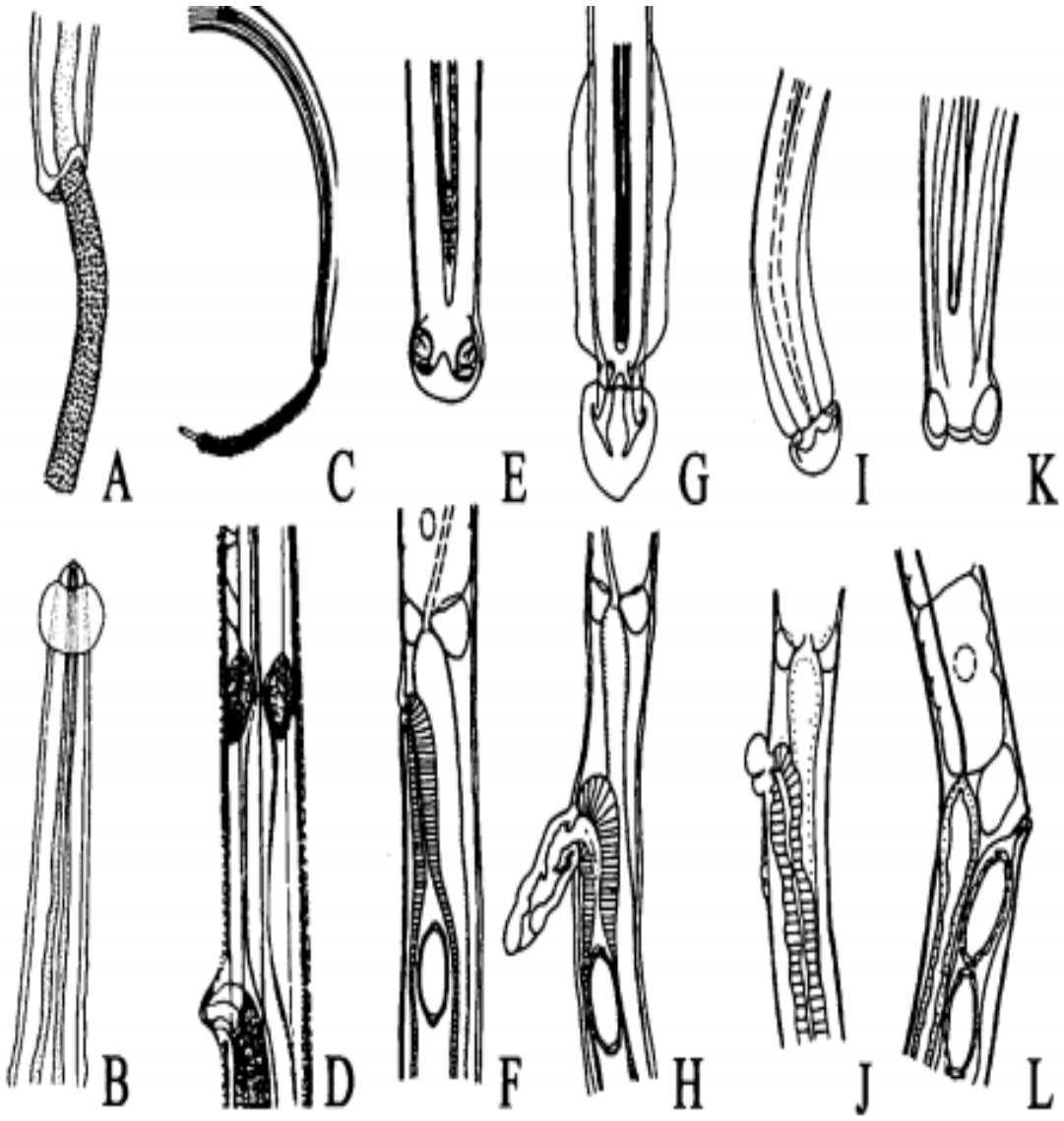

Figure 2. 11: Morphological structure of *Capillaria* spp. A, C, E, G, I and K represent the Male bursa. B represent the head of the Female. D, F, H, J, and L represent the Female vulva. A and B: represent *Capillaria annulata*, C and D: *C. contorta*, E and F: *C. obsignata*, G and H: *C. caudinflata*, I and J: *C. bursata*, K and L: *C. anatis*. (Source: Permin & Hansen, 1998)

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Capillaria species may experience either direct or indirect life cycle (Permin & Hansen, 1998; Belete, 2016). Their eggs are unembryonated and are deposited with the faeces into the environment and develop into first larval phase within 9 – 14 days. *Capillaria obsignata, C. anatis* and *C. contorta* have a direct life cycle (Belete, 2016) as shown in (Figure 2.12), meaning the eggs are infectious at embryonated infective L1 phase. After the eggs are ingested, they hatch at their predilection sites (oesophagus and small intestine) and mature into adult worms without migrating in the final host. Earthworms are the intermediate host of the species *C. caudinflata, C. bursata* and *C. annulata.* When swallowed by the intermediate host, within 14 – 21 days they develop into infective phases. Birds become infected when the earthworms are ingested. The *Capillaria* spp. prepatent time is about three weeks (Permin & Hansen, 1998; Butcher & Davis 2018).

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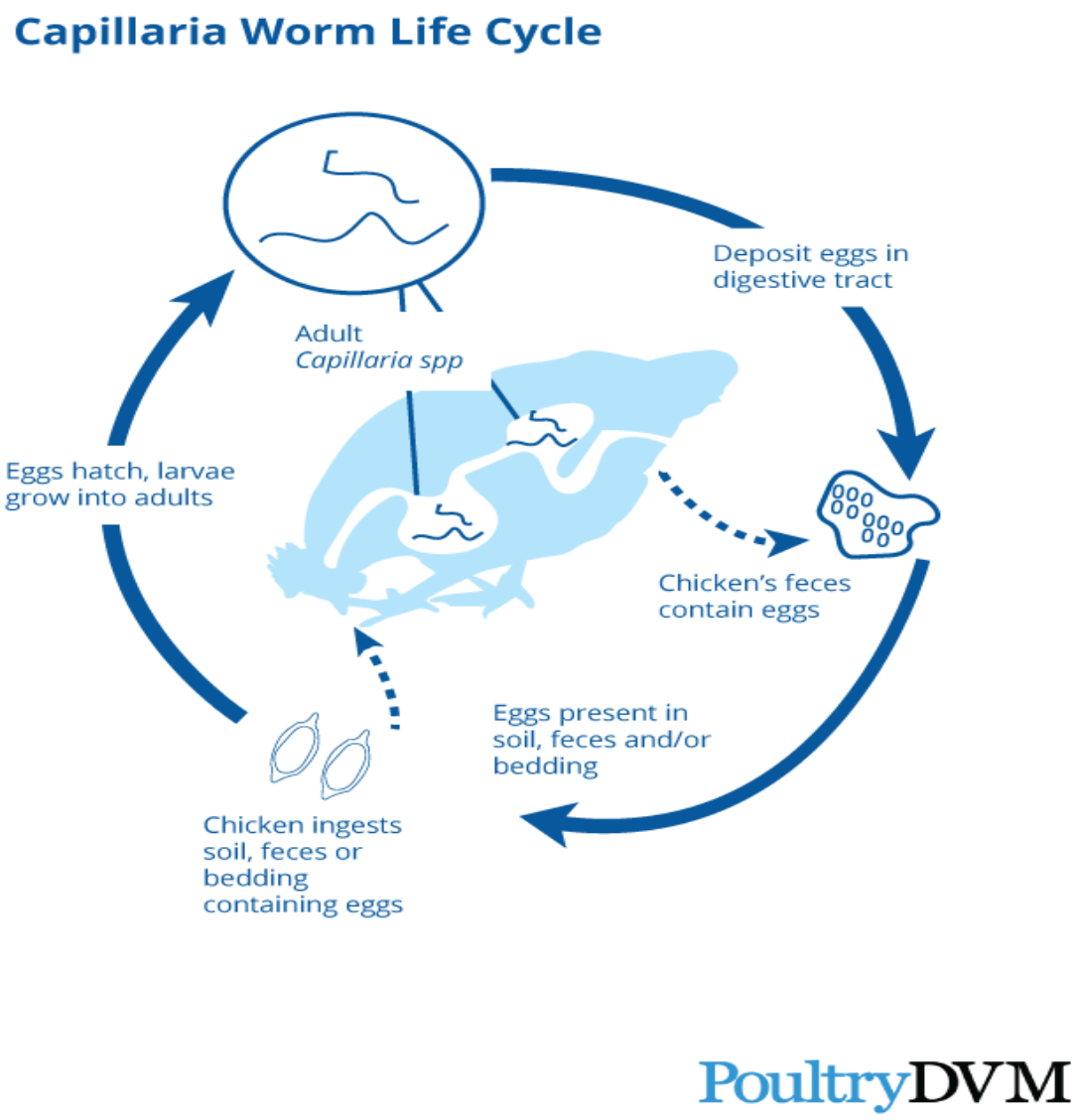

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Figure 2. 12: Diagram showing the life cycle of *Capillaria* spp. worm (Source: Poultry DVM, accessed on 20/11/2020)

Capillaria species can be very pathogenic for birds on free-range and those that are kept in deep-litter systems. Inflammation and thickening of the oesophagus and crop, birds become sluggish and anaemic, bloody diarrhoea and haemorrhagic enteritis due to severe infections are some of the clinical

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indications. This may cause high poultry mortality rate (Ngongeh et al., 2014; Chen et al., 2018; Lozano et al., 2019).

2.9.7. *Allodapta suctoria*

Molin in 1860 discovered *Allodapta suctoria* parasitic worm that affect poultry and other wild birds (Catelli et al., 1999; Permin et al., 2001; Faizullah et al., 2013). Another name for this parasite is *Subulura brumpti or Subulura suctorial*. *Allodapta suctoria* is very common in chickens and other domesticated poultry reported to be found in North and South America, Asia and Africa and has been reported in Ghana (Poulsen et al., 2000; Faizullah et al., 2013). The adult worm can be located in the lumen of the caeca (Hussen et al., 2012; Henry et al., 2019).

Morphologically, the male adult worm and female measures 7 – 10 mm and 9 – 18 mm, respectively. Eggs from this parasite is thin-shelled and spherical, and measures 52 – 64 × 41 – 49 µm (Permin, 1998). The adult worms are relatively similar in size and shape to *Heterakis* spp. but can be distinguished microscopically by examining the oesophagus and appearance of the spicules of the worm (Figure 2.13) (Permin & Hansen, 1998; McDougald, 2020).

The life cycle of this parasite is indirect with beetles and cockroaches as the intermediate hosts (Abdou & Selim, 1963; Nagarajan et al., 2012). They develop in the intermediate host after the eggs have been passed out with faeces and ingested, they develop in the intestinal wall after 7 – 8 days (Permin & Hansen, 1998). For another seven days in the intermediate host, the infective L3 larvae will be fully matured. Ingesting the cockroaches and beetles by birds causes

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infection and the larvae migrate to the ceca and matures into adult worm in six weeks (Permin & Hansen, 1998).

For *Allodapta suctorial* infection, clinical signs are rarely seen, but they are significantly import as *Heterakis* spp. (Permin & Hansen, 1998).

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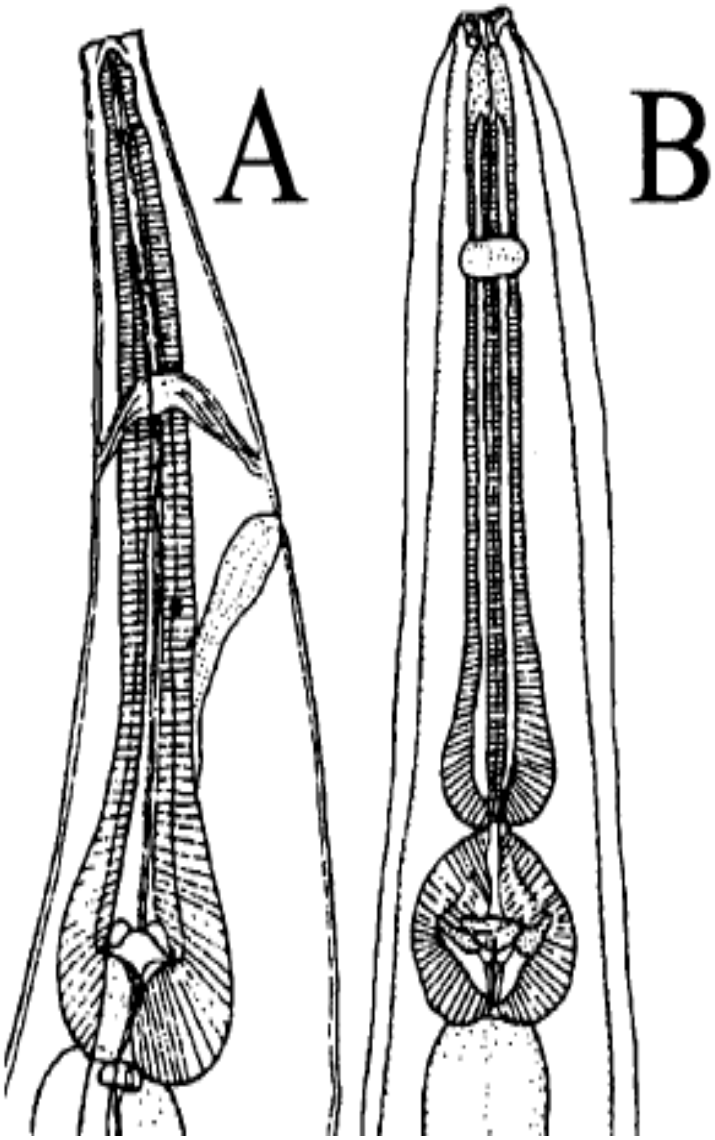

Figure 2. 13: (A) Anterior end of *Heterakis gallinarum* and (B) anterior end of *Allodapta suctoria* (source: McDougald, 2020)

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2.9.8. *Heterakis* spp.

Another common roundworm that infects poultry is the *Heterakis* spp. and cause a parasitic infection known as *Heterakidosis*. The predilection site of these nematodes is the ceaca (Clapham, 1934; Amundson et al., 2016). There are three *Heterakis* species that are of important to poultry, namely, *Heterakis gallinarum* (Schrank, 1788), *Heterakis isolonche* (Linstow, 1906) and *Heterakis dispar* (Schrank, 1790). *Heterakis gallinarum* has been reported to occur in chickens, turkeys, guinea fowls, quail, partridge, and also waterfowls in most part of the globe. *Heterakis isolonch* is very prevalent in pheasants, however there have been reports of its occurrence also in ducks, turkeys, grouse, prairie chicken and quail. Whilst *Heterakis dispar* has also been reported to affect ducks and geese in some parts of the word (Hoque et al., 2011; Nagwa et al., 2013; Wang et al., 2013; Bobrek et al., 2019).

2.9.8.1 Morphological Features of *Heterakis* spp.

Permin & Hansen, (1998) described the morphology of *Heterakis* spp. All *Heterakis* spp. are comparable in appearance, even though *H. dispar* is relatively larger than *H. gallinarum* and *H. isolonche* (Figure 2.14). The male measures 7 - 13 mm long and that of the female measures 10 –15 mm long. These worms are differentiated by the shape of the oesophagus and the length of the spicules. *Heterakis* spp. eggs are thick with a smooth shell surface. The egg characteristics makes it difficult to distinguish them from egg of *Ascaridia galli* (Belete, 2016). Their eggs measure 65 – 80 x 35 – 46 µm (Permin & Hansen, 1998).

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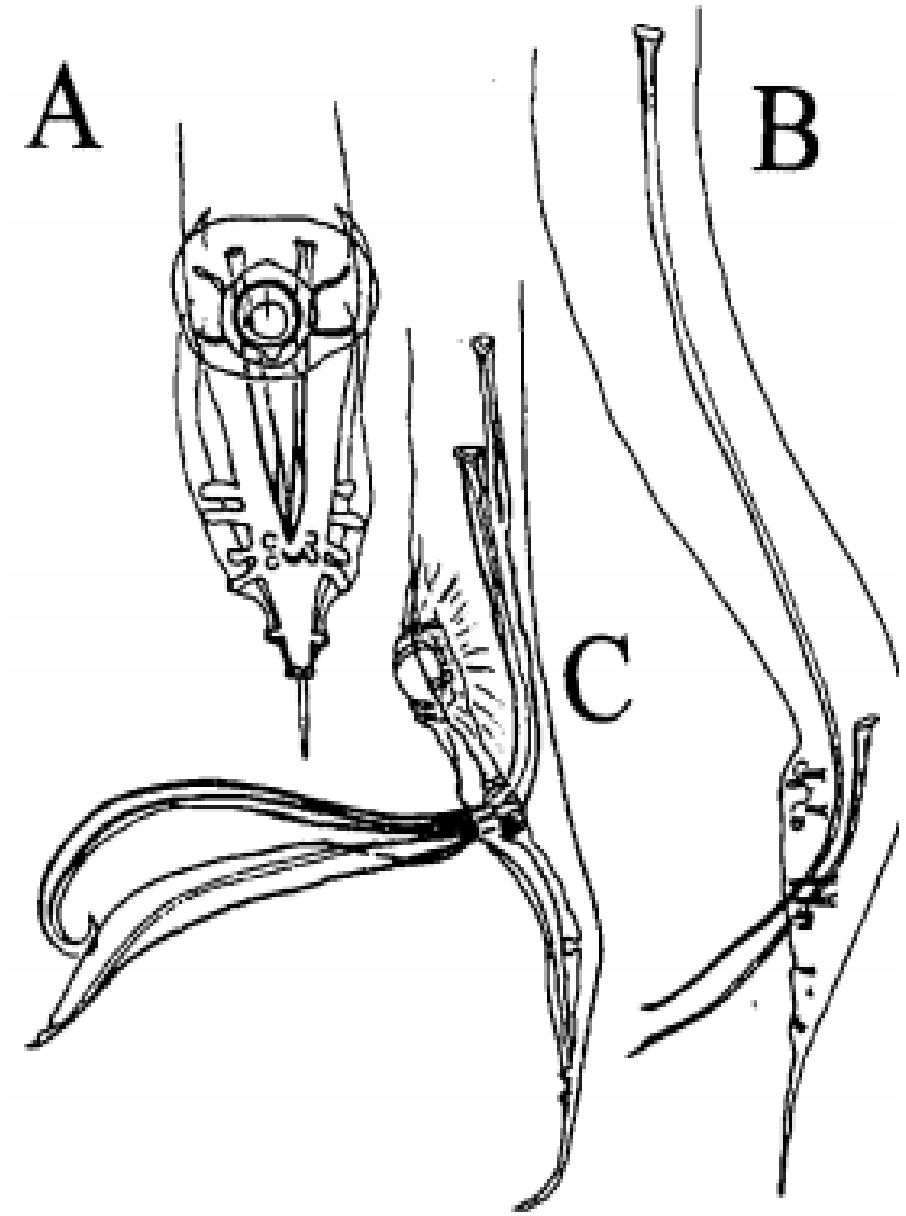

Figure 2. 14: Morphology of *Heterakis* spp. (A) represent the tail of *Heterakis dispar*; (B) Tail of *Heterakis gallinarum*, and (C) Tail of *Heterakis isolonche*

2.9.8.2. The Life Cycle of *Heterakis* spp.

Heterakis spp. have direct life cycle (Dorman, 1928; Stehr et al., 2018; Cupo & Beckstead, 2019) as shown in (Figure 2.15). Eggs passed with faeces are unembryonated and develop into infective stage within two weeks period depending on the environmental conditions. Houseflies and earthworms

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sometimes act as mechanical transport host or the unembryonated eggs are consumed directly by the host kept on deep litter or during scavenging (Permin & Hansen, 1998; Phiri et al., 2007; Bobrek et al., 2019). When susceptible host ingest the infective eggs, the eggs hatch in the small intestine and the larvae migrate to the caeca within 24 h period and mature into adult worm. Before *H. isolonche* larvae becomes an adult worm, it may have gone through a tissue stage that takes a prepatent time of 24 – 30 days (Permin & Hansen, 1998).

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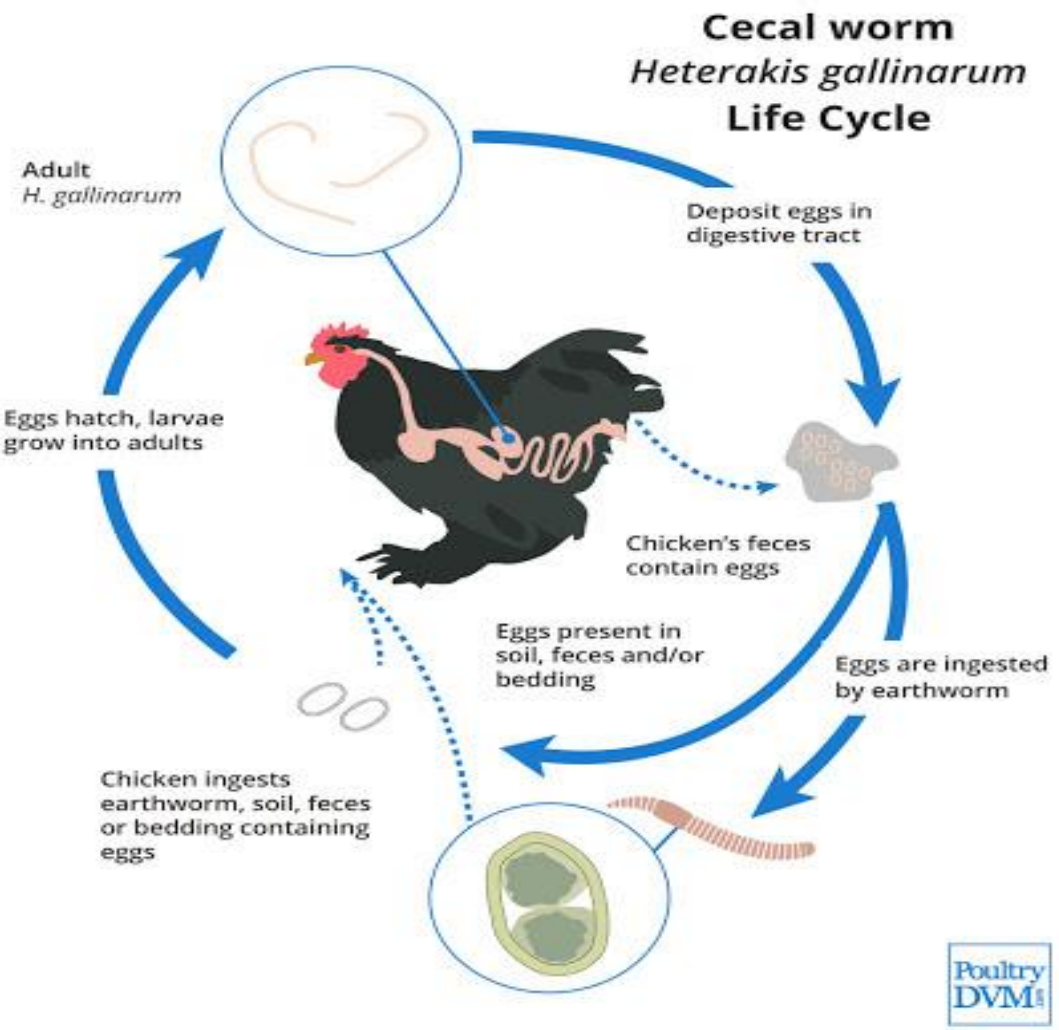

Figure 2. 15: Diagram showing the life cycle of *H. gallinarum* in chicken (Source: Poultry DVM. Accessed on 20/11/2020).

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2.9.8.3. Pathogenicity and Clinical Signs of *Heterakis* spp. Infection.

Heterakis gallinarum is the best species described among all the Heterakids that infects birds. They are able to infect gallinaceous birds and waterfowl, and cause inflammation of the caeca wall resulting in thickness, diarrhoea, emaciation and death (Park & Shin, 2010). They may also be a vector of *Histomonas meleagridis*, a protozoan which is the causative agent of a deadly disease in chickens and turkeys called histomoniasis (Lund & Chute, 1974; McDougald, 2005; Saif et al., 2008; Chute et al., 2011). Mostly, infection by *Heterakis gallinarum* are asymptomatic (Permin & Hansen, 1998).

2.9.8.4. Economic Importance of *Heterakis* spp. Infection in Poultry

Heterakis spp. are responsible for low eggs production, treatment cost, low growth rate and high mortality rate in chickens (Permin et al., 2006; Hinrichsen et al., 2016). Even though they are known to be none pathogenic, but they act as a vector of *Histomonas meleagridis*, a protozoan known to cause a deadly disease therefore causing economic losses to the farmers (Saif et al., 2008). In the United State of America, it was estimated that about $200 million are lost annually and $45 million are also used on medications and prevention of poultry diseases (Luka & Ndams, 2010).

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2.9.8.5. Control of *Heterakis* spp. Infection in Poultry

As described by Permin & Hansen (1998), *Heterakis* spp. could be controlled as follows:

1. Improving management system and sanitary condition
2. Application of prophylactic drugs and use of anthelmintic drugs recommended to use (such as imidazothiazoles, benzimidazoles and macrolides).

2.9.9. *Ascaridia* spp.

Ascaridia galli are also known as *A. lineata*, *A. perspicillum* (Ackert, 1931) has been reported to infect poultry globally. It is known to infect chicken, turkeys, duck, doves and geese where they are commonly found in the small intestine (Schmidt & Kuntz, 1970; Menezes et al., 2003). With unusual migration, they can be found occasionally in the oesophagus, crop, gizzard, oviduct, body cavity as well as being incorporated into hens egg (Yazwinski & Tucker, 2008; Bharat et al., 2017; Sharma et al., 2019). Other species like *A. columbae* have been reported to occur in pigeon and doves, *A. bonasae* in grouse and Bobwhite quail, *A. compar* found in grouse, partridge, pheasant and Bobwhite quail, *A. dissimillis* commonly found in turkeys (Saif et al., 2008).

2.9.9.1. History of *Ascaridia* spp

The name *Ascaris galli* was given by a German parasitologist called Schrank in 1788 (Ramadan & Abou Znada, 1992; Permin et al., 2006). According to Ramadan & Abou Znada, (1992), Schrank did not describe this worm, but the species were based on a composite species, *Ascaris teres*, this nematode occurred in chickens, dogs, cats and raptors. Description of the parasitic nematodes were

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provided by early researchers, including Bloch, Duardin, FrÖhlich, Gmelin, Linstow, Railliet, Rudolphi and Schneider (Skryabin et al., 1951; Fedynich, 2009).

In 1927, Cram regarded *A. perspicillum* to be a substitute of *A. galli.* Four species were listed as parasites that infects domestic fowls in India and they are; *A. lineata, A. galli, A. granulosa* and *A. compar*. *Ascaridia lineata* and *A. granulosa* were the two species that were considered as the synonyms of *A. galli* (Bhalerao, 1935; Baylis, 1936). A researcher in India also described three species, *A. galli*, *A. columbae* and *A. compar* and were all found in domestic poultry (Ramadan & Abou Znada, 1992). However, another investigator stated that, the characteristics of *A. perspicillum, A. lineata, A. granulosa* and *A. hamia,* which were previously described to differentiate from *A. galli* was unsound. Therefore, they were considered identical to *A. galli* (Nath, 1970).

Increasing interest in the 20th century discovered exchange of parasites between wild and the domestic populations. Also, the importance of parasites as a cause of infections and mortality in birds, led to a series of parasitic surveys in America and Europe (Peterson, 2004). As a result, early studies have made a significant contribution to the understanding of the occurrence and geographical distribution of these species (Atkinson et al., 2009).

2.9.9.2. Morphological Characteristic of *Ascaridia* spp.

The adult worms are semi-transparent, the male and female measures about 51 – 116 mm and 72 –116 mm, respectively (Permin & Hansen, 1998), making them the largest nematode in poultry (Bazh, 2013; Rahimian, 2016; Urbanowicz et al.,

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2018). The vulva of the female can be found in the anterior of the body. They have three prominent lips in the oral opening. Only the male can be identified accurately, with a preanal sucker as well as two equal spicules that measures 1 – 2.4 mm long (Permin & Hansen, 1998; Saif et al., 2008). Eggs of *Ascaridia* spp. have an ova shape with smooth surface and measures from 73 – 92 x 45 – 57 µm. *Ascaridia galli* and *Heterakis gallinarum* eggs are similar in appearance and shape except that eggs of *A.galli* are slightly smaller and parallel edges (Permin & Hansen, 1998) (Figure 2.16).

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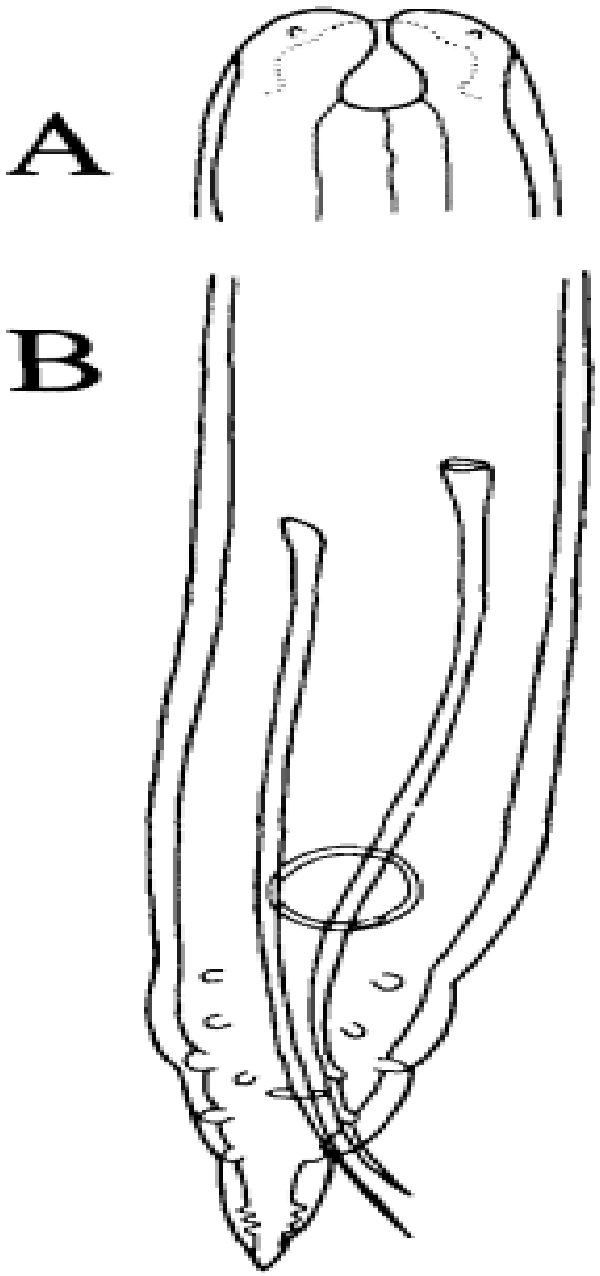

Figure 2. 16: Morphology of *Ascaridia galli* (A) Anterior end (B) Posterior end (Source: Permin & Hansen, 1998).

2.9.9.3. Life Cycle of *Ascaridia* spp.

According to Permin & Hansen, (1998), the life cycle of *Ascaridia galli* is simple and direct, involving the sexually adult worm in the GIT and the infective larvae stage in the form of an embryonated resistant eggs present in the environment

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(Figure 2.17). Infective eggs hatch in the upper or lower digestive tract of susceptible host after ingesting the eggs passed with faeces (Atkinson et al., 2009; Torres et al., 2019). After hatching, the second larvae phase are free in the mucous layer of the duodenum for some day after infection (Kaufmann et al., 2011; Daş et al., 2017) and reaches the infective stage in 10 – 20 days or longer, but depending on the environment condition. They molt to the fourth infective phase and developed within four weeks. Eggs may not survive after 22 h at a temperature of about -12℃ to -8℃, but might withstand winter and a moderate frost (Ackert, 1931; Cruthers et al., 1974; Chadfield et al., 2001). At all stages, temperatures above 43℃ are deadly for eggs to survive (Ackert, 1931; Boyko & Brygadyrenko, 2017). Environmental factors such as pH, humidity, temperature and concentration of ammonium can cause eggs to be infective in deep litter systems for a year (Sherwin et al., 2013; Katakam et al., 2014; Chen et al., 2015; Boyko & Brygadyrenko, 2017). Permin & Hansen, (1998), stated that, the life cycle is accomplished, when the infective eggs are consumed by new host through infected water or feed. The eggs which contain the third larvae phase (infective stage) are transported mechanically to the duodenum. Eggs arrive in the duodenum and hatch within 24 h after ingestion, and larvae are released into the lumen of the intestine (Tugwell & Ackert, 1952; Al-Daraji & Al-Amery, 2013). Histotrophic stage is initiated by most larvae after they have entered the mucous of the small intestine (Permin & Hansen, 1998; Norton et al., 1999; Dalgaard et al., 2015). Histotrophic stage length depends on the amount of infective eggs eaten (Nejsum et al., 2009; Ferdushy et al., 2013). The larvae then mature in the lumen of the intestine and passed onto the environment through faeces by the host, and the cycle continues (Permin & Hansen, 1998). It is widely

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agreed that several factors such as the age of the chicken, the volume of the ingested eggs, the age of the infective eggs, sex of chickens and the host diet, play a role in worms' establishment in the intestine (Clavijo & Flórez, 2018; Jha et al., 2019). *Ascaridia dissimilis* and other worms of this genus have a comparable life cycle (Hemsley, 1971).

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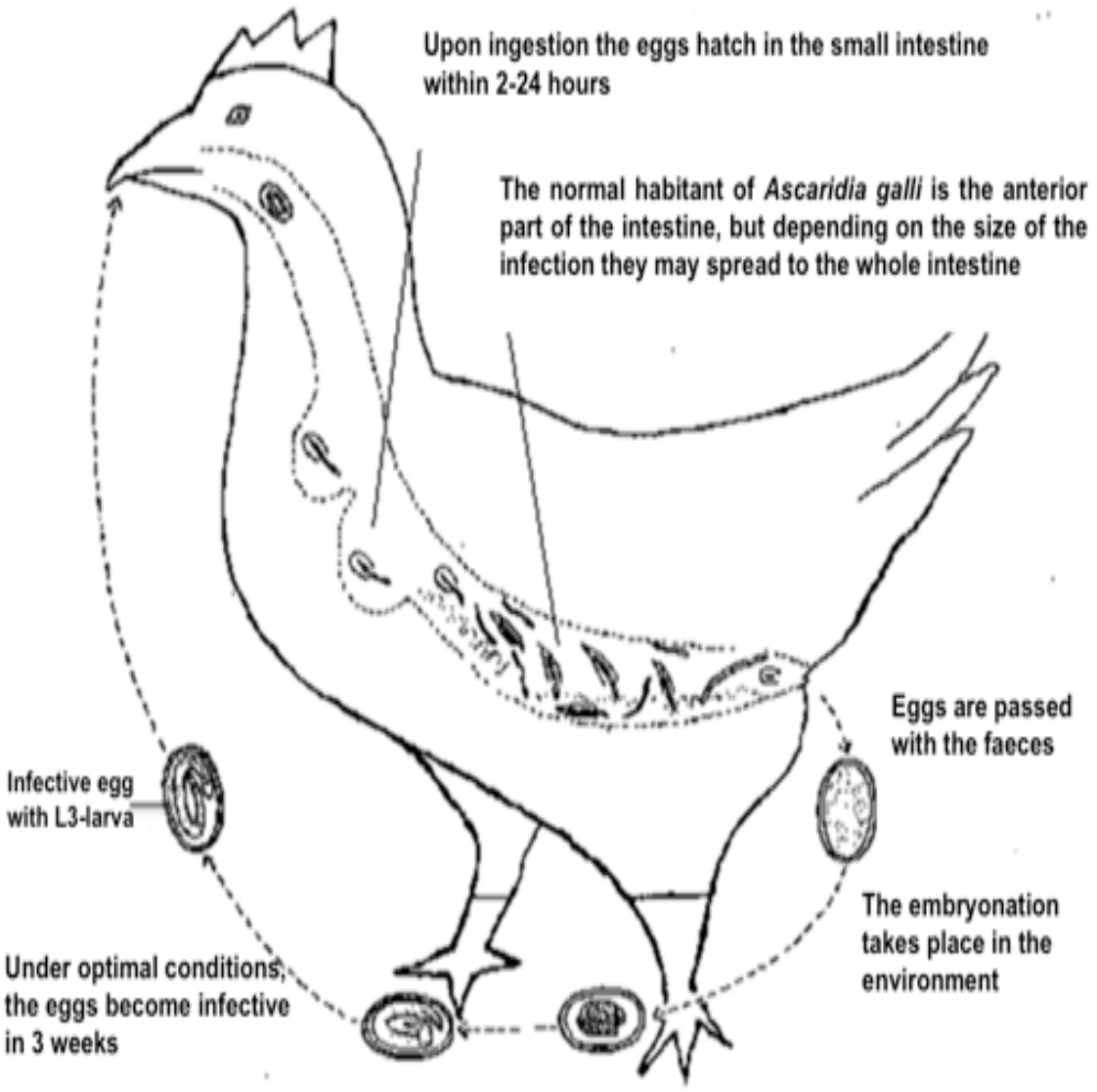

Figure 2. 17: Diagram showing the life cycle of *Ascaridia galli* (Source: Permin, 1997).

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2.9.9.4. Clinical Signs of *Ascaridia* spp. Infection in Poultry

Infection with *Ascaridia* spp. result in loss of weight in chicken which has a relation with heavy worm burden (Magwisha et al., 2002). Generally, the clinical signs showed by infected animals are; loss of appetite, ruffled feathers, wing drooping, weight loss, decrease in egg production, anaemia, diarrhoea and high mortality rate (Permin et al., 1997; Dahl et al., 2002; Ruhnke et al., 2017).

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2.9.9.5. Economic Importance of *Ascaridia* spp. Infection in Poultry

Despite the high occurrence of these parasites, the welfare and health of chickens have received little attention (Sherwin et al., 2013). The prevalence of *Ascaridia* spp. are responsible for low eggs production, treatment cost, low growth rate and high mortality rate in chickens (Permin et al., 2006; Hinrichsen et al., 2016; Ybañez et al., 2018). In Hungary alone, *Ascaridia galli* infection reported in about 40 million chickens caused annual economic losses of approximately 6 million U.S. dollars (Sarker et al., 2009). *Ascaridia galli* may also play a role in transmission of *Salmonella* infection and avian reoviruses resulting in disease and economic losses (Urbanowicz et al., 2018). They are known to occur worldwide,

2.9.9.6. Control of *Ascaridia* spp. Infection in Poultry

Parasitic control measures are purposely done to reduce the rate of parasitic infections at a lowest rate, which will prevent adverse clinical effect and production losses. According to Permin & Hansen (1998), the basic principles to the control of *Ascaridia* spp. infection in poultry include:

1. Improving management system and sanitary condition

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2. Application of prophylactic drugs and use of anthelmintic drugs recommended to use (such as imidazothiazoles, benzimidazoles and macrolides)

Mostly in rural areas, traditional concoctions have been used to control nematodes infection in backyard chickens (Mwale & Masika, 2011)

2.10. Molecular Characterization of *Ascaridia* spp.

Availability and use of molecular approaches for studying population genetics structure and taxonomy of animals have been employed in recent years (Li et al., 2013). Also, molecular markers such as Internal transcribed spacer genome (ITS) and Mitochondrial DNA (mtDNA) have been employed to study the relationship between *Ascaridia galli* infection in chicken population across the globe (Katakam et al., 2010; Bazh, 2013; Malatji et al., 2016). To distinguish worms and investigate transmission cycles of roundworms, sequence analysis of mitochondrial markers mainly *cox1* gene has been employed as well as sizing microsatellite containing loci (Höglund et al., 2012; Betson et al., 2013)

A recent study conducted in Sweden by Höglund et al. (2012), showed a genetic variation of *A. galli* from the same flock of chicken and between farms. Genetic methods open up prospect to trace how nematode infections are transmitted (epidemiological study) both within and between different host populations (Criscione et al., 2005). Geographical movement of parasitic nematode is understood by means of genetic markers (Fisher & Viney, 1998; Gilabert & Wasmuth, 2013; Malatji et al., 2016). Therefore, unlike most free-range animals, genetic variation of parasites is not only caused by their own reproductive and

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transmission patterns but also the host behaviour like migration (Schwensow et al., 2007; McGaughran et al., 2014). Direct sequencing of PCR-amplified mitochondria DNA has been employed to infer the population genetics and variation of a number of animal nematodes (Otranto et al., 2005; Liu et al., 2014). Malatji et al. (2016) reported *A. galli* genetic variation in South African chicken from the sequencing result of mitochondria gene. Population variation is significant for outlining control strategies, drug resistance and spread of parasitic nematodes disease**.**

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CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study Area and Study Population

This study was conducted in the Kumbungu, Savelugu and Tolon Districts in the Northern Region of Ghana. The Region's geographical coordinates are 9°29′59.99″North -1°00′0.00″ West. The Region experiences one rainy season from May to October that peaks in July and August with a mean annual rainfall of 110mm. The rest of the year is relatively dry. The vegetation is classified as grassland, especially savanna with clusters of drought resistance trees like baobabs. During the dry season the temperature reaches above 42 °C (Kranjac-Berisavljevic et al., 2014).

The study populations were composed of rural chickens, aged 6 to 10 weeks that were left to roam freely and scavenge from morning to evening for feed. Eighty-six chickens assembled from several villages within the three Districts were purchased from birds-dealers or owners for the period, from March to April 2019.

3.2. Sample Collection and Identification

The 86 chickens came from the three Districts as follows: Kumbugu n = 36 chickens, Savelugu n = 25 chickens and Tolon n = 25 chickens. On the days of procurement, all chickens of similar age category were bought for the study. Procured birds were transported in group cages with minimal stress, to the Biology Laboratory of the Spanish Laboratory Complex, University for Development Studies, Nyankpala campus for examination. Upon arrival, chickens were allowed to rest for 1 – 2 h and given water to stabilize.

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Prior to slaughter the sex of birds was noted using the rudimentary ring, comb, wattle and ear lobe, and identifiable codes were assigned to individual birds. Birds were slaughtered under minimal stress conditions: one person held secure the wings and legs to prevent flapping and kicking or jumping and a second person slaughter the chicken with sharp knife and allowed to bleed by holding upside down. Slaughtered chickens were carefully opened and the alimentary canal removed. Each part of the alimentary tract was dissected using surgical blades and thoroughly inspected for macroscopic adult parasites. Worms recovered were identified using the key by Moravec, (2009). Parasites were collected using forceps and were fixed/stored in 70 % ethanol at ambient temperature until molecular analysis.

The carcasses were burnt afterwards.

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3.3. DNA Extraction

DNA was extracted from individual roundworms that were recovered. For each worm, the tail end of the body equivalent to 200 mg was cut and chopped into smaller pieces and transferred into 1.5 ml Eppendorf tube containing 400 µl of lysis buffer pH 5.0 [2 % Cetyl trimethylammonium bromide (CTAB), 1 M Tris-HCl pH 8.0, 5 M Sodium Chloride (NaCl), 0.5 M Ethylenediaminetetraacetic acid (EDTA) pH 8.0, 1 % of polyvinyl pyrrolidone (PVP 40)]. Tissues – lyses buffer mixture was homogenized by vortexing and incubated in a water bath at 80 °C for 4 h to digest the cell to release its content. After the incubation, the lysate was centrifuged at 15,000 RPM for 7 min 20s. The supernatant was removed by pipetting into a clean 1.5 ml Eppendorf tube and 200 µl Chloroform: Iso Amyl alcohol (24:1) was added and carefully mixed by inversion for 60 s before then

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centrifuging at 15,000 RPM for 60 s. The upper aqueous phase was collected and transferred into a clean 2.0 ml Eppendorf tube. DNA was precipitated by adding 50 µl of 6.25 M Ammonium acetate, 500 µl of ice-cold absolute ethanol and kept at -20 °C for 1 h. The precipitated DNA was centrifuged at 15,000 RPM for 7 min. The supernatant was decanted gently without disturbing the DNA pellet. DNA pellet was washed with 500 µl of ice-cold 70 % ethanol to remove excess salt following 15,000 RPM centrifugation for 3 min. Ethanol was discarded gently and excess ethanol was removed by drying for only 15 min. to avoid over drying the DNA. DNA was suspended or re-dissolved in 50 µl sterile DNase/RNase-free molecular water. The mixture was incubated at 65 °C for 20 min. to destroy any DNase that may be present and afterwards stored at 4 °C until further molecular analysis.

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3.4. Amplification of Partial Mitochondrial *cox1* gene

Polymerase chain reaction was performed (PCR) on 20 samples based on DNA quantity and quality yielded from 72 individual worm's DNA extracted, using the previously designed primers by Katakam et al. (2010), GCox1F4 (forward: 5'-ATT ATT ACT GCT CAT GCT ATT TTG ATG-3') and GCox14 (reverse: 5'-CAA AAC AAA TGT TGA TAA ATC AAA GG-3') to amplify a partial fragment of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene (533 bp). PCR was performed in a total volume of 50 µl reaction mixture consisting of 10 µmol each of forward and reverse primers, OneTaq® Quick-load® 2x Master Mix with Standard Buffer [20 mM Tris-HCl (pH 8.9 at 25 °C), 22 mM KCl, 1.8 mM $MgCl_2$, 22 mM NH_4Cl, 0.2 mM dNTPs, 5 % Glycerol, 0.06 % IGEPAL® CA-630, 0.05 % Tween® 20, and 0.625 U One Taq® DNA polymerase] (New

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England BioLabs Inc.) and 3 µl of DNA. PeQlab thermal cycler (PEQLAB Ltd.) (appendix III) was used to perform the reaction.

The PCR cycling condition for the *cox1* gene consisted of an initial DNA denaturation at 95 °C for 15 min., followed by 35 cycles of 95 °C denaturation for 30 s, 55 °C annealing for 40 s and 72 °C extension for 60 s, and final elongation at 72 °C for 10 min. (Katakam et al., 2010). In all the runs, negative control (no-DNA) was included.

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3.5. Gel Electrophoresis

Agarose gel (1.5% w/v) was prepared and pre-stained with ethidium bromide. The 1.5 % agarose gel was prepared as follows: 0.75 g of agarose granules was weighed and dissolved in 50 ml of 1x Tris-Borate EDTA (TBE) buffer and dissolved in a microwave for 60 s. The mixture was poured in a gel tank. Gels were cast under room temperature and allowed to solidify for 40 min. The 1.5 % agarose gel was carefully placed into the electrophoresis gel chamber filled with 1x TBE buffer.

After the amplification, 7 µl of the PCR amplicons were mixed with 1 µl of 6x loading dye and were loaded into the wells of the gel. A molecular marker ranging from 100 – 5000 bp (FastRuler™ middle Range DNA ladder, ThermoScientific, UK) was used to estimate the size and movement of DNA bands. Gels were loaded with the samples and run at 80 v for 30 min. Gel visualization was done using the transilluminator.

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3.6. DNA Sequencing

The 20 PCR products from the three Districts were sequenced using the reverse primer used in the amplification. Sequencing reaction was performed using the Big Dye reaction reagents and detected using the ABI 3500XL Genetic Analyze ABI 3500XL Genetic Analyzer by Inqaba Biotec. Ltd, Pretoria (South Africa).

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3.7. Data Analyses

3.7.1. Statistical Analysis

The parasitological data collected were entered into Microsoft Excel 2016. Data analysis was done using Microsoft Excel and STATA v.15. The prevalence of gastrointestinal parasites infections was expressed as a percentage as animals positive per total number of animals examined (Permin, 1999). The data was subjected to the Chi-Square test and level of significance was considered as ($p < 0.05$). The mean intensity (worm count) was determined by dividing number of a particular parasite species by the number of infected animals containing that worm, and average abundance was determined by dividing the number of parasites of a particular worm species by the total number of chickens examined (Ben Slimane, 2016).

3.7.2. DNA Sequence Analysis

Sequence chromatograms were viewed, edited and trimmed using the GENtle software v.1.9.4 (Manske, 2003, University of Cologne) to attain the consensus bases of 475 bp of all the 20 sequences. The sequences were cut from a specific position without the noise portion. Basic Local Alignment Search Tool (BLAST) of GenBank was employed on each sequence to search for identical species in the

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GenBank of the National Centre for Biotechnology Information (NCBI). Nucleotide and haplotype diversities were determined and gene flow between populations using DnaSP software v.6 (Rozas et al., 2017) and PopART v.1.7 (http://popart.otago.ac.nz) was used to construct a haplotype network of *cox1*gene sequences based on TCS algorithm (Clement et al., 2002).

Level of differentiation within the populations and amongst the population was determined using Analysis of Molecular Variance (AMOVA), and Tajima's D (Tajima, 1989) and Fu's tests *Fs*, (1997) were performed in Arlequin v.3.5.2.2 (Excoffier & Lischer, 2010)**.** Moleculsar Evolution Genetics Analysis (MEGA) software version X (Kumar et al., 2018) was used to show relationship between the sequences of the current study and sequences from other countries based on the Hasegawa-Kishino Vano model (Hasegawa et al., 1985) and 1000 bootstrap replications used to test the tree.

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CHAPTER FOUR

4.0 RESULTS

4.1. Prevalence of Roundworms in Chicken

A total of 86 chickens were collected from several villages in the Kumbungu, Savelugu and Tolon Districts. Out of the total number of chickens examined for parasitic infection, 41 (47.67 %) were infected with gastrointestinal namatodes in this study. Two nematode species were identified as *Ascaridia galli* and *Heterakis* spp. with prevalence rate of 37.21 % and 20.93, respectively. Prevalence in relation to sex was 48 % in male and 47.54 % in female birds (Figure 4.1). Chi-square analysis showed no significant association of sex and infection ($x^2 = 0.147$, p = 0.929).

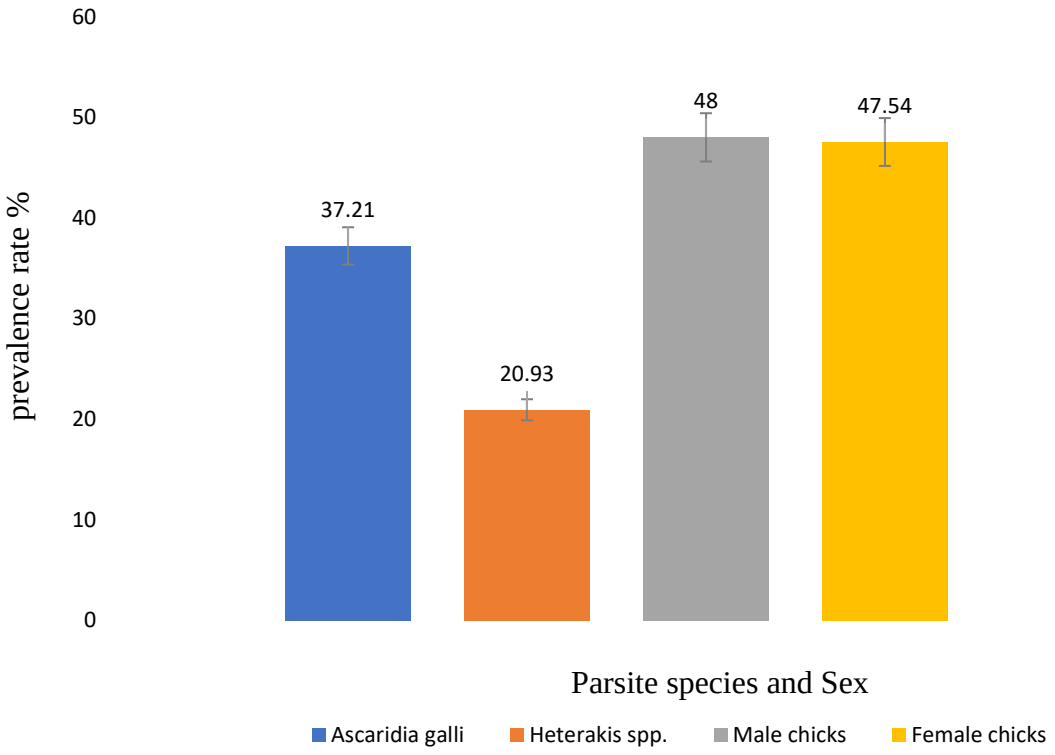

Figure 4. 1: Prevalence of roundworm infection in chicken

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4.2. Geographical Distribution of Parasites Infection

Amongst the 86 chickens sampled, 25 were from Savelugu District, 25 from Tolon District, and 36 from Kumbugu District (Table 4.1). Prevalence of *Ascaridia galli* was higher in Tolon District (56.0 %) and lower in Kumbungu District (25.0 %) Savelugu district was (36.0 %), Tolon and Kumbungu districts recorded and respectively. *Heterakis* spp. prevalence rate in Savelugu District was (28.0 %), Tolon and Kumbugu Districts also recorded (20.0 %) and (16.67 %), respectively. A statistical analysis by the Chi-square test showed a significant association in the distribution of *A. galli* (x^2 = 6.0907, p = 0.048) infection and location but not for *Heterakis* spp. (x^2 = 1.1635 p = 0.559).

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Table 4. 1**:** Prevalence of *Ascaridia galli* and *Heterakis* spp. in rural chicken from Kumbungu, Savelugu and Tolon Districts.

	Districts				
Parasite species	Savelugu (%) (n = 25)	Tolon (%) (n = 25)	Kumbungu (%) (n = 36)	x^2	p- Value*
Ascaridia galli	9 (36.0)	14 (56.0)	9 (25.0)	6.0907	0.048
Heterakis spp.	7 (28.0)	5 (20.0)	6 (16.67)	1.1635	0.559

*P values, Chi-square test p< 0.05

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4.3. Prevalence of Gastrointestinal Parasites Mixed Infections

Several chickens were infected with at least one or more parasites. Out of the chickens sampled in this study, 32 (37.21 %) were infected with one of the nematode species either *Ascaridia galli* or *Heterakis* sp. and 9 (10.46 %) chickens had a multiple infection of the two nematode parasites (Table 4.2).

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Table 4. 2: Prevalence of *Ascaridia galli* and *Heterakis* spp. mixed infections in rural chicken.

Parasite	Number of chicken infected	Percentage chickens infected (%)
Ascaridia galli or *Heterakis* spp.	32	37.21
Ascaridia galli and *Heterakis* spp.	9	10.46
Total	**41**	**47.67**

4.4. Worm Burden of *Ascaridia galli* and *Heterakis* spp. in Rural Chicken.

Worm burden of *Ascaridia galli* and *Heterakis* spp. infection in chicken from the three Districts was calculated (Table 4.3). Chickens from Tolon District were found to harbour *Ascaridia galli* more than any other chicken in the other Districts with the highest mean worm load of 9.14 ± 8.12 and a range of 3 – 36 worms. Savelugu chickens were found to habour *A. galli* more than *Heterakis* spp. with average worm burden of 8.78 ± 3.83 and a range of 6 – 12 worms. Chickens from

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Kumbungu District recorded the lowest mean worm load of 6.11 ± 2.20 and a range of 3 – 9 worms.

Table 4. 3: Worm burden of *Ascaridia galli* and *Heterakis* spp. infections in rural chicken.

District	Parasite species	Mean worm load		
		Mean ± SD*	Min**	Max#
Savelugu	*Ascaridia galli*	8.78 ± 3.83	4	13
	Heterakis spp.	9.57 ± 2.07	6	12
Tolon	*Ascaridia galli*	9.14 ± 8.12	3	36
	Heterakis spp.	9.80 ± 2.59	7	13
Kumbungu	*Ascaridia galli*	6.11 ± 2.20	3	9
	Heterakis spp.	9.00 ± 2.10	6	12

*Standard deviation

**Minimum number of worms in a chicken

Maximum number of worms in a chicken

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4.5. DNA Extraction and Amplification

DNA was successfully extracted from individual worms. Polymerase chain reaction (PCR) was performed on 20 DNA samples in a total volume of 50 µl. Amplicons of partial mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene showed a uniform DNA fragments size (approximately 533 bp) (Figure 4.2) .

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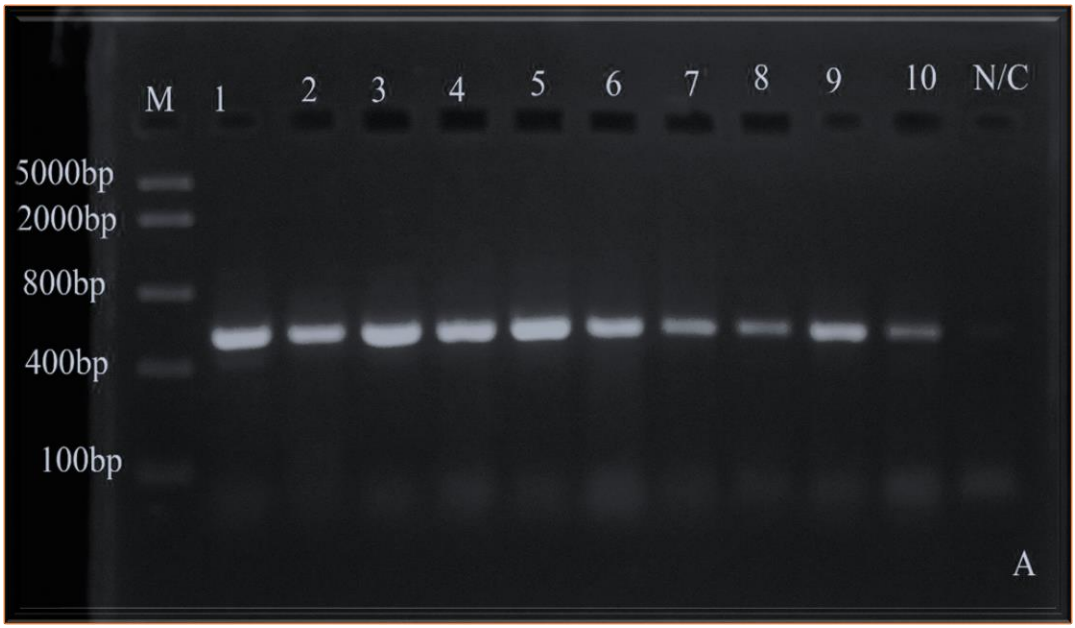

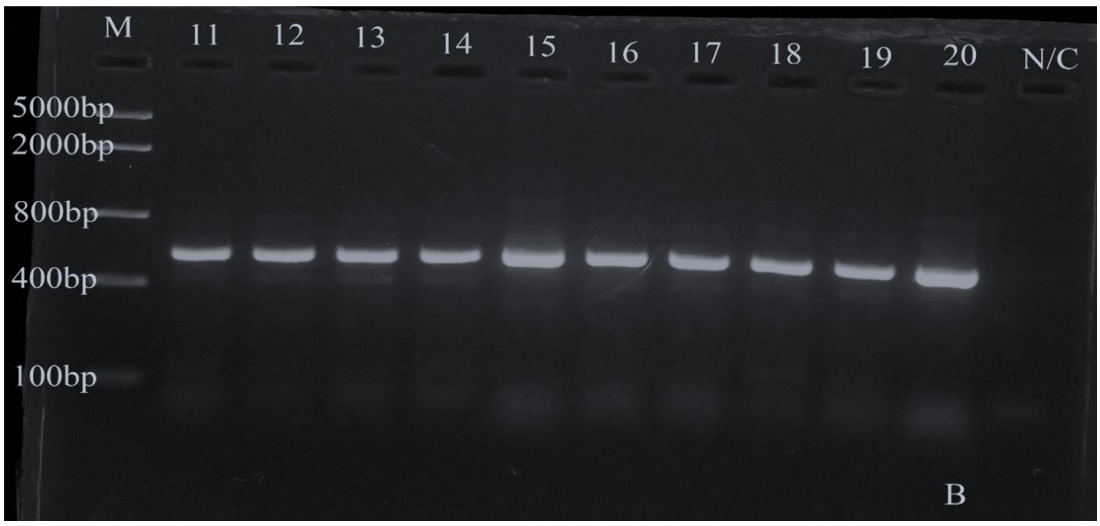

Figure 4. 2**:** Diagram A and B shows the gel electrophoresis of all the 20 samples amplified (approximately 533 bp), from lane 1 – 10 on A, and 11 – 20 on B, M and N/C indicate FastRuler^{TM} middle Range DNA ladder and negative control (no-DNA), respectively.

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4.6. Molecular Identification of *Ascaridia* spp

Overall, 20 parasitic roundworms sampled from Savelugu District (n = 7), Tolon District (n = 7), and Kumbugu District (n = 6) were sequenced and yield approximately 533 bp. The sequences were manually edited and cut at a consensual length of 475 bp. When the sequences were aligned, only one variable nucleotide site was observed in one sample from Salvelugu District (sample SAV 3). The remaining 19 samples from all the Districts were identical. The BLAST result from GenBank, showed all the roundworm isolate from the present study to be *Ascaridia galli* with a percentage identity of 99.79 – 100 % to previous deposits (Table 4.4).

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Table 4. 4**:** Identification of *Ascaridia galli* isolate in the present study to GenBank references

Ghana Isolate (Present Study)	**Accession numbers (Country of Origin)**	**Reference**	**Percentage Identity (%)**
***GHA1** (MW243593)	KP982856.1 (Brazil), GU138668.1, GU138669.1 (Denmark) FM178545.1 (Italy)	Gomes et al. (2015) Katamkam et al. (2010) Cerutti et al. (2008)	100
****GHA2** **(**MW243594)	KP982856.1 (Brazil)	Gomes et al. (2015)	99.79

*GHA1 represent isolates from Ghana that formed one group.

**GHA2 represent isolate from Ghana that formed a different group.

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Two haplotypes (GenBank accession number: MW243593 and MW243594) were identified from 20 partial *cox1* sequences of which 19 sequences of isolates from all the Districts formed MW243593 haplotype and one sequence of an isolate from Savelugu formed the second haplotype MW243594 (Table 4.4 and Figure 4.4). The haplotype MW243593 was 100% identical to the earlier reports from Brazil (KP982856), Denmark (GU138668, GU138669) and Italy (FM178545). Haplotype MW243594 was only 99.79 identical to haplotype KP982856 from Brazil. The change between the two haplotypes occurred at site 333 (as referenced from the complete gene sequence of KP982856) of a transition between Thymine and Cytosine (Fig 4.3). This mutational change resulted in only one singleton (T for C) but no parsimony-informative site.

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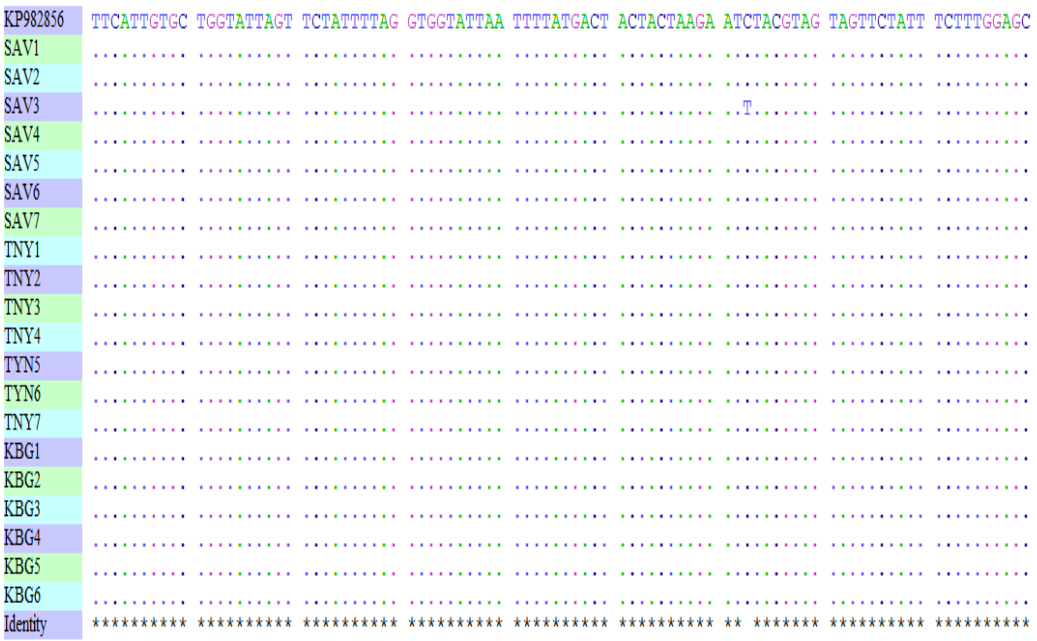

Figure 4. 3**:** Consensus region of a multiple sequence alignment of the 20 partial *cox1* sequences of *A. galli* in the present to the reference sequence KP982856 that shows the variable site (site 333 counting from the start codon).

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4.7. *Ascaridia galli* Partial *Cox1* Sequence (475 bp) Diversity and Neutrality Indices

The 20 partial *cox1* sequences revealed a nucleotide diversity of $\pi = 0.00021$, an average number of nucleotide differences, of k = 0.100 and haplotype diversity of Hd = 0.100 (Table 4.5).

Analysis of Molecular Variance (AMOVA) showed no significant difference between the populations (p = 0.05042) but with a negative fixation index (*F*st) of -0.01205. Neutrality test also revealed a negative Tajima's D (-1.16439) and Fu's Fs (-0.879) indices though not statistically significant. This means the population might have in historically gone through demographic expansion.

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Table 4. 5**:** *Ascaridia galli* parasite populations diversity indices based on partial *cox1* sequences (475 bp)

District/Population	n	H	Hd	π	k
Savelugu	7	2	0.286	0.00060	0.286
Tolon	7	1	0	0	0
Kumbungu	6	1	0	0	0
Total	**20**	**2**	**0.100**	**0.00021**	**0.100**

n = number of sequences used, h = number of haplotypes, Hd = Haplotype diversity, π = nucleotide diversity and k = average of nucleotide differences.

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4.8. Haplotype Network of Partial *Cox1* Sequences (369 bp)

There were two haplotypes (GenBank accession no. MW243593 and MW243594) identified in the present study. Haplotype MW243593 was shared among all three populations and MW243594 from the Savelugu population (Fig 4.4). These sequences were compared to other sequences from the GenBank and sequences were cut to 369 bp. From the haplotype network analysis (Figure 4.5), haplotype MW243594 was away from all clusters of sequences from the present study and other referenced sequences from the GenBank. The other haplotype MW243593 was seen to be widespread in Brazil, Denmark, and Italy.

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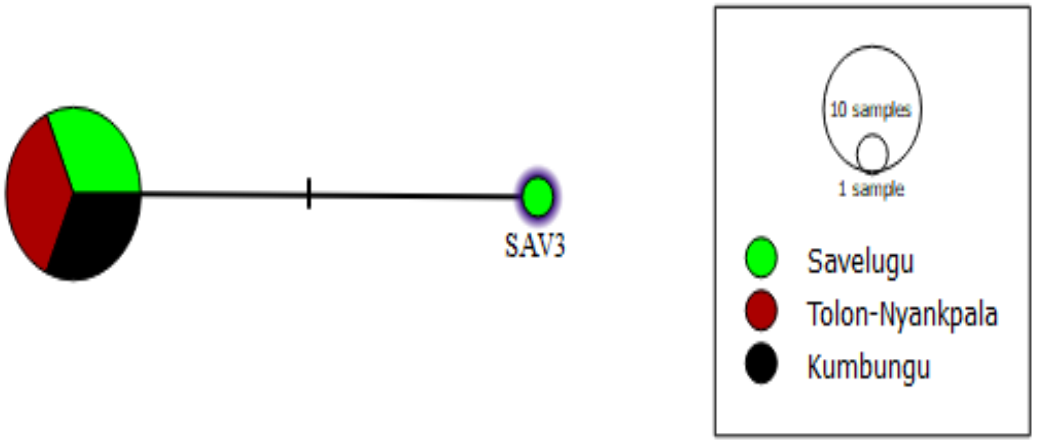

Figure 4. 4: Haplotype network of *Ascaridia galli* partial *cox1* gene (475 bp) from the present study population in Savelugu, Tolon and Kumbungu Districts.

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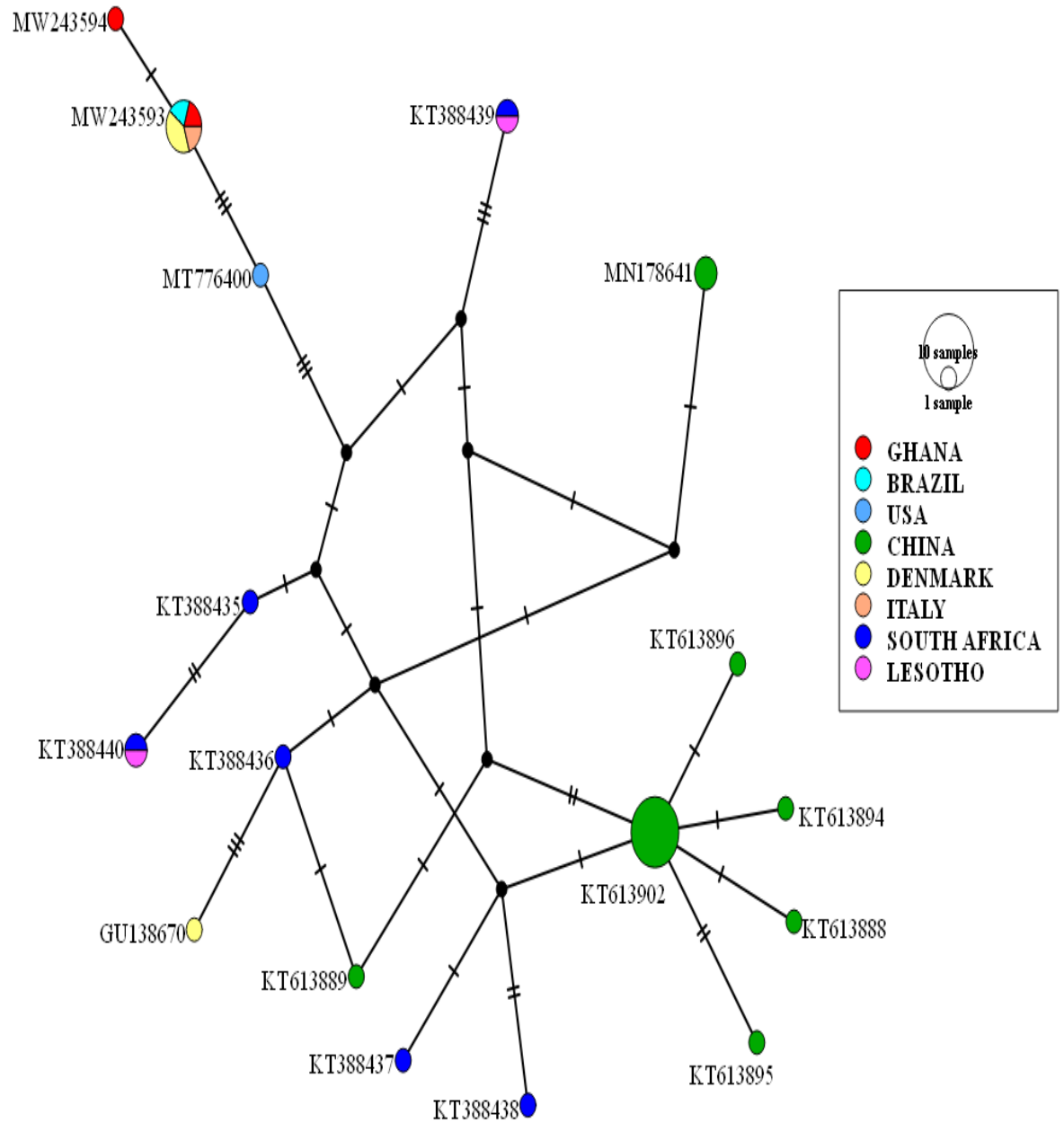

Figure 4. 5**:** Global haplotype network of *Ascaridia galli cox1* gene (369 bp) including those of the present study (GenBank accession no. MW243593 and MW243594).

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4.8. Phylogeny of *Ascaridia galli* Isolates Based on Partial *Cox1* (369 bp)

Phylogenetic analyses were made up of combined sequences of partial *cox*1 gene haplotype from present study and reference sequences (global) from the GenBank including *Ascaris suum* (KT282029) as an outgroup. Sequences of the present study (isolate from Ghana, MW243593 and MW243594) formed a monophyletic group with sequences from Brazil (KP982856), Denmark (GU138668, GU138669), Italy (FM178545) and USA (MT776400) (Figure 4.6).

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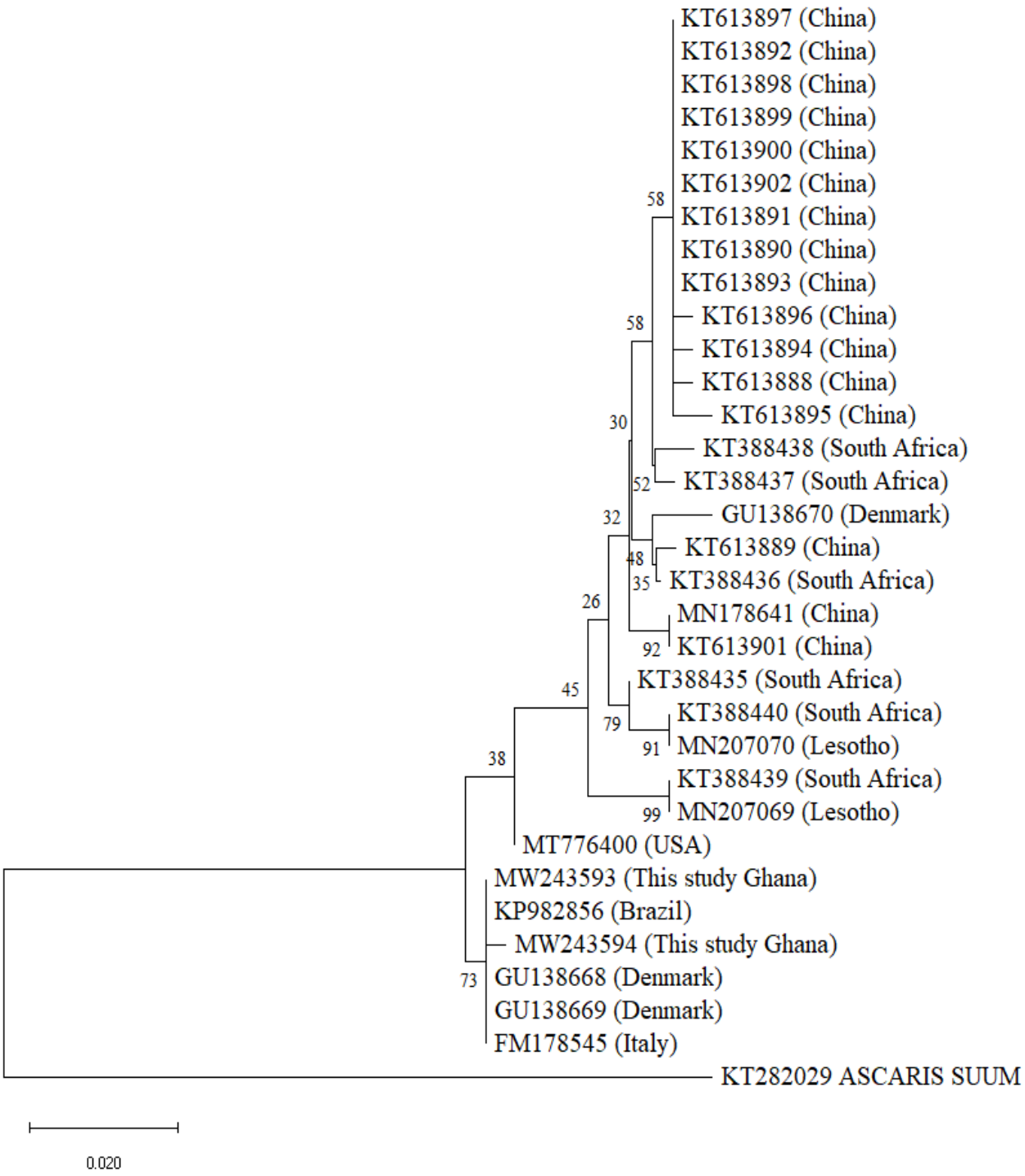

Figure 4. 6: Phylogenic relationship among *cox1* gene sequences of *Ascaridia galli* isolate from various part of the world including present study isolate (MW243593 and MW243594) with *Ascaris suum* (KT282029) as an outgroup.

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CHAPTER FIVE

5.0. DISCUSSION

This study characterized the occurrence of *Ascaridia galli* and *Heterakis* spp in rural chicken in the Northern Region of Ghana. *Ascaridia galli* and *Heterakis* spp. are generally cosmopolitan parasites in poultry (Abebe et al., 1997; Kaufmann et al., 2011; Ngongeh et al., 2014; Idika et al., 2016). The nematodes were observed in the small intestine and the caecum which confirms the predilection sites of the *Ascaridia galli* and *Heterakis* spp., respectively (Permin et al., 2006; Katakam et al., 2010; Ngongeh et al., 2019). Parasitic nematodes are known to cause economic losses as a result of low feed intake, low eggs production, weight loss and treatment cost. The predominance of the two nematode species in this study (47.67 %) indicates their importance in poultry production in northern just as it is known in many other places. Globally, nematodes are noted to be the most commonly occurring parasitic helminths infection in village chickens (Tesfaheywet et al., 2012; Thapa et al., 2015; da Silva et al., 2016; Khan et al., 2016; El-Dakhly et al., 2019). This is attributed to the system of poultry production where chicken scavenge for feed such as insect pests that serves as intermediate hosts of poultry nematodes (Reta, 2009; El-Dakhly et al., 2019). Multiple infection by *Ascaridia galli* and *Heterakis* spp. observed in this study was also recorded in previous studies in Ghana, Ethiopia and Thailand (Poulsen et al., 2000; Ahmed, 2017; Berhe et al., 2019; Wuthijaree et al., 2017) that supports the thought that mixed infection is common in birds kept under extensive system.

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Nematodes infection was also recorded in both sexes of the chickens. The prevalence of *Ascaridia galli* in the present study (36.0 – 56.0 %) is near to previous infection rates in village chickens in other parts of Ghana, thus 24.0 % in the Upper Eastern Region (Poulsen et al., 2000) and 54.3 % in the Ashanti Region (Tay et al., 2017). In other studies, Salam et al. (2015) reported 35.35 % in Tunisia. The variation in the prevalence rate may be as a result of differences in climatic conditions like rainfall pattern, temperature, and relative humidity. Climatic conditions are thought to affect *A. galli* transmission by differences in rainfall pattern and temperature which influence the survivability of the infectives eggs on the environment. The significant association of *A. galli* and geographical location in this study (x^2 = 6.0907, p = 0.048), is in agreement with previous research by Poulsen et al. (2000) in Ghana, Ben Slimane (2016) in Tunisia, and in Ethiopia by Eshetu et al. (2001) and Ashenafi et al. (2004). This confirms that differences in geographic area influences *A. galli* infection. In contrast, prevalence was higher in this study when compared with previous reports in Kenya (10 – 14.5 %) by Irungu et al. (2004) and in Nigeria 6.0 % and 17.2 % by Ola-Fadunsin et al. (2019) and Rufai & Jato (2017), respectively. However, the present observed prevalence was much lower than those reported in Germany by Kaufmann et al. (2011) (88 %) and in Ethiopia by Abebe et al. (1997) (71.58 %). The vast variation was attributed to management practices, poor biosecurity and also the characteristic properties of conventional farming that appear to provide favourable condition for helminths infection.

The prevalence of caecal nematode *Heterakis* spp. (16.67 – 28.0 %) in this study is similar to previous records of 16.0 – 31.0 % in Ghana (Poulsen et al., 2000)

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and 12.4 % in Nigeria (Idika et al., 2016). Higher prevalence was observed in previous report in Tunisia (100 %) (Ben Slimane, 2016). The differences could be as a result of sample size, where less chickens were sampled in the previous study in Tunisia (30 chickens) that may be contrary to this study. Alternatively, favourable environmental condition of the *Heterakis* spp. infective eggs could influence higher prevalence of this parasite.

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The chickens were found with heavy burden of gastrointestinal roundworm parasites in this study. This finding agrees with the total worm burden reported (12.5 ± 5.8) in rural chickens in Jordan (Abdelqader et al., 2007), and was attributed to continuous exposure of chickens to infection under the free-range production system. Rural chickens feed on a variety of feeds from one place to another and scavenge on the ground for worms, arthropods and insects (such as beetles, cockroaches, grasshoppers, and houseflies) that could serve as the possible intermediate host for poultry worms (Dube et al., 2010; Mwale & Masika, 2011; Khan et al., 2016). Birds used in this study were raised under extensive or free-range system with little or no feed supplement and lack of veterinary service attention. Moreover, the heavy worm burden in this study could also be attributed to the dry season during limited feed resources and birds have to scavenge on insects which the intermediate host of the nematode spp.

Ascaridia galli parasite has a pathogenic effect such as emaciation, anaemia, diarrhoea, coughing and death in chickens (Permin & Hansen, 1998). They are also able to cause lesion and necrosis of the mucosa wall which was observed in this study and also reported by previous authors (Permin & Hansen, 1998; Abdelqader et al., 2008). *Ascaridia galli* are also known to predispose infected chickens to

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secondary infection such as *Salmonella* infection and erratic migration of other parasitic worms which could cause zoonotic diseases (Chadfield et al., 2001; Permin et al., 2006; Schou et al., 2010). According to Dahl et al. (2002) and Ferdushy et al. (2016) concurrent infection of *A. galli* with other pathogens produce more severe pathological condition than a single infection with this pathogen. A study by Bharat et al. (2017), reported two viable adult *A. galli* worms in the albumin portion of an egg in India, this supports the claims that the erratic migration can lead to horizontal transmission of diseases.

Heterakis spp. are generally considered nonpathogenic parasites in chickens. However, *H. gallinarum* is known to have an association with *Histomonas meleagridis*, a protozoan responsible for causing blackhead disease of poultry predominantly in turkey (Norton et al., 1999; Gu et al., 2016). These parasites do well in the tropics therefore favourable environmental conditions in the study area enhance their survival.

All the 20 *Ascaridia galli* sequences were closely related when compared to referenced sequences in the GenBank, database with percentage identity of 99.97 – 100 %. This study observed a low haplotype diversity (0.100) when compared to other nematodes that infects poultry, humans and pigs. Malatji et al. (2016) reported 0.749 and 0.758 haplotype diversity in *A. galli* population from Limpopo and KwaZulu-Natal provinces, respectively in South Africa whereas, Zhou et al. (2011) noted in China 0.138 – 0.605 and 0.553 – 0.741 haplotype diversity in *Ascaris lumbricoides* and *Ascaris suum* in humans and pigs, respectively. The reduction in haplotype diversity in the present study was accompanied by a lower nucleotide diversity when compared with the account by Malatji et al. (2016)

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from South Africa (0.00060 vs. 0.013 or 0.014). Differences in *cox1* sequence length, thus 475 bp in the present study against 510 bp in the South African study may partly explain the differences in the diversity indices but the plausible explanation may be that the South Africa *A. galli* population predates the northern Ghana population. Analysis of more samples and the complete *cox1* sequence will be required to support this assertion.

AMOVA showed no genetic structuring between the three *A. galli* subpopulation in this study which is similar to what was observed within the South African *A. galli* subpopulation by Malatji et al. (2016). The absence of genetic structuring was not surprising since almost 20 sequences were identical. This was further attributed to gene flow between the subpopulations aided by human activities such as poultry marketing and migration. These human activities within the region might have helped in gene flow between the *A. galli* subpopulations. Thus, as exchange of birds within the region occurs, dilution of genes due to breeding with other populations will also occur resulting in the low genetic variability between the subpopulations in this study. Other factors like founder's effect and increased rate of selection pressure could contribute to the genetic similarity between the subpopulations as indicated by Jamieson, (2011). In Ghana, Osei-Amponsah et al. (2010) hypothesised based on microsatellite data that, the local chicken population probably dispersed from the Forest ecological zone to the Savannah. Since the present study was based in the Savannah region only, such dispersal route could not be observed. It will be useful in subsequent studies to include the sub-populations of *A. galli* from the various ecological zones in Ghana. Where populations are far apart and there is minimal to no exchange of

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gene flow, genetic structuring could be noticed. Example is the account of moderate genetic sub-structuring of *A. galli* populations in Sweden and Denmark (Höglund et al., 2012).

Negative Fu's Fs value and Tajima D value in the present study indicates the population might have gone through demographic expansion probably after suffering a recent bottleneck event that have limited the genetic diversity in the populations.

Two *cox1* haplotypes found were in this study, haplotype GHA1 was seen to be widely distributed in many countries including Brazil (Gomes et al., 2015), Denmark (Katamkam et al., 2010), and Italy (Cerutti et al., 2008, unpublished), suggesting frequent movement around the globe. Factors such as continental trade of domestic birds, and migration might have resulted in the geographical movement of this founder haplotype(s). Surprisingly, the same common haplotype was also found to infect wild mammal (*Cerdocyon thous*) in Brazil (Gomes et al., 2015). Parasitism of this species in mammals is very rare, as such this observation was attributed to species jump or the crab-eating foxes feeds on wild birds which are carriers of this nematode (Woolhouse et al. 2005; Gomes et al., 2015). While the novel GHA2 haplotype is believed to be a true natural variation, more samples need to be analysed to ascertain its geographic and/or host spread.

Haplotype network analysis and phylogenetic tree constructed from *cox1* sequences from this study and reference sequences from GenBank showed isolate from the present study to cluster with isolate from Europe and South America

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which indicates that they may share common ancestry. Why the Ghanaian isolated clustered from the South African isolates could not be readily explained with the few DNA sequences employed at the moment. More data are needed.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study revealed the prevalence of two nematode species *Ascaridia galli* and *Heterakis* spp. in village chickens in the Northern Region, Ghana to 37.21 % and 20.93 %, respectively. Molecular analysis revealed lower genetic variability in *A. galli* in non-differentiated subpopulations with prevailing two haplotypes (GenBank accession number MW243593 and MW243594). Haplotype GHA1 (MW243593) was more frequent and widespread than haplotype GHA2 (MW243594). The data generated by the present study revealed the importance of the *A. galli* and *Heterakis* spp. infection in rural chicken in Ghana and pave way for future epidemiological study and population genetics characterisation of the nematodes.

6.2 Recommendations

More genetic analysis is needed, perhaps, involving different genetic markers to elucidate the evolutionary trajectory and population genetic structure of the Ghanaian *Ascaridia galli* population. Molecular characterisation of *Heterakis* spp. in rural chicken will also be beneficial. Further, molecular epidemiology of parasitic nematodes in rural chicken in the various ecological zones is needed to explain various aspects *A. galli* and *Heterakis* spp. infection/transmission such as the disease burden and parasite dispersal routes.

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APPENDICES

Appendix I: List of Software and Data Bases Used for Statistical and Bioinformatics Analyses

Software/Database	Version	URL and Reference
STATA	15	
DnaSP	6	http://www.ub.edu/dnasp/ (Rozas et al., 2017)
Cluster Omega		https://www.ebi.ac.uk/tool/msa/clustalo/
GENtle	1.9.4	http://gentle.magnusmanske.de/ (Manske, 2003, University of Cologne)
Arlequin	3.5.2.2	http://cmpg.unibe.ch/software/arlequin35/(Excoffier & Lischer, 2010)
PopART	1.7	http://popart.otago.ac.nz
BLAST		https://www.blast.ncbi..nih.gov/Blast.cgi
MEGA	X	https://www.megasoftware.net/ (Kumar et al., 2018)

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Appendix II: *Cox1* Haplotypes from This Study 475 bp

GHA1 (Accession number: MW243593.1)

CTGCTCATGCTATTTTGATGATTTTTTTTATAGTGATGCCCACTATAA
TTGGGGGTTTTGGTAATTGAATGTTACCTTTGTTGTTGGGTGCTCCGG
ATATAAGGTTTCCGCGTTTGAATAATTTAAGTTTCTGGTTATTACCTA
CTGCTATAGTTTTGATTTTGGGTTCTACTTTGGTTGATAGTGGTTGTG
GTACTAGTTGAACTGTTTACCCTCCTTTGAGAACTAGTGGACATCCT
GGTAGGAGAGTTGATTTGGCTATTTTTAGTCTTCATTGTGCTGGTATT
AGTTCTATTTTAGGTGGTATTAATTTTATGACTACTACTAAGAATCTA
CGTAGTAGTTCTATTTCTTTGGAGCATATGAGTTTGTTTGTTTGAACT
GTGTTTGTTACTGTTTTTTTTGTTAATTTTGTCTCTTCCTGTATTGGCGG
GTGCTATTACCATGTTGTTGACTGATCGTAATTTAAATACTTC

GHA2 (Accession number:MW243594.1)

CTGCTCATGCTATTTTGATGATTTTTTTTATAGTGATGCCCACTATAA
TTGGGGGTTTTGGTAATTGAATGTTACCTTTGTTGTTGGGTGCTCCGG
ATATAAGGTTTCCGCGTTTGAATAATTTAAGTTTCTGGTTATTACCTA
CTGCTATAGTTTTGATTTTGGGTTCTACTTTGGTTGATAGTGGTTGTG
GTACTAGTTGAACTGTTTACCCTCCTTTGAGAACTAGTGGACATCCT
GGTAGGAGAGTTGATTTGGCTATTTTTAGTCTTCATTGTGCTGGTATT
AGTTCTATTTTAGGTGGTATTAATTTTATGACTACTACTAAGAATTTA
CGTAGTAGTTCTATTTCTTTGGAGCATATGAGTTTGTTTGTTTGAACT
GTGTTTGTTACTGTTTTTTTTGTTAATTTTGTCTCTTCCTGTATTGGCGG
GTGCTATTACCATGTTGTTGACTGATCGTAATTTAAATACTTC

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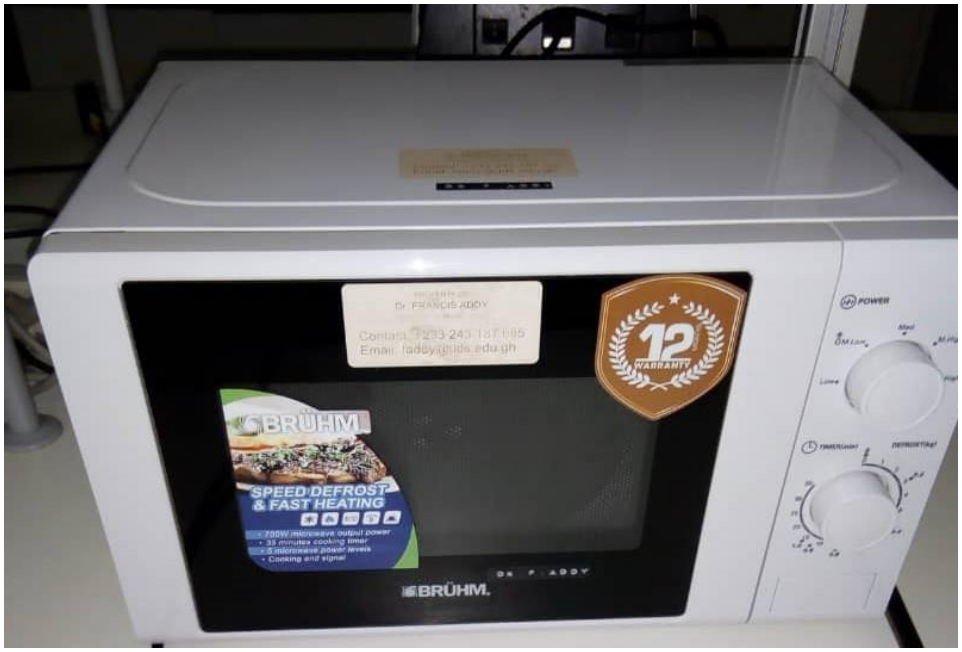
Appendix III: Laboratory Equipment Used



Microwave BRUHM

Electrophoresis Machine

PeQlab thermal cycler (PEQLAB Ltd.)

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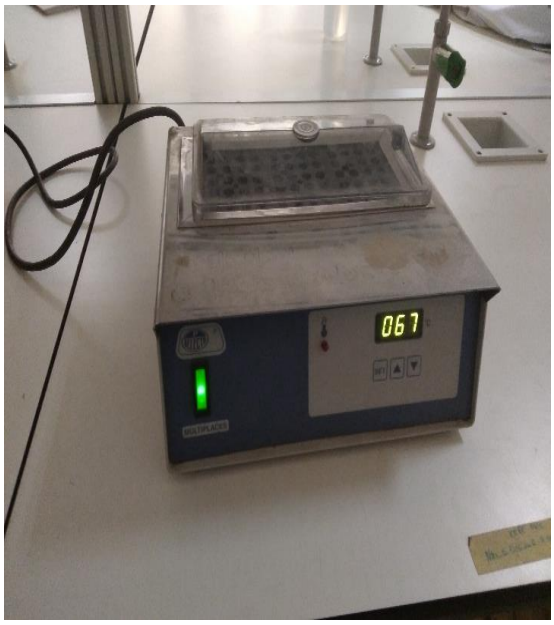
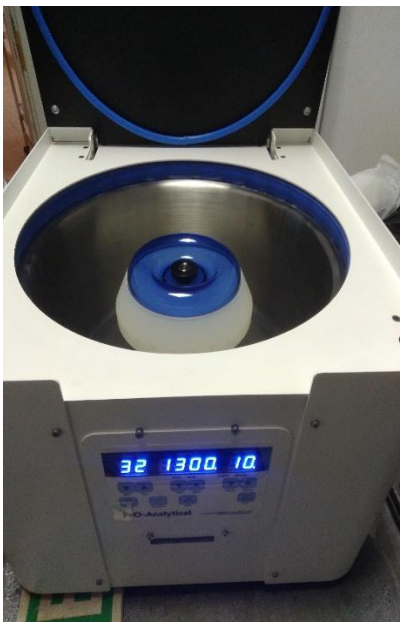


Water bath



PrO – Analytical Centrifuge

Clever UV transilluminator

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Appendix IV: Roundworm Isolates from This Study.

Roundworms fixed in 70% ethanol

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