Studies on Prevalence, Morphological and Molecular Characterization of Gastrointestinal Nematode Parasites of *Gallus gallus domesticus*

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Submitted By

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2021

DEDICATED

TO

MY PARENTS

DECLARATION

I hereby declare that the thesis entitled **"Studies on Prevalence, Morphological and Molecular Characterization of Gastrointestinal Nematode Parasites of *Gallus gallus domesticus*"** submitted to the Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow by me for the award of degree of the **Doctor of Philosophy in Applied Animal Science** is an outcome of my original work and the outcome of my own efforts under the supervision of **Prof. Kamal Jaiswal,** Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Lucknow. It has not been submitted in part or full to this or any other University for the award of any other diploma and degree. This is also declared that the thesis is essentially free from all kinds of plagiarism.

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on Prevalence, Morphological and Molecular Characterization of Gastrointestinal Nematode Parasites of *Gallus gallus domesticus*"** submitted by **Anjum Bee** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow, satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulations- 1999 as amended in 2008/2010/2013 and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the university.

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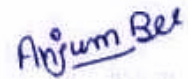

(Anjum Bee)

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ABBREVIATIONS

Abbreviations	Full Name
GDP	Gross Domestic Product
FAO	Food and Agriculture Organization
SPSS	Statistical Package for the Social Sciences
sp.	Species
spp.	Multiple species
Um	Micrometer
SEM	Scanning Electron Microscopy
OsO4	Osmium tetra oxide
Fig.	Figure
etc.	Et cetra
viz.	Namely
i.e.	That is
e.g.	For example
et al.	And others
pH	Potential Hydrogen
V/V	Volume by volume
Hrs	Hours
µM	Micromolar
Ng	Nanogram
ddH2O	Double distilled water
Min	Minute
NaCl	Sodium Chloride
PCR	Polymerase chain reaction
EDTA	Ethylenediaminetetraacetic acid
Tris-HCl	Tris-hydrochloride
TAE	Tris-acetate-EDTA
EtBr	Ethidium bromide
UV	Ultraviolet

O.D	Optical density
Bps	Base paires
NCBI	National Center for Biotechnology Information
UPGMA	Unweighted Pair Group Method with Arithmetic mean)
BLAST	Basic Local Alignment Search Tool
Acc. No.	Accession Number

SYMBOLS

SYMBOLS	MEANING
%	Percentage
°C	Degree Celsius
$\chi 2$	Chi- square
≤	Less than or equal to
≥	Greater than or equal to
@	At the rate

CHAPTER-1

General Introduction and Objectives

1.1 INTRODUCTION-

Livestock plays an important role in the Indian economy. Livelihood of 20.5 million people depends upon livestock. Livestock contributes to 16% of the income of small farm households as against an average of 14% for all rural households. Two-third of the rural community gets their livelihood from livestock. Livestock also provides employment to about 8.8 % of the population in India. India has vast livestock resources. The livestock sector contributes to 4.11% GDP and 25.6% of total Agriculture GDP. Poultry farming is the form of animal husbandry which raises domesticated birds such as chickens, ducks, turkeys and geese to produce meat or eggs for food. Poultry mostly chickens are farmed in great numbers. Chickens raised for eggs are known as layers, while chickens raised for meat are called broilers (Poultry Punch, 2021). More than 50 billion chickens arereared annually as a source of food for both their meat and eggs (Ciwf. org.uk, 2011). *Gallus gallus domesticus* have a great socio-economic importance than other animals domesticated by humans. The chickens *Gallus gallus domesticus* is believed to have descended from the wild Indian and South East Asian red jungle fowl (Permin and Ranving, 2001). High nutritional value and socio-economic benefit cannot be overemphasized (Mathur, 2002).

Poultry industry in particular received greater importance during the planning phase of the country. Various agencies initiated production of poultry on commercial line. The greater impact was seen around the thickly populated towns and cities where the demand for eggs in particular raised with increased level of income of the services class. Considering the demand for eggs, it comes to more than 100 billion eggs based on intake of half eggs per person per day, as per the recommendations by the Nutritional Advisory Committee of the Government of India. As against this, the

current level of egg production is less than 10 per cent of the estimated potential demand. The per capita annual availability of eggs in India was 15 eggs as compared to 200-250 eggs in many development countries of the world (McMichael *et al.,* 2007). Globally, production of the primary poultry product (meat and egg) has been rising rapidly. This reflects consumption in turn on consumer preference for these high quality products and the relatively low price because of efficiency of production. The statistics on poultry production do not consider the value added to poultry product, whether that is food processing and restaurants or alternative production approaches that attract higher prices. India has 3rd rank in egg production and 7thrank in chicken meat production in the world (Watt Executive Guide, 2015). About 3.4 million tons (74 billion) of eggs are produced from 260 million layers and 3.8 million tons of poultry meat is produced from 3000 million broilers per annum in India. Poultry is one of the most intensively reared domesticated species and one of the most developed and profitable animal production enterprises (Obiora, 1992).

Its importance in national economy of developing countries and its role in improving the nutritional and income of many small farmers and those with small land holdings as well as landless has been recognized by various scholars and rural development agencies in the last two decades (Food and Agriculture Organization of the United Nation (FAO, 1987; Creevey, 1991; Kitalui,1998). Poultry enterprise requires small space and less investment as compared to dairy and other such enterprises which can be combined with agriculture. That's why poultry is being given more importance in the country (Vetrivel *et al.,* 2013). The first and foremost global concerned today is the growth of population. Hunger and malnutrition remains the most alarming problem being faced by majority of the world's poor and it is serious problem in India also. Despite general improvement in food availability, health and social services,

hunger and malnutrition exist in some or the other form in our country. India made a commendable progress in agriculture after green revolution. The rate of growth in food production has surpassed by the rate of growth of population. Increasing population and globalization can play havoc in Indian food security if the necessary action is not taken (Hunger *et al.,* 2013).

Usually, poultry is criticized as a competitor to human food resources. The main energy sources maize, jowar comprises approximately 30% of the layer ration. A hen which eats 42 kg feed in its life produces 12 kg vitamins rich, mineralized protein. That means 12.6 kg maize i.e. 30% of 42 kg will be required to produce 12.00 kg egg protein. The other constituent in poultry ration are industrial by-products (Byron *et al.,* 1998). One gets a profit much sooner from poultry than from many other farm products. Financial returns may be expected from broilers in 8 to 10 weeks. From a white Leghorn flock, one should start to get eggs in five months (Bastien, 1997).

1.1.1 Nutritive value of poultry meat

Chicken is now the most popular meat in the country, finally toppling beef off its throne in 2014 (Chikpro, 2017). Chicken is a good source of protein and vitamins and minerals, such as iron, selenium, zinc, and B vitamins. It is also one of the main sources of vitamin B12. It is full of beneficial amino acids. According to the National Chicken Council, in each 100 grams of cooked skinless, boneless chicken breast, you will get 31 grams of protein which is more than half of the daily recommended amount. In addition, chicken's proteins contain all the important amino acids (simple organic compounds that compose proteins). For example, chicken provides an ample amount of the amino acid tryptophan. Another of chicken's amino acids, isoleucine helps in wound healing (including recovery after exercise), growth and the

stabilization of blood sugar levels. Threonine helps to maintain the central nervous system, cardiovascular system, and immune system (Chikpro, 2017). It has several advantages as half of the fat from chicken meat is made up of the desirable monounsaturated fats, and only one-third of the less healthy saturated fats. There are much higher proportions of saturated fats in most cuts of red meat, which also vary considerably in total fat. Chicken meat is therefore seen as a healthy meat. Chicken meat does not contain the trans-fats that contribute to coronary heart disease. Poultry meat is rich in the omega-3 fats and is an important provider of the essential polyunsaturated fatty acids (PUFAs), especially the omega (n)-3 fatty acids. Scavenging chickens are a particularly good source because of their varied diet. The amounts of these important fatty acids can be increased more easily in chicken meat than in other livestock meats. Poultry meat can be enriched with several of the important dietary nutrients like Selenium whose deficiency is becoming more widespread in humans because soils are becoming depleted and the foods grown on them are therefore lower in selenium (Department of Animal Husbandry, Dairying & Fisheries Ministry of Agriculture & Farmers Welfare Government of India, 2017).

With all these merits of poultry industry in India, the industry is yet to grow satisfactory. Poultry and eggs contribute as an important source of agricultural income to farmers in our country. With these aims the Department of Agriculture and Animal Husbandry has initiated a number of poultry projects around the cities in collaboration with Government of India. In the state main emphasis was laid on keeping improved breed of hens for the purpose of egg and flesh. Finance and subsidies has been provided to large number of poultry growers by Government in rural and semi urban areas to start poultry farming (Mehta *et. al.*, 2007).

1.1.2 Effects of Helminths on host

Parasitic infections are considered to be the major constraint to the economy of farmers by reducing the growth and production of livestock. Parasitism causes heavy economic losses to poultry industry in the form of anorexia, retarded growth, reduced weight gain, decreased egg production, diarrohea, intestinal obstruction and mortality (Anwar *et al*., 1991; Shah *et al*., 1999; Dube *et al.,* 2010; Katoch *et al.,* 2012). Parasitism has resulted 17% reduction of weight gain in growing chicken and 12.5% reduction in egg production in laying hens (Bhowmik *et al.,* 1982). Prevalence of gastrointestinal parasites in desi fowl has been reported by various workers from different parts of world (Permin *et al.,* 2002; Ashenafi and Eshetu 2004; Pinckney *et al.,* 2008, Yehualashet 2011; Percy *et al*., 2012) including India (Sundaram *et al.,* 1962; Devada and Sathianesan, 1989; Hange *et al*., 2007; Katoach *et al*., 2012). Parasitic infection or their concurrent infections also result in immune suppression, especially in response to vaccines against some poultry diseases (Nnadi and George, 2010).

The domestic chicken feeds on a wide range of food substances. This ranged from grains, fruits to insects which may harbour infective stages of parasites thereby predisposing them to parasites particularly gastro-intestinal parasites (Permin *et al*., 1999; Frantovo, 2000; Oniye *et al.,* 2001; Pennycott and Steel, 2001; Jansson *et al*., 2010; Kaufmann *et al.,* 2011; Sherwin *et al.,* 2013; Bestman and Wagenaar, 2014). The free ranging management system, humid tropical climatic conditions, availability of intermediate hosts, immune status of definitive host (Magwisha *et al.,* 2002) are favorable for faster propagation and development of the larval stages of helminth parasites (Matta and Ahluwalia, 1981; Malhotra, 1983; Kulkarni *et al.,* 2001). The domestic fowl are raised traditionally under free range management system in village

with little or no supplementary feeding and without any veterinary care thereby exposing them to parasitic infection (Gary and Richard, 2012). In India huge loss of birds due to disease is being faced by farmers due to management related problems. Poultry have heavy infection of diverse types of parasites i.e. helminth, protozoan, viruses and arthropods etc. Intestinal helminth infections have a serious impact on poultry health, productivity, quality and quantity of meat.

Helminth parasites of poultry birds are commonly divided into three main groups (cestodes, nematodes, trematodes). Nematodes constitute the most important group of helminth parasites of fowl both in number of species and extent of damage they cause; the main genera include Capillaria, Heterakis, and Ascaridia (Jordan and Pattison, 1996). Cestodes are of the two genera Railletina and Hymenolepsis. The most important cestodes of poultry are the *Raillietina* spp., *Hymenolepis* spp., *Choanotaenia* sp. *and Davainea* species. Some of the cestodes species penetrate into an intestinal mucosa deeply producing severe inflammation and hemorrhage of the intestine (Soulbsy, 1982). Trematodes infection is not very common in domestic fowl. Helminthiasis is considered to be one of the important problems in chicken (Pandey, 1989; Jansen and Pandey, 1989; Abebe *et al.,* 1997). Helminths, when present affects poultry performance, causing significant direct to indirect losses (Reid and McDougald, 1997; Silva, 2009). These worm infections may cause considerable damage and great economic loss to the poultry industry due to malnutrition, decreased feed conversion ratio, weight loss, lowered egg production, death in young birds and behavioural changes which could indicate reduced animals welfare in chickens (*Gallus gallus domesticus*) (Ried and Carmon, 1958; Toledo and Castell, 1981; Khater, 1993; Skallerup *et al.,* 2005; Gauley *et al.,* 2007; Cardona and Msoffe, 2009; Shahin *et al*., 2011; Sonune, 2012).

Some helminths act as carriers of pathogenic agents such as *Pasteurella multocida* (Dahl *et al*., 2002) and *Escherichia coli* (Permin *et al.,* 2006), salmonellosis, coccidiosis and some other pathological protozoa which are responsible for mortality (Badreddine Ben Slimane, 2016). Additionally helminth e.g. *Ascaridia galli, Heterakis* spp. can serve as vector for transmission of pathogenic infections. *Ascaridia galli* may also interfere with the development of immunity in chickens after vaccination against newcastle disease (Pleidrup *et al*., 2014).

Nematodes of the genus Ascaridia (Dujardin, 1845) infect many species of birds. These nematode species may cause serious and frequently fatal diseases in farm and exotic birds kept in captivity, parrots (Kajerova *et al.,* 2004). Nematodes have either a species-specific, direct life cycle with bird-to-bird transmission by ingestion of infective eggs or larvae or have an indirect cycle that requires an intermediate host (eg, insects, snails, or slugs). Eggs of many nematode species are resistant to low temperatures and disinfectants but may be more susceptible to heat and desiccation. Eggs of *A. galli* and *H. gallinarum* can survive up to two years in soil (Kenneth and Rüdiger, 2019). *Ascaridia galli* (Schrank, 1758; Freeborn, 1923) was synonymised with *A. lineate* (Schneider, 1866) *A. perspicillus* (Rudolphi, 1803). *Ascaridia galli, H. gallinarum* and *Ascaridia columbae* are common nematode parasites reported to infect domesticated as well as wild birds (Soulsby, 1982). *A. galli* is the most common nematode in all types of production system and has a worldwide distribution (Permin *et al.,* 1997; Ashenafi and Eshetu 2004; Abdelqader *et al.,* 2008).

1.2 LIFE CYCLE OF NEMATODES

Each species of roundworm tends to infect a specific area of the gastrointestinal tract. In general, the different species of roundworm have very similar life cycle. Adult roundworms lay eggs inside the infected birds. The eggs are voided from the host

through the feaces. Roundworm may or may not require intermediate host. The eggs of the roundworm must be consumed by the avian host. Once the eggs reached to infective sites, the cycle is complete (Pattison, 2007).

(A.) Primary species- Large roundworm (*Ascaridia galli*)

Location- Lumen of intestine

The life cycle of *A.galli* is direct, involving two principal populations; the sexually mature parasite in the gastrointestinal tract and the infective stage (L3) in the form of a resistant egg in the environment. Eggs of the *A.galli* are released with the faeces of the host and develop in the open environment. Infective L3 stage is completed in 10 to 20 days or longer based on temperature and humidity. Sometimes, earthworms can ingest *A.galli* eggs and transmit these to chickens, but this is not the primary route of transmission. The life cycle is completed when the infective eggs are ingested by new hosts through contaminated water or feed. The eggs containing the L3-larvae are mechanically transported tothe duodenum. The larvae are protected by the three layers coveringthe eggs until they reach the duodenum or jejunum, where they hatchwithin 24 hours. During hatching the mature coiled larvae protrude the anterior end of the egg through an opening, and the larvae live freely in the lumen of the duodenum for the first 9 days (Cvet.tu.edu.iq. 2021). Then they penetrate the mucosa, causing hemorrhages, return to the lumen by 17–18 days, and reach maturity in 28–30 days. Levels of infection are often underestimated, because early larval stages are barely visible and can remain for long periods within intestinal tissues, whereas adult stages in the lumen are generally fewer in number (Kenneth and Rudiger, 2019).

Symptoms- Ascaridiosis is caused by various species from the Ascaridia genus. *Ascaridia galli* is one of the most prevalent helminth parasites in fowl. It is found in

all age group of chickens, the greatest amount of damage is in young birds. In adult hens, eggs production can be reduced. In heavy infection *Ascaridia galli* can move up the oviduct and be found in hen's eggs and some large roundworm can be found in the faeces of birds. Infected birds are progressively emaciated, anaemic and sometimes diarrhoeic (Kenneth and Rudiger, 2019).

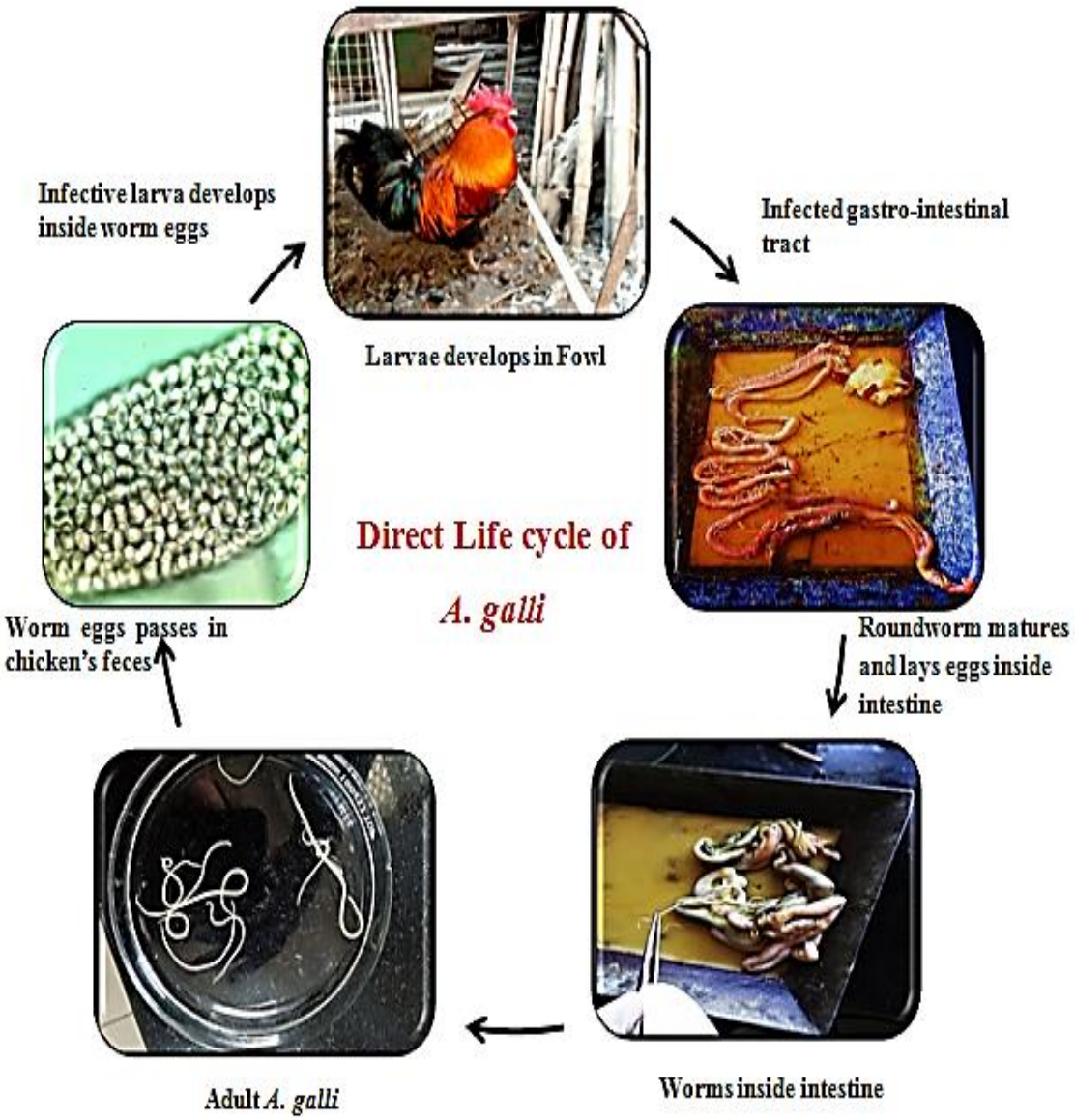


Figure 1.1- Direct life cycle of *A. galli* (Source: Author own**)**

(B.) Primary species- Cecal worm (*Heterakis gallinarum*)

Location- Lumen of cecal pouches

The life cycle of *H. gallinarum* is similar to that of *A. galli*. Earthworms may ingest the eggs of the cecal worm and serve as a source of infection when ingested by poultry. Darkling beetles may also serve as a mechanical vector (Kenneth and Rudiger, 2019). Infective stage of eggs develops in 12 to 14 days at 22 ℃ and can stay infective for 4 years in soil. Infection occurs when eggs are eaten. The second stage of larvae hatches in the gizzard or duodenum and passed to the ceca. Most of development is completed in the lumen, but some penetrate the mucosa, where they remain for 2 to 5 days without further development after returning to the lumen they mature, in 14 days after infection. If eaten by an earthworm, the juvenile may hatch and become dormant in the worm's tissues, remaining infective to chickens for at least a year (Cvet.tu.edu.iq. 2021).

Symptoms- *Heterakis gallinarum* Infection is mildly pathogenic. *H. gallinarum* plays the role of the life cycle of ***Histomonas meleagridis***, the causal pathogen of enterohepatitis “Blackhead”. Primary infections are usually not apparent. Secondary infections are characterized by the formation of nodules in the cecum and the submucosa of the cecum. During heavy infection espically in young birds, restlessness, depression and thickening of intestinal walls and exhibit marked inflammation (Pattison, 2007).

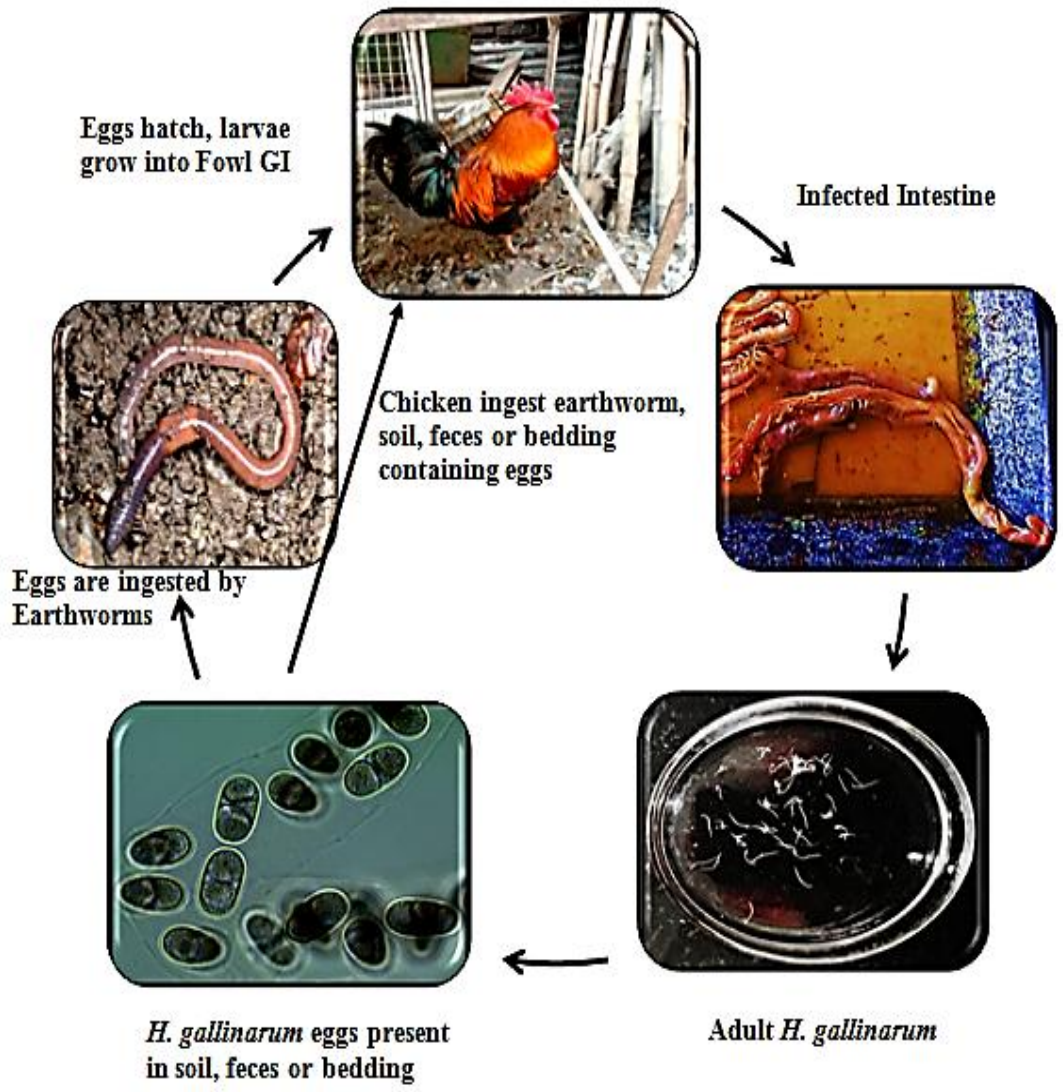


Figure 1.2- Life cycle of *H. gallinarum* (Source: Author own)

Regular deworming in backyard poultry improve the growth and subsequently the mean body weight gain in treated group, while the untreated group of birds were weak and emaciated (Katoch *et al.,* 2012). Helminthic infections are rarely fatal and are often neglected but they cause heavy economical loss to poultry farmers due to reduced productivity (meat and eggs), beside some helminths also act as carriers of pathogenic agents. Keeping in view above facts, this study explored the gastrointestinal nematode parasites of the backyard chickens (*Gallus gallus domesticus*) and the aim of the present study was planned to determine the prevalence,

morphology and molecular identification of gastrointestinal (GI) nematodes in traditional reared free ranging chickens at Lucknow, India.

1.3 OBJECTIVES-

1. To study theprevalence of gastrointestinal nematode parasites of *Gallus gallus domesticus* in Lucknow region.

2. To identify and characterise the gastrointestinal nematode parasites of *Gallus gallus domesticus* by molecular technique.

CHAPTER-2

Review of Literature

2.1 REVIEW OF LITERATURE-

Poultry is an important farm species in almost all countries. It is an important source of animal protein, and can be raised in situations with limited feed and housing resources. Chickens are 'waste converters', they convert a scavenged feed resource base into animal protein. They are therefore by far the most important species for generating income for rural families (Eekeren *et al.*, 2006). Poultry diseases are infectious, non infectious or parasitic, cause tremendous economic loss to the poultry industry. The damage is caused through high mortality; drop in egg production, poor performance, and medication cost. Parasitic diseases caused by helminth, arthropods protozoa are reported in almost all species of domestic fowls (Ghebremariam *et al.*, 2011). Parasitism is an important limiting factor that is responsible for deteriorating the health and productivity of livestock (Amin *et al.*, 1997).

Boyazoglu (1998) evaluated the Livestock farming as a factor of environmental, social and economic stability with special reference to research. Scientific advances and the present methods of technology for food production, preservation, processing, transport and marketing are forcing rapid changes throughout the developing and marginal areas of the world. On the topic of sustainable growth, without promoting less progressive approaches and uneconomical environmental policies, we run the risk today of over hastilyaccepting and applying new alternatives that might neglect the fundamental factors of adaptability and time; this could be adestructive experience for livestock production especially in marginal area.

Ruff *et al.*, (1999) studied the important parasites in poultry production systems. They briefly review the major protozoan, helminth, and arthropod species in poultry including pathogenesis as well as the importance of the interaction of other diseases

and parasites, and control of the infection by chemotherapy, management, and immunity.

Permin *et al.*, (1999) evaluated the prevalence of gastrointestinal helminths in different poultry production systems, the result confirm the higher risk of helminth infection in free-range and backyard systems but prevalence may also be high in deep litter systems.

Gasser *et al.*, (1999) evaluated the PCR-based technology in veterinary parasitology. DNA technology is having a major impact in many areas of veterinary parasitology. In particular, the polymerase chain reaction (PCR) has found broad applicability because its sensitivity permits enzymatic amplification of gene fragments from minute quantities of nucleic acids derived from limited amounts of parasite material. The focus is on PCR tools for the accurate identification of parasites and their genetic characterisation, the diagnosis of infections, the isolation and characterisation of expressed genes, the detection of anthelmintic resistance, and mutation scanning approaches for the high resolution analysis of PCR products.

Skantar *et al.*, (2004) studied the molecular characterization and phylogenetic evaluation of the Hsp90 Gene from selected nematodes, Hsp90 sequences may help strengthen branch support or clarify tree topologies when other molecules show ambiguous alignments, greater branch-length heterogeneity, or codon bias in certain taxonomic groups.

Gasser *et al.*, (2005) evaluated the molecular tools advances, opportunities and prospects. They concluded that modern molecular technologies are having a substantial impact in many fundamental and applied areas of parasitology. They focused mainly on tools for the accurate identification of parasitic nematodes and

protozoa of socio-economic importance, the diagnosis of infections and the detection of genetic variability using PCR-coupled mutation scanning technology.

Hange *et al.*, (2007) conducted a study in Maharashtra to examine desi birds (*Gallus gallus domesticus*). Overall prevalence was 63% of the gastrointestinal infection in Parbhani region. The species wise prevalence was higher for the nematodes (viz., *Ascaridia galli*, *Heterakis gallinarum*, *Subulura brumpti*, *Dispharynx nasuta*, *Cheilospirura hamulosa*, *Gongylonema ingluvicola* and *Tetrameres mohtedai*) as compared to cestodes (viz., *Raillietina echinobothridia*, *R. tetragona*, R. *cesticillus*, *Davainea proglottina*, *Amoebotaenia sphenoides*, *Choanotaenia infundibulum* and *Hymenolepis carioca*). Prevalence of single infection was higher than mixed parasitic infections. Seasonal prevalence was higher during winter followed by rainy and summer seasons.

Manaswini (2007) examined GIT of desi fowls in Bhubaneswar, India to study the incidence of gastrointestinal helminths. He found that all birds were infected with helminth parasites either as a single infection or mixed infection. Examination of bursa of fabricius and trachea of birds revealed *Prosthogonimus* spp. (39.70%) and *Syngamus trachea* (4.41%). Among nematodes *Heterakis gallinarum* (52.94%) was the highest prevalent parasite followed by *Tetrameres mohtedai* (45.59%), *Ascaridia galli* (42.65%), *Acuria hamulosa* (20.59%), *Acuria spiralis* (5.88%), *Syngamus trachea* (4.41%) and *Capillaria species* (2.94%). Whereas in cestodes *Railletina tetragona* (38.23 %) was the most prevalent parasite followed by *R.echinobothridia* (32.35%), *R.cesticillus* (10.29%), *Amoebataenia* spp. (27.94%), *Hymenolepis* spp. (22.06%), *Cotugnia digonophora* (16.18%) and *Davainea proglottina* (1 1.47%).

Only *Prosthogonimus* spp. (39.7%) and *Echinostoma revolutum* (2.94%) were identified trematode parasites.

Phiri *et al.,* (2007) reported a higher prevalence rate of helminths from the gastrointestinal tracts of free range chicken. The most common species were *Allodapa suctoria, Tetrameres americana, Ascaridia galli, Gonglonema ingluvicola, Raillietina* spp. and *Heterakis gallinarum*. No trematodes or Syngamus trachea have been found. Mixed infections were more common as compared to single infections. These findings confirmed the higher risk of helminth infections in free-range systems and can cause the harmful effects in chickens.

Puttalakshmamma *et al.,* (2008) studied the prevalence of gastrointestinal parasites of poultry in and around Bangalore, in which the presence of gastrointestinal parasites was screened for farm and desi birds. It was confirmed by the gross examination that desi bird had high infection of cestodes few were also infected with nematode and mixed infection was also observed.

Yegani (2009) reported the future of poultry science in student perspective. Poultry science education and research have resulted in excellent contributions to the poultry industry. The key message is that one of our main priorities should always be the development of the relationship between research centers and the poultry industry. Research initiatives need to be applicable to the industry in the short or long-term.

Maurer *et al.,* (2009) identified the poultry litter as a source of gastrointestinal helminth infections. The study reported the prevalence and worm burdens in tracer animals were low compared to a similar study with tracer kept in poultry runs. The reason is that the litter has a negative impact on the viability and infectiousness of helminth eggs.

Katakam *et al.*, (2010) reported the Molecular and parasitological tools for the study of *Ascaridia galli* population dynamics in chicken. Experiments were first conducted to compare and evaluate different methods *of Ascaridia galli* larval recovery from the chicken intestine. PCR-RFLP analysis of larvae revealed the same haplotype as that of their maternal parent. The identification of distinguishable cohorts may be a powerful tool in population studies of parasite turnover within the animal host.

Bhure *et al.*, (2011) studied thehaematological observations of *Gallus gallus domesticus* infected with *Cotugnia digonopora.* Out of 43 *Gallus gallus domesticus*, 28 were infected with cestode parasites. The significant increase in size of RBC and number of WBC; however reduction in the count of RBC, Hb, PCV, MCV in infected *Gallus gallus domesticu*s as compared with normal one. The haematological parameters of the infected bird *Gallus gallus domesticus* shows high infection cause macrocytic anaemia, lymphocytosis due to difficiency of related factors.

Kaufmann (2011) evaluated the helminth infections in laying hens kept in organic production systems in Germany. The hens were sacrificed and the gastrointestinal tracts were examined for the presence and intensity of helminth infections with standard parasitological methods. Three nematode (*Ascaridia galli, Heterakis gallinarum, Capillaria* spp.*) and* four cestodes *(Raillietina cesticillus, Hymenolepis cantaniana, Hymenolepis carioca, Choanotaenia infundibulum*) species were found. It can be concluded that the vast majority of hens are sub clinically infected with at least one helminth species. The prevalence as well as intensity of the helminth infections, particularly with tapeworms, considerably increases in summer. The results indicate that it is essential to adopt alternative control strategies in order to lower infection risk in organic production systems.

Shukla *et al.*, (2012) worked on the seasonal variation of intestinal tapeworms in *Gallus gallus domesticus* at Ahmednagar region. Higher prevalence of Raillietina parasite has occurred in winter season followed by summer season and low in the rainy season. This type of results indicates that environmental factors and feeding habitats are influencing the seasonality of parasitic infection either directly or indirectly.

Katoch *et al.*, (2012) examined free-range chickens in subtropical and humid zone of Jammu, India.They found 72% of prevalence of helminth parasites and the prevalence of nematodes and cestodes was 56.66% and 43.33%, respectively. Thirty seven and sixty eight per cent of birds were infected with mixed and single infection. The study revealed *Ascaridia galli* (29.6%) as the most prevalent helminth spp. followed by *Heterakis gallinarum* (24.0%), *Raillietina cesticillus* (19.2%), *R. echinobothridia* (13.6%), *R. tetragona* (9.6%), *Amoebotaenia cuneata* (4.0%), *Capillaria spp*. (2.4%) and *Cheilospirura hamulosa* (1.6%).

Sonune (2012) examined the gastrointestinal tracts of 50 local and farm birds in and around Chikhli, Buldana (M.S.) India. He revealed 72.0% of prevalence of 6 gastrointestinal parasites only in local birds. Out of 36 positive local birds, 19 (52.77%) were found positive for cestodes viz., *Cotugnia* sp., *Raillietina* sp., and 11 (30.56 %) for nematodes viz., *Ascaridia galli* and *Strongyloides avium*.

Dama *et al.*, (2012) conducted a cross sectional study in the Solapur district, Maharashtra, India for a period of one year to know the prevalence of cestode parasites in backyard poultry. The study revealed single (81.82 %) and mixed (18.18 %) infection of cestodes with an overall prevalence of 57.5%. *Raillietina tetragona, R. centropi, R. urogalli, Cotugnia kamatiensis and Davainea singhi* were noticed.

Naphade (2013) worked on the prevalence of helminth infection in desi poultry birds from Marathwada region of Maharashtra (India). The overall prevalence was 239 (75.40%) during the study period. Season-wise prevalence was higher during summer (83.96%), followed by rainy (77.66%) and lowest during winter (64.81%). Prevalence of cestode parasites was 72 (22.71%) and 114 (35.96%) for the nematode parasite desi birds, while the rest 53 (16.71%) were found to have mixed infection. Seasonal prevalence was as follows; cestode (Summer: 26.38%, Rainy: 22.32%, Winter: 19.42%), nematode (Summer: 39.63%, Rainy: 37.87%, Winter: 30.61%) and mixed infection (Summer: 17.91%, Rainy: 17.5%, Winter: 14.79%) The major helminth infection of parasites includes *Raillietina* sp. (21.01%) and *Ascaridia* spp. (32.78%).

Satish *et al.*, (2013) studied the gastrointestinal helminths parasites of *Gallus gallus domesticus* sample from tribal areas of Madhya Pradesh. Two hundred (200) gastrointestinal tracts of local and exotic breeds of chickenwere collected from the tribal areas including Seoni, Chhindwara, Dindori, Mandla, and Jabalpur (M.P). Six different gastrointestinal parasites were isolated and identified. *Ascaridiagalli* was found to be the most prevalent (51.60%) among the chickens. Other parasites encountered included; *Railleitina echinobothrida* (21.60%), *R. tretragona* (22.0%), *Hymenolepsis carioca* (23.00%), *Hetarakis gallinarum* (31.00%) and *Syngamus trachea* (1.50%). Females harbored more parasites than males.

Vetrivel *et al.*, (2013) evaluated the role of poultry industry in Indian economy. The advances in cereal technology can fill the empty stomach, but it may not help in the balanced growth of the human body. The chief ingredients of balanced diet also comprise proteins, fats, minerals and vitamins, which are essential for growth. The

supply of these items can easily be increased through increased production of livestock products.

Dar and Tanveer (2013) reported 85.83% prevalence of cestode parasites in free range backyard chicken of Kashmir valley, India by the examining of gastrointestinal tracts of 120 chickens. The prevalence of cestode species viz., *Raillietina tetragona, R. cesticillus, R. echinobothridia, R. spiralis, Choanotaenia infundibulum, C. gondwana, Amoebotaenia cuneata, A. domesticus* and *Davainea proglottina* was 65.0%, 22.5%, 33.33%, 26.66%, 18.33%, 10.83%, 20.0%, 15.0% and 18.33% respectively. Concurrent infection with other nematode parasites viz., *Ascardia galli, Heterakis gallinarum* and *Capillaria* spp. were also observed. Further it was reported that prevalence was higher in adults (88.29 %) and male (86.0 %) than in nestlings (76.92 %) and female (85.71 %) birds.

Jatoi *et al.*, (2013) examined 500 freshly slaughtered indigenous chickens maintained in four taluks of Larkana district, Pakistan. The parasites identified in the study area were *Raillietina tetragona* (318) (63.55 %), *R. cesticillus* (123) (24.57 %), *R. echinobothridia* (27) (5.39 %), *Cotugnia digonopora* (80) (16.10 %) and *Choantaenia infundibulum* (8) (1.69 %).

Bhat *et al.*, (2014) conducted a study to investigate the prevalence of helminth parasites in back yard poultry farming in Jammu region of northern India by the examination of faecal and gastrointestinal tracts of birds. The prevalence of helminth parasites in faecal and gut examination was 63.67% and 68.33% respectively. The study revealed 6.67% of mixed infection. The most common parasites identified by faecal and gut examination was *Ascaridia galli* (19.16 % and 20.0 %). The other helminth species identified were *Heterakis gallinarum, Capillaria* spp.,

Trichostrongylus tenuis, Raillietina spp., *Cheilospirura hamulosa* and *Eimeria* spp. The prevalence of cestodes was less as compared to nematodes. The infection was highly prevalent in monsoon (83.33 % and 72.0 %) and was lowest in post monsoon (50.0 % and 56.0 %) based on gut and feacal sample examination, respectively.

Hembram *et al.*, (2015) investigated the prevalence of gastrointestinal helminths in Banarajafowls reared in semi-intensive management system in Mayurbhanj district of Odisha and they found mixed infection with gastrointestinal helminths of different species was more common than infection with single species and season-wise prevalence was higher in rainy season followed by winter and summer. They found five different species of parasites such as *Ascaridia galli* (25.63%), *Heterakis gallinarum* (33.75%), *Raillietina tetragona* (46.25%), *Raillietina echinobothrida* (11.87%), and *Echinostoma revolutum* (1.87%). Chicks were found to be more prone to this parasitic infection and a slight higher prevalence among female birds was observed. Age-wise prevalence revealed that chicks were more susceptible than adults to gastrointestinal helminths infection.

Kumar *et al.*, (2015) conducted a study to investigate the gastrointestinal parasitic infections in chickens of upper gangetic plains of India with special reference to poultry coccidiosis and they found the prevalence of helminthic infections was higher in poultry farms of Uttarakhand (40.0%) as compared to Uttar Pradesh (11.62%) with higher prevalence in backyard poultry (36.4%), followed by layer farms (28.6%) and lowest in broiler farms (9.1%). *A. galli* was the most common G.I. helminth and it was recorded in free-range (backyard poultry) as well as intensive systems (broiler and layer farms).

Edith *et al.*, (2015) conducted a study to investigate the guinea fowl mortality associated with *Ascaridia numidae* infection and they found few adult parasites were

present in the lumen and were identified as *Ascaridia numidae* and they also found 3.5 % morality in the flock. Postmortem of chicken revealed the highly inflamed mucosa severely studded with ascarid larvae at the jejunum and ileum region.

Ayisha *et al.*, (2015) studied the helminth parasites of domestic fowl (*Gallus domesticus*) in Doda district of Jammu & Kashmir state, India. Study revealed that 67.85% of the birds were infected with endoparasites. Different types of helminth parasites recovered included *Raillietina tetragona*, followed by *Heterakis gallinarum* and *Ascaridia galli.*

Kumar *et al.*, (2015) studied fifty eight poultry farms for gastrointestinal parasites in two north Indian states viz., Uttar Pradesh and Uttarakhand. They observed that 81.03% were positive for *Eimeria* spp., 15.52% for *Ascaridia galli*, 3.45% for *Hetarakis gallinarum*, 1.72% for *Syngamus trachea*, 5.17% for *Capillaria* spp., 1.72% for *Raillietina* spp., 1.72% for *Trichostrongylus tenuis*, 1.72% for *Choanotaenia infundibulum* and 1.72% for *Strongyloides avium*. In broiler farms, the prevalence of *Eimeria* spp. was higher (88.24%) as compared to layer farms (71.43%) and backyard poultry (70.0%). The prevalence of helminthic infections was higher in backyard poultry (36.4%) followed by layer farms (28.6%) and lowest in broiler farms (9.1%). *A. galli* was the most common parasite recorded in free-range as well as intensive systems.

Solanki *et al.*, (2015) examined 3773 fresh poultry droppings revealed an overall prevalence of 31.65% (1194) of gastro-intestinal parasitism in backyard poultry in and around Navsari area of South Gujarat. Out of 1194 positive samples, 40.87% were found to be positive for nematodes viz., *Ascaridia galli* eggs (11.98%), *Strongyloides* spp. eggs (4.61%), *Capillaria* spp. eggs (3.02%) and other nematode eggs (21.27%), 3.52% for cestodes viz., *Hymenolepis diminuta* eggs (0.75%),

Choanotaenia infundibulum eggs (0.59%) including other tape worm segments (2.8%), 58.04% for Eimeria oocysts and 1.92% for mixed infection. The prevalence was higher in winter (35.91%) followed by monsoon (36.20%) and summer (24.68%) season.

Sofi *et al.*, (2016) studied the different organs of 137 slaughtered birds in Gurez valley Kashmir, India. They revealed 40.14% of helminthic infection in study area. Three species of parasites were identified viz., *Heterakis gallinarum* (35.76 %), *Ascaridia galli* (32.11 %) and *Raillietina tetragona* (27.00 %). Seasonal prevalence revealed higher prevalence in summer (34.88 %, 39.53 % and 41.86 %) followed by autumn (23.68 %, 31.57 % and 34.21 %) and winter (19.23 %, 23.07 % and 30.76 %) with three species respectively. Age-wise and sex-wise prevalence revealed no significant difference in the prevalence of infection.

Javaregowda *et al.*, (2016) conducted a study to investigate the prevalence of gastro-intestinal parasites of backyard chickens (*Gallus domesticus*) in and around Shimoga and they found mixed prevalence infection of cestodes and nematodes. Cestodes species are *Raillietina tetragona* (77.6%), *Raillietina echinobothrida* (12.8%) and *Raillietina cesticillus* (9.6%) whereas nematodes species are *Ascaridia galli* (22.6%) and *Heterakis gallinarum* (15.1%).

Sreedevi *et al.*, (2016) conducted a study to examine the prevalence of gastrointestinal parasites in desi fowl in and nearby villages of Gannavaram, Andhra Pradesh for a period of 1 year and they found different species of parasites in chickens such as *Davainea proglottina*, *Raillietina cesticillus* and *Raillietina echinobothrida* in cestodes (32.47%), *Ascaridia galli*, *Capillaria annulata*, *Heterakis gallinarum* in nematodes (39.87%), *Eimeria tenella*, *Eimeria acervulina* and *Eimeria necatrix* in *Eimeria* spp. (39.87%). *Ascaridia galli* and *R. cesticillus* and *A. galli* and

Eimeria spp. were common in mixed infection (12.86%). *Ascaridia galli* was the most prevalent species.

Rasdhid *et al.*, (2017) worked on epidemiology of cestode parasites in domestic fowl (*Gallus gallus domesticus*) of Kashmir valley. During the study, 576 hosts were examined for cestode parasites and prevalence rate was found to be 61.63% (355/576) with mean intensity load of 43.46 per infected host. The species found were *R. tetragona* (38.27%), *R. cesticellus* (20.82%), *R. echinobothrida* (28.07%), *C. infundibulum* (15.12%), *A. cuneata* (9.27%), *and D. proglottina* (10.4%). Highest prevalence was found to be more in summer (74.30%), followed by autumn (70.13%), followed by spring (54.86%), and followed by winter (47.22%). The Prevalence rate was found higher in females (69.09%) than in males (53.81%). Infection was found more in growers (77.44%) than in adults (43.77%). Female sex had higher prevalence (70.1%) than the male (60.2%). There was a statistical significant association among the sex group and occurrence of the infection ($p<0.05$).

Rao *et al.*, (2018) evaluated the prevalence of gastrointestinal helminth parasites in *Gallus domesticus* at different seasons. They reported mixed parasitic species during the study period. They observed 61.26% prevalence in Karimnagar district and 32.29% prevalence in Khammam district. In which two species of nematodes *Ascaridia galli* (43.66%), *Heterakis gallinarum* (62.07%) and two species of cestodes *Raillietina tetragona* (50.72%), *Raillietina echinobothrida* (46.48%) was reported. Prevalence of *Ascaridia galli, Heterakis gallinarum, Raillietina tetragona,* and *Raillietina echinobothrida* among helminths was 71.26%, 62.07%, 82.76%, 75.86% respectively. Results of this study showed that both nematodes and cestodes are highly prevalent in domestic chickens in the studied area.

Singh *et al.*, (2018) investigated the prevalence and species diversity of helminths indomestic fowl (*Gallus domesticus*) of Pipar city. They observed 53.33% helminth infection. They recovered three types of helminth parasites from the gastrointestinal tract of infected fowls. Throughout the investigation one cestode *Raillietina tetragona* and two nematodes *Heterakis gallinarum* and *Ascaridia galli* were recovered. The most common nematode parasite was *A. galli* in the checked gastro-intestinal tracts of fowls. The prevalence of helminth species were, *Raillietina tetragona* (9.16%), *Heterakis gallinarum* (13.33%), and *Ascaridia galli* (30.83%). Females (42.5%) were more infected than males (10.83%). Single type of infection was common than double and triple infection.

Kumari *et al.*, (2018) determined the prevalence of gastrointestinal helminthic infections in birds reared under deep litter and free range system in Durg, Chhattisgarh. The prevalence of gastrointestinal helminthic infection in deep litter system was 25% out of which 12 (48%) were found positive for *Ascaridia galli*, 11 (44%) harboured *Raillietina* spp.as single infection and remaining 2 (8%) had mixed infection. Among local birds helminthic infection was 67 (67%), in which following species were observed; *Raillietina* spp. 24 (35.82%), *A. galli* 12 (17.91%), *Heterakis gallinarum* 3 (4.48%) and remaining mixed infection 28 (41.79%).

Das *et al.*, (2020) studied the prevalence, species diversity and intensity of gastrointestinal (G.I.) parasitic infections in the backyard poultry of Meghalaya. Fecal samples were collected from different age groups and examined by flotation, sedimentation and modified McMastertechniques. Overall prevalence of G.I. parasitic infections was 37.97%. Eight species viz. *Eimeria* sp. (30.12%), *Heterakis gallinarum* (14.09%), *Ascaridia galli* (21.22%), *Strongyloides avium* (12.46%), *Capillaria* sp. (7.57%), *Raillietina* sp. (8.61%), *Syngamus trachea* (3.56%) and

Choanotaenia infundibulum (2.37%) were recorded. Highest and lowest infections were recorded in October (52.88%) and February (26.34%), respectively. Seasonal prevalence was higher during monsoon (44.71%) followed by autumn (44.34%), winter (27.22%) and spring (36.62%). *Eimeria* sp. was highestin monsoon (33.87%), winter (27.78%) and spring (12%) seasons while *A. galli* (31.63%) was higher in autumn season. Age wise prevalence was observed higher in < 8 (25.24%), 8-28 (48.17%) and > 28(38.77%) week old birds. *Eimeria* sp. was seen highest in both < 8 (68.18%) and 8-28 (25.86%) weeks. *A. galli* (27.38%) was seen highest in > 28 weeks old birds.

Bandi *et al.*, (2020) studied the desi birds' samples (faeces, gastrointestinal tracts) collected from various villages in Krishna district, Andhra Pradesh. They found 74.22% overall prevalence.The identified species was *Amoebotaenia sphenoides, Cotugnia digonopora, Davainea proglottina, Hymenolepis carioca, Raillietina cesticillus, R. echinobothridia and R. tetragona in cestodes, Ascaridia galli, Capillaria* spp., *Heterakis gallinarum, Strongyloides avium, Subulura brumpti, Tetrameres mohtedai* and *Dispharynx spiralis* in nematodes and *Eimeria* spp. in protozoa. The prevalence of infection was higher in higher age. There was no significant relationship between the prevalence of infection and age, sex and seasons (P>0.05). *Cotugnia digonopora* was the highest prevalent parasite in studied area. *Dispharynx spiralis* was exclusively found in chicks and in summer. *Tetrameres mohtedai* was exclusively identified in female bird. Mixed infection was higher (70.39 %) and cestodes were the common parasites in all infected birds. No trematode parasites were seen in studied area.

2.1.2 International researches-

Shah *et al.*, (1999) studied the prevalence of cestode parasites in indigenous and exotic layers of Faisalabad, Pakistan and observed an overall prevalence of 59.4% and 16.0% respectively. In indigenous layers, *Raillietina echinobothridia* (13.2%), *R. tetragona* (10.6 %), *R. cesticillus* (12.8 %), *Choanotaenia infundibulum* (6.8 %), *Amoebotaenia cuneata* (1.8%), *Hymenolepis carioca* (9.0%) and *H. cantaniana* (5.2 %) were observed. While in exotic layers *Raillietina echinobothridia* (2.0 %), *R.cesticillus* (3.6 %), *R.tetragona* (3.0 %), *Choanotaenia infundibulum* (3.4 %) and *Hymenolepis carioca* (4.0%) were identified.

Eshetu *et al.*, (2001) examined 267 scavenging chicken gastrointestinal tracts, in which 243 (91.01 %) were found to be infected with gastro-intestinal helminths. The study revealed that the highest prevalent parasite was *Raillietina tetragona* (45.69 %) followed by *Amoebotaenia sphenoides* (40.45 %), *Ascaridia galli* (35.58 %) and *R. echinobothrida* (25.84 %).

Irungua *et al.*, (2004) studied helminth parasites in the intestinal tract of indigenous poultry in parts of Kenya and they found different species of parasites such as *Raillietina* sp. (47.53 %), *Heterakis gallinarum* (21.33 %), *Ascaridia galli* (10.03 %), *Strongyloides avium* (9.96 %), *Choanotaenia infundibulum* (4.61 %), *Cotugnia digonopora* (3.6 %), *Capillaria* sp. (1.5 %), *Trichostrongylus tenius* (1.04 %) and *Syngamus trachea* (0.40 %). Most of the parasites are found in mid and hind guts and *Syngamus trachea* and *C. digonopora* were only found in the foregut and midgut, respectively.

Rabbi *et al.*, (2006) studiedthe prevalence of gastrointestinal helminth parasites and the gross pathologica lesions produced by them in broiler, layer and backyard

indigenous chicken in Mymensingh district Bangladesh. The prevalence was 100% in backyard poultry. The species observed were *Raillietina tetragona* (100 %) and *Amoebotaenia sphenoides* (40.0 %) in cestodes, *Ascaridia galli* (87.50 %), *Heterakis gallinarum* (80 %) and *Capillaria annulata* (5.0 %) in nematodes and *Catatropis verrucosa* (16.25 %) in trematodes were recorded in backyard poultry.

Schou *et al.,* (2007) conducted a study to investigate the gastrointestinal helminths in indigenous and exotic chickens in Vietnam. Association of the intensity of infection with the Major Histocompatibility Complex (MHC) and they found the most prevalent helminths such as *Ascaridia galli, Heterakis beramporia, Tetrameres mothedai, Capillaria obsignata, Raillietina echinobothrida* and *Raillietina tetragona* in a comparison between 2 breeds of chicken i.e, indigenous breed Rhode Island and the exotic breed Luong Phuong. Indigenous breed have more intensity of mixed helminthes infection and exotic breed was more infected with *A. galli* and *C. obsignata.*

Mungube *et al.,* (2008) studied three hundred and sixty (360) local chicken of semi arid area of Kenya from March 2005 to August 2006 to identify and estimate the prevalence of parasites. An overall prevalence of 93.3% was observed. The prevalence of nematodes was recovered in 268 (74.4 %) chicken viz., *Tetrameres americana* (37.7 %), *Ascaridia galli* (33.3 %) and *Heterakis gallinarum* (22.8 %). Cestodes were recorded in 245 (68.1%) chicken viz., *Raillietina echinobothrida* (33.3 %) and *Davainea proglottina* (19.4 %). Two coccidia species, *Eimeria necatrix* (6.7 %) and *E. tenella* (16.7 %) were isolated. Endo-parasites (helminths and coccidia) occurred in significantly ($p<0.05$) higher frequencies during the wet season than the dry season. Male exhibited higher frequencies than female birds.

Pinckney *et al.,* (2008) reported 66.9% prevalence of gastrointestinal parasites in free-range poultry in Grenada, West Indies. The common helminth parasites identified were *Raillietina tetragona* (38.6 %), *Gongylonema ingluvicola* (29.2 %), *Ascaridia galli* (10.3 %), *Heterakis gallinarum* (4.7 %) and *Capillaria contorta* (2.83 %).

Kurt *et al.,* (2008) investigated the cross-sectional survey on helminth infections of chickens in the Samsun region, Turkey and they found 16 different species of parasites in a cross-sectional study. The helminth species found were: *Davainea proglottina* (23%), *Raillietina echinobothrida* (13%), *Raillietina cesticillus* (12%), *Hymenolepis carioca* (10%), *Raillietina tetragona* (6%), *Choanotaenia. infundibulum* (2%), *Amoebotaenia cuneata* (2%), *Echinoparyhium recurvatum* (1%), *Echinostoma revolutum* (1%), *Heterakis gallinarum* (29%), *Ascaridia galli* (16%), *Capillaria caudinflata* (12%), *Capillaria retusa* (6%), *Capillaria bursata* (4%), *Capillaria annulata* (1%) and *Syngamus trachea* (2%).

Abdelqader *et al.,* (2008) assessed the prevalence and burden of gastrointestinal helminthes among local chickens, in northern Jordan. A cross-sectional seasonal study was conducted to determine the prevalence of gastrointestinal and tracheal helminthes among local chickens in northern Jordan. The inspection of trachea and gastrointestinal tract of each bird was done for the presence of helminths parasites. Three nematode and eight cestode species were present. No trematodes were found.The prevalences of *A. galli* and *R. cesticillus* were higher in male than female hosts while those *H. carioca* were higher in females.

Mukaratirwa and Hove (2009) conducted a survey to know the prevalence of cestode parasites in indigenous free-range chickens in selected districts of rural Zimbabwe. The cestode parasites included *Raillietina tetragona* (84.4%), *R.*

echinobothrida (32.2%), *R. cesticillus* (27.3%), *Davainea proglottina* (4.1%), *Cotugnia digonopora* (1.9%), *Hymenolepis* spp. (31.9%), *Amoebotaenia cuneata* (28.9%) and *Choanotaenia infundibulum* (24%) were recorded.

Mukaratirwa *et al.,* (2010) conducted a study to investigate the prevalence of helminth parasites in free-range chickens from selected rural communities in KwaZulu-Natal province of South Africa. The sixteen helminth species were recorded comprising of 12 nematode and 4 cestode species from the 4 localities and the most prevalent nematode species was *Heterakis gallinarum* (prevalence range 80–94.4%), *Gongylonema ingluvicola* (43.3–86.7%), *Tetrameres Americana* (53.3–66.7%) and *Ascaridia galli* (22.2–43.8%) and for cestode species, *Raillietina tetragona* (16.7–40%) and *Skrijabinia cesticillus* (3.3–13.3%).

Adang *et al.,* (2010) reported the histopathology of *Ascaridia galli* infection on the liver, lungs, intestines, heart, and kidneys of experimentally infected domestic pigeons (*C. l. domestica*) in Zaria, Nigeria. As these are vital organs of the body, impact on then may lead to elevated morbidity or mortality, or may lead to secondary infections or complicate the course of other domestic pigeon diseases.

Amer *et al.,* (2010) isolated and identified the oocysts of *Eimeria* spp. in 7-8 weeks old naturally infected native breed chicken flocks in Giza, Egypt. The sporulated oocysts were identified based on morphological features, micrometry and shape index as *Eimeria tenella*, *E. necatrix*, *E. acervulina* and *E. praecox.*

Dube *et al.,* (2010) studied the endoparasites in free range chickens of Matebeleland North and South and observed endoparasites viz., *Tetrameres americana*, *Acuaria hamulosa, Ascaridia galli*, *Heterakis gallinarum*, *H. dispar*, *Allodapa suctoria*, *Capillaria annulate, Raillietina echinobothrida* and *R. Tetragona,*respectively.

Nnadi and George (2010) conducted a cross-sectional survey to identify and estimate prevalence of endoparasites of village chicken in Enugu state, Nigeria. Examination of faecal samples of 1038 birds of different age group revealed an overall prevalence 35.5% each of helminths and coccidian infection. The infection was highly (92.6%) prevalent in adults compared to growers (76.3%) and chicks (33.3%). Among helminth species, *Ascaridia galli* (17.2%) was the most prevalent parasite.

Ghebremariam (2011) reported the prevalence of helminth parasites in indigenous fowls of Zoba Anseba of Eritrea, North-East Africa. The helminth parasites recorded were: *Ascaridia galli*, *Subulura* sp., *Heterakis* sp., *Tetrameres* sp., *Cheilospirura* sp., *Raillietina* sp. and *Amoebotaenia* sp. The rate of infection is higher for nematodes than cestodes. Mixed infections of two to three species were prevalent. Cloacal swabs of fowls collected were found positive for different types of ova, i.e., *Ascaridia galli*, *Heterakis* sp., *Tetrameres* sp., and *Raillietina* sp.

Mwale and Masika (2011) conducted a study to determine the prevalence of gastrointestinal parasites in village chicken of Centane district, South Africa and observed 99.0% of various gastro-intestinal parasites. The parasites recorded were nematodes viz., *Ascaridia galli* (45.71%), *Heterakis gallinarum* (52.86%), *Subulura brumpti* (1.43%), *Syngamus trachea* (1.43%), *Capillaria spp.* (51.43%) and *Strongyles* (51.43%), cestodes viz., *Choanotaenia infundibulum* (1.43%), *Amaebotaenia sphenoides* (1.43%), *Raillietina cesticillus* (2.86%) and *Davainea proglottina* (1.43%), trematodes viz., *Postharmostomum gallum* (38.57%), *P. communtatum* (21.43%) and Prosthogonimus species (2.86%) and coccidia species (67.14 %).

Kaufmann *et al.*, (2011) examined the gastrointestinal tracts of laying hens kept in organic free range system in Germany revealed 99.6% of prevalence of helminth infection. Mixed infections accounted for 97.0% while 4.9% birds had single infection. The most prevalent species were nematodes viz., *Heterakis gallinarum* (98.0%), *Ascaridia galli* (88.0 %) and *Capillaria spp*. (75.3%) compared to that of cestodes viz., *Raillietina cesticillus* (17.8%), *Hymenolepis cantaniana* (8.2%), *Hymenolepis carioca* (3.8%) and *Choanotaenia infundibulum* (0.5%). The study also revealed that total prevalence did not significantly differ between summer and winter season but total worm burden was significantly higher in summer compared to winter.

Baboolal *et al.*, (2012) evaluated the intestinal helminths in commercial broiler chickens and estimated their prevalence in Trinidad. Among the counties a significant difference was found in the prevalence of helminth infection. Inspite of the short life span and rearing under intensive farm management, broiler chickens in Trinidad harbour several intestinal helminths.

Paul *et al.*, (2012) examined the gastrointestinal tracts collected from indigenous birds in Bangladesh revealed 72.47% of prevalence of helminth parasites. The prevalence of trematodes, cestodes and nematodes was 5.50, 41.48 and 72.47% respectively. The parasites identified were *Catatropis verrucosa* (5.50%), *Echinostoma revolutum* (3.66%), *Raillietina tetragona* (36.69%), *R. echinobothridia* (38.53%), *R. cesticillus* (24.77%), *Ascaridia galli* (42.20%) and *Heterakis gallinarum* (67.88%). Significantly higher prevalence was noticed in adults (78.37%) and males (78.43%) than in young (60.0%) and female (67.24%). The infection was more prevalent in dry season (79.55%) than in wet season (67.97%).

Radfar *et al.*, (2012) studied the prevalence and associated risk factors in free-range backyard chickens of Sistan region, Iran. Examination of gastrointestinal tracts of 59 birds, 55 (93.22%) were found positive for helminthic (nematode and cestode) infection. Three species of nematodes and four species of cestodes were identified. *Subulura brumpti* (67.79%) was the most prevalent parasite followed by *Choanotaenia infundibulum* (40.67%), *Raillietina tetragona* (35.59%), *R. Echinobothrida* (27.11%), *Heterakis gallinarum* (23.72%), *Ascaridia galli* (16.94%) and *R. cesticillus* (15.25%). There was no significant difference in prevalence of nestling (83.33%) and adult (95.74%) birds and also in male (96.29%) and female (90.62%) birds.

Percy *et al.*, (2012) studied the seasonal prevalence of different endoparasites in Murehwa District, Zimbabwe and observed significantly higher intensity of infection in *Ascaridia galli* (93.33%) and *Choanotaenia infundibulum* (100%) during summer. There was no significant difference in the intensity of infection of *Allodapa suctoria* (96.67%), *Heterakis gallinarum* (95.00%), *Capillaria obsignata* (100%), *Tetrameres americana* (56.67 %), *Amoebotaenia cuneata* (30 %) and *Hymnolepis* spp. (33.3 %).

Al-Gawad *et al.*, (2012) investigated the seasonal prevalence of *Eimeria* spp. in Egyptian Balady breed chicken in Cairo. Screening of 711 birds samples comprising faecal samples and gastrointestinal tracts revealed 21.24% of *Eimeria* spp. The species identified were *Eimeria necatrix* (58.27%), *E. tenella* (25.82%), *E. acervulina* (19.20%), *E. mitis* (10.59%) and *E. maxima* (4.66%). The high rate of infection was noticed in 64 to 84 day old chicken and during winter season. Rate of infection was higher (45.13%) during winter season and was lowest during summer season (1.86%).

Hussen *et al.*, (2012) reported 89.5% of prevalence of helminths in chicken raised under traditional management system in two selected districts of Eastern Shewa Zone, Ethiopia based on cross sectional survey on gastrointestinal tracts of 124 slaughtered birds. The overall prevalence of cestodes and nematodes was 83.0 and 58.0% respectively. The different helminth species viz., *Raillietina echinobothrida* (63.7 %), *R. tetragona* (56.5%), *R. cesticillus* (40.3%), *Choanotaenia infundibulum* (13.7%), *Davainea proglottina* (8.1%), *Hymenolepis contaniana* (17.1%) and *H. carioca* (17.7%) whereas nematodes viz., *Heterakis gallinarum* (37.9%), *Ascaridia galli* (32.0%), *Gongylonema ingluvicola* (25.8%), *Dispharynx nasuta* (4.0%) *Heterakis isolonche*, *Allodapa suctoria* (7.35%) *Capillaria anatis* (3.2%) and *Heterakis dispar* (6.5%) were identified. There was no significant difference between the prevalence of infection based on the age and sex of birds.

Molla *et al.*, (2012) examined gastrointestinal tract of local backyard chicken of North Gondar Administrative Zone, to know the prevalence of gastrointestinal helminth infections. The overall prevalence was 79.62% with nematodes (60.38%), cestodes (54.62%) and mixed infection (56.52%). Among cestodes, *Raillietina echinobothridia* (29.62%), *R. tetragona* (12.31%) and *Hymenolepis cantaniana* (10%) were most prevalent while among nematodes, *Ascaridia galli* (39.23%), *Heterakis gallinarum* (39.62%) and *Dispharynx nasuta* (3.46%) were prevalent parasites.

Ekpo *et al.*, (2013) reported 100% of prevalence of helminth parasites in gastrointestinal tracts of birds in Abeokuta, Ogun State. The identified species were two nematodes viz., *Ascaridia galli* (60.0%) and *Heterakis gallinarum* (60.0%) and one cestode i.e, *Raillietina echinobothridia* (80.0%). There was no significant

difference in prevalence between sexes of birds. Mixed infections accounted for 75% while 25% birds had single infection.

Medjouel *et al.*, (2013) worked on cestode parasites of free-range chickens (*Gallus gallus domesticus*) in the North-Eastern of Algeria. The overall prevalence rate was 88.19%, in the El-Tarf poultry. At least one species of cestodes was found on every chicken examined. Seven species of cestodes were identified; *Raillietina echinobothrida* (83.33%), *Raillietina tetragona* (68.75%), *Raillietina cesticillus* (29.16%), *Hymenolepis carioca* (12.5%), *Choanotaenia infundibulum* (11.8%), *Davainea proglottina* (11.11%) and *Amoebotaenia cuneata* (4.16%). This study showed that there was no significant difference ($p>0.05$) between the prevalence of cestodes in relation to age and sex.

Abdullah and Mohammed (2013) conducted a study on 65 local adult chickens in Sulaimani region to determine the prevalence of ecto and endoparasites. Examination of gastrointestinal tracts of birds revealed an overall prevalence of 89.23% of endoparasites including nematode viz., *Heterakis gallinarum* (81%), *Ascaridia galli* (31%), *Cheilospirura hamulosa* (8.62%), *Capillaria spp.* (1.72%) and cestodes viz., *Raillietina* spp. (55.17%), *Choanotaenia infundibulum* (31.0%), *Amoebotaenia sphenoides* (10.34%), *Hymenolepis carioca* (6.9%) and *Davaniea proglottina* (3.54%). *Heterakis gallinarum* was the most prevalent species among nematodes and *Raillietina* spp. was the most prevalent species among cestodes.

Ilyes and Ahmed (2013) studied the 144 free-range chickens in the El-Tarf, North-Eastern, Algeria to determine the prevalence of cestode parasites in the intestinal tracts of chicken. The overall prevalence was 88.19% with seven species of cestodes viz., *Raillietina echinobothridia* (83.33%), *R. tetragona* (68.75%), *R. cesticillus*

(29.16%), *Hymenolepis carioca* (12.5%), *Choanotaenia infundibulum* (11.8%), *Davainea proglottina* (11.11%) and *Amoebotaenia cuneata* (4.16%).

Uhuo *et al.*, (2013) examined the 150 faecal samples from the intestinal tract, lumen and gizzard of already slaughtered local chicken in some selected eatery centres in Abakaliki, Ebonyi State, Nigeria. All birds examined were found positive for different helminth parasites giving an overall prevalence of 100%. Out of 150 infected birds, 127 (86.6%) were found to be positive for mixed infection and 23 (15.3%) for single infection. There was no significant difference in infection of male (48.7%) and female birds (51.3%). Species of gastrointestinal helminth parasites viz., *Ascaridia galli* (48.7%) and *Heterakis gallinarum* (28%) in nematodes, *Choanotaenia infundibulum* (36.7%) and *Raillietina echinobothridia* (20.1%) in cestodes and *Prosthogonimus species* in trematodes were identified.

Banaja *et al.*, (2013) evaluated the ultrastructural and genetic characterization of the two nematode *Ascaridia galli* and *A. columbae* from birds in Taif, Saudi Arabia. RAPD-PCR analysis has revealed the presence of differences between the two nematodes including a high polymorphism alongwith the percentage. A total of 50 and 42 different specific markers were detected for *A. columbae* and *A. galli* respectively. The markers obtained in the present study might be used for identifying, tracking and the lineage of the two nematode worms. So, these markers might have potential applicationsof a successful control of ascarid nematode worms in poultry industry.

Okafor-Elenwo and Elenwo (2014) examined the 250 domestic fowls that were raised in deep litter in poultry farms of Nigeria revealed co-infections with helminths and protozoan parasites in the gastrointestinal tracts. Cent percent of the birds were found to be infected with either helminth or protozoan paraistes or both. There was

high prevalence of co-infection of helminth and protozoa infection in the birds (64.8%) compared to that of the single infection (35.2%) with either helminth (18.3%) or protozoa (16.4%). The parasites noticed were *Ascaridia galli*, *Heterakis gallinarium*, *Capillaria* sp. and *Eimeria* spp.

Agbolade *et al.*, (2014) examined the faeces and gastrointestinal tracts of domestic fowl (110 exotic and 23 local) from Ijebu North, Southwestern Nigeria and revealed 37.6% prevalence of gastrointestinal parasites. The prevalence of endoparasites in local chicken (87.0%) was significantly higher than that of exotic breed (27.3%). Mixed infection was noticed in infected birds. The parasites recorded were *Ascaridia galli* (10.5%), *Capillaria spp.* (9.0%), *Heterakis gallinarum* (6.8%), *Syngamus trachea* (0.8%), hookworm (6%), *Raillietina* spp. (10.5%), *Giardia* spp. (12.0%), *Trichomonas gallinae* (8.3%), and *Eimeria* spp. (6.8%).

Opara *et al.*, (2014) examined blood and gastrointestinal tracts of chickens (5600) and turkeys (560) reared in Owerri, Southeastern Nigeria to know the prevalence of haemoprotozoa and gastrointestinal parasites. Out of total chicken examined 8.9% were infected with *Leucocytozoon* spp. and 30.0% were infected with helminth parasites viz., *Ascaridia* spp. (24.4%) and *Raillietina* spp. (4.4%).

Ben Slimane (2014) studied the prevalence of the gastrointestinal parasites of domestic chicken *Gallus domesticus* Linnaeus, 1758 in Tunisia according to the agro-ecological zones. A significant difference ($p<0.01$) was found between the prevalence rates of helminth parasites in the different agro-ecological zones. The highest prevalence was observed in lowland areas of northern Tunisia (Siliana district). This suggests that agro-ecology has a major influence on the distribution of helminth parasites. Recovered nematodes included *Heterakis* spp. (100%), *Ascaridia*

galli (53.33%) and *Acuaria hamulosa* (37%). The principal cestode species encountered were *Hymenolepis* spp. (73.33%) and *Raillietina* spp. (33.33%).

Butt *et al.*, (2014) studied the prevalence of cestode parasites in the intestine of local chicken (*Gallus gallus domesticus*) from Hyderabad, Sindh, Pakistan. Over all prevalence of infection was 94.5%. Three species of cestode parasites were recovered from the intestine of infected chickens. The identified cestode species and their prevalence were *Cotugnia digonopora* (94.5%), *Choanotaenia infundibulum* (89.5%), and *Raillietina cesticillus* (83.5%). The results of present study revealed that sub-standard poultry farming is a major factor for parasitic infection in local chicken which ultimately causes heavy loss.

Junaidu *et al.*, (2014) studied the prevalence of gastrointestinal helminth parasites of the domestic fowl (*Gallus gallus domesticus*) slaughtered in Giwa market, Giwa local government area Kaduna State, Nigeria. Total prevalence was 81.5% of the study population. Six helminth parasites were (*Gallus gallus domesticus)* encountered including *Raillietina tetragona* 48 (24.0%), *Raillietina echinobothrida* 22 (11.0%), *Raillietina cesticillus* 7 (3.5%), *Hymenolepis carioca* 79 (39.5%), *Ascaridia galli* 34 (17.0%) and *Heterakis gallinarum* 41 (20.5%). *Hymenolepis carioca* was the most abundant cestode parasite while *Heterakis gallinarum* was the most abundant nematode parasites recovered from the domestic chickens. Seventy nine (39.5%) of the birds had single infection, 68 (34.0%) had double infection, 14 (7.0%) had triple infection and 2 (1.0%) harboured four parasites. No single trematode parasite was recorded. Site preferences by the parasites in the gastrointestinal tract of the birds were small intestine, large intestine and caeca. There was no statistically significant difference ($p<0.05$) in the infection rate between sex.

Marciniak *et al.*, (2014) studied the prevalence and intensity of parasitic gastrointestinal infections in free-range chickens from the West Pomerania province and they observed gastrointestinal parasites in 9 farms out of the 10 farms. The prevalence of infections on these farms with protozoa of *Eimeria* spp. was on average 32.7%, while for nematode species they amounted to 9.6% for *Ascaridia galli*, 5.7% for *Heterakis gallinarum* and 12.5% for *Trichostrongylus tenuis.*

Beyene *et al.*, (2014) reported the effect and occurrence of gastrointestinal (GI) nematode parasites in chickens in and around Bahir Dar, northwest Ethiopia. There was higher infection rate recorded in extensive management system compared to intensive management system. There was no statistically significant difference among gender category of chickens. Mixed infections with two or more parasite species were also observed. This study strongly suggested that nematode parasites are a serious problem of backyard chickens. Appropriate control strategies are needed in the study area.

Alam *et al.*, (2014) examined the prevalence of gastrointestinal helminths in indigenous chicken Barisal district, Bangladesh. The gross pathological lesions were produced by them. Six species of helminth parasites viz., *Ascaridia galli* (41.56%), *Heterakis gallinarum* (15.62%), *Capillaria spp.* (4.68%), *Acuaria hamulosa* (8.75%), *Dispharynx spiralis* (1.56%) and *Raillietina tetragona* (19.68%) were identified. Higher prevalence of *A. galli* (45%), *H. gallinarum* (30%) and *A. hamulosa* (5%) were recorded in rainy season followed by summer and winter season. However, higher prevalence of *R. tetragona* infection was observed in summer season and *Dispharynx spiralis* infection was noted only in summer (1.58%) and winter season (4.0%).

Bsrat *et al.,* (2014) examined the chickens from four Kebeles of the Enderta district, Ethiopia to record clinical signs, gross and histopathological changes on common local chicken diseases. Endoparasites viz., *coccidian* spp. (40%), *Ascaridia galli* (35.1%), *Heterakis gallinarum* (10%) and *Raillietina* spp. (20.0%) were recorded.

Jegede *et al.,* (2015) examined the 280 faecal samples and 50 intact whole intestines of birds in Gwagwalada Guinea Savannah zone of Nigeria. The overall prevalence was 42.5% of gastrointestinal parasites. The most prevalent parasite based on faecal examination was *Ascaridia* sp. (36.1%) followed by *Eimeria oocysts* (34.5%), *Heterakis* spp. (12.6%), *Raillietina* spp. (10.1%), *Capillaria* sp. (4.2%) and *Syngamus* sp. (2.5%). Gut examination revealed presence of mixed infections (5.0%) with helminths and protozoan parasites. The prevalence was significantly ($P<0.05$) higher in growers than that of chicks and adults. No statistical significance was observed between the prevalence of male (46.8%) and female (40.8%) birds.

Thapa *et al.,* (2015) studied the prevalence and magnitude of helminth infections in organic laying hens (*Gallus gallus domesticus*) across Europe. Data on flock-level management factors (e.g. nutritional factors, litter quality, housing system, opening- and closing hours of popholes, pasture rotation and provision of occupational materials) were collected during a farm visit when the hens were on an average 62 weeks old. Results showed that *A. galli* was highly prevalent across Europe with an overall mean prevalence of 69.5% and mean worm burden of 10 worms per hen. The overall mean prevalence and worm burden for *Heterakis* spp. were 29.0% and 16 worms per hen, respectively, with a large variation between countries. The mean prevalence of *Raillietina* spp. was 13.6%. A positive correlation was found between mean *A. galli* worm burden and ascarid EPG. Of the analysed management factors,

only pasture access time had a significant negative association with *A. galli* worm burden.

Lawal *et al.*, (2015) examined the prevalence of gastrointestinal nematodes in village chickens (*Gallus gallus domesticus*) slaughtered in Gombe Metropolis Poultry Dressing Slabs. A total of seven Nematode species were identified in this present study with an overall prevalence of 20.1% in the Nothern markets and 19.5% in the Southern markets of the study area. Three nematode species recovered from the intestine were *Heterakis gallinarum* (365), *Ascaridia galli* (267) and *Gongylonema ingluvicola* (21), *Subulura brumpti* (123) was found in the caecum while *Dispharynx nasuta* (34) and *Cheilospirura hamulosa* (34) were found in the gizzard and *Syngamus trachea* (6) was recovered from trachea. There was no statistical significant association between the occurrence of the infection and the two zones of the study area ($p>0.05$), except for *Syngamus trachea* having a statistical significant association ($p<0.05$) between its occurrence and the two zones of the study area with the odd of occurrence having a value of 13.265 in the Northern zone. The study also indicated that female sex had a higher prevalence (70.1%) than the male (60.2%). There was a statistical significant association among the sex group and occurrence of the infection ($p<0.05$). The odd of occurrence was about twice in the female than male.

Sotudehalireza and Yagoob (2015) studied the prevalence of intestinal helminthic parasites in domestic chicken of Abhar city, Iran during 2015. The overall prevalence was 57%. Cestodes had the highest prevalence (36%) viz., *Reillietina tetragona* (15%), *R. echinobothrida* (12%) and *Choanotaenia infundibulum* (9%) followed by nematodes (21%) viz., *Heterakis galinarum* (21%). No trematode was encountered and (14%) birds had mixed infections.

Khan *et al.*, (2016) examined the adult backyard chickens of both sexes from August to November 2012 to determine the prevalence of gastrointestinal cestodes in Tando Allahyar district of Sindh province, Pakistan. They found an overall prevalence of 72.2% with five cestodes species viz., *Davainea proglottina* (10%), *Raillietina tetragona* (31.1%), *R. cesticillus* (14.45%), *Choanotaenia infundibulum* (7.78%) and *R. echinobothridia* (17.78%), while 18.89% of the birds were infected by more than one species of cestodes parasites.

Aziz (2016) studied theprevalence of gastrointestinal helminths of *Gallus gallus domesticus* (Linnaeus, 1758) in free-range system at Upper Egypt. Overall prevalence of infection was 84.4% (114/135). The percent of cestodes was 96.5%, but nematodes percent was 73.7%. Two nematodes species*Heterakis gallinarum* (56.14%) (64/114), *Ascaridia species* were identified. Cestode *Raillietina tetragona* (39.47%) (45/114) were also found.

Silva *et al.*, (2016) examined the helminthic parasites of chickens (*Gallus domesticus*) in different regions of São Paulo State, Brazil. The following helminth species were identified in chickens reared in 17 municipalities of the state of São Paulo: nematodes (*Ascaridia galli, Capillaria* sp., *Cheilospirura hamulosa, Heterakis gallinarum, Oxyspirura mansoni,* and *Strongyloides* sp.*),* cestodes (*Amoebotaenia cuneata, Choanotaenia infundibulum, Hymenolepis* sp., *Raillietina cesticillus, Raillietina echinobothrida,* and *Raillietina tetragona),* and trematodes (*Zygocotyle lunata* and *Postharmostomum commutatum*).

Slimane (2016) investigated the prevalence of the gastro-intestinal parasites of domestic chicken *Gallus domesticus* Linnaeus, 1758 in Tunisia according to the agro-ecological zones. He found the highest prevalence rate of infection in lowland

areas of northern Tunisia (Siliana district) with different species of nematodes such as *Heterakis* spp. (100%), *Ascaridia galli* (53.33%) and *Acuaria hamulosa* (37%) and the principal cestode species encountered were *Hymenolepis* spp. (73.33%) and *Raillietina* spp. (33.33%).

Malatji *et al.*, (2016) examined chicken faecal samples and intestinal tracts to determine the prevalence of gastrointestinal parasites in village chicken of Limpopo and Kwa Zuli-Natal provinces of South Africa. Species identified were *Ascaridia galli* (18.77%), *Heterakis gallinarum* (15.56%) and *Capillaria* spp. (4.00%) in nematodes; *Choanotaenia infundibulum* (2.10%) and *Raillietina cesticillus* (6.00%) in cestodes and *Eimeria* spp. (29.46%) in protozoa. Mixed infections were noticed in infected birds with helminths and protozoa parasites.

Rehman *et al.*, (2016) studied thedomestic chicken of Mardan District, Pakistan. They observed that 18.14% birds were found to be infested with *Raillietina cesticillus*. The prevalence rate in female was 21.2% while 14.33% was recorded in male. With respect to seasons, the prevalence rate in winter was 46.97%, spring 6.7%, summer 21.1% while in fall 30.37% was recorded.

Asumang *et al.*, (2019) studied the the prevalence of gastrointestinal parasites in local and exotic breeds of chickens in Pankrono–Kumasi in the Ashanti Region of Ghana. Two hundred (200) cloacae of slaughtered birds were collected and the faecal samples were examined for the presence of eggs/cysts of gastrointestinal parasites by using the simple flotation technique and microscopy. Prevalence of nematodes and cestodes was 65.5%. *Ascaridia galli* recorded as the most prevalent parasite. Some of the nematodes include *Ascaridia galli* 65 (32.5%), *Heterakis gallinarum* 38 (19.0%), and *Capillaria* spp. 29 (14.5%). Some cestodes were

Raillietina spp. 19(9.5%) and *Choanotaenia infundibulum* 5 (2.5%) with *Prosthogonimus* spp. 3 (1.5%) being the only trematode recovered.

Berhe *et al.*, (2019) investigated the infection rate and identify helminth parasite species in chickens managed under different production systems, in Mekelle, Ethiopia. Postmortem (N=138) and fecal (N=410) samples of chicken were considered for necropsy and coproscopic examination to see both adult and eggs of helminth parasites, respectively. The study attested high prevalence (87.7%) of mixed infection with helminth parasites of chicken. *Heterakis gallinarum* (72.5%) and *Ascaridia galli* (68.8%) were found as the most prevalent species (necropsy). During coproscopic examination cestode (89%) infections represent a higher prevalence than nematodes (84.4%), even though no difference was observed during the necropsy examination results.

Ola-Fadunsin *et al.*, (2019) investigated the prevalence, species diversity, intensity, and risk factors associated with the gastrointestinal helminths of intensively raised poultry in Kwara Central senatorial district of Kwara State. Seven helminth species were detected but *Heterakis gallinarum* (10.2%) and *Ascaridia galli* (6.0%) were most prevalent, while *Capillaria* species was the least prevalent (0.8%). Many factors significantly ($p<0.05$) influence the prevalence of helminth parasites like; physiological status, bird type, production purpose, farm age (years), presence of other animals in the farm, flock size (birds), farm size (acres), housing type, farm type, frequency of anthelmintic use, distance to waste area (meters), level of biosecurity, and frequency of cleaning the pen.

Van *et al.*, (2020) investigated the prevalence/burden of gastrointestinal helminths in small-scale commercial flocks in the Mekong Delta region and the association

between worm burdens and birds' weight and disease status. A total of 54.2% and 54.4% normal and diseased chickens contained helminths. Among colonised birds, the diseased ones harboured a higher mass of helminth worms than normal (healthy) birds (3.8 ± SD 8.6 g vs. 1.9 ± SD 6.3 g, respectively). During the study period they found eight species, including nematodes (*Ascaridia galli*, *Cheilospirura hamulosa* and *Heterakis gallinarum*), cestodes (*Hymenolepis, Raillietina cesticillus, Raillietina echinobothrida, Raillietina tetragona*,) and one trematode (Echinostomatidae). *Heterakis gallinarum* was the most prevalent helminth (43.3% and 42.2% in normal and sick chickens, respectively), followed by *A. galli* (26.7% and 41.1%). Colonised chickens weighed 101.5 g less than non-colonised birds. Colonisation was higher during the rainy months (May–November) for both *H. gallinarum* and *A. galli.*

CHAPTER-3

Prevalence of Gastrointestinal Nematode Parasites of *Gallus gallus domesticus* in Lucknow Region

3.1 INTRODUCTION-

The chicken *Gallus gallus domesticus* is believed to have descended from the wild Indian and South East Asian red jungle fowl (Permin and Ranving, 2001). Birds are an important part of the ecosystem as they play vital role in ecological, medicinal, nutritional and economical point of view. Poultry farming is the process of raising domesticated birds, such as chickens and ducks for the purpose of producing meat or egg for food. India has 498 million poultry population with an average growth rate of 8–10% per annum. India ranks third in egg production and sixth in broiler meat production (USDA, 2011).

Poultry farming developed enormously in recent years and has become one of the most demanding forms of animal husbandry activities. Though the impact of parasitic diseases in farm birds, reared on cage systems have diminished due to modernization in poultry farming and bio security measures, farm birds maintained on deep litter system and backyard free ranging birds still remain susceptible to parasitic infection via litter droppings and scavenging habits. The domestic chicken feeds on a wide range of food substances. This range from grains, fruits to insects which may harbour infective stages of parasites thereby predisposing them to parasites particularly gastro-intestinal parasites (Oniye *et al.*, 2001; Frantovo, 2000). These parasitic infections may cause considerable damage and great economic loss to the poultry industry due to malnutrition, decreased feed conversion ratio, weight loss, lowered egg production and death in young birds. Improved poultry management practices are responsible for the reduction in incidence of parasitic infection.

Helminths constitute the most important group of gastrointestinal parasites of fowl both in number of species and extent of damage they cause; the main genera of

nematodes responsible for infection in fowl include *Capillaria* sp., *Heterakis* sp., and *Ascaridia* sp., (Jordan and Pattison, 1996) additionally, cestodes of significant importance are of the two genera Railletina and Hymenolepis. The trematodes infection is not very common in domestic fowl. In villages free range management system is used to raise domestic fowl with little or no supplementary feeding and without any veterinary care thereby exposing them to parasitic infection (Gary and Richard, 2012). Parasitism ranks high among factors that serve as a threat to chickens, the presence of a few parasites do not usually cause a problem; however, a large number can have a devastating effect on growth, egg production, and overall health. The Helminths are the most important group of parasites that affect the chickens both in terms of number and extent of damage caused to the gastrointestinal tract in the chickens.

So, keeping in view the importance of these parasites in chickens, this study was undertaken to find out the prevalence of gastrointestinal nematode (helminths) parasites of the chickens (*Gallus gallus domesticus)* especially in the Lucknow, Uttar Pradesh, India, so that treatment strategies can be adopted accordingly and provide guidelines in adopting the preventive measures to control the parasitic infection.

3.2 MATERIALS AND METHODS-

3.2.1 Study area-

The study was conducted in Lucknow, stands at an elevation of approximately 123 meters (404 ft) above sea level. Lucknow district covers an area of 2,528 square kilometres (976 sq mi) (Lucknow District Population Census, 2011; Lucknow Population statistics, map and location 2014), bounded on the east by Barabanki, on the west by Unnao, on the south by Raebareli and in the north by Sitapur. Lucknow

sits on the north western shore of the Gomti River. This city has a humid subtropical climate with cool, dry winters from mid-November to February and dry, hot summers with thunderstorms from late March to June. The rainy season is from July to September when the city gets an average rainfall of 896.2 millimetres (35.28 inches) from the south-west monsoon winds, and occasionally frontal rainfall will occur in January. In winter the maximum temperature is around 25 °C (77 °F) and the minimum is in the range of 3 °C (37 °F) to 7 °C (45 °F) (The Times of India, 2012) (Fig. 3.1).

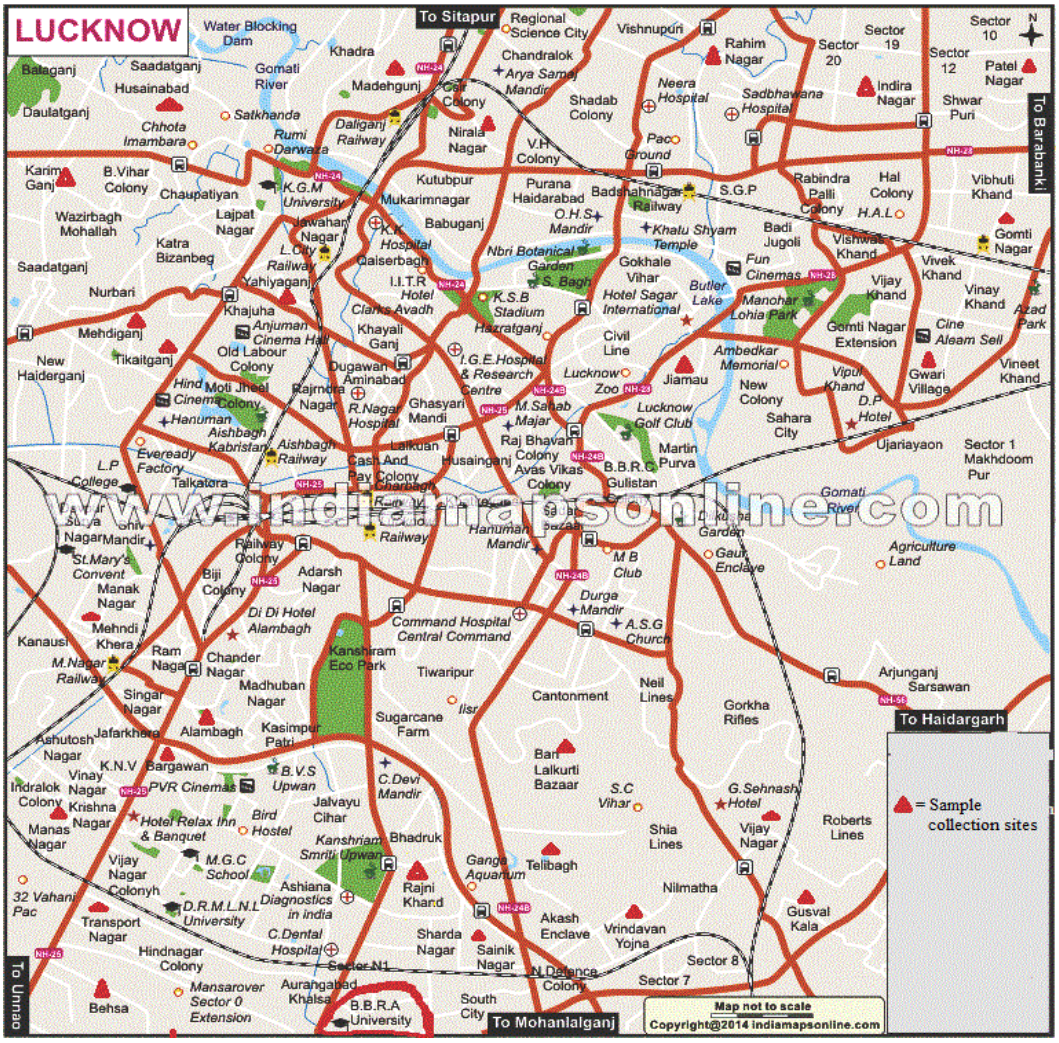


Figure 3.1- Map of study area showing different sample collection sites (source: IND map.com)

3.2.2 Study Population-

Study population includes 557 domestic chickens managed under unorganized backyard systems. The age of the studied birds was determined through information collected from owners. Growers were 12-24 weeks old and adult aged 32 weeks were collected during study period. Chickens that were old enough to fend for themselves called as growers but had not started reproducing, while adults included cocks that were mating and hens that had at least one clutch of chicks.

3.2.3 Study period-

Study was conducted during January, 2017 to December, 2019 in Lucknow to determine the prevalence of gastrointestinal helminth parasites of domestic chicken.

3.2.4 Parasitological examination-

During the present study, the domestic fowls were collected from January, 2017 to December, 2019 from different regions of study sites. The hosts were then brought to the parasitology laboratory of Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow for the parasitic examination. For the collection of endoparasites, the gastro intestinal tracts of *Gallus gallus domesticus* were dissected thoroughly to investigate the presence of parasites, according to the procedure as described by Fowler, 1990.

3.2.5 Preservation-

Nematodes were collected from host with the help of forceps, washed in saline water and killed in hot 70% alcohol, and stored in the glycerine alcohol solution and thick parasites kept in lectophenol. Cestodes were also collected from the same host and preserved in Carnoy's fluid (25 ml of glacial acetic acid and 75 ml of Absolute

ethanol) for the identification. After fixation, all parasite specimens were washed in distilled water for 2 to 4 times to remove fixative. The parasites were then processed through 30% → 50% → 70% grades of alcohols and finally preserved in 70% alcohol for further use. The preserved parasites (in 70% alcohol) were hydrated through descending grades 70% →50 % → 30 % of alcohol, washed in distilled water for 2 to 4 times and then placed in aqueous Borax Carmine (a staining reagent) for 10 to 30 minutes depending on the thickness of the parasites and then the parasites were destained using acid water (1-2 ml of concentrated HCl in 100 ml distilled water) to achieve the desired intensity. When the parasites took appropriate stain then they were washed in distilled water and dehydrated with ascending grades 30 % → 50 % → 70 % → 90 % → 100 % of alcohol for 10 to 30 minutes (depending on the size of the parasites) in each grade. After complete dehydration, parasites were transferred into Xylene (clearing agent) for 2 to 5 minutes. The Xylene treated parasites were then mounted with DPX (Dextrin Plasticized Xylene) and a coverslip was carefully lowered over them (Cable, 1957; Pandey, 2019).

Parasites were observed under light bright field microscope (10X, 40X, 100X) and photographs were taken by Evos XL imaging microscope. Parasites were identified according to the keys and description given by Soulsby, 1982. The prevalence of helminthiasis was recorded as per formulae described by Margolis *et al.,* 1982.

3.2.6 Definitions

The ecological terms used in this study are-

$$\text{Prevalence (\%)} = \frac{\text{Total number of hosts infected}}{\text{Total number of hosts examined}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{Total number of Parasites}}{\text{Total number of hosts infection}}$$

$$\text{Relative Density or Abundance} = \frac{\text{Total number of Parasites}}{\text{Total number of hosts examined}}$$

3.2.7 Data analysis

The most commson measurements of parasite population levels in hosts are prevalence, mean intensity and mean abundance (Bush, 1997). Prevalence refers to the percentage of organisms infected by a particular species of parasite. Mean intensity is the number of parasites of a given species per infected host. Mean abundance refers to the number of parasites of a given species per host examined, infected and uninfected. The nomenclature used to define ecological parameters is in consistency with that of Margolis *et al.,* 1982. The information obtained from laboratory test and observations were entered in the IBM SPSS version 20 for analysis. Chi-square ($\chi 2$) test was used to analyse the sample data. Chi-square test was used to assess whether there is a statistically significant difference in gastrointestinal parasitic infection between seasons, genderand age. A statistically significant association between variables was considered to exist if the calculated p-value is less than 0.05 with 95% confidence level.

3.3 RESULTS-

3.3.1 Prevalence of helminth infection

During the present study, different helminth parasites belonging to two classes; cestoda and nematodes were observed. A total of 557 specimens of fowls were

examined during the present study, which revealed 45.96% (256/557) of infection by helminthes in the study area (Table 3.1 and Fig. 3.2). Different types of helminth parasites were recovered during the study, including two nematodes *Ascaridia galli* (41.7%) *and Heterakis gallinarum* (7.36%) and three cestodes i.e., *Cotugnia diagnopora* (17.6%), *Raillietina tetragona* (11%), and *Raillietina cesticillus* (6.64%).

Table 3.1- Overall prevalence of gastrointestinal helminths in *Gallus gallus domesticus* (2017 - 2019)

Total no of hosts examined	No of infected hosts	Prevalence of infection (%)
557	**256**	**45.96**

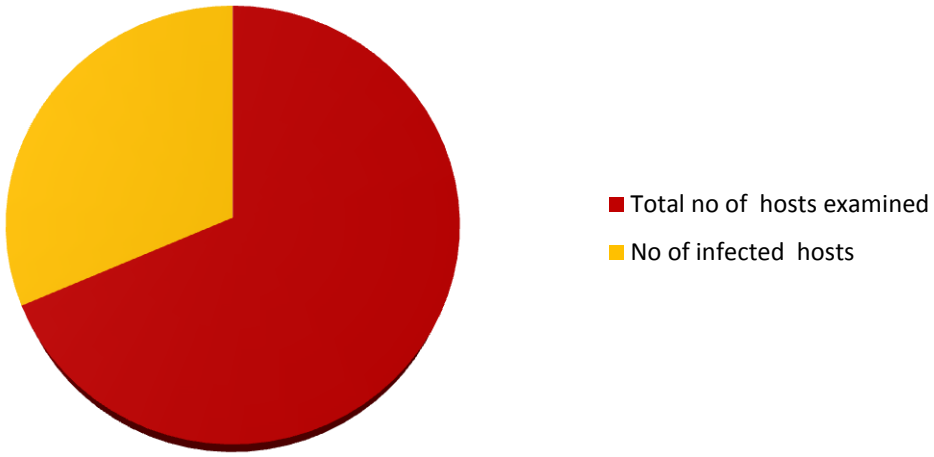


Figure 3.2- Overall prevalence of gastrointestinal helminths in *Gallus gallus domesticus* (2017- 2019)

3.3.2 Seasonal prevalence

The study showed that the prevalence of parasites in fowls was throughout the year but the prevalence varied from season to season. The highest prevalence was observed during monsoon 65.40% (121/185) followed by summer (50.53%) (94/186) and least in winter (22.04%) (41/186). During summer, 186 fowls were examined, out of which

90 (48.4%), 16 (8.6%) and 29 (15.6%), 20 (10.8%), 12 (6.5%) were found infected with *Ascaridia galli, Heterakis gallinarum, Cotugnia diagnopora, Raillietina tetragona, Raillietina cesticillus,* respectively. Similarly, during monsoon out of 185 specimens examined, 102 (55.1%), 20 (10.8%), 45 (24.3%), 26 (14.1%) and 18 (9.7%) were infected with *Ascaridia galli, Heterakis gallinarum, Cotugnia diagnopora, Raillietina tetragona, Raillietina cesticillus,* respectively. However lowest prevalence of these helminth parasites was observed during the winter. Out of 186 specimens examined 40 (21.5 %), 5 (2.7 %), 24 (12.9%), 15 (8.1%) and 7 (3.8%) were infected with *Ascaridia galli, Heterakis gallinarum, Cotugnia diagnopora, Raillietina tetragona, Raillietina cesticillus,* respectively (Table 3.2 and Fig. 3.3). *Ascaridia galli* was found to be most prevalent in both single and multiple type of infection, thus the order of prevalence in the study area was monsoon >summer >winter

Table 3.2- Season wise prevalence of gastrointestinal helminth parasites in *Gallus gallus domesticus* (2017 - 2019)

Season	Total no. of Intestines	Infected intestines	Non Infected intestines	Prevalence (%)	No. infected intestines with particular Parasitic spp. (% prevalence)				
					Nematodes		Cestodes		
					A.G	H.G	C.D	R.T	R.C
Winter	186	41	145	22.04	40 (21.5)	5 (2.7)	24 (12.9)	15 (8.9)	7 (3.8)
Summer	186	94	92	50.53	90 (48.4)	16 (8.6)	29 (15.6)	20 (10.8)	12 (6.5)
Monsoon	185	121	64	65.05	102 (55.1)	20 (10.8)	45 (24.3)	26 (14.1)	18 (9.7)
Total	557	256	301	45.96	232 (41.7)	41 (7.3)	98 (17.6)	61 (11)	37 (6.64)
				χ^2 **P**	48.37 0.001 P<0.05	9.605 0.008 P<0.05	9.117 0.010 P<0.05	3.423 0.181 P>0.05	5.340 0.069 P>0.05

(A.G- *Ascaridia galli,* **H.G-** *Heterakis gallinarum,* **C.D-** *Cotugnia diagnopora,* **R.T-** *Raillietina tetragona,* **R.C-** *Raillietina cesticillus***)**

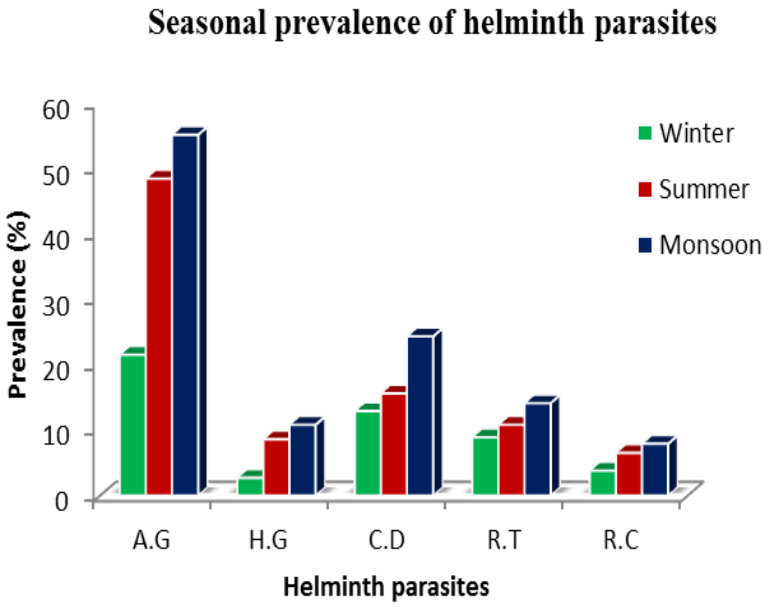


Figure 3.3- Season wise prevalence of gastrointestinal helminth parasites in *Gallus gallus domesticus* (2017 – 2019)

(A.G- *Ascaridia galli,* **H.G-** *Heterakis gallinarum,* **C.D-***Cotugnia diagnopora,* **R.T-** *Raillietina tetragona,* **R.C-** *Raillietina cesticillus***)**

3.3.3 Month-wise prevalence

Month-wise prevalence of various gastrointestinal parasites is presented in Table 3.3 and Fig. 3.4 and the results revealed that the highest incidence rate of these parasites was recorded in the month of July (68.08%) followed by August (73.33%) whereas the lowest was reported in the month of January (15.780%) followed by December (19.94%). In addition, the data presented in the Table 3.4 and Fig. 3.5 indicating the month–wise prevalence study of different parasitic species. The data revealed that the nematode parasite namely *Ascaridia galli*, *Heterakis gallinarum* showed an increase in the prevalence rate from December to November. Whereas cestodes namely *Cotugnia diagnopora, Raillietina tetragona, Raillietina cesticillus* parasites had shown a dynamic position in each month (Table 3.4 and Fig. 3.5).

Table 3.3- Month wise prevalence of gastrointestinal helminths in *Gallus gallus domesticus* (2017 - 2019)

Month	Total No. of Intestines	Total no of infected Intestines	% Prevalence
Nov	32	8	25.00
Dec	36	7	19.44
Jan	38	6	15.78
Feb	33	7	21.21
March	47	13	27.65
Winter	186	41	22.04
April	61	29	47.54
May	65	32	49.23
June	60	33	55.00
Summer	186	94	50.536
July	47	34	72.34
August	45	31	68.88
Sep	46	30	65.21
Oct	47	26	55.31
Monsoon	185	121	65.05
Total	557	256	45.96

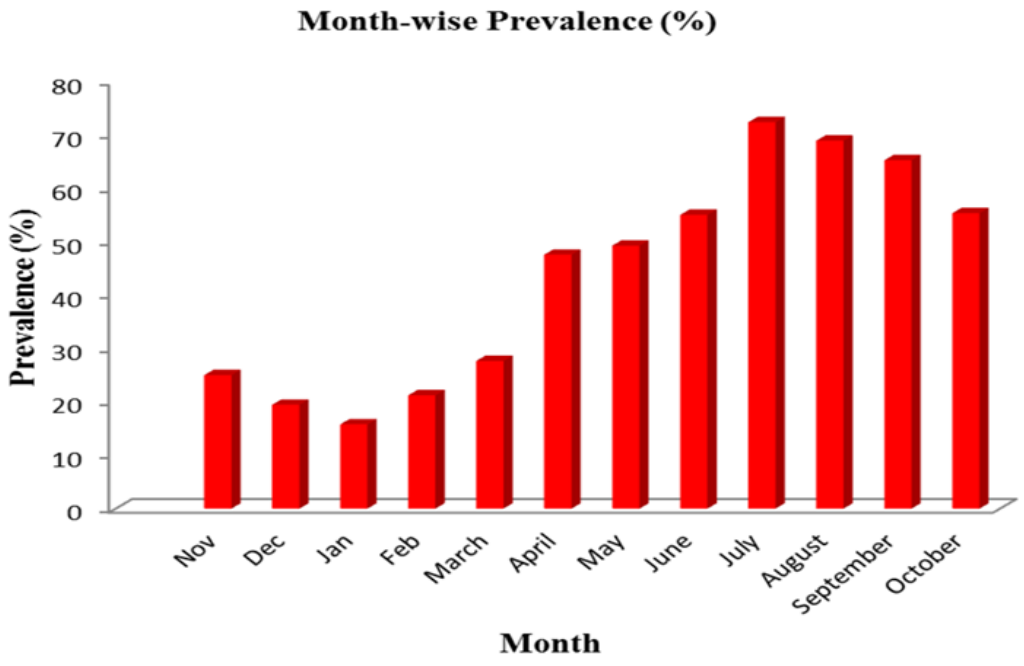


Figure 3.4- Month wise prevalence of gastrointestinal helminths in *Gallus gallus domesticus* (2017 - 2019)

Table 3.4- Month wise prevalence of gastrointestinal helminths species in *Gallus gallus domesticus* (2017 - 2019)

Month	Total No. of intestines	Total no of infected intestines	No. infected intestines with particular Parasitic spp. (% prevalence)				
			A.G	H.G	C.D	R.T	R.C
Nov	32	8	8 (25)	2 (6.25)	5 (15.6)	4 (12.5)	1 (3.1)
Dec	36	7	7 (19.44)	0 (0)	4 (11.11)	2 (5.5)	0 (0)
Jan	38	6	5 (13.15)	0 (0)	3 (7.8)	3 (7.8)	1 (2.6)
Feb	33	7	7 (21.12)	1 (3.0)	6 (18.18)	3 (9.0)	2 (6.06)
March	47	13	13(27.65)	2 (4.25)	6 (12.7)	3 (6.3)	3 (6.38)
Winter	186	41(22.04)	40 (21.5)	5 (2.7)	24 (12.9)	15 (8.06)	7 (3.8)
April	61	29	28(45.90)	4 (6.55)	8 (13.11)	5 (8.1)	3 (4.91)
May	65	32	31(47.69)	5 (7.69)	10 (15.38)	7 (10.76)	4 (6.1)
June	60	33	31(51.66)	7(11.66)	11 (18.33)	8(13.3)	5 (8.33)
Summer	186	94(50.53)	90(48.4)	16 (8.6)	29 (15.6)	20 (10.8)	12 (6.45)
July	47	34	32 (68)	8(17.02)	16(34.04)	10 (21.27)	7 (14.89)
August	45	31	28 (62)	6 (13.3)	12(26.66)	7 (15.5)	5 (11.88)
Sep	46	30	22 (47)	4 (8.69)	8 (17.39)	6 (13.0)	3 (6.50)
Oct	47	26	20 (42)	2 (4.25)	8 (17.02)	3 (6.38)	3 (6.30)
Monsoon	185	121 (65.05)	102 (55.1)	20 (10.8)	45 (24.3)	26 (14.1)	18 (9.7)
Total	557	256 (45.96)	232 (41.7)	41 (7.3)	98 (17.6)	61 (11)	37 (6.64)

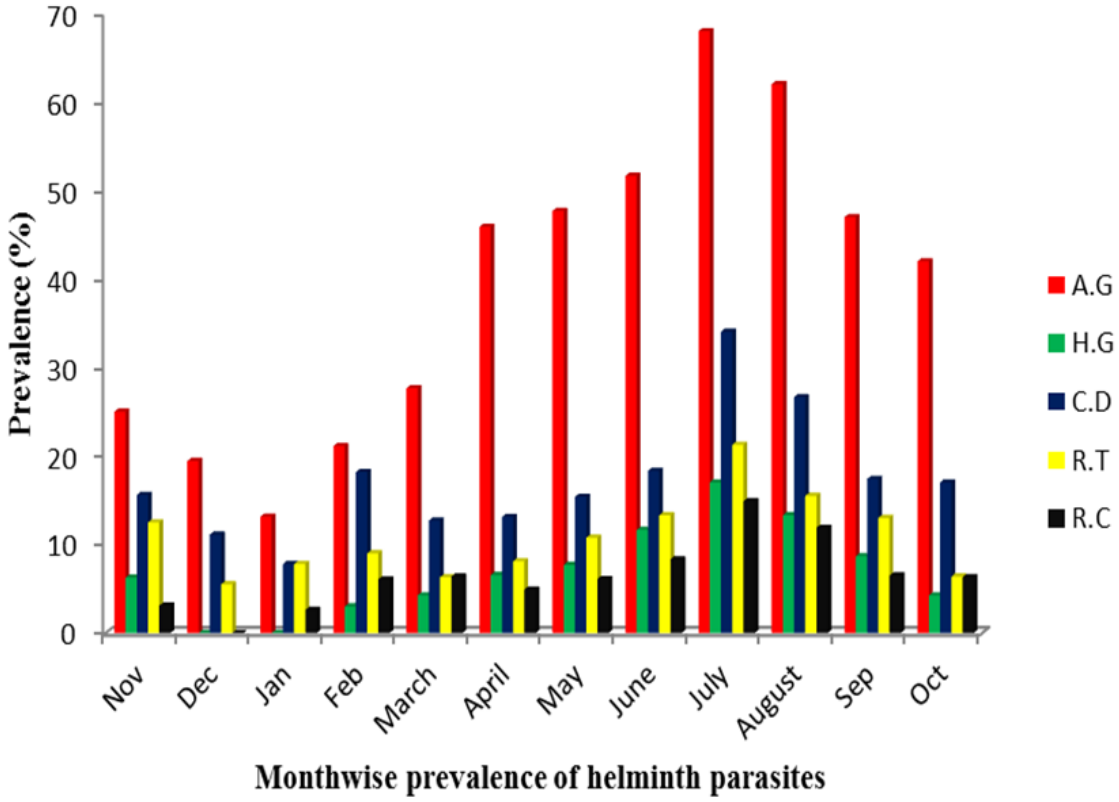


Figure 3.5 - Month wise prevalence of gastrointestinal helminths species in *Gallus gallus domesticus* (2017 – 2019)

3.3.4 Monthwise overall Mean Intensity and Relative Abundance-

Furthermore, in the season-wise study, the mean intensity (MI) and relative abundance (RA) were recorded highest during the monsoon (MI=37.60; RA=24.59) followed by summer (MI=33.40; RA= 16.88) and winter (MI= 30.73; RA= 6.77). Present study also reported higher mean intensity in the month ofJuly (MI= 45.00) and high relative abundance in the month of July (RA= 32.55). Monthwise MI and RA were as follows; Nov (MI 31.2, RA 7.81), Dec (MI 28.57, RA 5.55), Jan (MI 25, RA 3.94), Feb (MI 32, RA 6.96), March (MI 33.07, RA 9.14), April (MI 33.10, RA 15.73), May (MI 33.43, RA 16.46), June (MI 33.63, RA 18.5), July (MI 45.00, RA 32.55), August (MI 42.90, RA 29.55), Sep (MI 33.33, RA 21.73), Oct (MI 26.53, RA 14.68) (Table 3.5, Fig. 3.6).

Table 3.5 - Month-wise and season-wise mean Intensity and Relative Abundance of gastrointestinal helminths parasites in *Gallus gallus domesticus* (2017 - 2019)

Month	Total number of intestines examined	Number of the infected intestines	Total number of parasites	Mean Intensity (MI)	Relative Abundance (RA)
Nov	32	8	250	31.2	7.81
Dec	36	7	200	28.57	5.55
Jan	38	6	150	25	3.94
Feb	33	7	230	32	6.96
March	47	13	430	33.07	9.14
Winter	186	41	1260	30.73	6.77
April	61	29	960	33.10	15.73
May	65	32	1070	33.43	16.46
June	60	33	1110	33.63	18.5
Summer	186	94	3140	33.40	16.88
July	47	34	1530	45.00	32.55
August	45	31	1330	42.90	29.55
Sep	46	30	1000	33.33	21.73
Oct	47	26	690	26.53	14.68
Monsoon	185	121	4550	37.60	24.59

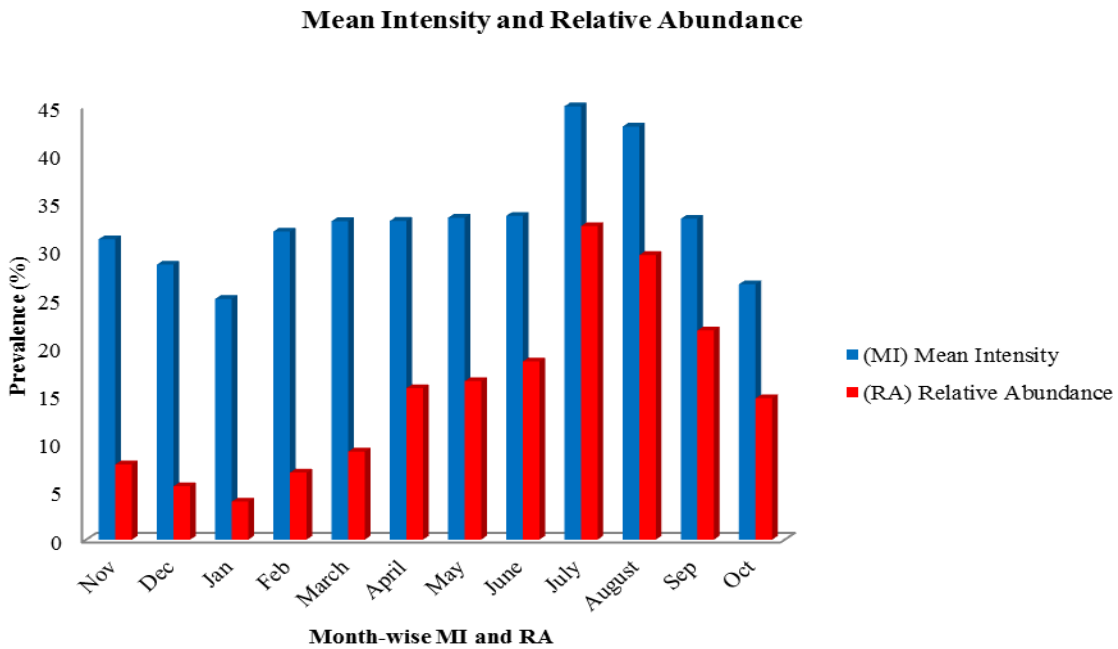


Figure 3.6 - Month-wise mean Intensity and Relative Abundance of gastrointestinal helminths parasites in *Gallus gallus domesticus* (2017 - 2019)

3.3.5 Species-wise Mean Intensity and Relative Abundance-

The Mean Intensity (MI) of the parasites was recorded maximum for *Raillietina Tetragona* (47.86) followed by *Raillietina cesticillus* (40.59), *Cotugnia diagnopora* (26.02), *Ascaridia galli* (15.30), *Heterakis gallinarum* (13.41). At the same time Relative Abundance (RA) was highest for *Ascaridia galli* (6.37) followed by *Raillietina Tetragona* (5.24), *Cotugnia diagnopora* (4.21), *Raillietina cesticillus* (2.69), and *Heterakis gallinarum* (0.98) (Table 3.6, Fig. 3.7).

Table 3.6- Species-wise Mean Intensity and Relative Abundance of gastrointestinal helminths parasites in *Gallus gallus domesticus* (2017 - 2019)

Parasitic species	Total Number of intestines examined	Number of The infected Intestines	Total number of parasites	Mean Intensity (MI)	Relative Abundance (RA)
Ascaridia galli	557	232	3550	15.30	6.37
Heterakis gallinarum	557	41	550	13.41	0.98
Cotugnia diagnopora	557	98	2550	26.02	4.21
Raillietina tetragona	557	61	2920	47.86	5.24
Raillietina cesticillus	557	37	1502	40.59	2.69

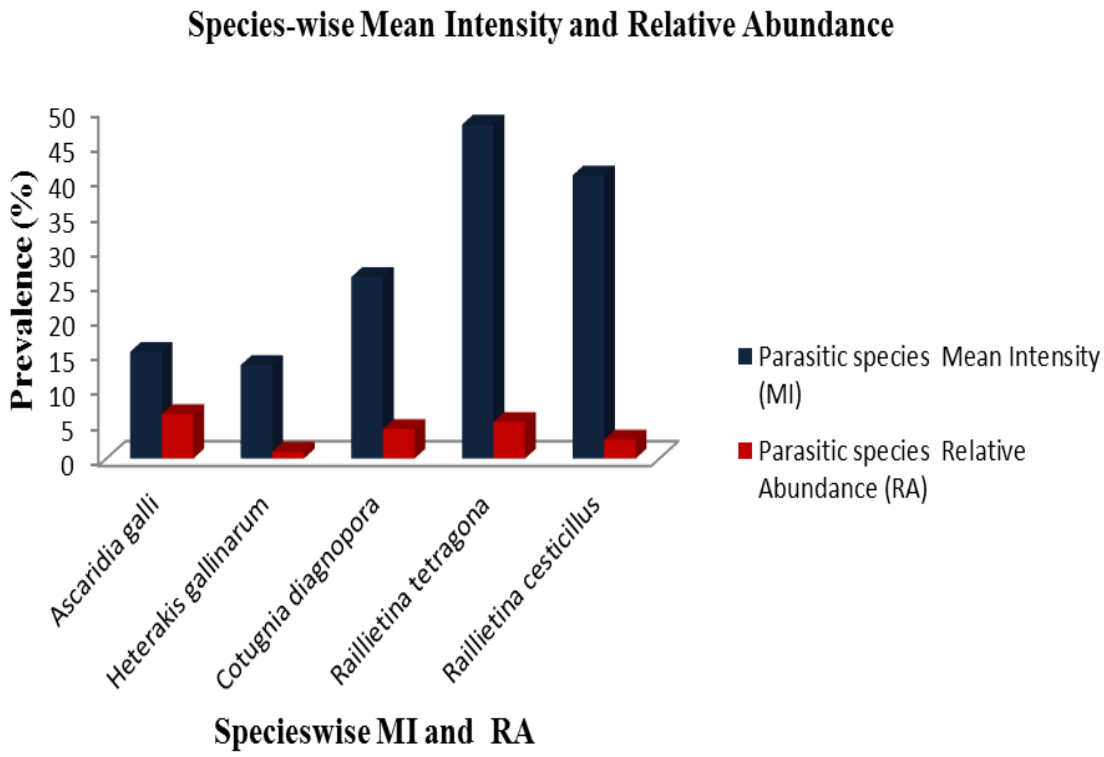


Figure 3.7 - Species-wise Mean Intensity and Relative Abundance of gastrointestinal helminths parasites in *Gallus gallus domesticus* (2017 - 2019)

3.3.6 Age-wise prevalence

Domestic fowls of different age groups were examined. Out of 557 specimens, 268 were growing age fowl and of which, 119 (44.4%) were infected with helminth parasites, similarly in the 289 adult fowl specimens, 137 (47.40%) were infected with helminth parasites. Results clearly indicate that, there is no significant age resistance shown by the hosts against nematode infection. Thus the hosts of any age group may be exposed to helminthic infections with a slight resistance shown by the growing age fowl because they are kept inside the houses (Table 3.7 and Fig. 3.8).

Table 3.7- Age-wise prevalence of Gastrointestinal helminths infection in *Gallus gallus domesticus* (2017 - 2019)

Age	Total no. of Intestines	Non infected Intestines	Infected intestines	Prevalence (%)
Grower	268	149	119	44.4
Adult	289	152	137	47.40
Total	557	301	256	45.96
				χ^2= 0.505
				P= 0.478
				P> 0.05

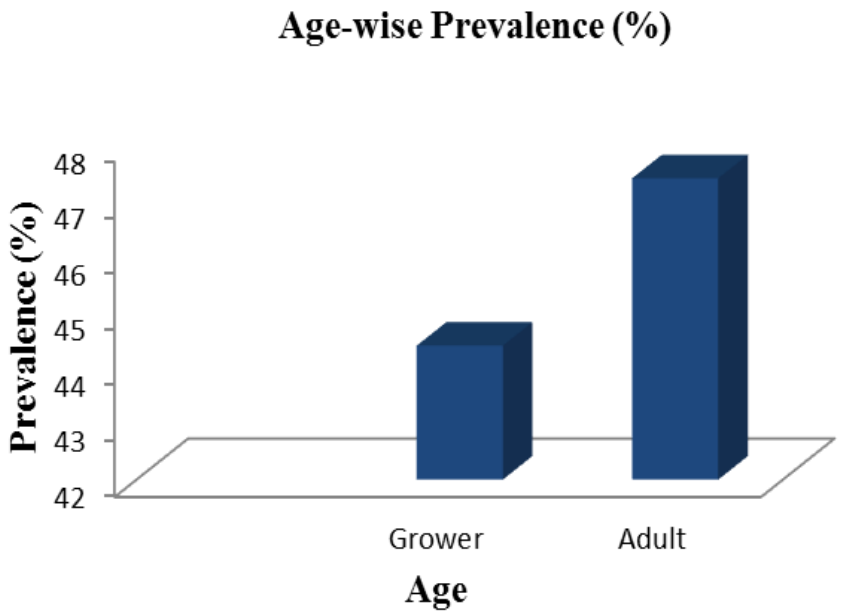


Figure 3.8- Age-wise prevalence of gastrointestinal helminths infection in Gallus gallus domesticus (2017 - 2019)

3.3.7 Gender-wise prevalence

Out of 557 specimens of *Gallus gallus domesticus* examined during the present study, 270 were females and 287 were males. A prevalence of 50.37% (136/270) was found in females and in males, 41.81% (120/287) respectively was observed during the study

period. The results shows that there is no marked but a slight resistance shown by males as compared to females (Table 3.8 and Fig. 3.9).

Table 3.8- Gender wise prevalence of gastrointestinal helminths infection in *Gallus gallus domesticus* (2017 - 2019)

Gender	Total no of Intestines	Non infected intestines	Infected intestines	Prevalence (%)
Female	270	134	136	50.37
Male	287	167	120	41.81
Total	557	301	256	45.96 χ^2= 4.10 P= .043 P< .05

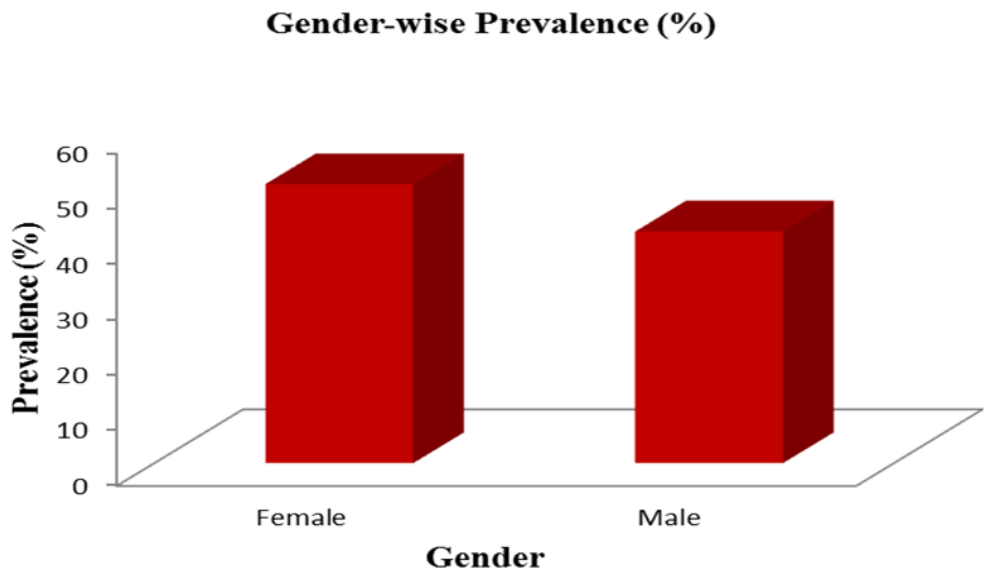


Figure 3.9- Gender-wise prevalence of gastrointestinal helminths infection in *Gallus gallus domesticus* (2017 - 2019)

3.4 DISCUSSION-

During the study period, overall prevalence of infection was found to be 45.96%, more or less similar to the prevalence (37.6%) as reported by Agbolade *et al.*, 2014. Among

all identified nematode parasites, *A. galli* (57.31 %) was highly prevalent parasite, similar prevalence of *A. galli* is also reported by Puttalaksshamamma *et al.,* 2008; Katoch *et al.,* 2012; Sreedevi *et al.,* 2016. Although mortality from *A. galli* is not significant, may lead to death of infected birds due to the obstruction of intestinal lumen (Sreedevi *et al.,* 2016). The prevalence of *H. gallinarum* (7.4%) was lower as compared to *A. galli* (41.7%) infection, but *H. gallinarum* play an important role as a carrier of protozoan parasite, *Histomonas meleagridis* causes fatal disease in birds. But in Goromonzi District in Zimbabwe (Permin, 2002) and Bhubaneswar (Manaswini, 2008) regions *H. gallinarum* was the common nematode identified with 64.62% and 52.94% infection, respectively. Whereas in Greneda (Pinckney, 2008) and Bangalore regions (Puttalaksshamamma *et al.,* 2008) *Raillietina tetragona* was the common parasite than the other cestode parasites. Trematode parasites were not detected during the study period. It might be due to lack of favourable environment for the perpetuation of the vectors of trematodes. Similar finding in desi fowl were also observed earlier (Puttalaksshamamma *et al.,* 2008; Katoch *et al.,* 2012; Rabbi, 2006; Magwisha *et al.,* 2002; Mungube *et al.,* 2008).

The present study shows that, single type infections of nematode were more prevalent than multiple types of infections with the cestodes. Contrary, multiple typesof infections with helminths in domestic fowl was observed by various researchers (Yadav and Tandon, 1989; Mpoame, and Agbede, 1995; Permin, 1997; Magwisha, 2002; Phiri, 2007).

Overall age-wise prevalence of endoparasites during the study period was 44.4% and 47.40% in growers and adults, respectively. No significant relationship was found between the prevalence of infection among chicks and adults ($P>0.05$). Significant differences were observed by Paul *et al.,* 2012 and Momin *et al.,* 2014 in Bangladesh

and the infection was highly prevalent in adults. High prevalence of endoparasites in adult birds could be due to their gregariousness as compared to chicks, therefore, exposing them to be more intermediate hosts than the former. Moreover, chicks were kept inside to protect them from predators. Contrary, Dar *et al.,* 2013; Hembram *et al.,* 2015 observed more prevalence in chicks and Molla *et al.,* 2012 observed more prevalence in growers than the adult birds.

The gender-wise prevalence of endoparasites in study area was 48.14% and 40.28% in female and male birds, respectively. Females were more susceptible to endoparasites than males with a statistical significance. The results are in contrary with other researchers where no statistical difference was reported (Paul, 2012; Hembram *et al.,* 2015; Rehman, 2016) in backyard poultry. This could be due to the voracious feeding habits of female birds especially during egg production, than that of males which are largely selective in nature (Paul, 2012; Hembram *et al.,* 2015; Rehman, 2016). In contrary Radfar *et al.,* 2012; Dar *et al.,* 2013; Momin *et al.,* 2014 and Sheikh *et al.,* 2015 reported more prevalence of parasitic infections in males than female birds.

Overall seasonal prevalence of endoparasites during study period in Monsoon, summer and winter seasons was 65.05%, 50.53% and 22.04%, respectively. Though the prevalence was high during rainy season, significant ($P<0.05$) relationship between the season and prevalence of endoparasites was observed in the present study. The environmental conditions of the study region are hot and humid which are favorable for development and survival of preparasitic stages of parasites and for insects, which in turn act as vectors for helminths leading to increased availability of infective stages for backyard poultry (Dube *et al.,* 2010), especially during the process of searching the feed.

Climatic conditions mainly temperature and humidity may alter the population dynamics of the parasites, resulting in variations in the prevalence and intensity of helminthic infections (Magwisha *et al.,* 2002). Significant relationship between the seasons and prevalence of gastrointestinal parasites was observed during rainy season being more favourable for the prevalence of parasites by Dube *et al.,* 2010 and Sreedevi *et al.,* 2016 in rural area of Zimbabwe and Gannavaram (Andhra Pradesh) respectively. The present findings are also in agreement with Mungube *et al.,* 2008; Alam *et al.,* 2014; Hembram *et al.,* 2015 who reported higher prevalence of infection during rainy season in semi-arid zone of Eastern Kenya, Bangladesh and Odisha, India respectively. Contrary, Hange *et al.,* 2007; Solanki *et al.* 2015 and Rehman*et al.,* 2016 reported highest prevalence of helminth infection in winter season compared to summer and rainy seasons.

High prevalence of endoparasites during summer season than winter and rainy seasons in free ranging birds was reported by Paul *et al.,* 2012; Naphade *et al.,* 2013 and Sheikh *et al.,* 2015. The high prevalence rate of gastrointestinal parasitism in desi fowl in the present study could be attributed to the fact that the desi fowls were free ranging and had free access to infective stages in the environment and to their respective intermediate hosts like beetles, earth worms, ants etc. in search of feed as they act as intermediate hosts for helminth parasites.

In the present study, nematodes *viz*., *Ascaridia galli* (41.7%) *and Heterakis gallinarum* (7.4%) and three cestodes i.e., *Cotugnia diagnopora* (17.6%), *Raillietina tetragona* (11%), *Raillietina* sp. (6.64%) were observed. In case of ascaridiosis the lumen of the intestine was filled with thick white pasty mucus, intestinal blockage due to numerous *Ascaridia galli* worms of varying sizes, thickening of intestinal wall with velvety appearance of mucosa and enteritis was noticed and the findings were in accordance

with the reports of Bsrat *et al.*, 2014 and Salam, 2015 and increased goblet cell activity was clearly evident. However Bsrat *et al.*, 2014 also observed diffuse haemorrhages on mucosal layer, mucoid frothy intestinal fluid, ulceration and mild enteritis with foci at different areas of intestine.

In heterakiosis, the caecum revealed thickening of caecal mucosa with small slender worms in the lumen causing nodular typhlitis similar to the reports of Rabbi *et al.*, 2006. Microscopically cross sections of parasites in the lumen along with cellular debris, infiltration of lymphocytes, heterophils and macrophages were also found by Salam, 2015a. The lesions recorded were similar to the observations made by Salam, 2015a and Salam, 2015b in backyard poultry.

Chemical control of helminth parasites is simple, low-priced and can be used both therapeutically and prophylactically against helminths. Helminth parasites treated with chemical showed several drawbacks like weakening of natural immunity and presence of drug residues in food products and in environment (Thamsborg *et al.*, 1999; Vercruysse and Dorny, 1999). Chemical anthelmintics (Piperazine, albendazole, levamisole, Ivermectin, benzimidazoles and fenbendazole) can stimulate resistance, so there is need of alternative ways to control helminths (Raza, 2016).

There are several medicinal plants which have anthelmintic activity and slow rate of resistance. Medicinal plants which show invitro anthelmintic activity include *Anacardium occidentale, Allium sativum*, *Tribulus terrestris*, *Bassia latifolia*, *Piper betle*, *Morinda citrifolia L.I*, *Cassia occidentalis L.* and *Aloe secundiflora* , whereas invivo studies include the usage of *Psorelia corylifolia*, *Piper betle*, *Pilostigma thonningi*, *Caesalpinia crista*, *Ocimum gratissimum* and *Anacardium occidentale* (Raza, 2016). In UK, nematode parasites were treated with chenopodium

oil from many years, obtained from *Chenopodiumam brosioides*. As well as, male fernsof *Dryopteris filix-mas* and *Artemisia* spp. plants have tendency against cestodes such as *Moniezia spp.* and nematodes, such as *Ascaridia* spp. (British Veterinary Codex, 1965). There are several medicinal plants which have good anthelmintic potential can be used as preventive mesure in poultry and may be a good alternative of synthetic drugs, and their use will not cause drug resistance in pathogen populations and drug residues in poultry meat.

3.5 CONCLUSION-

- Gastrointestinal helminth parasites were studied in domestic fowls. The aim of this chapter is to study the Nematode parasites, but it is not possible without the study of all helminths. Two species of nematodes and three species of cestodes were identified but trematodes were not detected during present study.
- The study revealed that the prevalence of parasites in fowls was throughout the year but highest prevalence was observed during monsoon followed by summer and least in winter.
- The highest prevalence of these parasites was recorded in the month of July and August whereas the lowest was reported in the month of December and January.
- The mean intensity (MI) and relative abundance (RA) were recorded highest during the monsoon followed by summer and winter.
- The Mean Intensity (MI) of the parasites was recorded maximum for *Raillietina Tetragona.* At the same time Relative Abundance (RA) was highest for *Ascaridia galli.*

- Growing age fowls were more infected with helminth parasites than adult fowls. Female fowls were found more prone to infection than male fowls.
- Pathologically gross lesion was observed so; further studies should be conducted to know the pathology and such gastrointestinal helminth parasites.
- This study on prevalence of gastrointestinal parasites in desi fowls suggests ways and means to formulate the appropriate strategies as one of the control measures to get the maximum benefit by rearing of backyard poultry in rural areas.
- Proper anthelmintic drugs in proper dose and hygienic environment can minimise the risk of helminth infection. Economic losses per year should be estimated to explain the better control program caused by these helminth parasites.

CHAPTER-4

Morphological Characterization of Gastrointestinal Nematode Parasites of *Gallus gallus domesticus*

4.1 INTRODUCTION-

Poultry farming has become one of the most demanding forms of animal husbandry activities. It has developed enormously, throughout the world in recent years. Most affordable sources of high protein are eggs and poultry meat which are popularly included in the diet. Poultry meat and eggs compensate the increasing demands of consumer for livestock products in the developing countries (Upton, 2007). Poultry meat and egg production is one of the fastest growing livestock industries. An average growth of 8% per annum and approximately US $7,500 million annual turnover was estimated by the poultry industry (Mehta and Nambiar, 2007). Though the impact of parasitic diseases in farm birds, reared on cage systems have diminished due to modernization in poultry farming and bio security measures, but farm birds maintained on deep litter system and backyard free ranging birds still remain susceptible to parasitic infection via litter droppings and scavenging habits. The domestic chicken feeds on a wide range of food substances. This ranged from grains, fruits and insects which may harbour infective stages of parasites thereby predisposing them to parasites particularly gastro-intestinal parasites (Oniye *et al*., 2001; Frantovo, 2000). Poultry production is persistently hampered by helminthic infections. Ascarid worms can be categorized as follows; phylum Nematoda; Class Secernentia; order Ascaridida; family Ascaridiidae.

Nematodes represent phylum Nematoda and are invertebrate round worms found in marine, freshwater, and terrestrial environments. Due to its large number of species, nematodes are present in the gastrointestinal tract of poultry and can cause damage to the poultry birds. These parasites are cylindrical, elongated in shape with un-segmented body. They are covered with the cuticle and have a well-developed alimentary tract. Most species of nematodes are bisexual (Butcher, Hogsette and

Jacobs, 1997). In terms of size, they appear small in size, inconspicuous and seemingly unimportant to humans. However, some nematodes can cause diseases of great importance to humans and domestic, wild plants and animals, whereas some are beneficial in attacking insect pests, mostly sterilizing, or otherwise debilitating their hosts (Gerald and Larry, 1996). Gerald and Larry 1996 stated that non parasitic nematodes can find their path and become short lived, pathogenic into a vertebrate host. Bilateral symmetry occurs in most of the nematode species (Croll, 1976). Several species of nematodes are oviparous, but some are viviparous or ovoviviparous. Adult stage occurs after four larval stage molts. Female are larger in size as compared to male (Croll, 1976). Six stages occur in nematodes life cycle; egg, four juvenile stages and adult (Croll and Matthews, 1977).

Normally elastic and tough cuticle occurs in nematodes in contrast to cestodes. Generally cuticle is smooth, scaled or scattered with bosses, seldom spined, and transversely, longitudinally or not often obliquely striated (Yamaguti, 1963). Nematodes body wall contains cuticle, hypodermis, and body wall musculature. Cuticle is the outermost covering and has a great functional and structural significance to the animals. Excretory pore, vagina, proctodeum, and stomodeum are lined by cuticle (Blaxter *et al.,* 1992). The cuticle is originated from hypodermis, an underlying sub-cuticle layer. Longitudinal lines are formed by cuticle, situated dorsally, ventrally and laterally. Longitudinal canals of the excretory system occur in the lateral lines. Muscular layer lines the body cavity, consists of a transversely striated cells having a basal contractile portion, and a cytoplasmic portion which contains the nucleus. Longitudinal lines divide this muscular layer into four quadrants (Soulsby, 1968).

Ascaridia galli (Schrank, 1788) and *H. gallinarum* are the members of the genus Ascaridia Dujardin, 1845, and a major intestinal helminth parasite which cause health problems in guinea fowl, geese, turkey and to a several wild birds; the main host is chicken (Ackert, 1931; Permin and Hansen, 2003). The adult form of *A. galli* resides in the lumen of small intestine and *H. gallinarum* occurs in ceacum of the *Gallus gallus domesticus*, which feeds on mainly the ingesta of host. *Ascaridia galli* (Shrank, 1788) functions as a vector for the transmission of *Salmonella enteric* and *H. gallinarum* is the carrier of *Histomonas meleagridis* in poultry. Thus nematodes are responsible for major problems and cause economic losses to poultry especially in free-range and floor production systems (Permin *et al.*, 1997; Ponnundurai and Chellappa, 2001) by causing poor growth rate and weight loss of fowl (Ramadan and Znada, 1991; Ramadan and Znada, 1992) and increased mortality due to secondary infections (Permin, 1999; Permin, 2006). They induce damage to the intestinal mucosa, leading to blood loss (Ackert and Herrick, 1928), partial or complete obstruction of the intestine.

Heavy infection may be responsible for diarrhoea, droopiness of wings, bleaching of the head and emaciation. A heavy infection causes diarrhoea which is responsible for anaemia and intestinal obstruction (Griffiths, 1978). Efficiency of feed utilization becomes reduced in the primary damage, but death occurs in heavy infection. Common symptoms in broiler chickens are weight loss, and reduced egg production. High prevalence of infection is found in young birds and higher resistance against these parasites is showsby the heavier breeds than the lighter one such as white minorcas and leghorns (Ackert, 1940). Transmission of infection can be very fast because of the direct life cycle of nematode and the environmental resistance of its eggs (Permin and Ranvig, 2001). Absorption of nutrients and enzyme systems in the

intestinal mucosa is adversely affected by the toxins of *A. galli* (Vassilev *et al.*, 1973). Significant behavioural changes caused by the Ascaridos infected birds, showed lower locomotion activity during the patent and prepatent periods (Gauly, 2007). All these factors are responsible for the mortality and losses of the flock due to disease outbreak in backyard (traditional) poultry production system. The aim of the present study is to determine a highly reliable and finer detail of ultra-structure of *Ascaridia galli* and *Heterakis gallinarum* by using light microscopy and scanning electron microscopy.

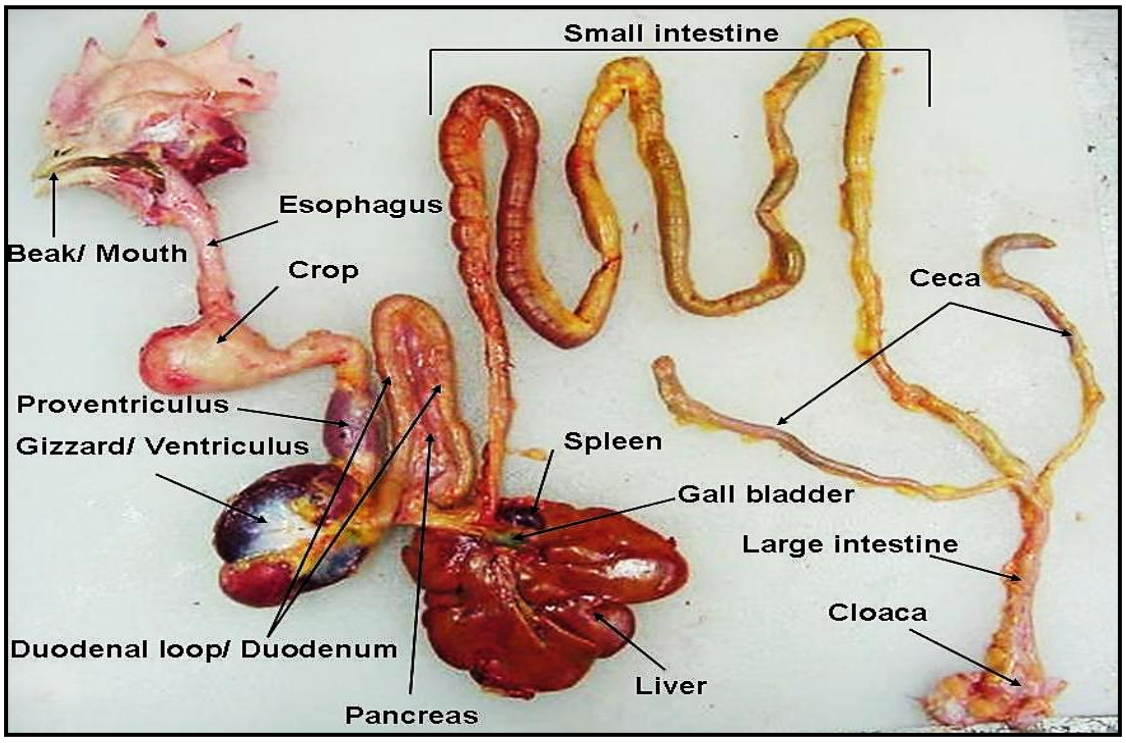


Figure 4.1- Gastrointestinal tract of domestic fowl (Jacob and Pescatore, 2011)

4.2 MATERIALS AND METHODS-

4.2.1 Study area-

Lucknow is the capital of Uttar Pradesh and situated on the Northern Gangetic plains of India. Geographically, Lucknow is situated between latitudes 26"30' to 27° 10 from the Northern side and longitudes 80°30' to 81° 13' from Eastern side and elevation

location at the height of 123 meters above sea level. Lucknow is surrounded by so many villages such as Chinhat, Bakshi ka talab, Malihabad, Rahimabad, kakori, Alamnagar, Jugganr, Bijnaur, Banthia, Utrathia, Mohanlalganj, Nagram, Gosainganj, Nigoha etc. The city is bounded by Barabanki on the east, Unnao on the west, Raebareli on the south whereas Sitapur and Hardoi towards north. Warm and humid subtropical climatic condition prevails in Lucknow during three seasons: 1. Torrid and steamy summers (April to June), 2. Cool, shrivelled winters (November to March), 3 .Monsoon (July to October) with an average annual rainfall of 896.2 millimetres. An extremely hot temperature (40 °C to 45 °C) is recorded during the months of May to June and very low temperature (3°C to 7 °C) in the month of January. The average annual percentage of humidity is 59.0 % which is higher in the month of August, September and low in the month of April (Singh *et al.*, 2012).

4.2.2 Study Population-

Study population included 557 domestic chickens managed under unorganised backyard systems. The age of the studied animals was determined through information collected from owners.

4.2.3 Study period-

Study was conducted during January 2017 to December 2019 in Lucknow to determine the morphology of gastrointestinal helminth parasites of domestic chicken.

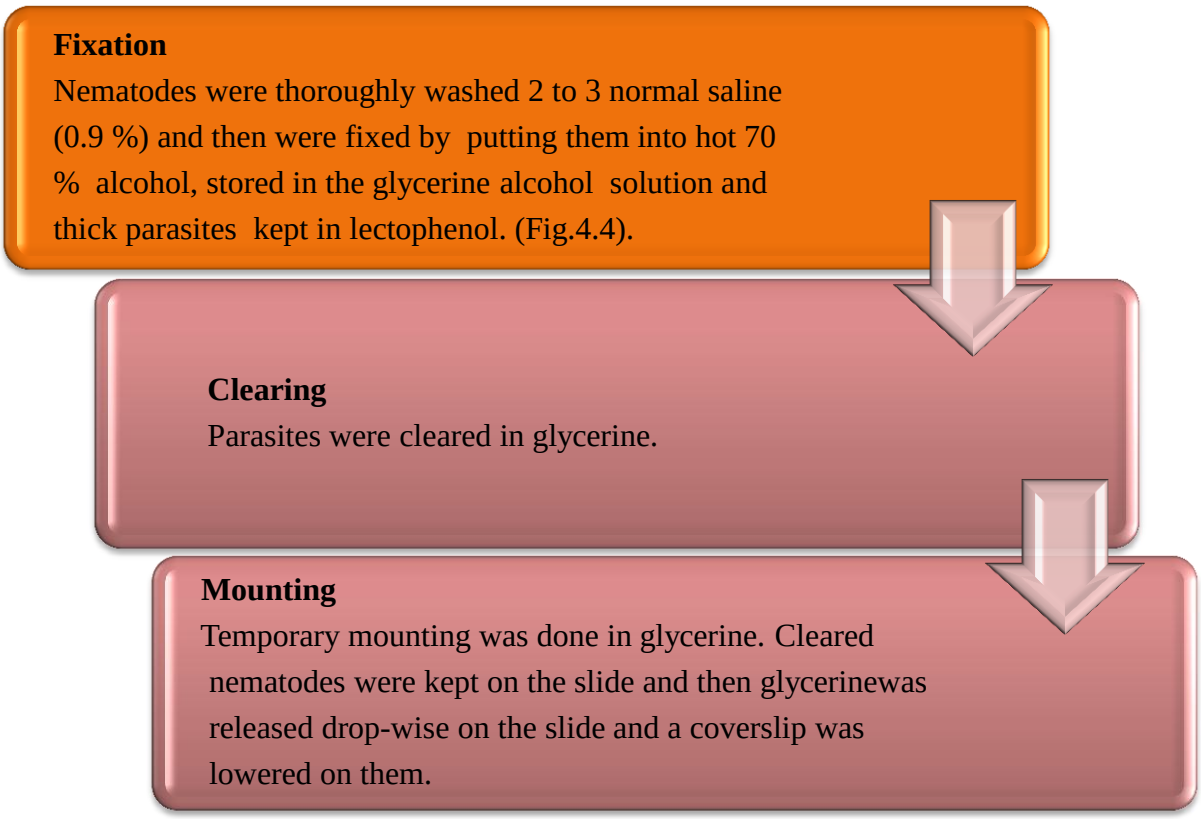
4.2.4 Parasitological examination-

During the present study the domestic fowls were collected from different regions of study sites (Fig. 4.2). The hosts were then brought to the parasitology lab of Department of Zoology, Babasaheb Bhimrao Ambedkar University Lucknow for the parasitic examination. For the collection of endoparasites the gastrointestinal tracts of

Gallus gallus domesticus were dissected thoroughly to investigate the presence of parasites, according to the procedure as described by Fowler, 1990 (Fig. 4.3).

4.2.5 Processing of gastrointestinal nematode parasites-

Nematodes were processed through the following steps:



4.2.6 Light Microscopy-

Microscopic studies were carried out by using Light bright field microscope (Olympus CH20i) under 10X, 40X magnification. Photographs were taken using EVOS XL imaging microscope.

❖ **Identification**

All the parasites were identified by their morphological characters as described by Soulsby, 1982.

4.2.7 Scanning Electron Microscopy (SEM)-

Fixation

Parasites were fixed in 2.5% glutraldehyde (v/v) in 0.1M phosphate buffer pH 7.3 for 3-5 hours and then washed in a phosphate buffer prior to post fixation in 1% osmium tetra oxide in 0.1 M-phosphate buffer for 24 hours followed by washing in the same buffers.

Dehydration

SEM samples were dehydrated by immersing for 15 min each in fresh solution of 30%, 50%, 70%, 95% and 100% acetone and dried till the critical point.

Mounting

The dried samples were mounted on specimen's stubs using a double side adhesive tape and coated with gold.

Observation

Coated samples were viewed under a JSM-6490LV scanning electron microscope at 15 KV, measured and photographed (Ptaszynska, 2014).

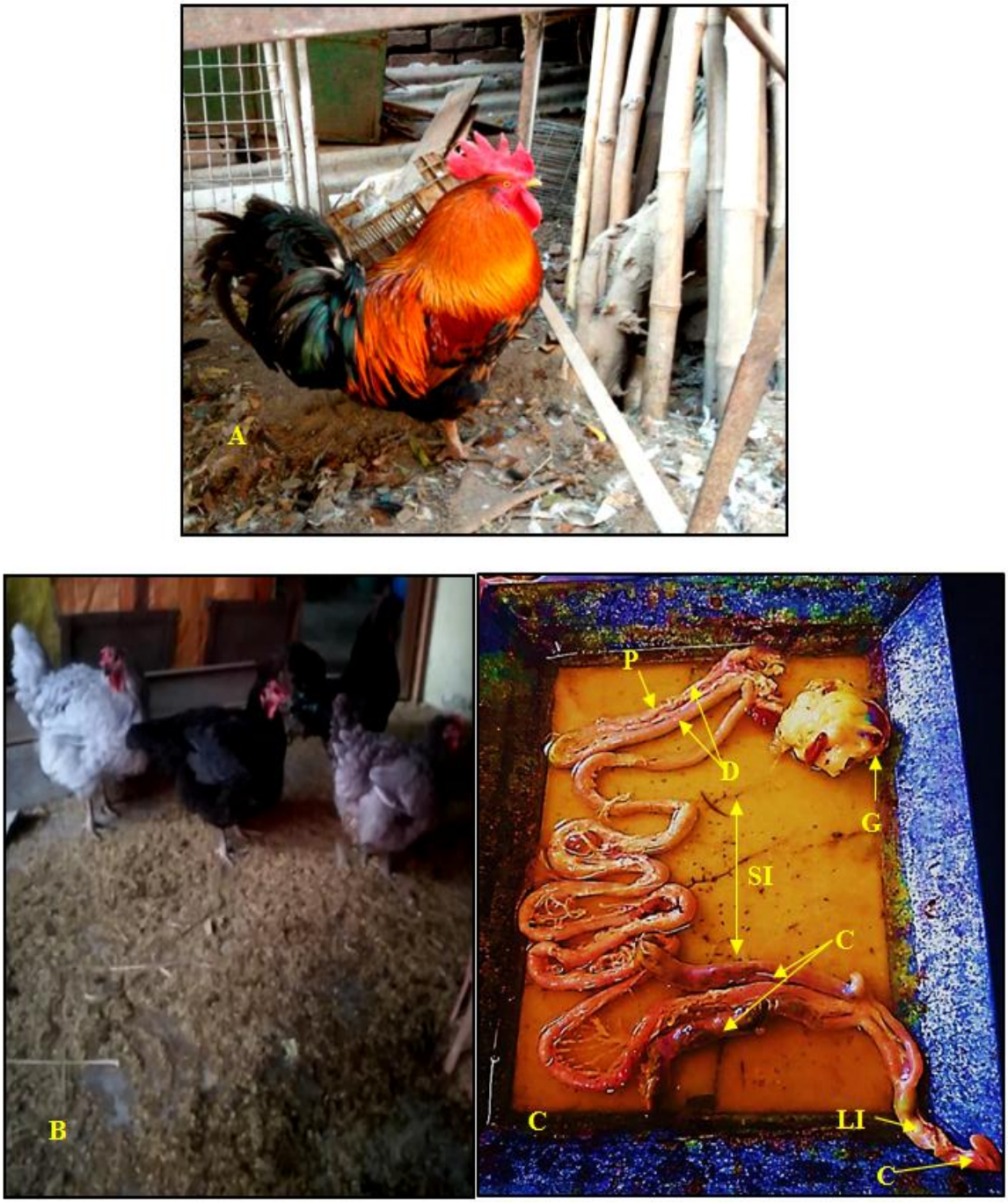


Figure 4.2- Domestic fowls and collected gastrointestinal tract of *Gallus gallus domesticus*

(A-B). Domestic fowls, **(B).** Collected gastrointestinal tract, **(G-** Gizzard. **P-** Pancreas, **D-** Deodenum, **SI-** Small Intestine, **C-** Ceca, **LI-** Large Intestine, **C-** Cloaca**)**

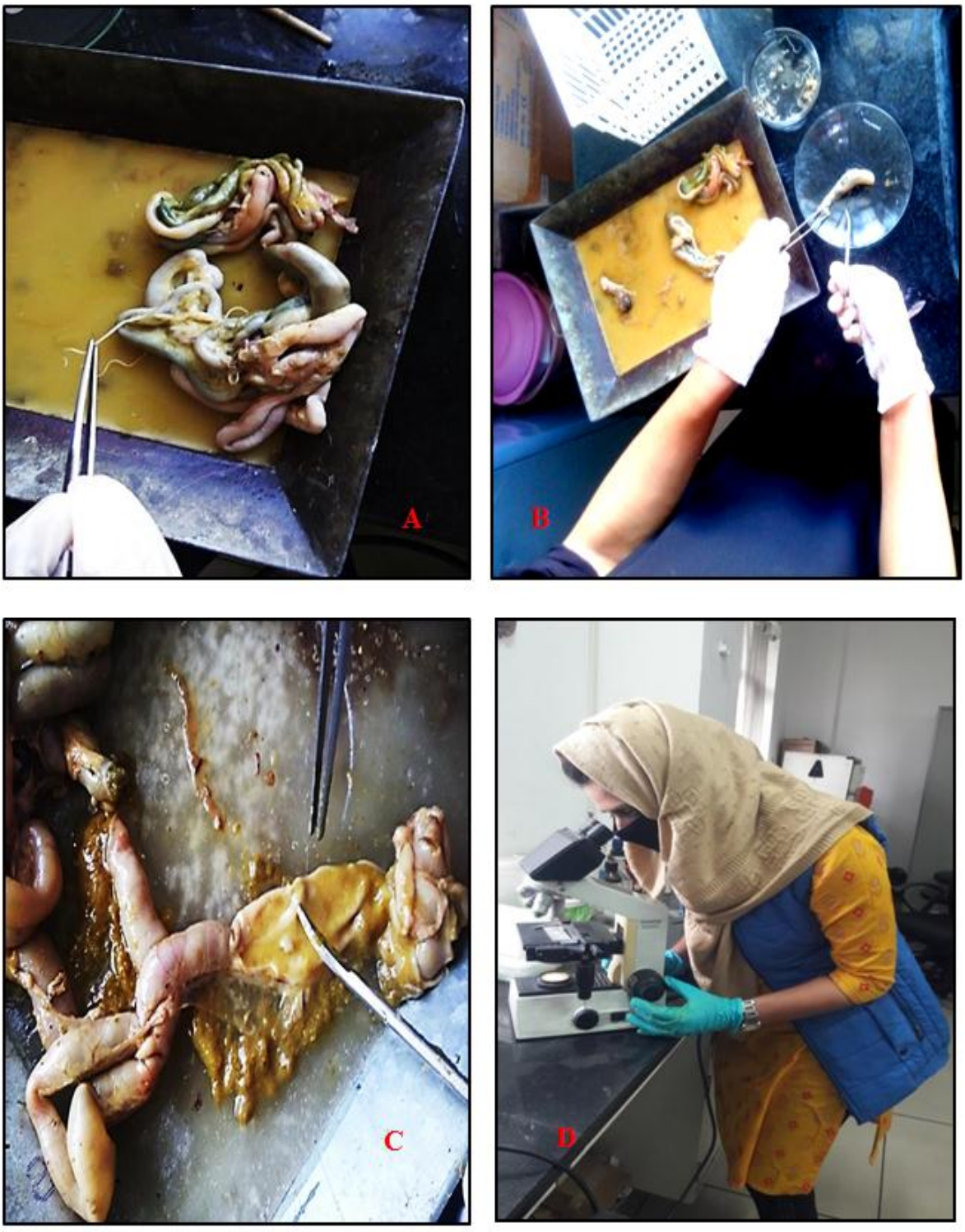


Figure 4.3- Dissection of GI tract of *Gallus gallus domesticus* and observation of gastrointestinal parasites (A-D).

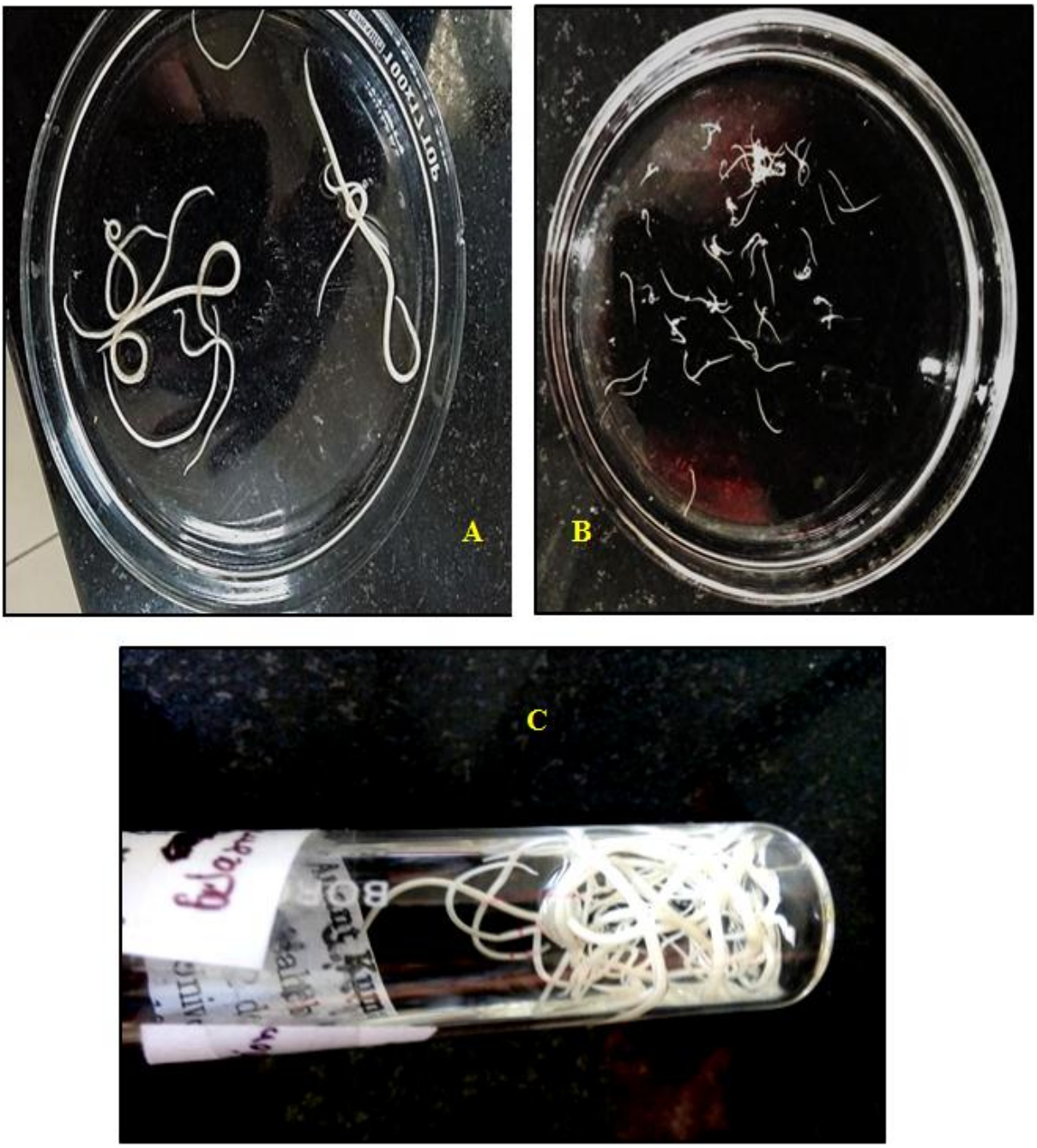


Figure 4.4- Collection and preservation of gastrointestinal nematode parasites of *Gallus gallus domesticus.*

(A). *Ascaridia galli*, **(B).** *Heterakis gallinarum*, **(C).** Preservation of parasites in glycerol-alcohol

4.3 RESULTS-

4.3.1 Identification of different gastrointestinal helminths parasites

On the processing, following gastrointestinal helminth parasites were identified in the samples collected for the research. The identification, systemic position, site of infection and morphology of each parasite is detailed to ensure chalking out prior strategy for their control.

4.3.1.1 Light and Scanning Electron Microscopy of Large roundworms- *Ascarida galli*

Site of location: *Ascarida galli* occurs in the lumen of small intestine of gastrointestinal tract of domestic fowl.

(a)- *Ascarida galli*

Scientific classification

Kingdom	:	Animalia
Phylum	:	Nematoda
Class	:	Secernentea
Order	:	Ascaridida
Family	:	Ascaridiidae
Genus	:	Ascaridia
Species	:	*A. galli*

(https://en.wikipedia.org/wiki/*Ascaridia_galli*)

Morphology: *Ascaridia galli* had yellow-whitish, slightly semi-transparent, elongated and cylindrical body with tapering ends at both side. The whole body covered by a tough proteinaceous cuticle. Mouth is triangular opened at the extreme anterior end (Fig. 4.5, 4.6). Continuous ridge along the longitudinal axis marked the dorso-ventral margin of the body.

Three trilobed lips surround the mouth, anchord with each other and having the smooth cuticle (Fig. 4.7). Two types of lips are found, one is mid-dorsal which is broadly elliptical and two latero-ventral oval lips. Lips work as a mechanical organs to ingest food materials. Three distinct lobes are present in each lip, one median lobe

in center, and two lateral lobes at the sides. A cup like structure is formed by the lobes. A single dentigerous ridge in the inner surface and minute denticles in a single line make up the median lobe of each lateral lip (Fig. 4.7). Prominent cuticular protuberance present on the external surface of the cuticle of the latero-ventral lip known as labial papilla. Club-shaped esophagus without posterior bulb measures 1.0–3 mm in length (Fig. 4.8). A series of continuous transverse annulations occurs in cuticle with diverse striations from cephalic region to posterior region of the body (Fig. 4.9). Parallel concentric rings of fine transverse grooves of striations are found to be running around the cylindrical body. A segmented appearance is given by the deep transverse grooves (annulations) present at the regular intervals of the body (Fig. 4.9).

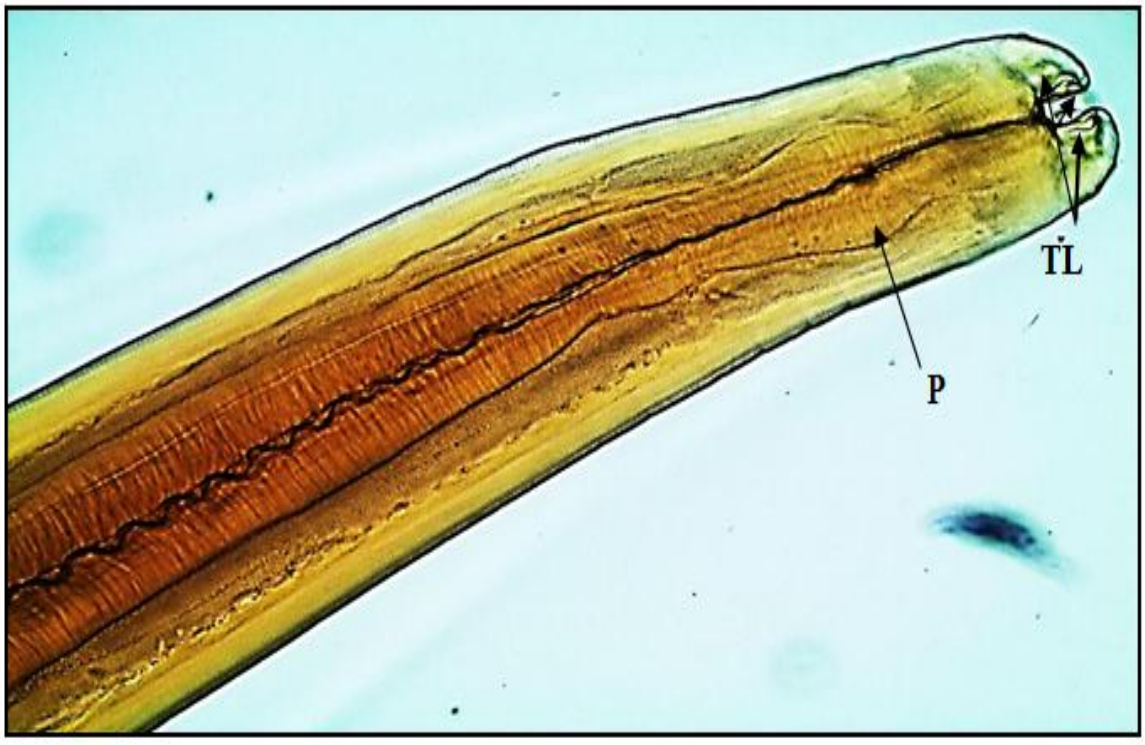


Figure 4.5- Light micrograph of the anterior portion of *A. galli* with prominent Three-lips (TP) and Pharynx (P) end in esophagus.

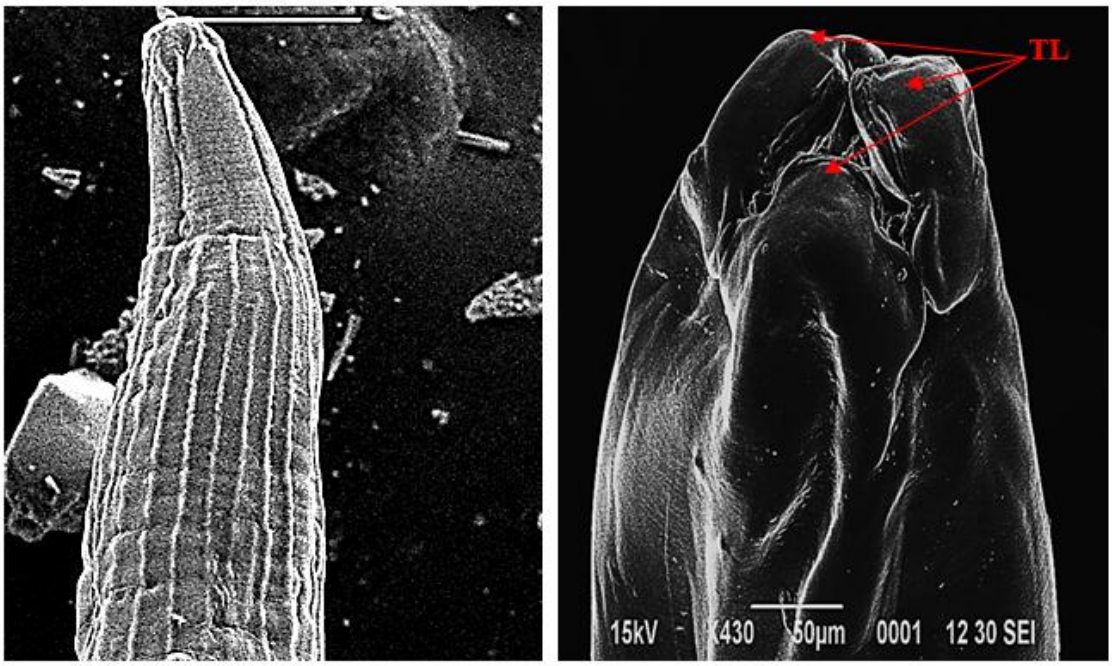


Figure 4.6- Scanning Electron Micrographs of the anterior end of an adult *A. galli*. Three large trilobed lips surrounded the mouth opening and smooth cuticular plate covered the inner surface of each lip and the outer surface is covered with cuticle.

Figure 4.7- Scanning Electron Micrographs of enlarged view of lips showing the rim that bears a series of denticles, and the Sensory Papillae (SP) which seems eye-like oval structures.

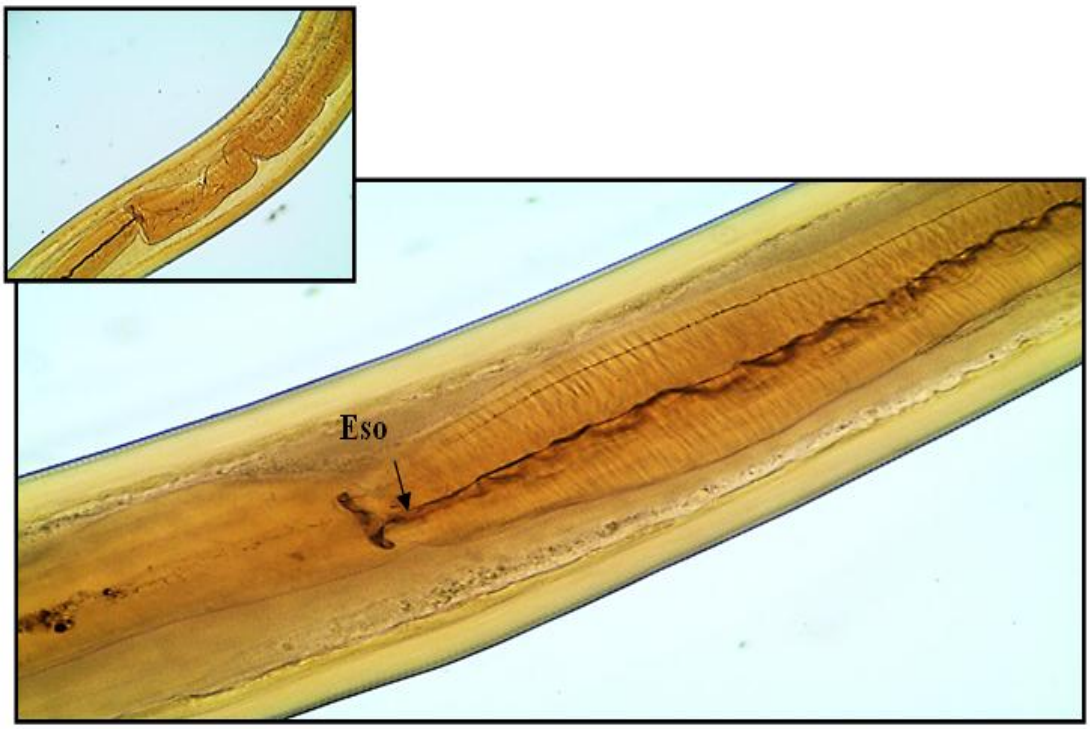


Figure 4.8- Light micrograph of the middle portion of the *A. galli* showing club-shaped esophagus.

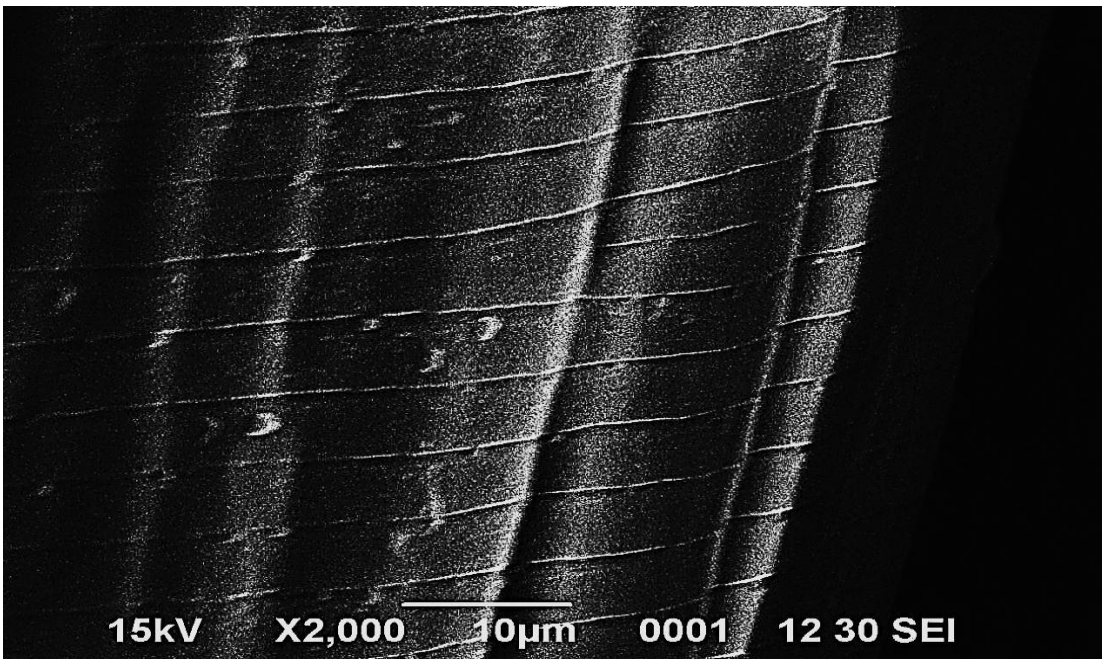


Figure 4.9- Scanning Electron Micrographs of middle portion of an adult *A. galli* body with fine corrugated cuticle arranged in a series of unique transverse striations named annulations, which forms the continuous ring around the body.

Sexual dimorphism in *Ascaridia galli*-

***Ascaridia galli* female**- Females have a blunt and rounded posterior end and males have pre cloacal sucker and ventrally coiled tail. Posterior region of female have a single large anal opening just before the tip of tail with a pair of papillae just near to its tip. Tail is straight with blunt end (Fig. 4.10). The length of female is 40–66 mm and 0.31–0.58 mm in width at the anterior end and 1–1.63 mm at the level of vulva. Vulva is situated a little posterior to the middle of the body. Distance of vulva from the anterior end varies from 19 to 35 mm. Distance of anus from the tip of tail varies from 0.51 to 0.73 mm. Tail is straight with a caudal spine and measures 0.73–0.97 mm in length (Fig. 4.10, 4.11, 4.12). Eggs are large, oval in shape measures 0.035–0.08 mm in length and 0.01–0.06 mm in width, and covered by thick albuminous shells that are more resistant to desiccation and perserve for a long time in the environment (Fig. 4.13).

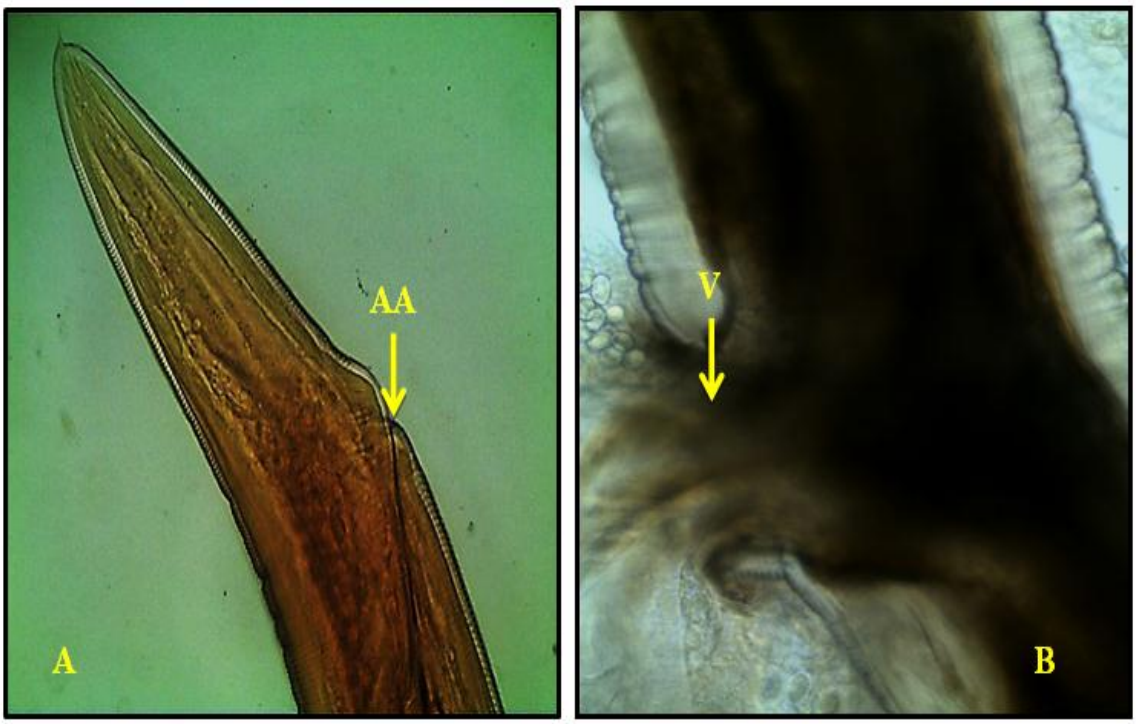


Figure 4.10- Light micrograph of posterior end of the female (A). Straight and pointed tip of tail and anal aperture (AA) (B). Vulva

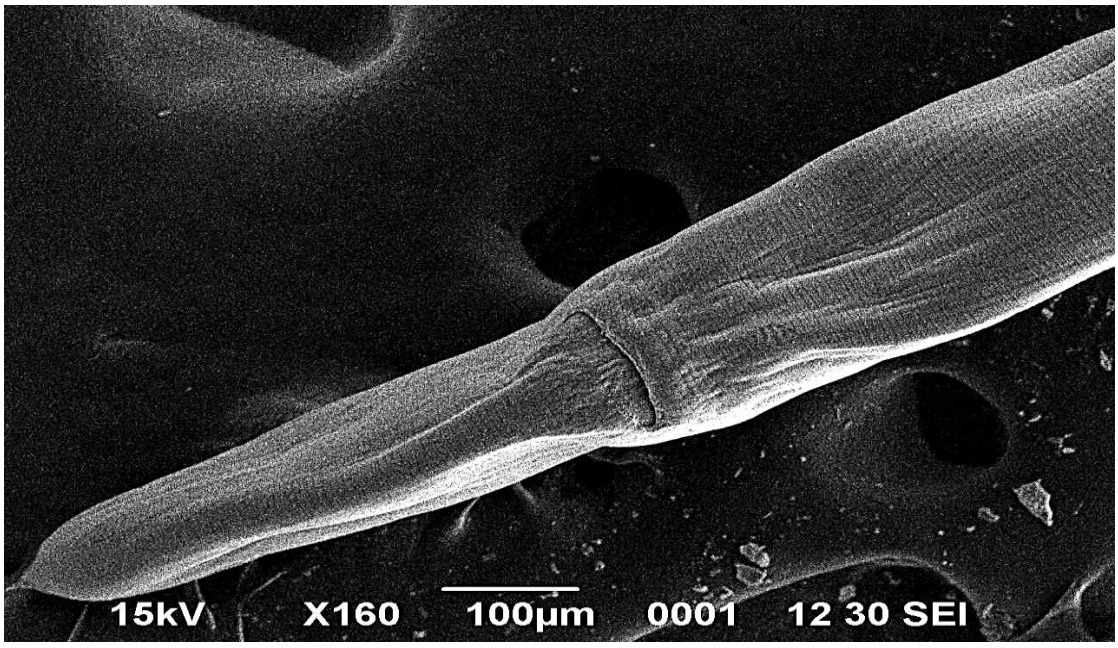


Figure 4.11- Scanning Electron Micrographs of an adult *A. galli* female with blunt and rounded posterior end and pre cloacal sucker.

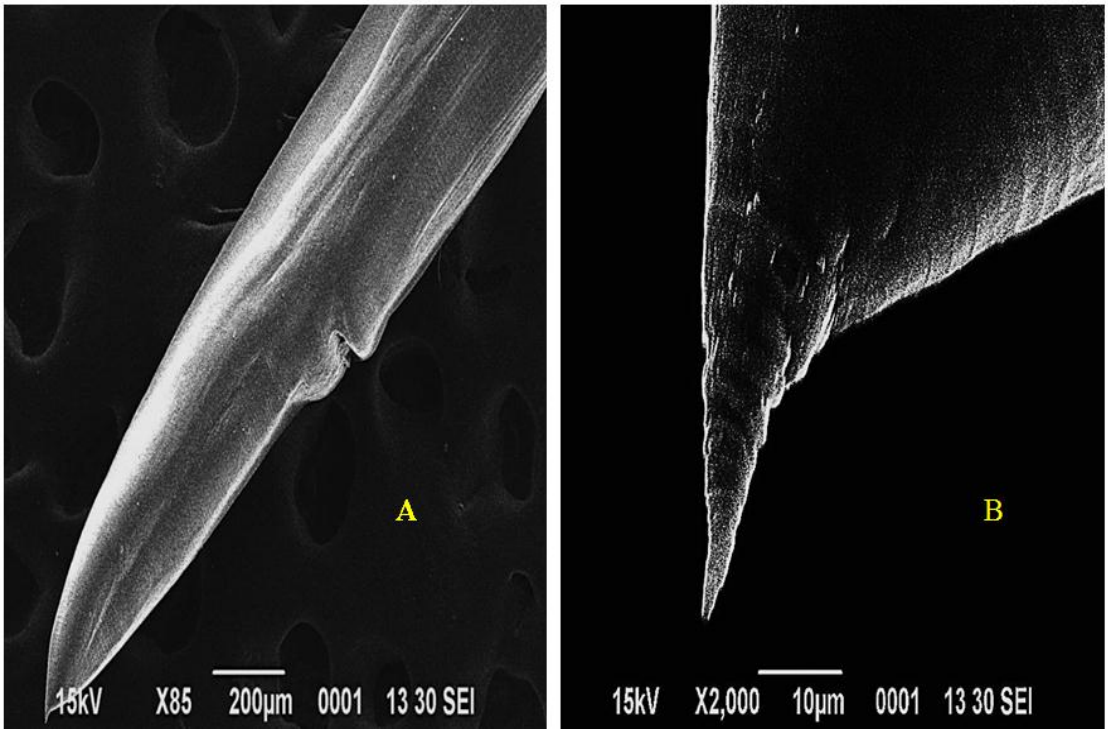


Figure 4.12- Scanning Electron Micrographs: (A) Single, large anal opening of female *A.galli* just before the tip of tail. (B) The end region of tail cuticle is without annuli.

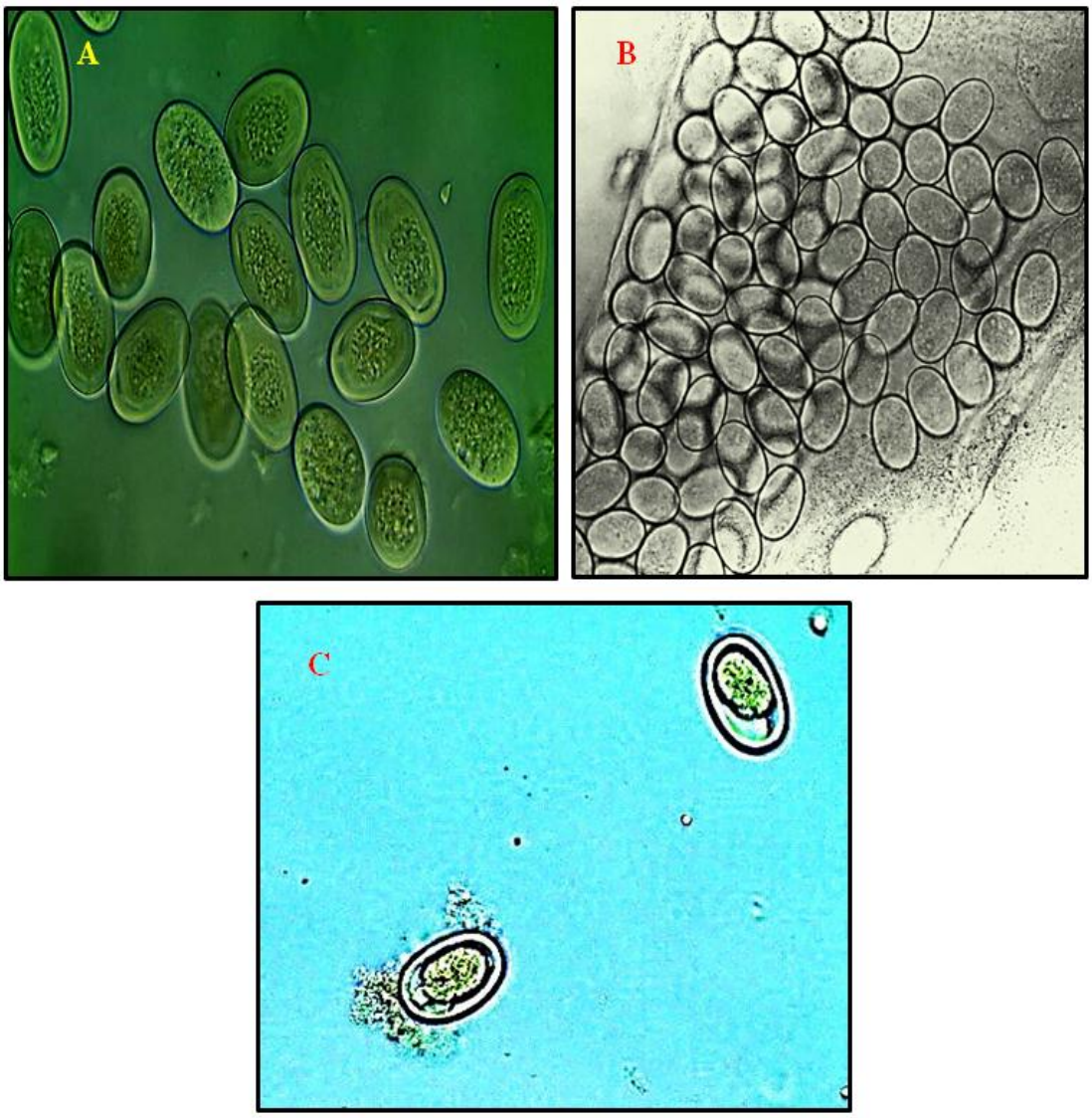


Figure 4.13- Light micrograph of eggs of *A. galli* which are ovoid characterized by a thick and smooth shell (A-C)

***Ascaridia galli* male-** Comparatively elaborated and more complex posterior end was present in male. Two major apertures were present in male, anus located at the posterior end and anterior to it a precloacal sucker is present (Fig. 4.14, 4.15, 4.16). Extreme terminal tip was pointed and slightly expended at the base. Sclerotized ring support the precloacal sucker and this sucker helps in the attachment during copulation (Fig. 4.17). Expanded region of caudal alae is the lateral longitudinal region around the anus, significantly extended on both sides. On the ventral side of

the tail region numerous bulges or caudal papillae or phasmids were seen on the either side of the anus (Fig. 4.15, 4.16). These caudal papillae functions as sensory organs of the male tail. Circular protrusions were formed by the anus with a central anal opening. Males are smaller than females and more slender, measuring about 40–47 mm in length and 0.40–1.20 mm in maximum breadth. Ventral part of body composed of thickened cuticular bosses, spicula without alae; tail length is 0.49 to 0.87 mm. Posterior to the pre cloacal sucker, cuticular vesicles and cloacal papillae are present. Preanal sucker is oval shaped with chitinous rim measuring about 0.10 to 0.29 mm in length and 0.085–0.19 mm in diameter, located at the distance of 0.25–0.57 mm in front of cloaca. Distance of cloaca from the tip of tail is 0.5-0.77 mm. **Ten pairs** of caudal papillae are present in the male in the following four groups (i) pre anal three pairs (ii) adanal one pair (iii) postanal three pairs (iv) subterminal three pairs. Spicules are almost equal in size, measuring 1.65–1.9 mm in length. Proximal end of spicules are expanded. All the generic characteristics shows by the *A. galli* (Yamaguti 1961; Hodova*et al.,* 2008) are as follows: generally leteral cuticular flanges present in Ascaridiinae; interlabia absent in lips; club-shaped oesophagus present without the posterior bulb. Male: a chitinous rim in the precloacal sucker; spicules are unequal or subequal in shape, caudal alae narrow, relatively larger papillae; no gubernaculums was seen (Fig. 4.14, 4.15, 4.16).

Symptoms: Large roundworms are found in all ages of chickens, the young birds are more prone to the infection (under 12 weeks of age). Unthriftiness, drooping of the wings, bleaching of the head and emaciation are described. In adult hens, egg production can be reduced. In heavy infections, large roundworms may move up the oviduct and be found in hens' eggs, and sometimes large roundworms can be found in the birds' feces.

Autopsy: Large number of worms can penetrate the mucosa during growth causing injury, loss of blood and permitting bacterial infection.

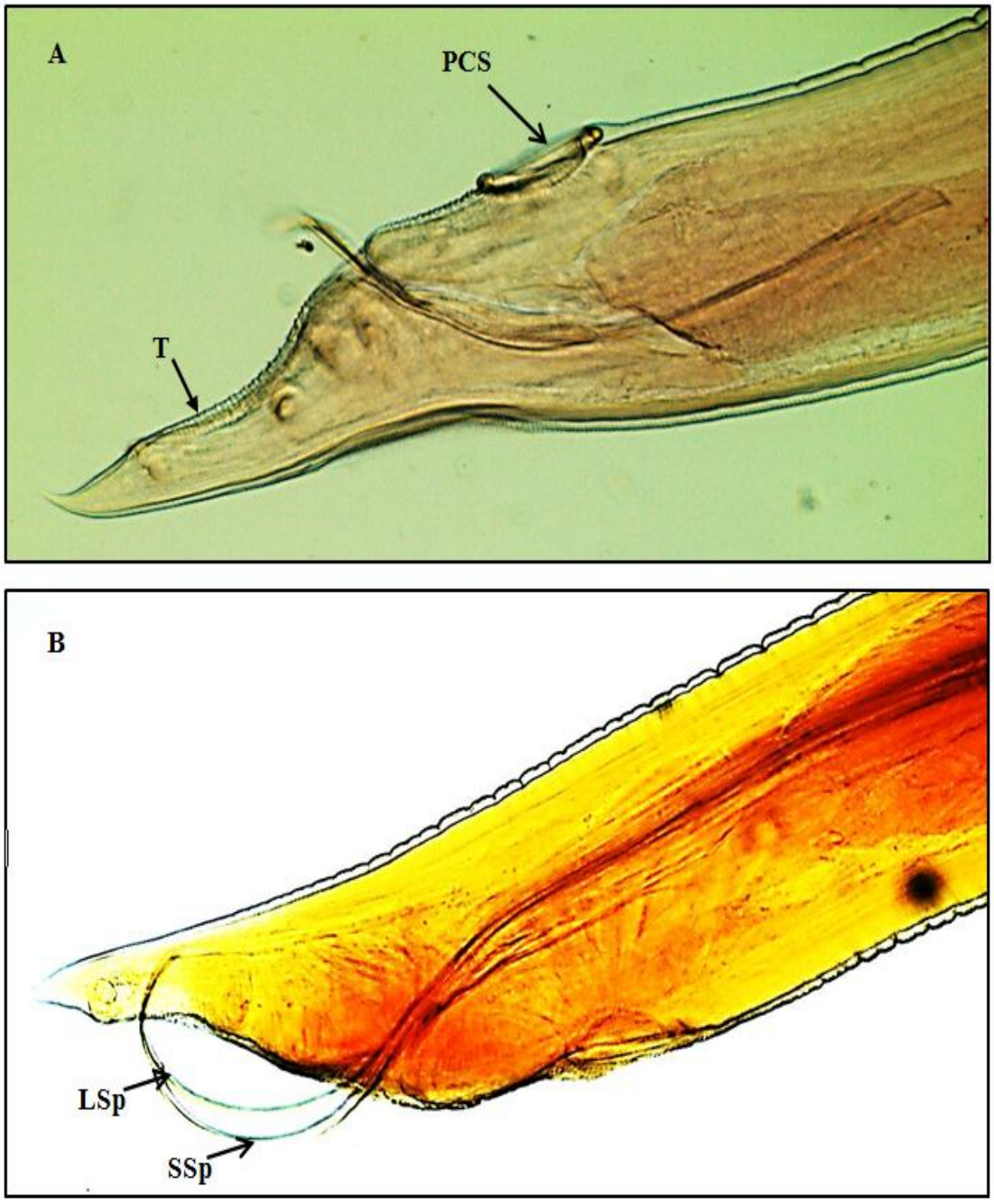


Figure 4.14- Light micrographs of posterior portion of the male *A. galli*: Reproductive organs: (A). Ventral side shows circular pre-cloacal sucker **(PCS) and Tail (T)**, **(B).** Dorsal side shows two caudal spicules; Large Spicule **(LSp)** and small Spicule **(SSp)**.

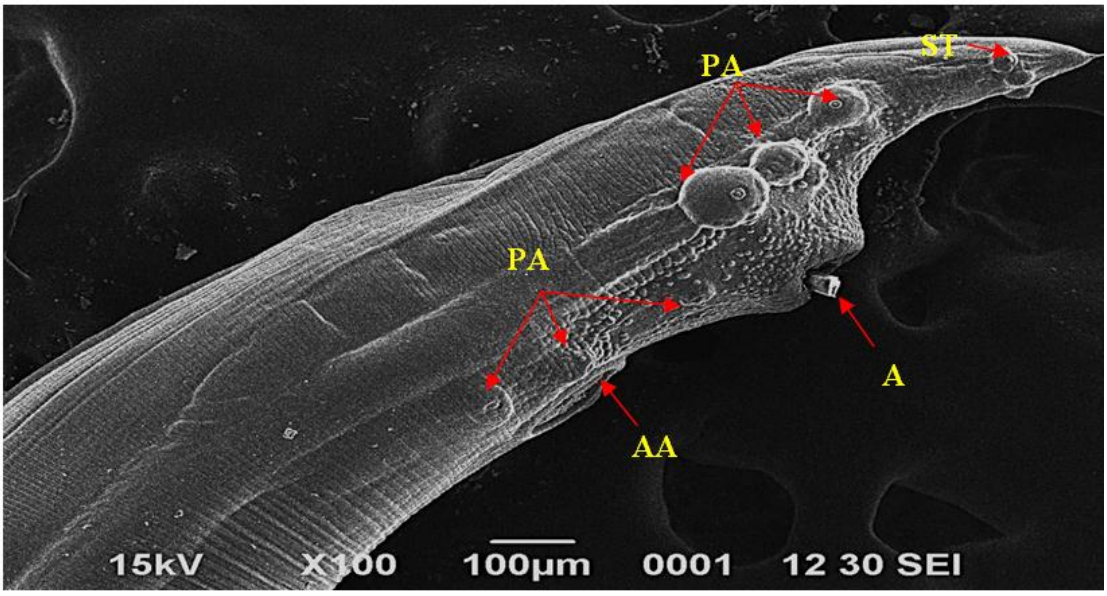


Figure 4.15- Scanning Electron Micrograph of an adult *A. galli* male with a fine pointed tail, anus and caudal papillae. Caudal papillae emerge on either side in the form of small knob like structure. (i) Pre anal (PA) three pairs (ii) Adanal (AA) one pair (iii) Postanal (PA) three pairs (iv) Subterminal (ST) three pairs

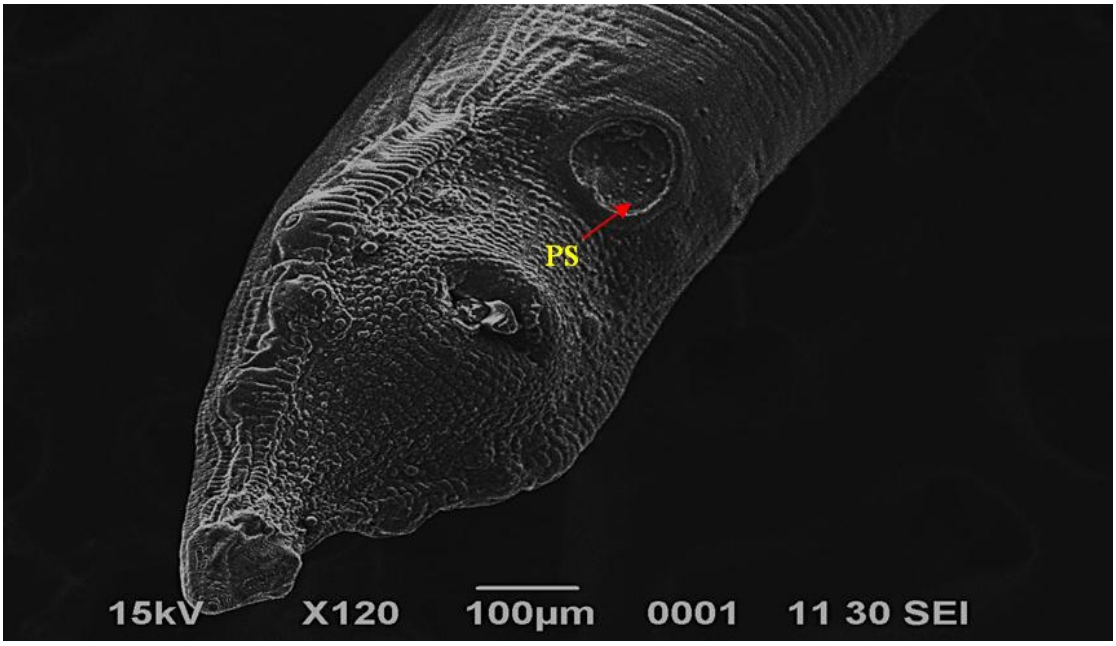


Figure 4.16- Scanning Electron Micrographof *A. galli* male with a ring-like structure Precloacal Sucker (PS) which helps in grasping during copulation. Anal region expanded both side to form a flap-like structure caudal alae. The anal opening is surrounded with numerous small blisters like structure.

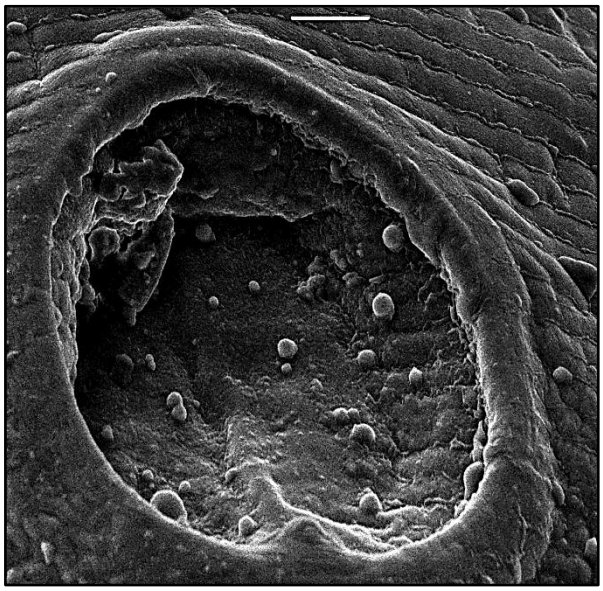


Figure 4.17- Scanning Electron Micrograph of enlarged preanal sucker bounded by a double-walled sclerotized rim.

4.3.2 Light and Scanning Electron Microscopy of cecal worm, *Heterakis gallinarum*

Primary species: *Heterakis gallinarum*

Location: Lumen of cecal pouches

(b)-Cecal Worm- *(Heterakis gallinarum)*		
Scientific Classification		
Kingdom	:	Animalia
Phylum	:	Nematoda
Class	:	Secernentea
Subclass	:	Rhabditia
Order	:	Ascaridida
Family	:	Ascarididae
Genus	:	Heterakis
Species	:	*H.gallinarum*

(https://en.wikipedia.org/wiki/*Heterakis_gallinarum*)

4.3.3 Morphology-

Heterakis gallinarum were taken from the lumen of the cecum from chickens (*Gallus gallus domesticus*). After microscopic examination it was observed that when the nematodes were taken freshly has muddy white color, and the anterior end isdorsally curved. The body is thinned at both ends, but more prominent at the posterior extremity.

Heterakis gallinarum has typical roundworm morphology with characteristics such as a cuticle, an esophagus ending in a valved bulb, and three papillae-lined lips and alae (Fig. 4.18, 4.19). Each lip is not globular but it has a wide base with gentle tapering towards apex.

Each main lip is provided with two small accessory ones at lateral sides. Three paired cephalic papillae and two amphids are found on the outer surface of the lips. The amphidial surface is provided with several pores. Small papillae were seen scattered on the cervical area of the worm. Alae, which run almost the entire length of the body, are ridges formed by the thickening of the cuticle that may act as receptors for molecules which stimulate reproduction. On the body's lateral sides, almost on itswhole length, it has two cuticular allae. *Heterakis gallinarum* is probably the most known nematode parasites that occure in a wide range of poultry, being commonly identified in households. The three small lips, equal as dimension, surrounding the oral opening. *H. gallinarum*, have chemosensory organs called amphids. Located anteriorly, these invaginations of the cuticle are made of many neurons which interpret and transmit incoming chemical signals. *Heterakis gallinarum* also has papillae, which are sensory structures surrounding the lip region (Fig. 4.18). Behind the lip region are peg-like sensory structures which function both as chemoreceptors to detect chemicals, as well as mechanoreceptors to detect motion. Chemoreceptors are likely used in finding a mate, and sexual pheromones have been identified for over 40 nematode species.

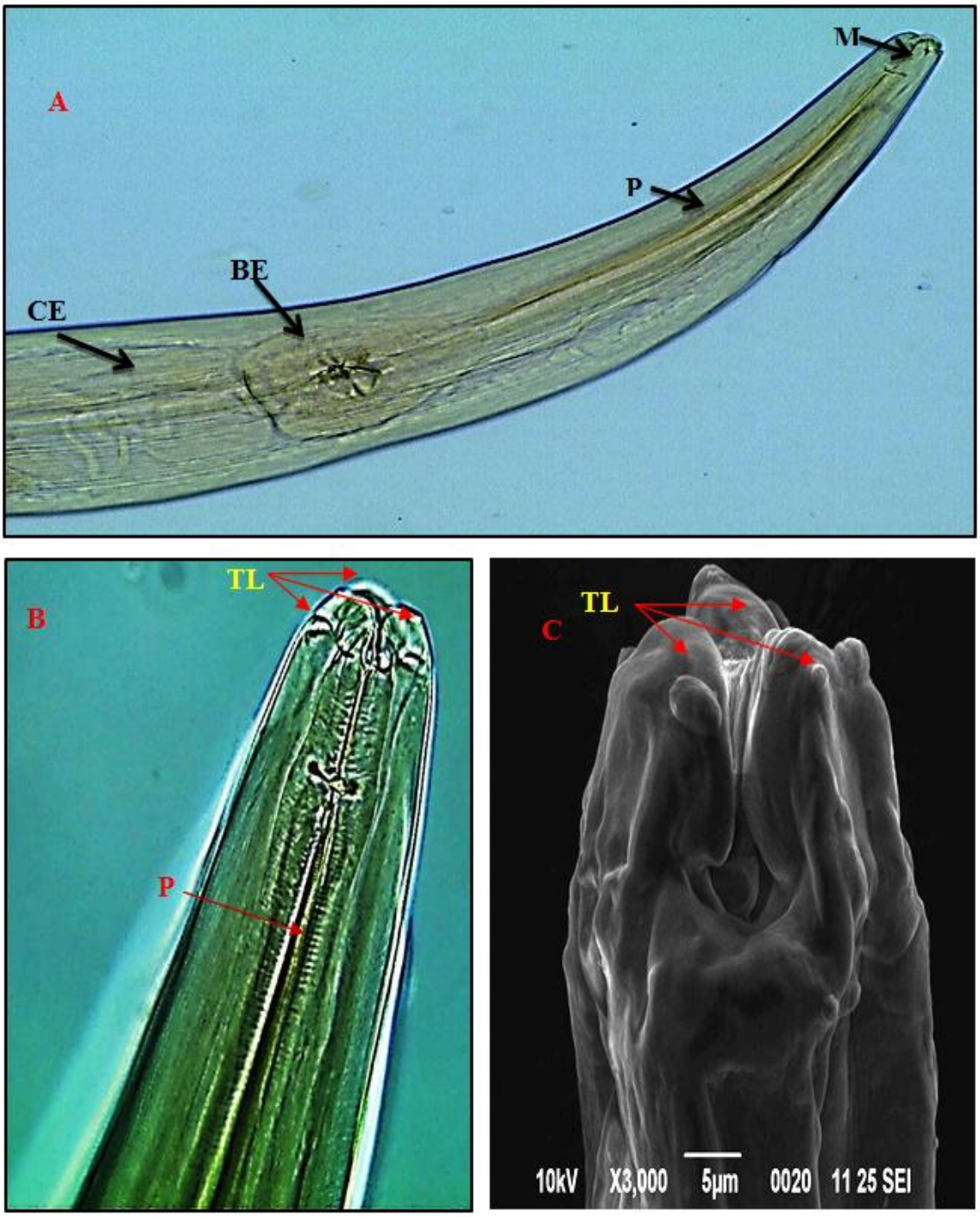


Figure 4.18- Light and Scanning Electron micrograph of *Heterakis gallinarum* anterior portion. (A). M –Mouth, **P –** Pharynx, **BE** – Bulbus esophagus, **CE –** Cylindrical part of esophagus. **(B). TM-** Three Lips. **(C). SEM of *Heterakis gallinarum*-** anterior end showing the mouth opening of the worm surrounded by three main large lips; one dorsal and two subventral.

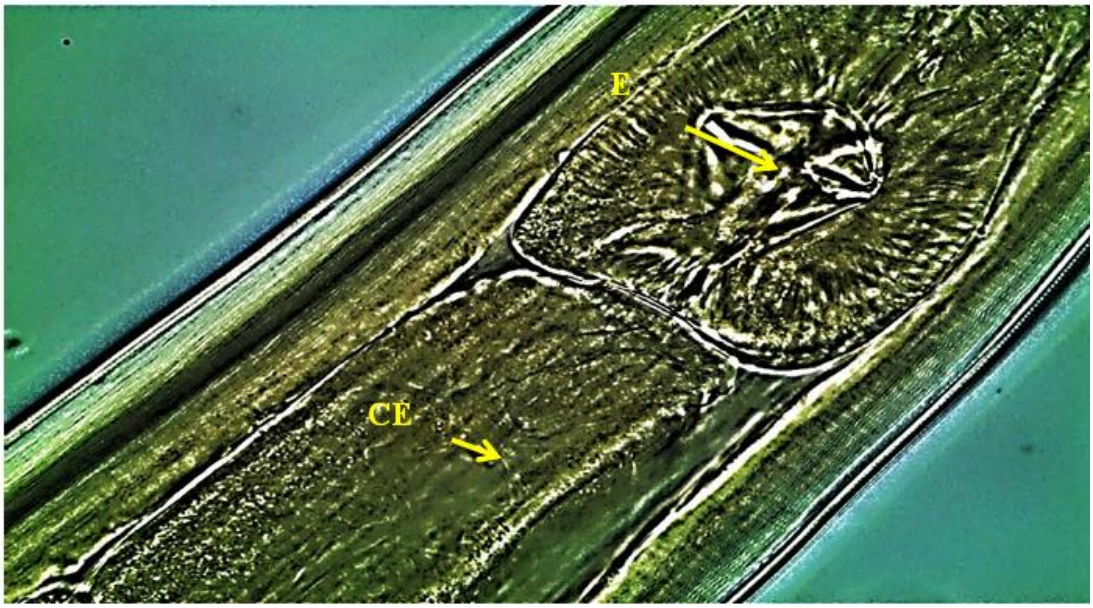


Figure 4.19- Light micrograph of *Heterakis gallinarum* middle portion with bulbus esophagus (E) ends in cylindrical esophagus (CE)

Male- Males measure 4.73–6.72 mm in length and 0.28–0.36 mm in diameter. The cuticular striations are extremely fine. The oesophagus along with the bulb measures 0.76–1.2 mm. The esophageal bulb is 0.19–0.23 mm in diameter (Fig. 4.19). Males have specificity of spicule size and structure, and numbers and position of tail papillae. The male *H. gallinarum* have stylet-like tail end and pseudobursa with well-developed lateral "wings" that smoothly taper. The spicules are unequal, left spicule is significantly longer than the right, and has a tapered sharp end, and the gubernaculum is absent (Fig. 4.20, 4.21, 4.22). The left spicule is longer and 0.99–2.5 mm long. The right spicule is 0.18–0.67 mm long. The proximal end of the spicule is alate 0.02-0.03 mm in diameter and the distal end is pointed (Fig. 4.20). Twelve pairs of tail papillae are located at the tail end (four postanal pairs, six adanal pairs, two preanal pairs) and one medial unpaired precloacal papilla (Fig. 4.22). The two pairs at the sides of the sucker may be called

the para sucktorial papillae, the group surrounding the cloacal aperture the paracloacal papillae, and the group near the posterior end are called caudal papillae. The caudal alae of the male are well developed and about 0.03–0.07 mm in diameter. The tail is about 0.4–0.75 mm long and tapers beyond the alae to a fine filament. The sucker is situated at a distance of 0.08–0.12 mm from the cloacal aperture. The preanal sucker is easily seen, round, well-developed, surrounded by a chitinized ring (Fig. 4.23). The precloacal sucker is rounded, measure 40-48 μm in length and 14.60-15.02 in width, being surrounded by a chitinous inner ring.

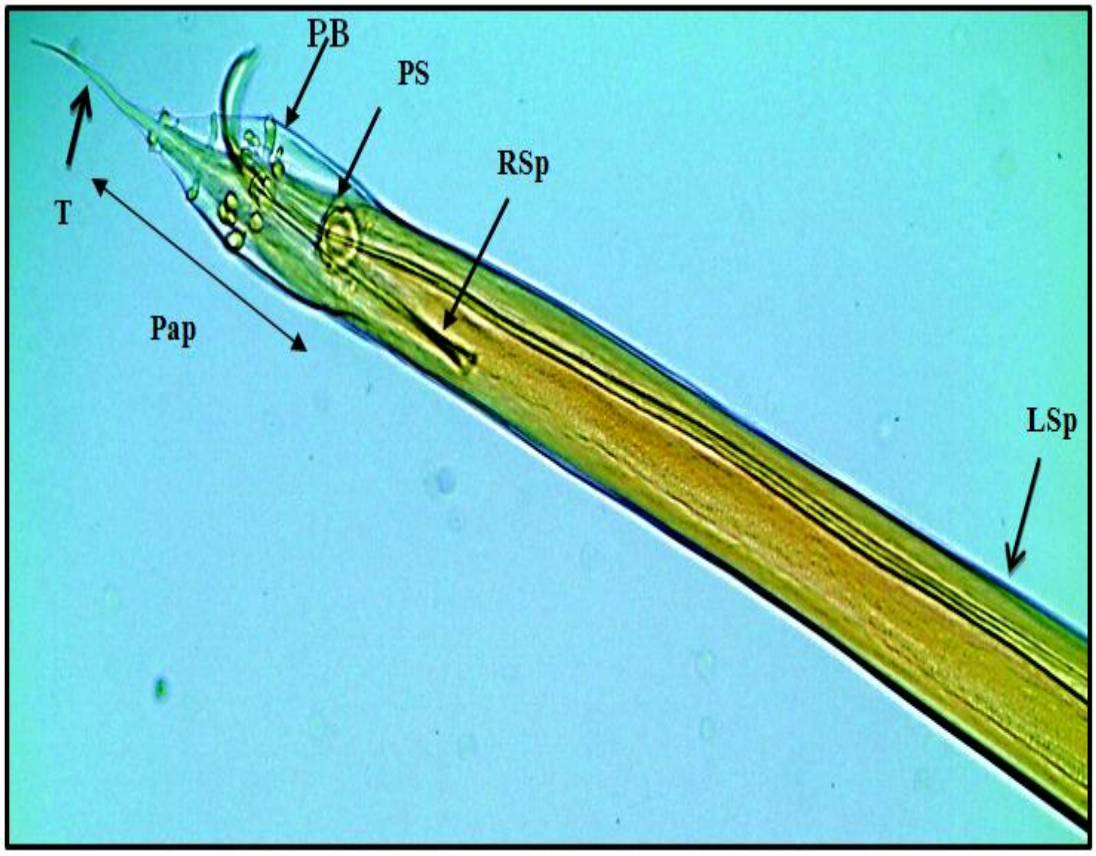


Figure 4.20- Light micrograph of male *H. gallinarum* tail end: LSp- Left Spicule, **RSp**-Right short Spicule, **PS**- Pre anal Sucker, **Pap, (four postanal pairs, six adanal pairs, two preanal pairs) and one medial unpaired precloacal papilla, PB**- Pseudobursa, **T**- Tail

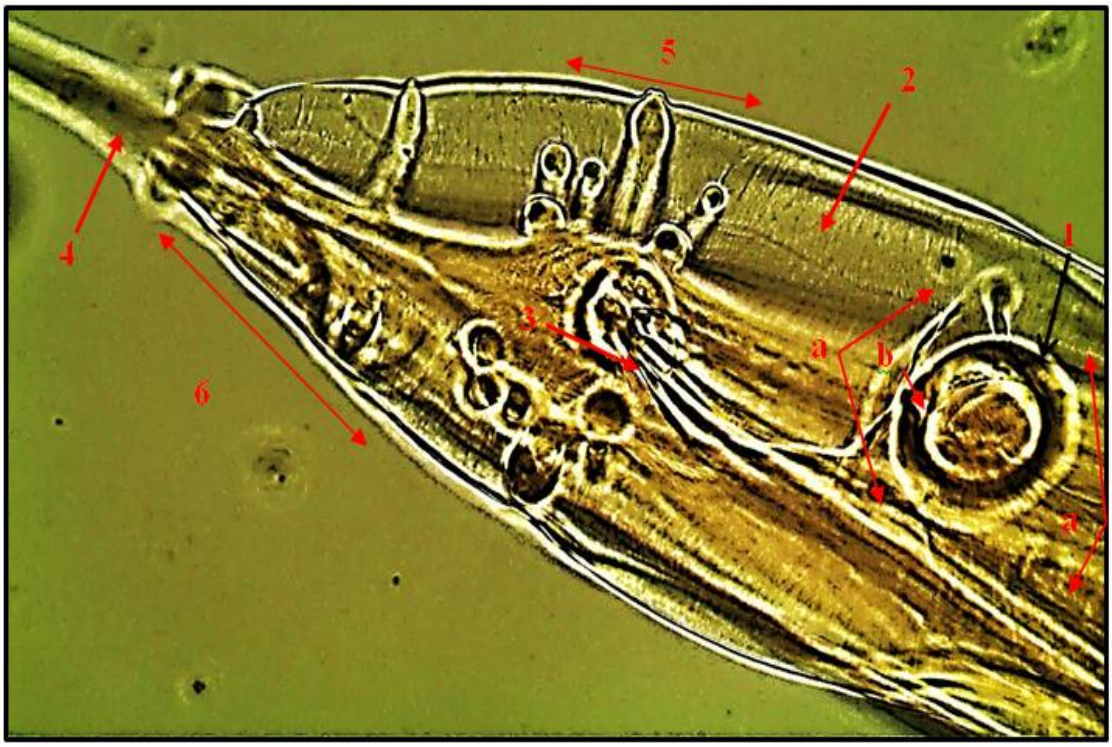


Figure 4.21- Light micrograph of male *H. gallinarum* tale (closer view) :1 – preanal sucker; **a –** position of two preanal pairs of papillae; **b –**medial unpaired papilla; **2 –** wings of pseudobursa; **3 –** anus; **4 –** stylet-like protrusion; **5 –** position of six pairs of adanal papillae; **6** –position of four pairs of postanal papilla.

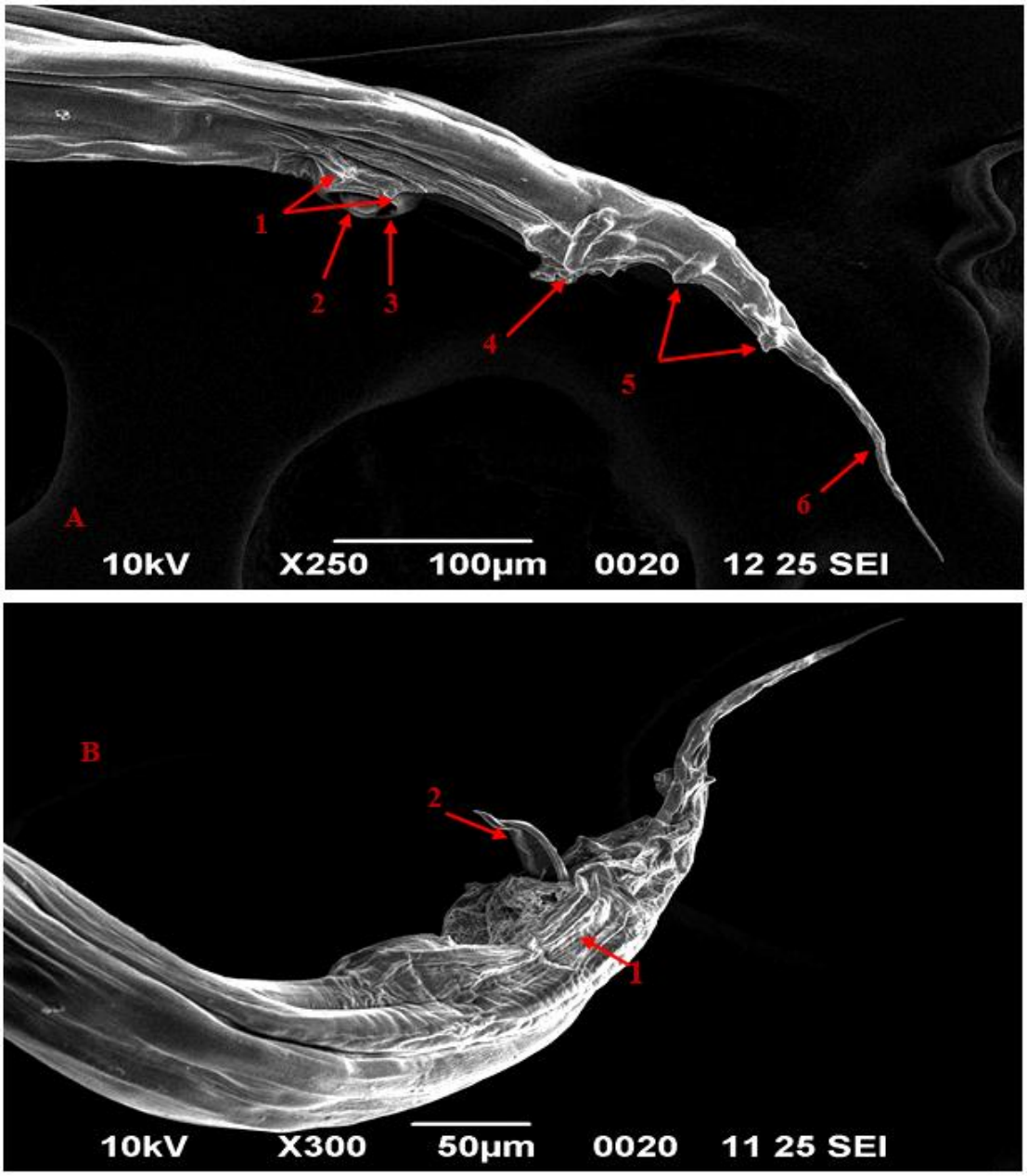


Figure 4.22- Scanning Electron Micrographof tail end of *Heterakis gallinarum* male: (A). 1- Two preanal pairs of papillae, 2- Preanal sucker, 3- Medial unpaired papilla, 4- Six adanal pairs of papillae, and 5- Four pairs of postanal papillae. Total twelve pairs of tail papillae are located at the tail end. **(B).**Ventral side of male have stylet-like tail end and pseudobursa (1) with well-developed lateral "wings" that smoothly taper (2). The left spicule is significantly longer than the right, and has a tapered sharp end. The right short spicule is has wing-like protrusions, its distal end is partly coarse, gradually tapered to a pointy hook.

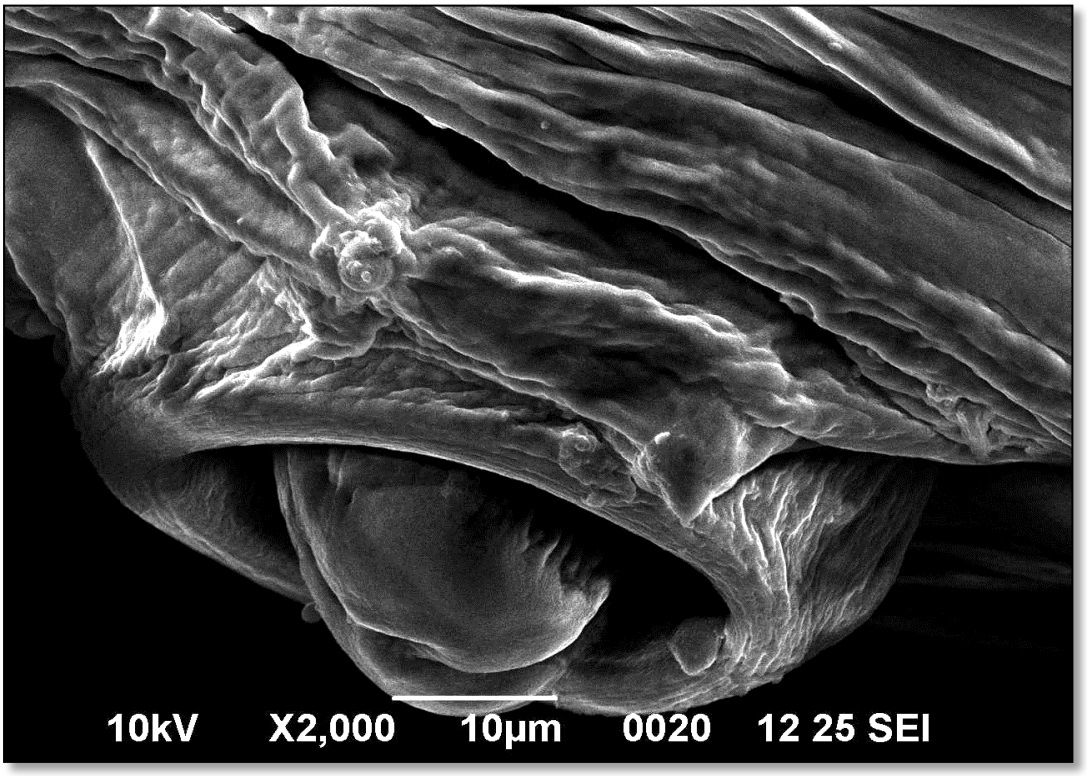


Figure 4.23- Scanning Electron Micrographof preanal sucker (Enlarge): The preanal sucker is easily seen, rounded, well-developed, surrounded by a chitinized ring.

Female- Body length is 7.1–9.79 mm and 0.31–0.41 mm in width. Females were visually identical. The tail end is elongated, gradually tapered, sharply pointed and measures about 0.81–0.96 mm from the posterior end. The anal opening is at the posterior part of body (Fig. 4.24). The vulva is situated slightly behind the middle body at a distance of 3.54–4.66 mm from the tip of the tail. There are three bends in the vagina after the vulva, angled posteriorly, anteriorly and once again posteriorly (Fig. 4.24). The vagina is connected to two opposing uteri. The uteri contain oval eggs with distinct two-contour shells, the light microscopy revealed the embronationof eggs (Fig. 4.25).

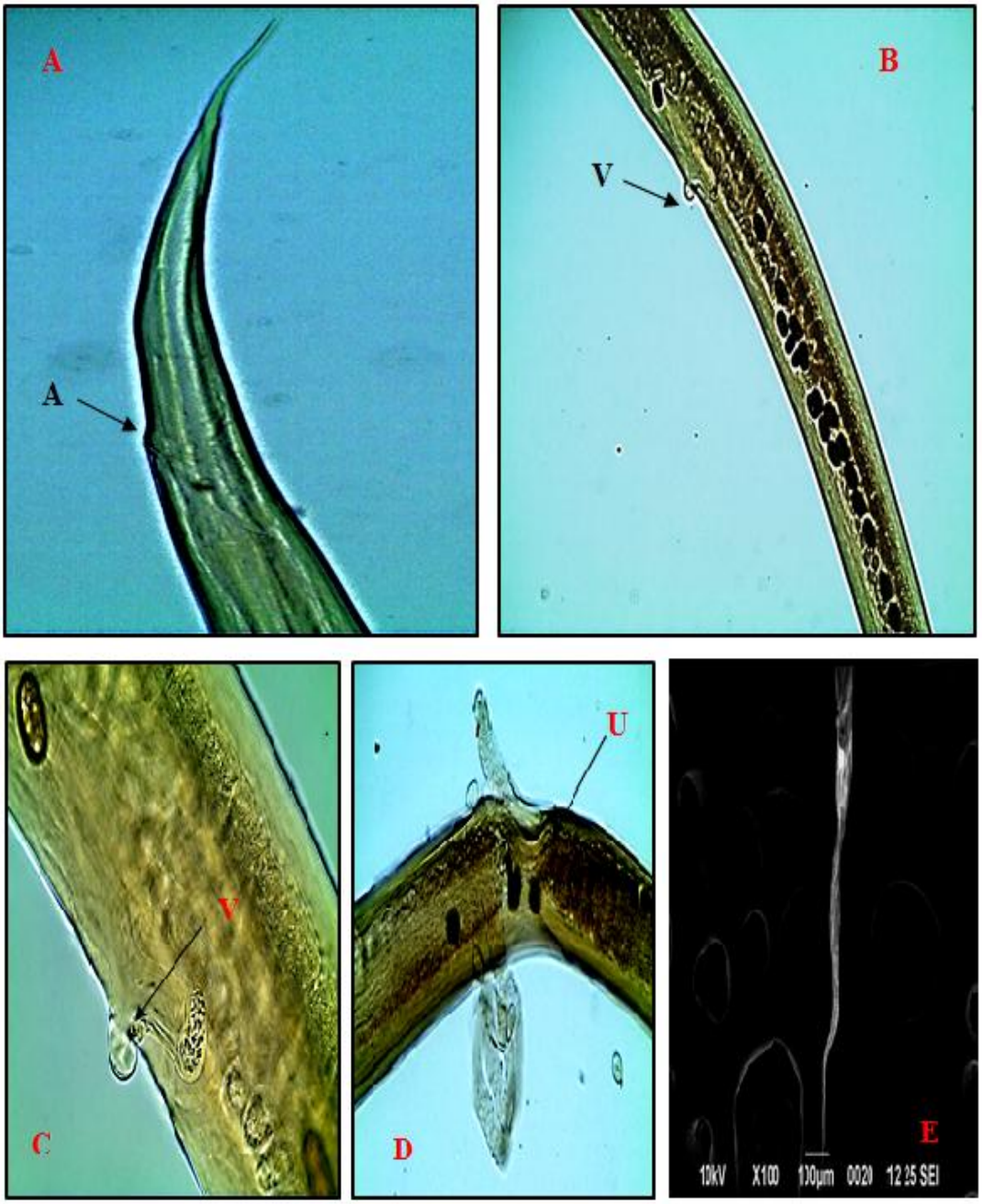


Figure 4.24- Light micrograph of female *Heterakis gallinarum*. The tail end is elongated and gradually tapered (A, E).

(A)-The anal opening is at the posterior part **(B, C)** - Vulva **(V)** at mid of the body **(D)-** Uterus ruptured **(E)** - SEM of the posterior region of female *H. gallinarum.*

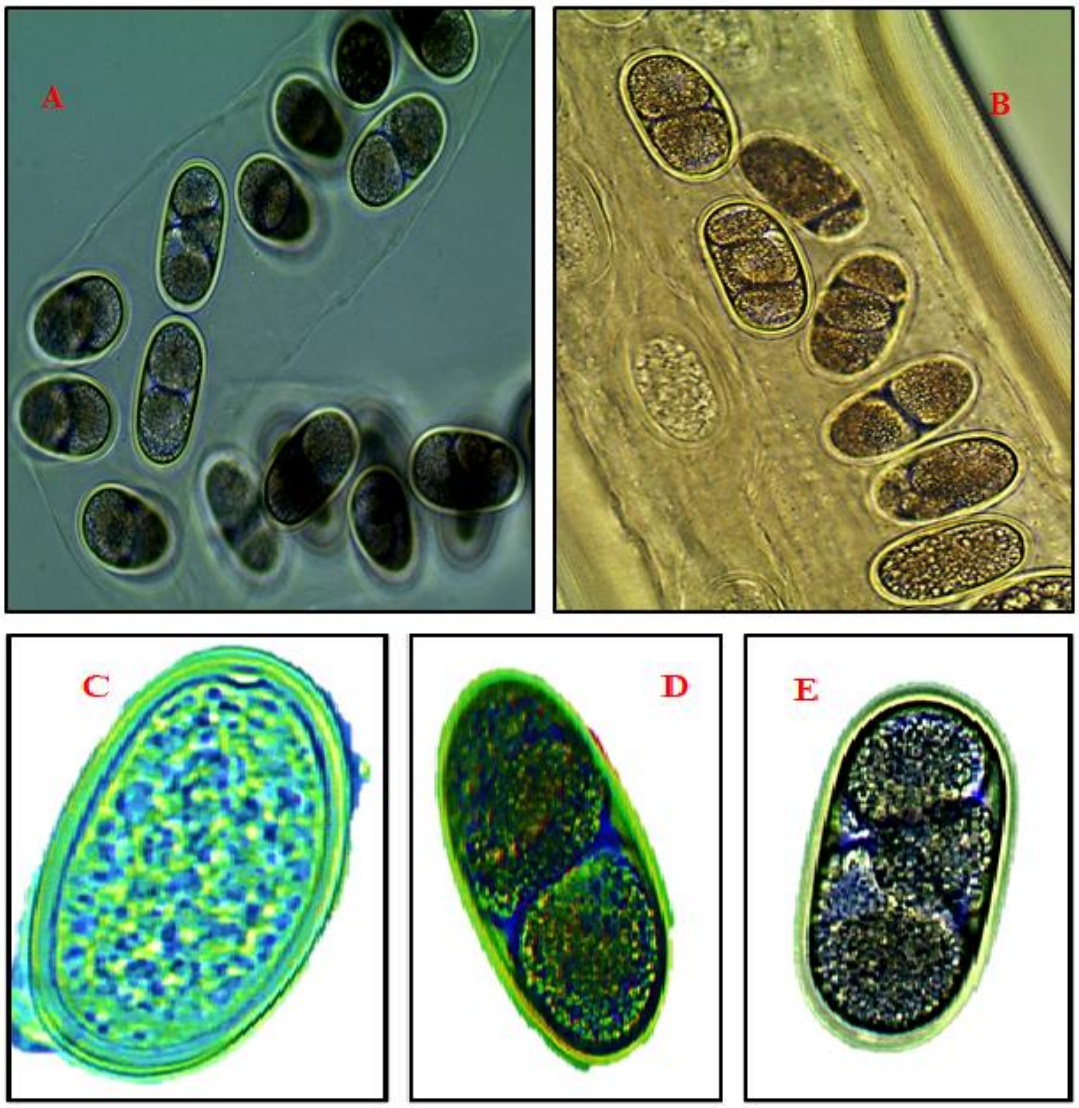


Figure 4.25- Light micrograph of *Heterakis gallinarum* eggs. Embronation of eggs (A-E)

4.4 DISCUSSION-

The nematode parasites *A. galli* and *Heterakis gallinarum* reduce the weight gain, egg production, and immunity as well as blockage and damage of the intestinal tract in hens when high worm burdens are present but no major changes were seen in blood variables or behavioural activities (Sharma *et al.*, 2019). High worm burden lowers stored energy reserves such as liver lipids in laying hens as

compared to uninfected hens (Sharma *et al.,* 2018). These energy reserves are used by the infected hens to maintain the production at the time of infection. According to some studies, neither artificial nor natural infection of *A. galli* was found to influence external and internal egg quality, regardless of infection intensity (Sharma *et al.,* 2018; Sharma *et al.,* 2019).

Ascaridia galli- Position of cephalic papillae is also an important feature in *A.galli*. Occurrence of four papillae, wherein two occur on the dorsal lip, and other one on the both side of latero ventral lips (Ashour, 1994). In the Ascaridian species presence of three lips is one of the characteristic features, but all lips are not similar to each other. All lips are trilobed and consist of a broad median lobe edged at each side by two small lateral lobes. Major structure of the lip is composed by the median lobe, while the small extensions at the base formed by the lateral lobes. Contrary to other species like *A. hermaphrodita* and *A. platyceri* these three lobes are almost equal in shape and size. Minute teeth or denticle in the inner surface of the medial lobe of each lip is also a characteristic feature of some species of *A. galli* like *A.australis, A. hermaphrodita* and *A. platyceri* (Hodova, 2008). According to Ashton, 1996, outer surface of lips has three paired cephalic papillae and two amphids. Major chemosensory organs of nematodes are amphids which helps in host finding and alsoin controlling the development (Ashton and Schad, 1996).Subannuli in *A. galli* are first reported and explaine by Hassanain *et al.*, 2009. During embryonic development of *A. galli* larvae to adult circular or transverse annuli divide into subannuuli. Such unique cuticular organization in *A. suum* seems to be the characteristic feature of ascarids (Fagerholm *et al.,* 1998; Fagerholm *et al.,* 2000). In the present and previous studies presence of the longitudinal ridge appears to form differentiation between the dorsal and ventral

side which is one of the well-known observations in *A. galli* (Lalchhandama *et al.*, 2009; Lalchhandama, 2010). Hassanain *et al.,* 2009 also exposed this ridge in *A. galli* by SEM, and failed to detect it as a different structure and uncertainly explained it as a median centroid (Hassanain *et al.*, 2009).

Females are longer with straight and blunt tail end, while males are shorter than females with curved and elaborated tail end (Ackert, 1940). Kung 1949 stated that spicules and papillae at the posterior end are the recognizable characteristic between different species (Kung, 1949). According to Cheng 1986 sexual dimorphism among females include thick shelled eggs, oviparous, vulva near middle of the body. Different members of the phylum nematode, show a stark similarity in the exoskeleton of cuticle. Basal lamina at the interior and epicuticle to the exterior is also recognised in many cases. It covers the digestive and reproductive systems as well as body surface (Cheng, 1986). Cuticle has criss-crossed makeup from carbohydrates, soluble and insoluble proteins like cuticulins, collagens and lipids (Page, 2001; Lee, 1966). The cuticle is a primary target site of anthelmintic drugs (Alvarez *et al.,* 2007).

Males have **ten pairs** of caudal papillae which are in different order, i.e., subterminal on ventral surface of caudal end, pre-anal, post-anal; **three pairs of preanals** – first pair anterior to pre-anal sucker, second close to the first pair and at level of pre-anal sucker; **sub-terminals-three pairs**, first smallest of all anal pairs, present near to second pair, second and third pairs comparatively more prominent surrounded by small cuticle raised structures giving them rosette-like appearance and second pair occur laterally and third one lying ventrally on the extreme tail region (Permin and Hansen, 2003; Permin *et al.,* 1997). Well developed and equal spicules are present, which are enclosed in spicular sheath, and protruding out at

anal opening (Permin and Hansen, 2003; Permin *et al.*, 1997). Hassanain *et al*, 2009 stated that cervical area of the worm have small papillae. The posterior region of *A. galii* is furnished with more complex structures, distended tail with poorly developed caudal alae on either side. Preclocal sucker and normal protrusion occurs on the ventral surface, situated near the cloacal opening and bounded by poor circular rim (Hassanain, 2009). Thickened cuticular bosses at the ventral part of rear end of body with cuticular ornamentation, spicula without alae, tail length is 0.45 to 0.80 mm, i.e. 0.96 to 1.14% of total body length, spicula length is 0.65 to 2.40 mm; and for female: tail length is 0.40 to 1.54 mm, i.e. 0.61 to 1.88% of total body length (Kajerova *et al.*, 2004). The entire generic characteristic shown by the *A. galli*is as follows: generally lateral cuticular flanges are present in Ascaridiinae; interlabia are absent in lips; club-shaped oesophagus present without the posterior bulb. Male: a chitinous rim in the precloacal sucker; spicules are equal or subequal in shape, caudal alae narrow, relatively larger papillae; no gubernaculums was found (Yamaguti, 1961).

Heterakis gallinarum is a parasite of galliform birds, feeding upon their fecal contents. Soulsby, 1982 reported that *Heterakis gallinarum* has three well-defined lips, the esophagus with a short narrow anterior portion (pharynx) and a long posterior part ending in a bulb. Normally, the cuticle has lateral flanges. Esophagus has a short narrow anterior portion (pharynx) and a long broader posterior portion ending in a well-developed bulb containing a valvular apparatus. The worm is small and white in colour. Three lips, a small buccal or pharynx, cover the mouth. Esophagus ends in a well-developed bulb containing a valvular apparatus. The length of the adult female and male cecal worms varies, with the female (10 to 15 mm) being typically larger than the male (7 to 13 mm). Both sexes have a pointed

tail, precloacal sucker present at the posterior end of males. The eggs of *H. gallinarum* are about 65-77µm by 35-48 µm, with visibly thick, smooth shells like the findings of Kaufmann, 1996; Olsen, 1986. Embryonated eggs of *H. gallinarum* are ingested by their definitive host by either direct uptake from the soil or by ingestion of an earthworm or insect which has eaten an egg. No data are available on the lifespan of adult worms, but the eggs of *H. gallinarum* have been observed to live up to five years in the soil, although this is likely rare (Lund and Chute, 1974; Lund, 1960).

As in other nematodes, *H. gallinarum* has longitudinal muscles which in combination with the cuticle and pseudocoelom form a hydrostatic skeleton. By utilizing the force that the contraction of the longitudinal muscles creates, the cuticle shortens on one side then lengthens on the other, creating the diagnostic S-shaped movement of nematodes. Juveniles of *H. gallinarum* normally reside in the lumen, but occasionally will travel to and enter either the cecal wall or cecal glands (Anderson, 2000; Olsen, 1986; Roberts and Janovy, 2008).

Nematodes, including *H. gallinarum*, have chemosensory organs called **amphids**. *Heterakis gallinarum* also has **papillae,** which are sensory structures surrounding the lip region. Behind the lip region are peg-like sensory structures which function both as chemoreceptors to detect chemicals, as well as mechanoreceptors to detect motion (Hui, 1976; Wright, 1977; Roberts and Janovy, 2008; Wright). *Heterakis gallinarum* feeds on the cecal contents of the bird in which it resides (Anderson, 2000). *Heterakis gallinarum* is not directly preyed upon, but eggs which have been released into the soil can be eaten by other bird species, earthworms, and insects such as flies and grasshoppers (Anderson, 2000; Lund, 1960). Earthworms can serve as paratenic hosts for juveniles, allowing them to move from the soil to a

bird's gut. Eggs of *H. gallinarum* can be a carrier of the disease causing protozoan *Histomonas meleagridis*. Birds can ingest infected *H. gallinarum* eggs and acquire *H. meleagridis*, resulting in blackhead disease. Blackhead disease affects mainly the liver and cecum of infected birds, causing lesions and ulcers that are eventually fatal (Olsen, 1986; Kaufmann, 1996).

According to Soulsby 1982, the tail of male has large caudal alae extending some distance down the sides of the body, bearing a number of caudal papillae and a prominent pre-cloacal sucker. The spicules are well developed, unequal, protruding out at anal opening. The tail of female is also elongated, narrow and pointed. The vulva of the *Heterakis gallinarum* is situated at the middle of the body.

4.5 CONCLUSION-

- Light and Scanning Electron Microscopy proved both are most feasible techniques for investigating nematodes in *Gallus gallus domesticus*. Objective of this chapter was only on morphological charecterization of nematodes. So the focus was only on nematodes of studied area. Three dimensional morphology of male and female parasite was captured by Scanning Electron Microscope which revealed prominent surface stuructures.
- All the samples analysed for nematodes investigation were collected from Lucknow that gave clear insight of ultrastructure of nematodes in *Gallus gallus domesticus* in Lucknow region of Uttar Pradesh, India.
- The present form agrees to the characters of *Ascaridia galli, Heterakis gallinarum,* as regards the number of caudal papillae and their shape, arrangement and number of spicules, the size of spicules. However, there are variations in the size ratio of various organs. Since these variations are

intraspecific, hence the present specimens are assigned to *A. galli* and *H. gallinarum.*

- The present study of an ultra-structure of gastrointestinal nematode parasites i.e. *Ascaridia galli* and *Heterakis gallinarum* in desi fowls, suggests the taxonomical status, ways and means to formulate the appropriate strategies as one of the control measures for getting the maximum benefit by rearing of backyard chickens in rural areas.

CHAPTER-5

Molecular Characterization of Gastrointestinal Nematode Parasites of *Gallus gallus domesticus*

5.1 INTRODUCTION-

Poultry industry plays a major role in supplying the animal protein to man and take part in the national economy as an income provider. One of the most important reared fowl species is chicken, which is highly developed and beneficial for animal production enterprise (Nnandi and George, 2010).

Poultry birds can be affected by viral, bacterial and protozoan infection which leads to death in birds. Helminths are responsible for the reduced egg production, poor weight gain (especially in young growing chickens) and other diseases caused by the helminths as carriers of pathogenic agents (Jordan, 1990). However, the less obvious, all these factors are responsible for reduced productivity to the poultry industry (Ssenyonga, 1982).

Ascaridia galli is the largest round worm of the small intestine of domestic fowl and wild birds. According to studies, *A. galli* is the most common nematode in all types of production systems and has a worldwide distribution which can cause reduced growth rate, poor weight gain, pathological lesions and occasionally serious illness, these factors are responsible for economical losses in poultry birds such as hens, turkeys, geese and some other birds (Ramadan and Znada, 1991). As well as sometimes damages and obstruction are occur in the intestinal mucosa due to the high worm burden which are responsible for the blood loss and secondary infection (Soulsby, 1982). Parasitic nematodes are responsible for serious illness in human beings and animals. Moreover, these helminth parasites cause a huge economic loss to the poultry producers. *Ascaridia galli* (Shrank 1788) is a potential vector for *Salmonella enterica* in poultry.

Heterakis gallinarum act as a carrier for *Histomonas meleagridis,* which is a causative agent for black head (enterohepatitis) in turkeys. The protozoan can remain viable in the egg of *H. gallinae* for a long time, maybe as long as the egg remains viable (Soulsby, 1982). The 15% occurrence of *Heterakis gallinarum* in birds was reported in previous studies (Orunç and Bicek 2009; Camacho-Escobar et al. 2008). Numerous diagnostic methods have been suggested, primarily polymerase chain reaction (PCR), because it depends on the amplification of the minute DNA materials present (Bazh, 2013). For the morphological identification of well-defined adults of *Toxocara canis, Toxocara cati* and/or *Toxascaris leonina,* the sequences of the first and second inner transcribed spacers (ITS-1 and ITS-2) can be used effectively (Jacobs *et al.,* 1997; Zhu *et al.,* 1998).

Information of the genome provides a broad resource to the scientific community and supports the development of new and urgently needed intervention (drugs, vaccines and diagnostic tests) against ascariasis and other nematodiasis (Jex *et al.,* 2011). Various molecular approaches are existing to provide a better understanding of the characterization of nematodes. Polymerase chain reaction reduces the possibility of misdiagnosis of parasites. It requires minute nanogram to pictogram amount of DNA to provide a suitable DNA target sequence (Gasser *et al.,* 1993).

Many studies have shown that the internal transcribed spacers (ITS) region of nuclear ribosomal sequences work as a genetic marker and gives accurate information about the parasites nematodes. (Hoste *et al.,* 1995; Chilton *et al.,* 1997). Interspecies phylogenetic analysis and discrimination between nematodes in order Ascaridata, ITS-1 ribosomal DNA (rDNA) is a appropriate molecular marker (Lin *et al.,* 2012).

The Internal Transcribed Spacer (ITS) region is a versatile genetic marker, placed between the repeating array of nuclear 18S and 28S ribosomal DNA genes. In eukaryotes like protozoa, plants, vertebrates, and fungi, information of ITS region have been used in phylogenetic analysis, structure of genetic population, assessing the populationlevel, information of evolutionary processes, and help in the taxonomic identity. Structure of cistron of the rDNA has a broad application. Cistron further divided into domains that evolve at the different rate, so these regions used in the diagnosis of evolutionary problems at the distinct level of divergence. Middle repetitive family of the nuclear DNA genome have the rDNA. PCR amplification becomes easy due to the presence of multiple copies of these genes in the genome of single juvenile and adult nematodes. Concerted evolution happened due to the rDNA genes,ITS and intergenic spacer (IGS) so that copies of these genes from a single individual tend to be similar to one another, although generally being distinct from those of other species (Elder and Turner, 1995). Nematologists mainly focuses on the applicability of the ITS region (Campbell *et al.,* 1995; Cherry *et al.,* 1997; Chilton *et al.,* 1995; Epe *et al.,* 1996; Fallas et al., 1996; Ferris *et al.,* 1993, 1994, i995; Gasser and Hoste, 1995; Hoste *et al.,* 1995; Ibrahim *et al.,* 1994, 1997; Joyce *et al.,* 1994; Kaplan, 1994; Nasmith *et al.,* 1996; Orui, 1996; Reid, 1994; Stevenson *et al.,* 1995; Szalanski *et al.,* 1997; Thiery and Mugniery, 1996; Vrain *et al.,* 1992; Vrain and McNamara, 1994; Wendt *et al.,* 1995 and Zijlstra *et al.,* 1995,1997).

So, it was anticipated that the ITS-1 and ITS-2 would be the useful genetic markers targets for the specific identification of nematodes. The object of the present study was to identify the *Ascaridia galli* and *Heterakis gallinarum* round worm from Lucknow by DNA sequencing study and phylogenetic relationship with other nematodes of Gen Bank.

5.2 MATERIALS AND METHODS-

5.2.1 Necropsy and parasites-

Nematodes were collected from the gastrointestinal tract of *Gallus gallus domesticus.* Morphological identification of adult worms was done by using the light and scanning electron microscopy according the key of soulsby 1982. Collected worms were rinsed by using normal saline and stored in 70% ethanol at -20˚C till the extraction of genomic DNA.

5.2.2 Purification of samples:

- A mortar and pestle was used for grinding of the nematode parasite.
- The Nematode parasites (after microscopic investigation) preserved at -20°C deep freezer (REMI.RQV-200 PLUS) were homogenised and washed with 1ml ddH2O and vortexes (Fig. 5.1) at high speed in 1.5ml eppendorf tubes (each sample with four replicates) and centrifuged (REMI-R-24 centrifuge) at 3000 rpm for 15 minutes.

Figure 5.1- Vortexing of homogenised sample

- The pellet was collected and supernatant was discarded and further 1ml of ddH20 was added to the pellet, vortexes and centrifuged.
- The process was repeated thrice to get a clear homogenate.

5.2.3 DNA Extraction: Extraction of genomic DNA of nematodes was carried out with slight modifications in SDS based PCI method (Donn *et al.*, 2008).

- Sample of nematode parasite was lysis in SDS based1000µl of lysis buffer in 2ml eppendorf tube.
- **Components of SDS based lysis buffer:** 100mM Tris-HCl, pH=8.5; 100mM NaCl; 50mM EDTA; 1% w/v SDS; 1% (V/V) β-mercaptoethanol was added to the extraction buffer just prior to use (Donn *et al.*, 2008).
- Mixture was prepared by vortexing and kept aside for five minutes.
- Sample was incubated at 50-60^{0}C for 60 minutes with occasional vortexing and then cooled at room temp.
- 10µl of Proteinase K and 10µl of RNase were added to the mixture and vortexed and incubated at 37^{0}C-45^{0}C for 10 minutes.
- After lysis buffer treatment samples were vortexes and centrifuged for 15 minutes at a speed of 14000 rpm.
- The supernatant was collected in fresh 2ml eppendorf tube (1ml in each tube).
- Equal volume of Phenol, Chloroform and Isoamyl-alcohol (P:C:I) were added in the ratio of 25:24:1 (Fig. 5.2).

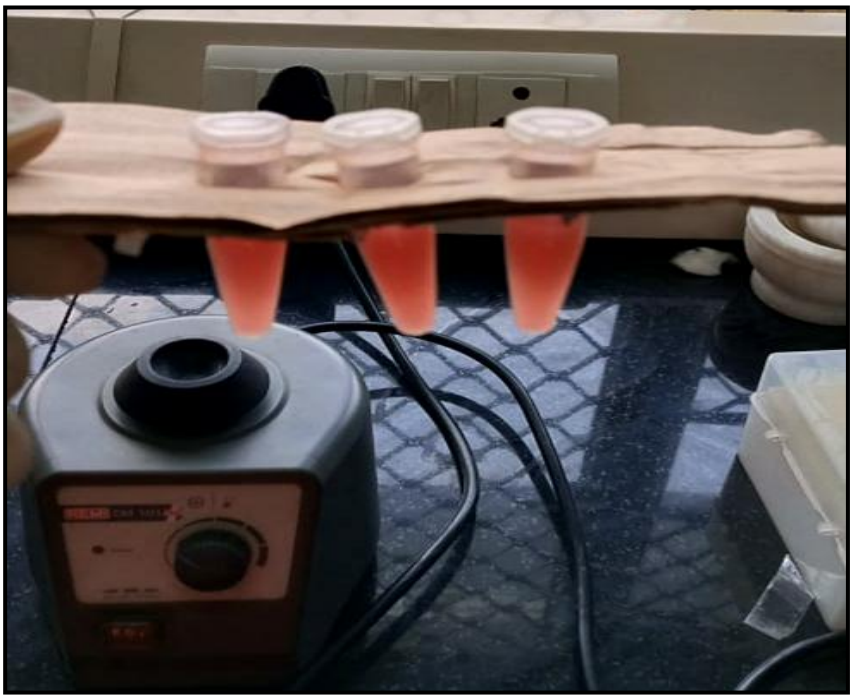


Figure 5.2 Addition of phenol:chloroform:isoamyl alcohol in supernatent

- After adding P:C:I, the eppendorf tubes were inverted several times. It was then centrifuged at 14000 rpm for 15 mins (Fig. 5.3).

Figure 5.3- Centrifugation of sample at higher rpm (A-B)

- Tubes were kept on ice for 2 mins.
- Resulted in the formation of two layers (Fig. 5.4).

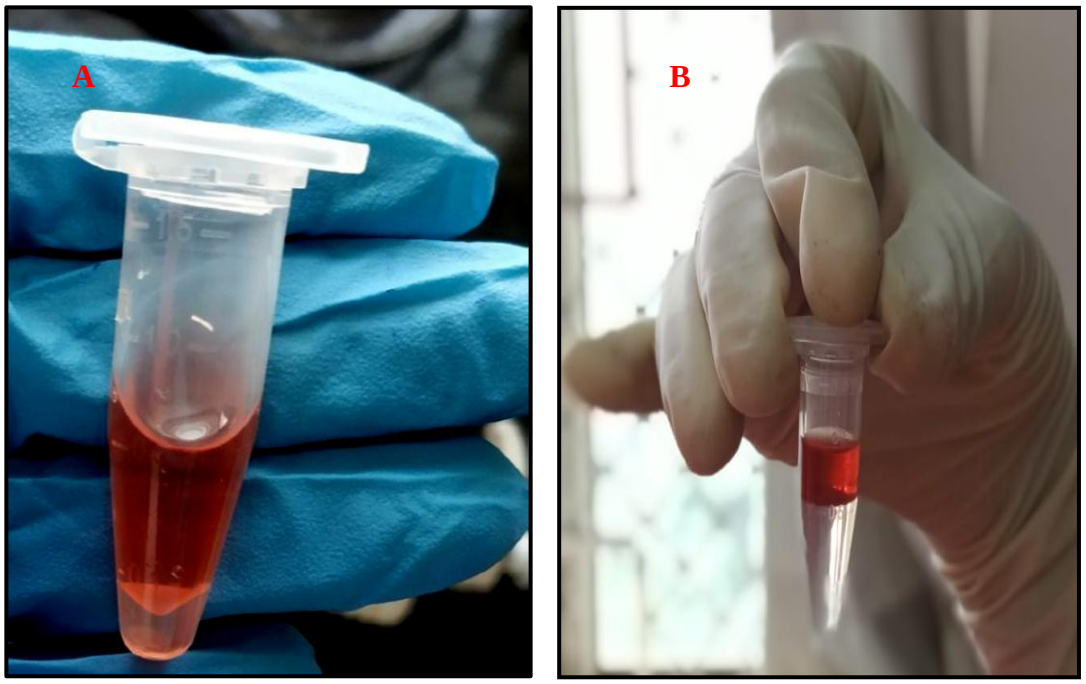


Figure 5.4- Formation of two layers after adding P:C:I (A-B)

- Top aqueous phase was transferred to clean 2ml eppendorf tubes.
- 0.2 volumes of 5M Nacl and 1 volume of cold absolute ethanol were added to the above.
- Vortexed to mix well and incubated at 4°C for 60 mins or overnight.
- After incubation above was centrifuged at 14000 rpm for 15 mins to precipitate the DNA.
- DNA pellet settled and the supernatant was discarded carefully (Fig. 5.5).
- DNA pellet was washed with 1ml of 70% ethanol and centrifuged at 14000 rpm for 5 mins.

Figure 5.5- Settling down of DNA pellet

- The supernatant was discarded.
- The tubes with collected DNA pellet were left to air dry.
- 30-50µl of autoclaved TE buffer or ddH2O was added and mixed by thawing.
- Preserved at -20°C deep freezer for further use.

5.2.4 Agarose Gel Electrophoresis:

- Agarose gel (0.8%) was prepared [*0.8g agarose powder (BR Biochem) by dissolving in 100ml of 1X TAE buffer and heated on hot plate, allowed to cool around 60°C temperature, 2-3µl of EtBr (*10mg dissolved in 1000µl sterile distilled water) was added and mixed properly and poured in a casting tray with comb in place.
- The 0.8% gel when solidified was transferred to electrophoretic unit filled with 0.5X TAE buffer and 5µl of DNA with 1-2µl of 6X DNA loading dye (Bromophenol blue; Xylene cyanol, Thermofisher Scientific) loaded (Fig. 5.6) and run at a voltage of 75V.

- The DNA band was observed under UV transilluminator (GeNei TM) at 256-260nm (Fig. 5.6C).

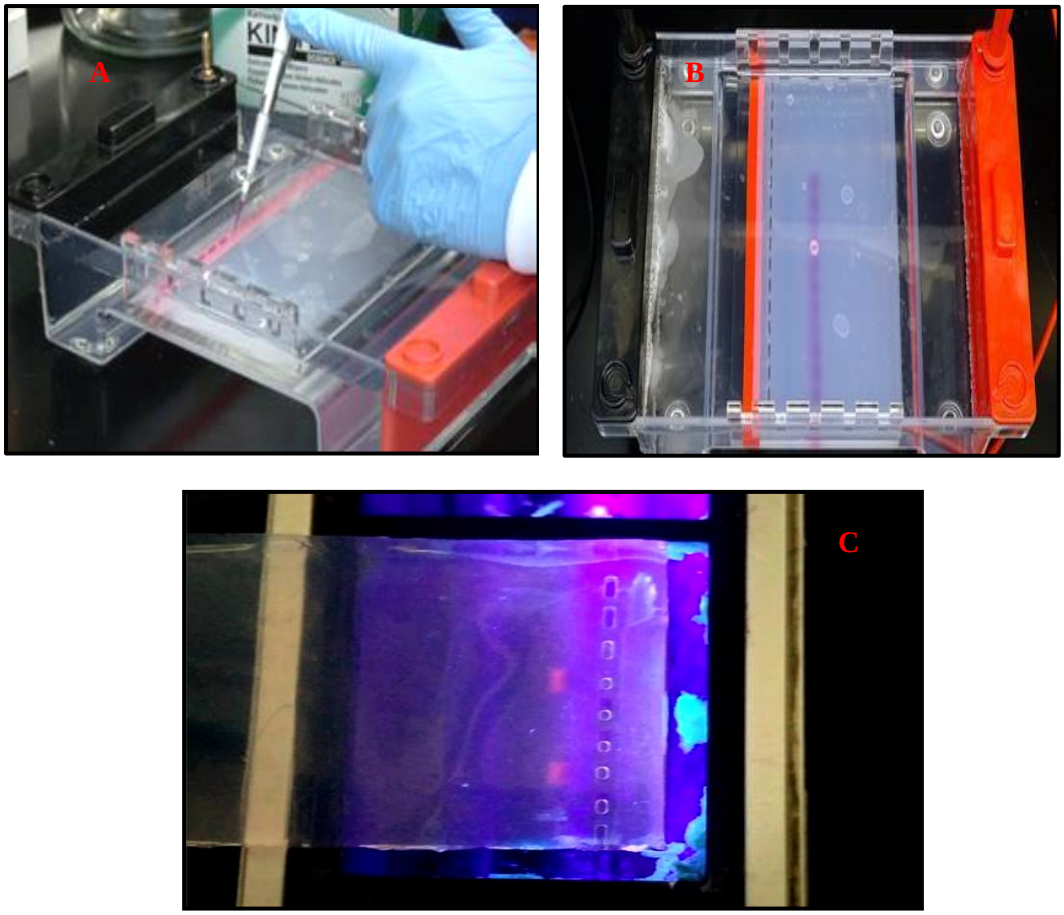


Figure 5.6- Agarose gel electrophoresis; (A). Pouring, (B). Running of genomic DNA, (C). Detection of genomic DNA bands under transilluminator

5.2.5 DNA Quantification:

- Genomic DNA Quantification was done by spectrophotometer (Biophotometer D-30).

5.2.5.1 Protocol for Spectrophotometer reading:

- 1µl of DNA was diluted in 99µl of ddH2O was used as control for calibration. Readings were taken at 260nm and 280nm 1O.D at 260nm for dsDNA=50ng/µl.

- Concentration was checked by the formula:

Concentration= O.D260×50ng/µl × dilution factor)

- Purity of DNA was checked by **O.D260/O.D280** ratio that has value between1.7-1.8.

5.2.6 Polymerase Chain reaction:

- PCR was carried out by amplifying ITS region between of 18S to 28S rRNA gene.

Primer: Reported Primer of *Ascaridia galli* and *Heterakis gallinarum* (Eman K. A. Bazh**)** were taken for PCR amplification.

Ascaridia galli-

Forward primer sequence: **(5'GTTTCCGTAGGTGAACCTGC3')**

Reverse Primer Sequence: **(5'ATATGTTAAGTTCAGCGGGT3').**

Heterakis gallinarum-

Forward primer sequence: **(5'GTTTCCGTAGGTGAACCTGC3')**

Reverse Primer Sequence: **(5'ATATGTTAAGTTCAGCGGGT3')**.

5.2.6.1 Polymerase Chain reaction procedure:

- PCR reactions were performed by using 25 µl of Ampli Taq Gold® Fast PCR Master Mix (2×) containing 2 µl of each primer (Table 5.1).
- The PCR cyclic conditions were as follows:

- **For *Ascaridia galli*-** Initial denaturation (enzyme activation) at 95 °C for 10 min, followed by 35 cycles of 30 s at 95 °C (denaturation), 60 sec (annealing) at 59 °C, 60 sec (extension) at 72 °C, followed by a final extension of 72 °C for 10 min (Table 5.2).
- **For *Heterakis gallinarum*-** Initial denaturation (enzyme activation) at 95 °C for 10 min, followed by 35 cycles of 30 s at 95 °C (denaturation), 60 sec (annealing) at 61 °C, 60 sec (extension) at 72 °C, followed by a final extension of 72 °C for 10 min (Table 5.2).
- Amplified PCR products from the reaction were run on 1.5% Agarose gel (mixed with 2µl EtBr [10mg/ml]) with 7µl of 1Kb plus DNA marker (Invitrogen) and product band size (with 6X loading dye Bromophenol blue) was checked under UV transilluminatorand photographs were taken by placing the gel in Gel doc (Gel Imaging and analysis system).
- If results are positive then it is followed by sequencing.
- Sequencing is made to the good identified PCR product by using forward and reverse primers on GATC Company by direct sequencing with an automated DNA sequencer (ABI 3730XL; Applied Biosystems, Thermo Fisher Scientific Foster City, CA, USA).

Table 5.1- PCR set up:

Components	Volume(µl)
DNA (10ng/µl)	**10**
ddH2O	**1**
Forward primer	**2**
Reverse primer	**2**
PCR Master Mix	**10**
Total Reaction Volume	**25**

Table 5.2- PCR Programme: Carried out in PCR thermocycler (AB Biosystems)

	Temperature (°C) *A. Galli*	Temperature (°C) *H. gallinarum*	Time in Seconds
Initial Denaturation	**95**	**95**	**600**
Final Denaturation	**95**	**95**	**30**
Annealing	**59**	**61**	**60**
Extension	**72**	**72**	**60**
Final Extension	**72**	**72**	**600**
Number of Cycles: 35			

5.2.6.2 Purification of PCR product:

Purification of the amplified PCR product (band of interest) has been performed by PCR purification kit (Pure link Quick Gel Extraction and PCR Purification Combo Kit).

Manual Protocol:

- Gel with band of interest was excised and weighed.
- Gel solubilisation buffer was added and centrifuged for a minute.
- Incubated at 50°C.
- Dissolved gel collected in a collection tube.
- Column washed with elution buffer (E1) and centrifuged for 2 minutes.
- DNA eluted into Purelink elution tube (Kashyap, 2018).

5.2.7 Sequencing:

- The pure amplified PCR product had been sent for sequencing (Progen Biolab). Nucleotide sequencing was done by using 'big dye terminator v 3.1 cycle sequencing kit' (ABI USA).
- Single set of primers (Forward/Reverse) were used for amplifying and single stranded amplified product were purified by using 125mM EDTA and 3M Sodium acetate and Further purification was done by absolute alcohol and 70% alcohol.
- Purified product was air dried and mixed with 13µl of formamide solution.
- Finally samples were fixed in machine for capillary run (Kashyap, 2018).

5.2.8 Phylogenetic tree:

- Received sequences of *A. galli, H. gallinarum* were deposited in the gene bank database under the Accession no **MW301652, MW661165** as an Internal Transcribed Spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and Internal Transcribed Spacer 2, partial sequence.
- Phylogenetic analysis was performed based on the ITS1- 5.8S-ITS2 rRNA region between the newly obtained sequences and the superfamily Ascarioidea and the genus Ascaridia available in GeneBank.
- Sequences generated after sequencing was blasted with Highly Similar Sequences (NCBI) and phylogenetic tree was constructed accordingly that showed similarity with other known sequences.
- Multiple sequence analysis was done by using CLUSTAL W Bioedit software version 7 computer programmes.
- Mega7.0 software was used to estimate identity and variability of sequences and multiple sequences aligned by UPGMA.

- Nucleotide sequences were aligned by using the CLUSTAL-W and MEGA7 software.
- We make tree view for the resulted nucleotide sequence on the data base by the Neighbour Joining (N.J) method by using BLAST programme.

5.3 RESULTS-

Table 5.3- DNA Quantification of *Ascaridia galli* by Spectrophotometer:

Sample	Absorbance at 260nm (A260)	Absorbance at 280nm (A280)	Stock DNA concentration (µg/ml)	Absorbance ratio at 260/280
Ascaridia galli	**0.499**	**0.292**	**2.495**	**1.708**

5.3.1 PCR results and molecular analysis-

The ITS1-5.8S-ITS2 rRNA product of *A. galli* was about 1000 bps (lane A-D) amplified by the species specific primer (Fig. 5.7) and was similar to existing sequences of *A. galli* in the Gene Bank (Fig. 5.8). The PCR reaction amplified the ITS1-5.8S-ITS2 rRNA region turned out to be a good tool for the differentiation between the nematodes, after that sample was sequenced (Fig. 5.8).

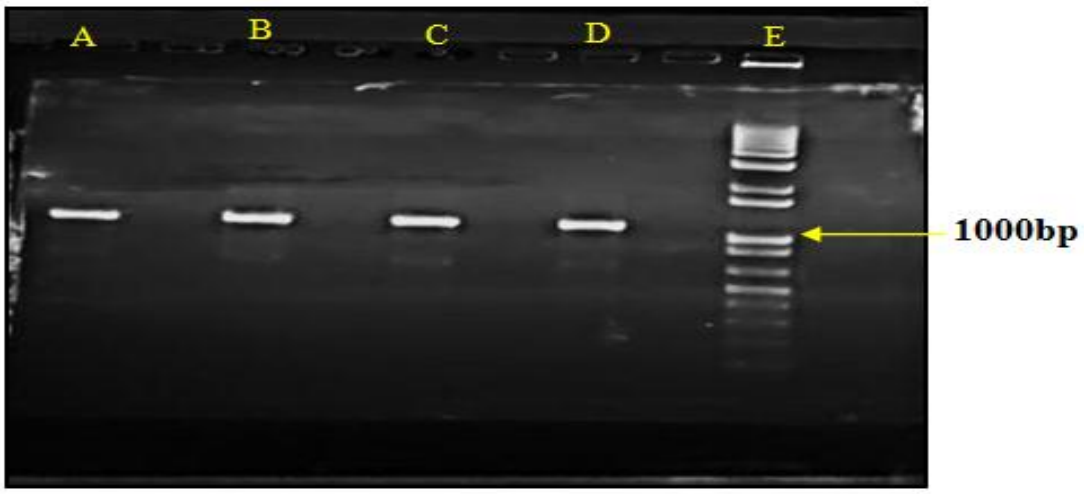


Figure 5.7- PCR product of *A. galli* were positive (Lanes A, B, C, D) with the Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder (Lane E) at ~1000 bp.

>MW301652 *Ascaridia galli isolate* AB1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GGGGATAATCCGAAGCTTTTAAAATAATCTTAATACTGTGCATACGCAATAATTTGCAC
AGATTTTTTCACACTACCATTGTATACTATATAATTTATGGTCGCTAGCTGTTATTGGCTT
GCAATGACTAATAGTATACATTAATAACGTCGTGATTGTGTTACTGGGTGATATACACTG
CAACTGGTATATCGCTAGAGCTCAGTAACGCGTAAATTTTAACAACGGTGTCACGGTTG
GCGTCTATGCTCCACCGAGTTGCTGCCCGACCGTCGGTAACGATGAAAAGTGGAGAATA
ATAAAAGCTTACTTGTAAAAACTTGATCAACTTTTACAAGTGAAGTAGACTTAATAAGC
GTCCAGCAAGTGCGCTGCCAACAAGAAATTTTTTTGCATCATAAAAGTGTATTATTATGT
AATTTGAATACGATATGATCAATTATGATGATGATGATGATGATACGTTATTAAATTCAA
ATATTATCACTCTTAGCGGTGGATCACTCGGCTCGTGGATCGATGAAGAACGCAGCTAG
CTGCGATAACTAGTGCGAATTGCAGACACATTGAGCACTAAGATTTTGAACGCAAATTG
CGCCATTGGGTTCATTCCCAATGGCACGTCTGGTTGAGGGTCGAATTTCTAATGCTACTG
CTTGATTGCTATTGCCATCGCTTGTTTAGTGGCACATATGCGCGCAAGATAATATAATAA
TCAAGTGTATGTGTATGTGCGTATGCGTTTGTTTACGTTGTACTACTTGATAAAAGCGTT
TTATAATAGCTCGTTTTATAAAACATGCTTTAAATCAGGGACTACCATTAGATGATGGAG
ATGGTGAAGATGGATGATGCCGCTTTTTTTTTTTTTTTATTGGAGGTGCTAGGGTGGCGG
GGGGGGCCCCACCCCTCGGTATAATAAATATTAAATATATAGGGGGAGAAAAGAGGGG
AGGAGTCGGCGGAGGAACCGCGCCAAAAAAAAAAAATTTTGTCTTTG

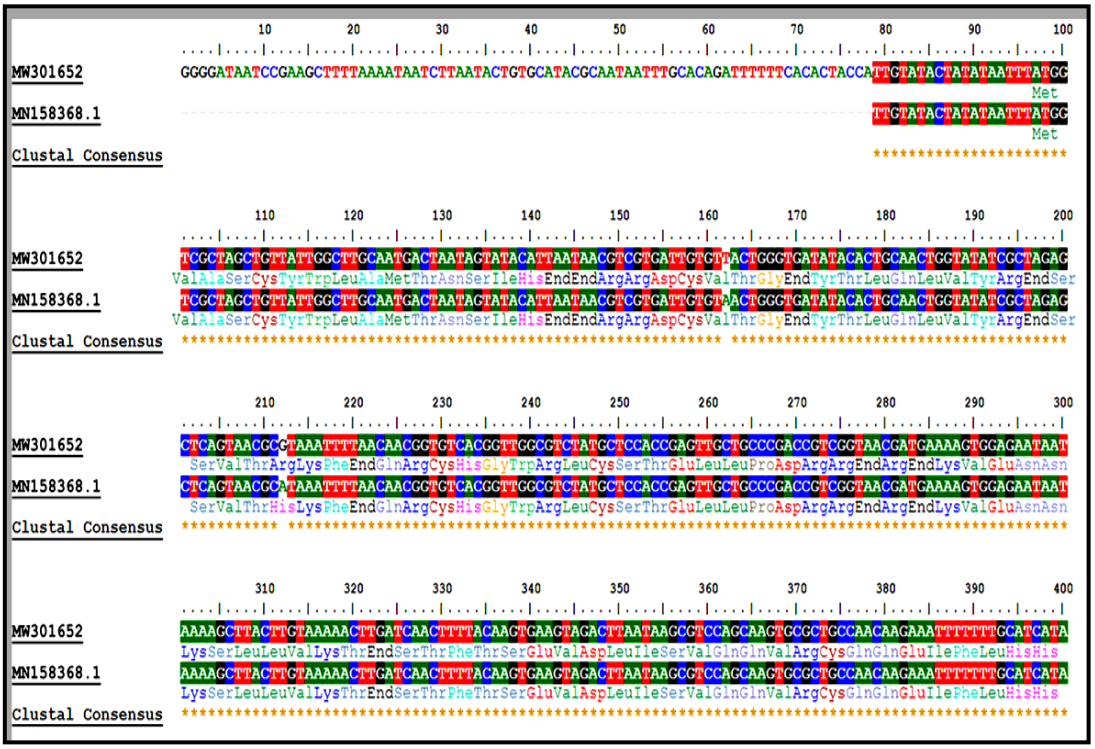
Figure 5.8- Sequence generated after sequencing of PCR product (1000bps) by primer of *Ascaridia galli* in *Gallus gallus domesticus.*

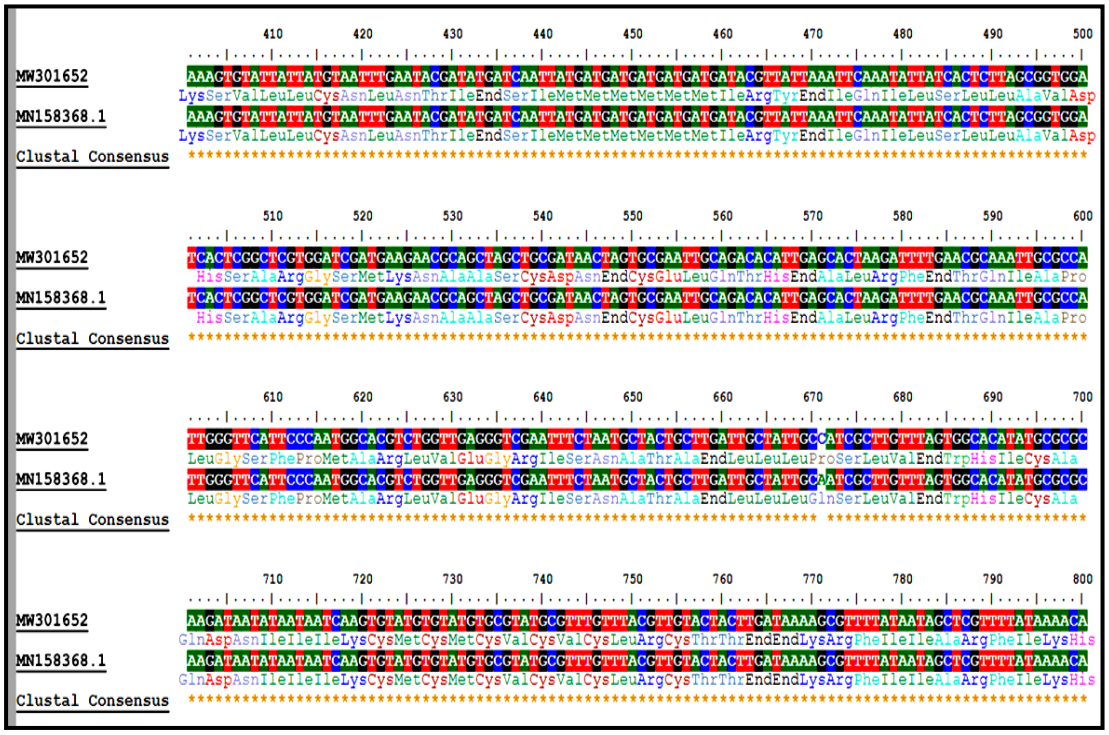
5.3.2 Gene sequences and phylogenetic analysis –

Gene sequences of the *A. galli* (MW301652) samples from Lucknow region were analysed in comparison with another one of accession number of *A. galli* (MN158368) represented in Fig. 5.9.

BLAST analysis of ITS region in the obtained region of *A. galli* showed a lower similarity with *Heterakis spumosa* (80.95%) and higher similarity with *A. galli* (AJ007451) (99.58%), *A. galli* (AM408550) (99.36%), *A. galli* (MN158368) (98.57%). The differences between the ITS regions are very close so it can be concluded that this is *A. galli* in Lucknow region. To make the phylogenetic relationship of Luckhnavi *A. galli* Neighbour Joining (NJ) method was used on the

NCBI GenBank database based on ITS-1 5.8S, ITS-2 regions of *A. galli* species and some related species from Ascariodea (Fig. 5.10).





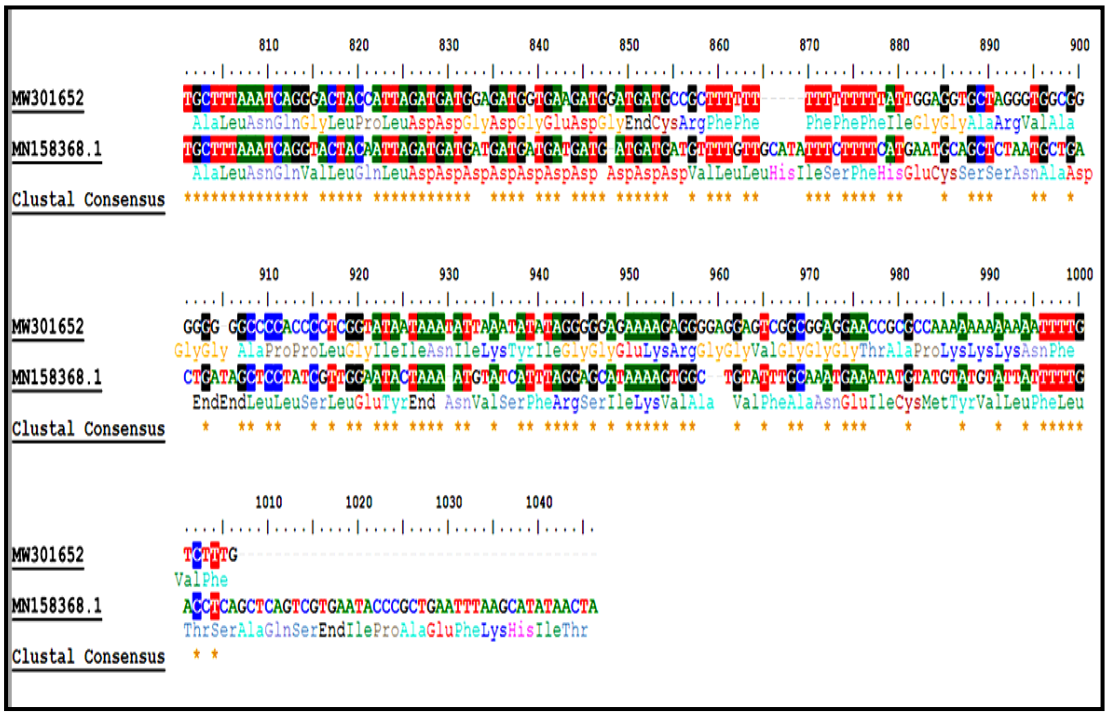


Figure 5.9- A comparison of the sequences of *A. galli* (MW301652) with another one (reference of accession number, MN158368)

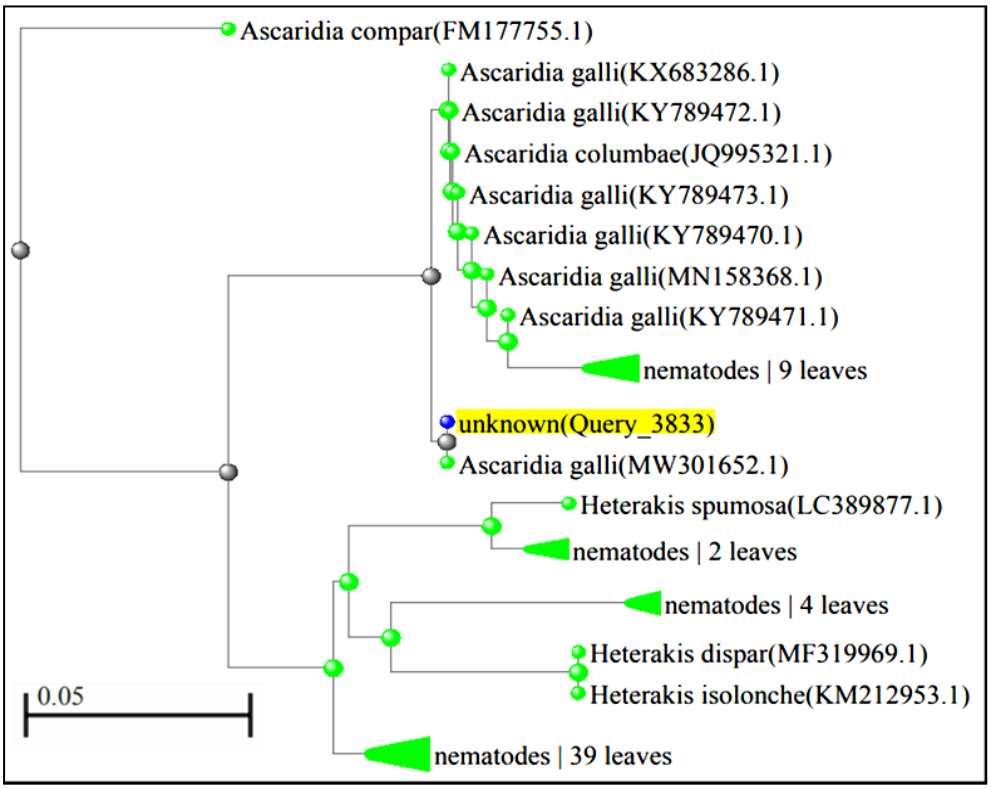


Figure 5.10- Phylogenetic relationships of *A. galli* (MW301652.1) by the neighbour joining using the BLAST programme

Table 5.4- DNA Quantification of *Heterakis gallinarum* by Spectrophotometer:

Sample	Absorbance at 260nm (A260)	Absorbance at 280nm (A280)	StockDNA concentration (µg/ml)	Absorbance ratio 260/280
Heterakis gallinarum	**0.459**	**0.263**	**2.295**	**1.745**

5.3.3 Results of PCR

The amplified genomic DNA of *H. gallinarum* was positive for ITS-1, 5.8S, ITS-2 regions with the specific primer at ~1000 bps by using PCR (Fig. 5.11).

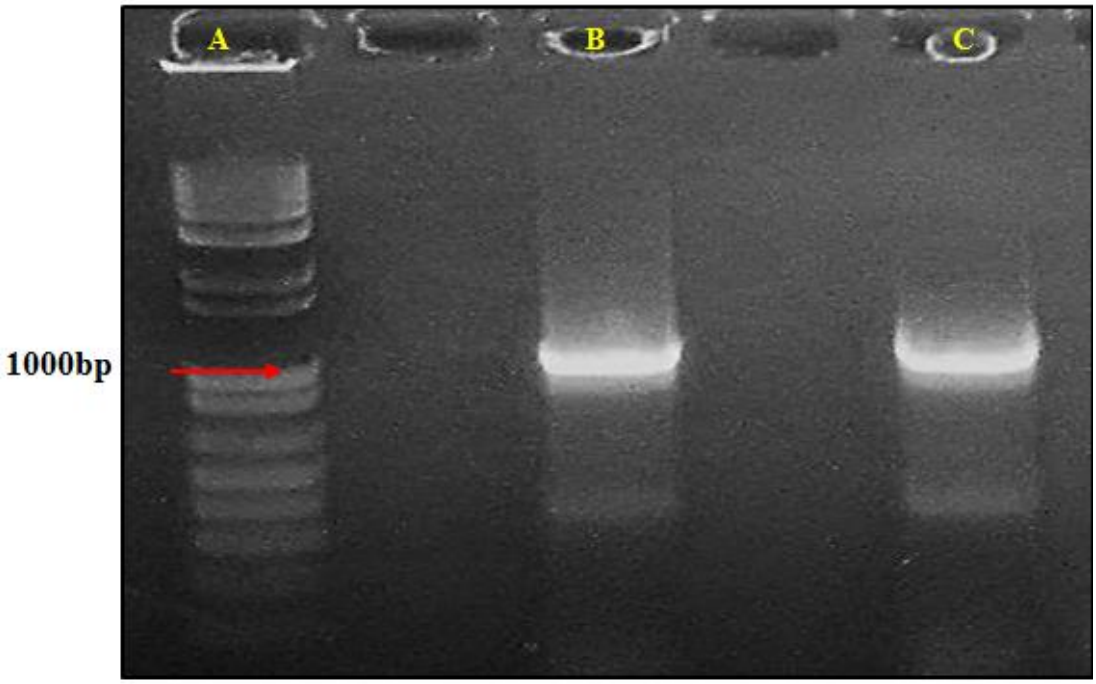


Figure 5.11- PCR product of *H. gallinarum* was positive (Lanes B, C) with Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder (Lane A) at ~1000 bps.

5.3.4 Sequencing-

Finally, sequencing was made to the identified PCR product by using primer on GATC Company by direct sequencing with an automated DNA sequencer and (ABI 3730XL; Applied Biosystems, Foster City, CA, USA) (Fig. 5.12).

> MW661165 *Heterakis gallinarum* isolate Hetrakid worm internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

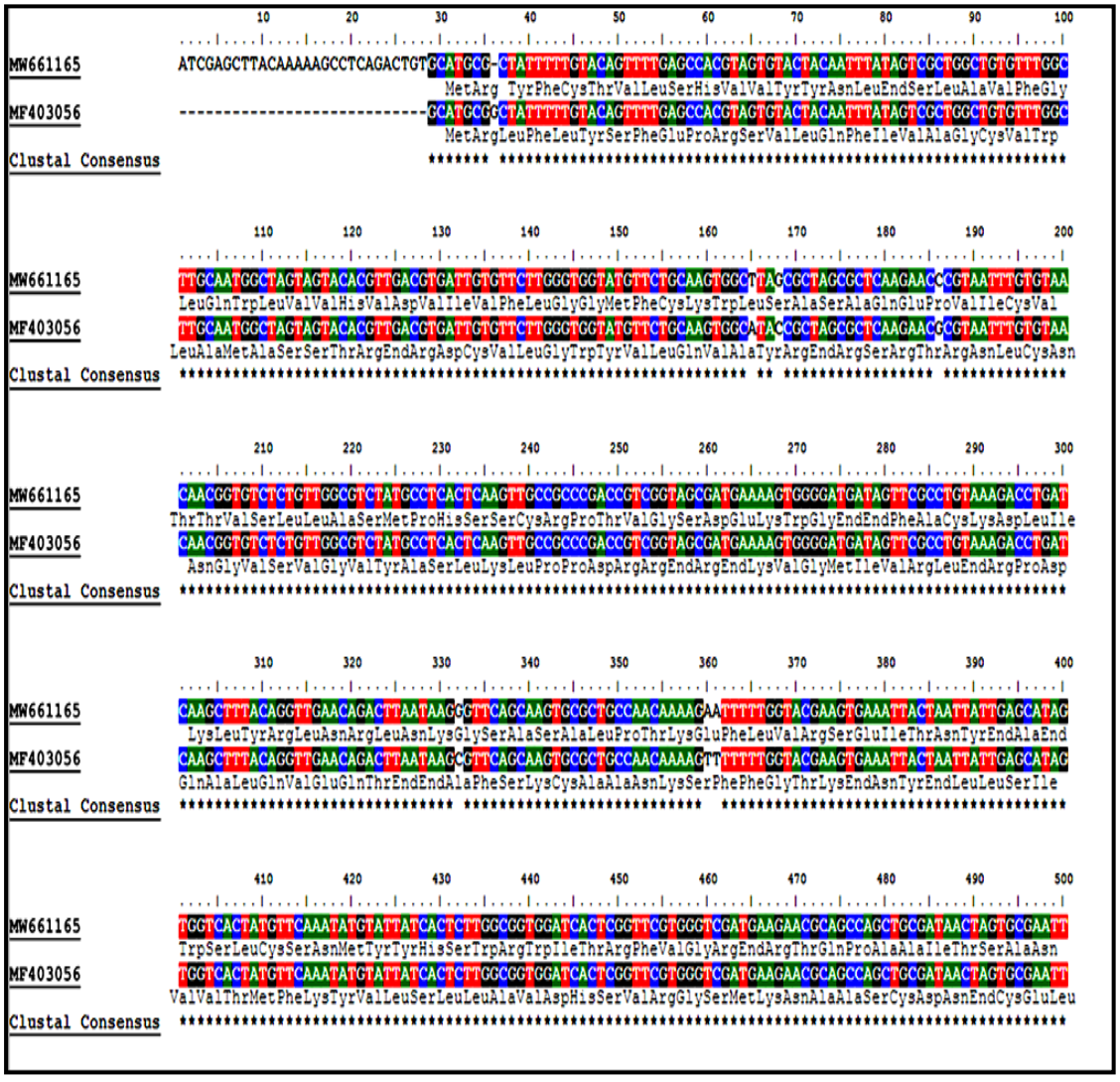
ATCGAGCTTACAAAAAGCCTCAGACTGTGCATGCGCTATTTTTGTACAGTTTTGAGCCA
CGTAGTGTACTACAATTTATAGTCGCTGGCTGTGTTTGGCTTGCAATGGCTAGTAGTAC
ACGTTGACGTGATTGTGTTCTTGGGTGGTATGTTCTGCAAGTGGCTTAGCGCTAGCGCT
CAAGAACCCGTAATTTGTGTAACAACGGTGTCTCTGTTGGCGTCTATGCCTCACTCAAG
TTGCCGCCCGACCGTCGGTAGCGATGAAAAGTGGGGATGATAGTTCGCCTGTAAAGAC
CTGATCAAGCTTTACAGGTTGAACAGACTTAATAAGGGTTCAGCAAGTGCGCTGCCAAC
AAAAGAATTTTTGGTACGAAGTGAAATTACTAATTATTGAGCATAGTGGTCACTATGTT
CAAATATGTATTATCACTCTTGGCGGTGGATCACTCGGTTCGTGGGTCGATGAAGAACG
CAGCCAGCTGCGATAACTAGTGCGAATTGCAGACACATTGAGCACTAAAATTTTGATCG
CAAATTGCGCCGTCGGGTTCTTTCCCAACGGCACGTCTGGCTGAGGGTCGATATTTTAT
ACTGCTGCTCATTCATTGCGCTTGCGTTGGCTATTTGACGTATGCGCATGCTAGAAATTA
GAAAAGTATTTGAGTGCTCACGTTGCACTACTTGATTAAAGGACTTTTGCAATCGCTAG
TTTTGCAATGGTTCTTTTGATTGGGTACTGCAGCTAGTGTTAATTTGAGTTTGCATAACA
GCCCAAATATCGTGCTAGCTGATAAGCTGGGTGTGATATGCGAATTGTTACATCTCGAT
TCAATACCATACGCGCTAATGGGATGTGTATAGCTCCTAATGTGTTAAATGCAAGAAAC
ATTTTGGAGCTTATAACGCTAGAATACATCTTTTGCGCGTATGCGCATCTGTGTTGAAC
GCGTGTAACAATGATGCGACTGTATCTGTGTTGAACGCGTGTAACAATGATCGGAT

Figure 5.12- Sequence generated after sequencing of PCR product by primer of *H. gallinarum*.

5.3.5 Gene sequences and phylogenetic analysis-

The gene sequences obtained from *H. gallinarum* (MW661165) samples from Lucknow were analysed in comparison with a gene sequence of *H. gallinarum* accession number (MF403056) using the BioEdit software version 7 computer

programme (Fig. 5.13). Also, the MEGA4 software was used to estimate the identity and variability of sequences (Tamura *et al.*, 2007). It shows that the sequence was greatly identical to each other (98.62%). Nucleotide sequences homology was aligned using the nucleotide sequence search in the National Center for Biotechnology Information (NCBI) Gen Bank database using the Basic Local Alignment Search Tool (BLAST) programme. Also from the BLAST programme, a tree view for the resulted nucleotide sequence on the database by neighbour joining method was made. The BLAST programme on NCBI GenBank database was used with the other nematode species showing that it was similar to *H. gallinarum* ITS-1, 5.8S, ITS-2 isolates with maximum homology (Fig. 5.14)



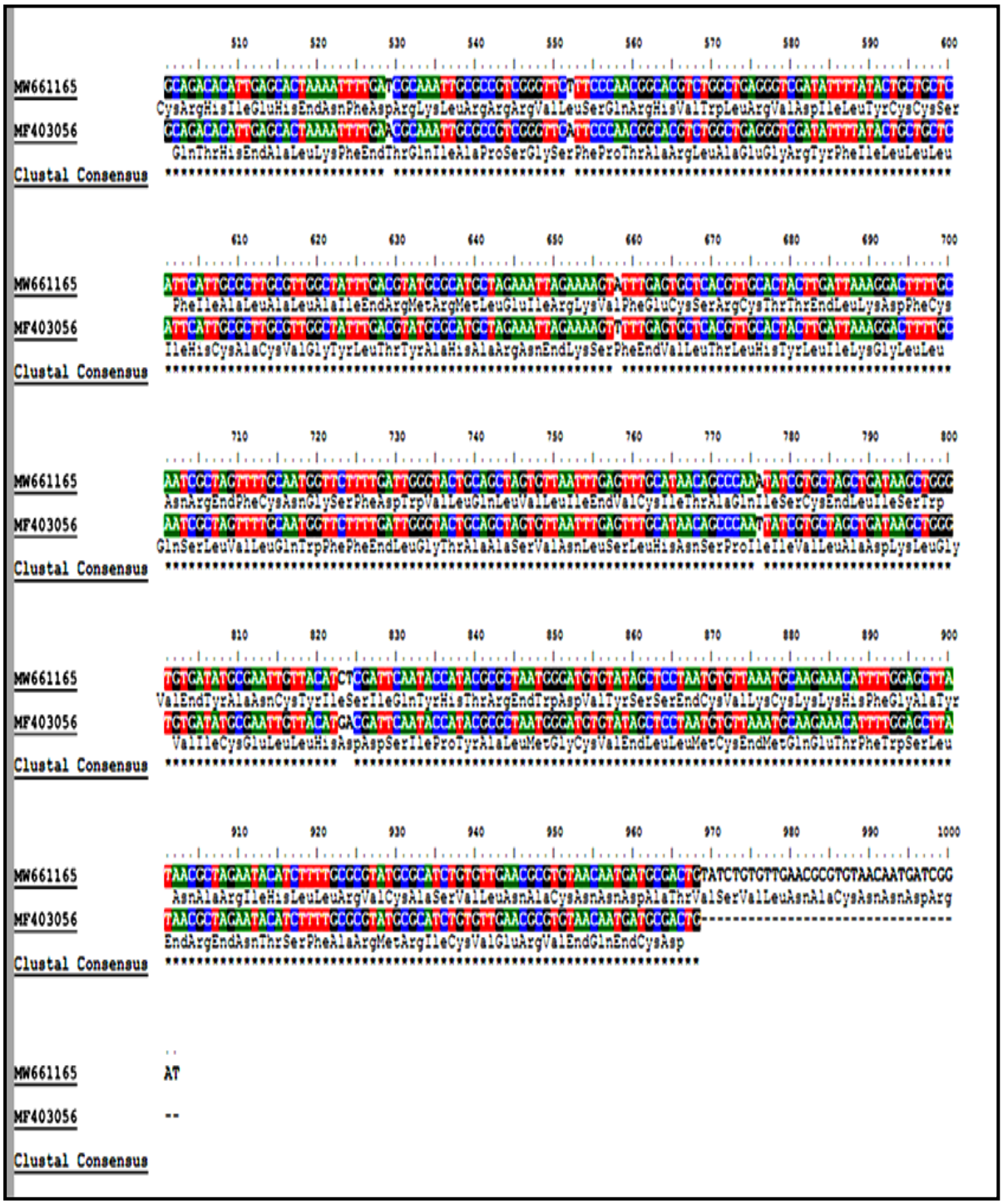


Figure 5.13- A comparison of the sequences of *H. gallinarum* (MW661165) with another one (reference of accession number, MF403056)

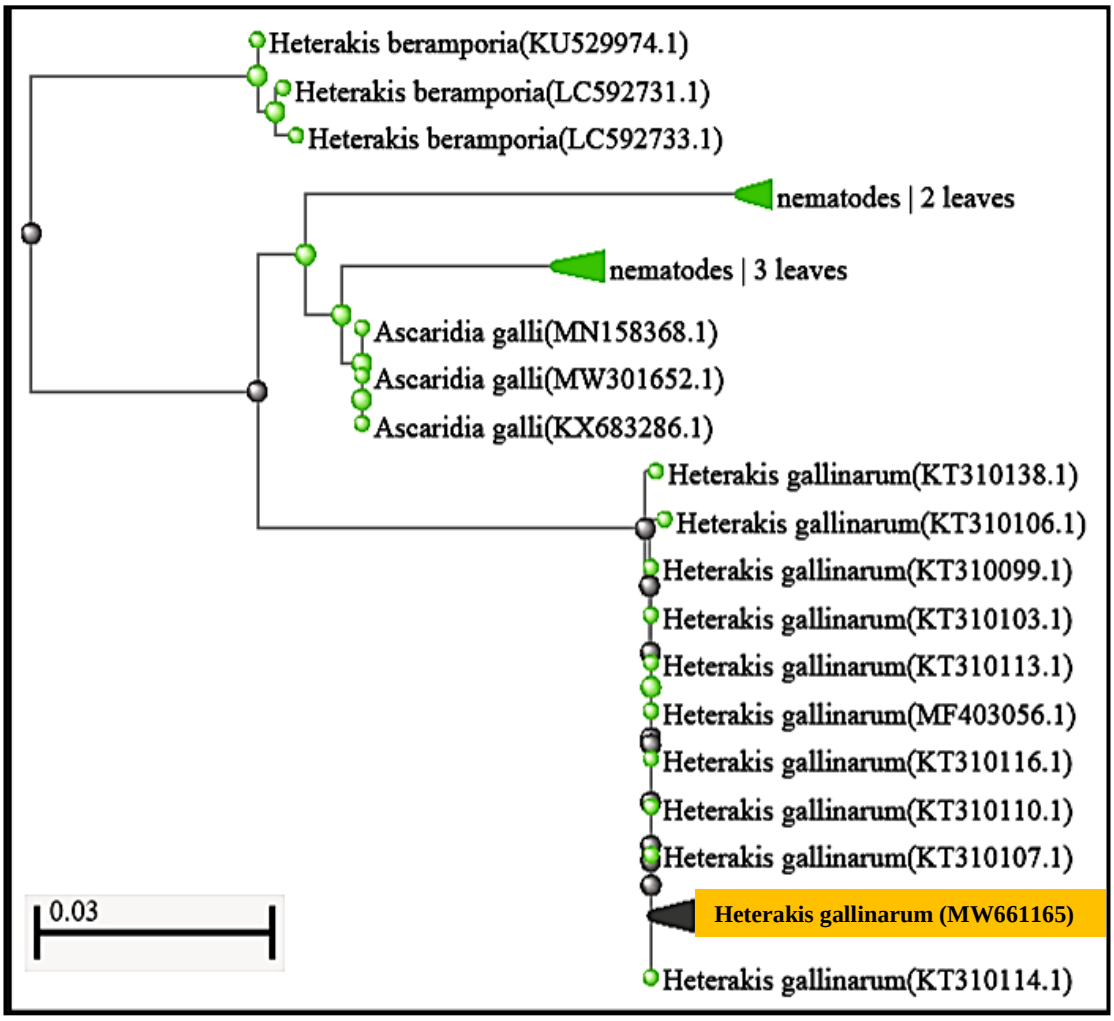


Figure 5.14- Phylogenetic relationships of *H. gallinarum* (MW661165) by the neighbour joining.

5.4 DISCUSSION-

H. gallinarum and *A. galli* are the most common round worms which infect the domestic chicken, leading to severe problems. Programmes of mass drug administration minimise the frequency and severity of infections. They may also, however, induce selection pressure on the parasites to develop resistance. Several studies have shown that the widespread and repeated use of anthelmintic drugs increases the resistance in nematodes (Wolstenholme *et al.*, 2004; Coles *et al.*, 1995). According to Lee *et al.*, 2008 genome analysis of *A. galli* and *H. gallinarum* will

identify such genes whose inactivation by the drugs will kill the parasites but did not harm the host because end parasites can not survive outside the host (Lee *et al.,* 2008) and this analysis has effective targets for nematocides (Campbell *et al.,* 2011).

During present study SDS based Phenol–Chloroform Isoamyl extraction method was used for genomic DNA extraction. By manual method good band of genomic DNA of nematodes (*A. galli, H. gallinarum*) was obtained as compared to DNA extraction kit. Spectrophotometer analysis was done to check quantity and quality of DNA sample for further analysis. The O.D ratio of 260/280 was used to check the purity of genomic DNA. The ratio between ~1.7 -1.8 indicated that UV absorbance was due to nucleic acid. The quantified DNA samples of *A. galli* (1.708) and *H. gallinarum* (1.74) were suggesting the above fact.

In the present study, PCR was used for amplification of the nuclear DNA which helped in the adequate DNA yield for the sequencing reactions. The PCR technique has significant implications for the diagnosis of parasite infections. As stated by Zhu *et al.*, 1998, PCR provides a high-resolution mode for the analysis of variation in the sequenes. The reported species specific primer of *A. galli* and *H. gallinarum* annealed PCR product of band size 1000bps, 1001bps respectively. Though, Bazh 2013 reported 818 bps in *A. galli* and 914bps in *H. gallinarum* with the same primer. Obtained sequences was submitted in gene bank as internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.

The variation between species occurs with in ITS region. Lin *et al.,* 2012 stated that the ITS-1 rDNA functions as an appropriate molecular marker for the identification of nematodes in order Ascaridata and the inter-species phylogenetic analysis. The

accurate identification of different nematode species has important role for studying their life cycles, population biology, epidemiology, taxonomy as well as controlling the diseases they cause (Zhu *et al.,* 1998).

During the study analyzed ITS-1, 5.8S, ITS-2rRNA sequences of *A. galli* and *H. gallinarum* was similar to existing sequences in GeneBank database. The phylogenetic tree based on these sequences shows a close relationship between Ascarids parasitizing poultry, which are grouped into separated clade, and between *Heterakis* spp. isolated from domestic fowl forming second clade. Similarly, Bayesian phylogenetic analysis based on mitochondrial cytochrome c oxidase subunit 1 (cox1) gene exposed *Setaria cervi* (a round worm of Italian red deer) seems to be monophyletic with other Setaria species and sister speciesto *Setaria labiato papillosa* and *Setaria digitata* (Alasaad *et al.,* 2012). Moreover, analysis of restriction enzymes of rRNA gene complex shows that the variation among species take place in ITS region. It is possible that some intraspecific variation may occur at the spacer regions and difference may exist between the rRNA copies within an individual. *Hysterothylacium aduncum* were collected from different marine fishes of the Tunisian coasts and characterised by sequences of the first (ITS-1), the5.8S and second (ITS-2) ITS of nuclear rDNA which allows the definition of genetic markers for their unambiguous identification confirming that all the samples belong to a single species (Amor *et al.,* 2011). The ITS-1, 5.8S, ITS-2 rRNA subunits representes genetic markers for the specific identification of species. In the present study, the identity between the accession numbers (*H. gallinarum* MW561275, MF403056), (*A. galli* MW301652, MN158368) may be due to the similarity of the ribosomal ITS copies.

In the present study phylogentic analysis of *A. galli* showed a higher similarity with submitted sequences in Genbank database in Poland (Acc. No. KY789472, 99.11%; KY789470, 99.10%; KY789473, 98.98%; KY789471, 98.85%) and in China too (Acc. No.MN158368, 98.72). Amplified sequences of *Heterakis gallinarum* showed higher homology with sequences submitted in Genbank database in Poland (Acc No. MF403056, 98.62%) and in Sichuan (Acc No. KT310104, 98.46%) and in USA too (Acc No. JQ 995320, 98.41%). The differences and identity in some nucleotide sequence between the *A. galli* and *H. gallinarum* of Lucknow region and the reference species may be due to different number of primers used (Jordanova *et al.,* 2005) as well as due to the variation between individual worms or variation between the multiple ribosomal ITS copies within the genome of a single worm, and due to the geographical conditions resulting to several identity nucleotides that occurred in different points. Previous studies on nematodes demonstrated that the nucleotide variation in the ITS between species significantly increases the differences within a species.

Humbert and Cabaret, 1995 demonstrated that random amplified polymorphic DNA has been used for the identification of species and phylogenetic study of ruminant trichostrongylid nematodes as a source of markers. The interspecific distances were often larger and did not very much differ from the intraspecific ones (between 0.8 and 0.9). This was established through the use of PCR for the rapid identification of genetic variation within *H. gallinarum*. Such work paves the way for future fundamental molecular explorations genomic, and post genomic approaches provide new hope for the discovery of intervention strategies, with major implications for improving the global health (Bazh, 2013).

5.5 CONCLUSION-

- In the present chapter, molecular characterization is based on the Internal Transcribed Spacer (the spacer DNA situated between the small-subunit ribosomal RNA and large-subunit rRNA genes in the chromosome) for the evolution and classification of nematodes.
- BLAST of the nucleotides sequence has a significant similarity (99%) with *Ascaridia galli* and *Heterakis gallinarum.*
- Molecular characterization of *Ascaridia galli* and *Heterakis gallinarum* is the first attempt from Lucknow.
- The further study on molecular and epidemiological study of all helminth species of *Gallus gallus domesticus* is essential for detailed investigation.

Significance

And

Future Prospects

SIGNIFICANCE AND FUTURE PROSPECTS-

Poultry farming is the form of animal husbandry which raises domesticated birds such as chickens, ducks, turkeys and geese to produce meat or eggs for food. Poultry mostly chickens are farmed in great numbers. Chickens raised for eggs are known as layers, while chickens raised for meat are called broilers. Poultry play an important role for mankind through food supply and income generation, but a range of different parasitic infections are re-emerging with increased poultry system.

- The prevalence study of gastrointestinal nematode parasite in commercial, semi-intensive and traditionally reared chicken can be used to plan strategies against parasitic disease.
- The morphological and molecular characters will be useful in the future diagnostic and taxonomic studies. Moreover, molecular studies reveal specification of the prevalent parasites in the given area.

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Appendix

PAPERS PUBLISHED IN JOURNALS-

S. No.	Title of the paper	Authors	Journal Name/Volume (Issue)/Page Number	Year
1.	Scanning electron microscopy of *Ascaridia galli*in *Gallus gallus domesticus* in Lucknow, U.P, India	Kamal Jaiswa1, Suman Mishra, **Anjum Bee**	Indian Journal of Science and Technology 13(19): 1944-1954. https://doi.org/ 10.17485/IJST/v13i19 .102	**2020**
2.	Prevalence of Gastrointestinal Helminth Parasites in *Gallus gallus domesticus* in Lucknow, U. P, India	Kamal Jaiswa1, Suman Mishra, **Anjum Bee**	Advances in Zoology and Botany, Vol. 8, No. 5, pp. 422 - 430, 2020. DOI: 10.13189/azb.2020.08 0506.	**2020**

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RESEARCH ARTICLE

Scanning electron microscopy of *A scaridia galli* in *Gallus gallus domesticus* in Lucknow, U.P, India

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Abstract

Background/objectives: *Ascaridia galli* (Schrank) is a well-known nematode parasite of poultry birds. Heavy infection causes an economic loss to the poultry farmers. Information related to its detailed morphological and anatomical structure is inadequate. A study of ultrastructure has revealed the possibility of differentiating the nematodes. **Methodology:** *Ascaridia galli* were collected from the domestic fowl (*Gallus gallus domesticus*) from Lucknow, UP. Scanning electron microscopy was used for the detailed identification of *Ascaridia galli*. **Findings:** Scanning electron microscopy exposed that mouth was surrounded with three trilobed lips. Internal rim of each lip was surrounded with fine teeth, and sensory papillae on the outside. Annulations of cuticle occur on the body surface, which is further divided into subannuli. Posterior end of male was pointed and curved having a precloacal sucker and anus. Females had a blunt and straight tail with an anus at ventral side. Caudal papillae and the lateral caudal alae are the tiny outgrowths which surrounds the each side of posterior opening. A sclerotized ring bounded to precloacal sucker was present. These signs of *Ascaridia galli* attained in the present study could be helpful in the taxonomical studies of nematode worms. Development of control strategies is very essential by understanding the host–parasite relationships. For this purpose, identification of nematode parasite will be very useful. **Novelty/ contribution:** The present study was designed to understand the morphology of *A. galli*. Therefore, these markers could be helpful in the taxonomical status of ascaridia nematode worms in domestic fowl.

Keywords: Domestic fowl; A galli; Precloacal sucker; Trilobed lips; Caudal papillae; Scanning electron microscopy

1 Introduction

The chicken *Gallus gallus domesticus* is believed to have descended from wild Indian and South East Asian red jungle fowl[(1)]. Birds are an important part of the ecosystem

as they play vital role ecologically, medicinally, nutritionally and economically. Poultry farming is the process of raising domesticated birds such as chickens and ducks for the purpose of meat and egg for human welfare. India has 498 million poultry population with an average growth rate of 8–10% per annum. India ranks third in egg production and sixth in broiler meat production [2].

Poultry farming has become one of the most demanding forms of animal husbandry activities. It has developed enormously, throughout the world in recent years. Most affordable sources of high protein are eggs and poultry meat which are popularly included in the diet. Poultry meat and eggs compensate the increasing demands of consumer for livestock products in the developing countries [3]. Poultry meat and egg production is one of the fastest growing livestock industries. An average growth of 8% per annum and approximately US $7,500 million annual turnover was estimated by the poultry industry [4]. Though the impact of parasitic diseases in farm birds, reared on cage systems have diminished due to modernization in poultry farming and bio security measures, but farm birds maintained on deep litter system and backyard free ranging birds still remain susceptible to parasitic infection via litter droppings and scavenging habits. The domestic chicken feeds on a wide range of food substances. This ranged from grains, fruits and insects which may harbour infective stages of parasites thereby predisposing them to parasites particularly gastro-intestinal parasites [5,6]. Poultry production is persistently hampered by helminthic infections. Ascarid worms can be categorized as follows; phylum Nematoda; Class Secernentia; order Ascaridida; family Ascaridiidae.

Nematodes represent phylum Nematoda and are invertebrate round worms found in marine, freshwater, and terrestrial environments. There are large number of species of nematodes present in the gastrointestinal tract of poultry and can cause damage to the poultry birds. These parasites are cylindrical, elongated in shape with un-segmented body. They are covered with the cuticle and have a well-developed alimentary tract. Most species of nematodes are bisexual [7]. In terms of size, they appear small in size, inconspicuous and seemingly unimportant to humans. However, some nematodes can cause diseases of great importance to humans, domestic and wild plants and animals [8], whereas some are beneficial in attacking insect pests, mostly sterilizing, or otherwise debilitating their hosts [9]. Gerald and Larry stated in 1996 that non parasitic nematodes can find their path into a vertebrate host and become short lived and pathogenic [8]. Bilateral symmetry occurs in most of the nematode species [10]. According to Lee, single testis occurs in male nematode and ovarian tubules are present in females. Several species of nematodes are oviparous, but some are viviparous or ovoviviparous. Adult stage occurs after four larval stage moults. Female are larger in size as compared to male [11]. Six stages occur in nematodes life cycle; egg, four juvenile stages and adult [12].

Normally elastic and tough cuticle occurs in nematodes in contrast to cestodes. Generally cuticle is smooth, scaled or scattered with bosses, seldom spined, and transversely, longitudinally or not often obliquely striated [13]. Nematodes body wall contains cuticle, hypodermis, and body wall musculature. Cuticle is the outermost covering and has a great functional and structural significance to the animals. Excretory pore, vagina, proctodeum, and stomodeum are lined by cuticle [14]. The cuticle is originated from hypodermis, an underlying sub-cuticle layer. Longitudinal lines are formed by cuticle, situated dorsally, ventrally and laterally. Longitudinal canals of the excretory system occur in the lateral lines. Muscular layer lines the body cavity, consists of a transversely striated cells having a basal contractile portion, and a cytoplasmic portion which contains the nucleus. Longitudinal lines divide this muscular layer into four quadrants [15].

Ascaridia galli (Schrank, 1788) is the member of the genus *Ascaridia Dujardin*, 1845, and a major intestinal helminth parasite which cause health problems in guinea fowl, geese, turkey and to a several wild birds; the main host is chicken [16,17]. The adult form of *A.galli* resides in the lumen of the small intestine of *Gallus gallus domesticus*, which feeds mainly ingesta of host. *Ascaridia galli* (Shrank, 1788) functions as a vector for the transmission of Salmonella enteric in poultry. Thus ascaridia are responsible for major problems and cause economic losses to poultry specially in free-range and floor production systems [18,19] by causing poor growth rate and weight loss of fowl [20,21] and increased mortality due to secondary infections [22,23]. It also induces damage to the intestinal mucosa, leading to blood loss [24], partial or complete obstruction of the intestine.

Heavy infection may be responsible for diarrhoea, droopiness of wings, bleaching of the head and emaciation. A heavy infection causes diarrhoea which is responsible for anaemia and intestinal obstruction [25]. Efficiency of feed utilization becomes reduced in the primary damage, but death occurs in heavy infection. Common symptoms in broiler chickens are weight loss, and reduced egg production. High prevalence of infection is found in young birds and higher resistance against these parasites shows by the heavier breeds than the lighter one such as white minorcas and leghorns [26]. Transmission of infection can be very fast because of the direct life cycle of nematode and the environmental resistance of its eggs [27]. Absorption of nutrients and enzyme systems in the intestinal mucosa is adversely affected by the toxins of *A. galli* [28]. Significant behavioural changes caused by the Ascaridos infected birds, showed lower locomotion activity during the patent and prepatent periods [29]. All these factors are responsible for the mortality and losses of the flock due to disease outbreak in backyard (traditional) poultry production system. The aim of the present study is to determine a highly reliable and finer detail of ultra-structure of *Ascaridia galli* by using scanning electron microscopy.

2 Materials and methods

2.1 Study area

The study was conducted in and around Lucknow, standing at an elevation of approximately 123 meters (404 ft) above sea level. Lucknow district covers an area of 2,528 square kilometres (976 sq mi)[30,31]. Bounded on the east by Barabanki, on the west by Unnao, on the south by Raebareli and in the north by Sitapur, Lucknow sits on the northwestern shore of the Gomti River. This city has a humid subtropical climate with cool, dry winters from mid-November to February and dry, hot summers with thunderstorms from late March to June. The rainy season is from July to September when the city gets an average rainfall of 896.2 millimetres (35.28 in) from the south-west monsoon winds, and occasionally frontal rainfall will occur in January. In winter, the maximum temperature is around 25 °C (77 °F) and the minimum is 3 °C (37 °F) to 7 °C (45 °F) range[32].

2.2 Study Population

Study population includes domestic chickens managed under unorganised backyard systems. The age of the studied fowl was determined by asking the owners.

2.3 Study period

Study was conducted during January 2017 to December 2019 in and around Lucknow (U.P) to determine the finer details of gastrointestinal nematode parasites (*Ascaridia galli*) of domestic fowl.

2.4 Parasitological examination

During the present study the gastrointestinal tract (GI) of domestic fowls were collected from January 2017 to December 2019 from different regions of study sites. The GI tracts were tied at both the end (to prevent the leakage of internal material) and kept in the polythene bags and brought to the Parasitology Laboratory of Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow for the parasitological examination and dissect thoroughly to investigate the presence of parasites, according to the procedure as described by Cable RM 1958[33].

2.5 Preservation

Nematodes were collected from host with the help of forceps, washed in saline water and killed in hot 70% alcohol, and stored in the glycerine alcohol solution.

2.6 Scanning Electron Microscopy (SEM

Parasites were fixed in 2.5% glutraldehyde (v/v) in 0.1M phosphate buffer pH 7.3 for 3-5 hours and then washed in a phosphate buffer prior to post fixation in 1% osmium tetra oxide in 0.1 M-phosphate buffer for 24 hours followed by washing in the same buffers. SEM samples were dehydrated by immersing for 15 min each in fresh solution of 30%, 50%, 70%, 95% and 100% acetone and dried till the critical point. The dried samples were mounted on specimen's stubs using a double side adhesive tape and coated with gold. Coated samples were visualised under a JSM-6490LV scanning microscope at 15 KV, measured and photographed[34].

3 Results

Ascaridia galli had yellow-whitish, slightly semi-transparent, elongated and cylindrical body with tapering ends at both sides. The whole body was covered by a tough proteinaceous cuticle. Mouth is triangular, opened at the extreme anterior end [Figures 1 and 2]. Continuous ridge along the longitudinal axis marked the dorso-ventral margin of the body. Three trilobed lips surround the mouth and anchored with each other with smooth cuticle [Figures 1 and 2]. Two types of lips are found, one is mid-dorsal which is broadly elliptical and two latero-ventral oval lips. Lips function as mechanical organs to ingest food materials. Three distinct lobes are present in each lip, one median lobe in center, and two lateral lobes at the sides. A cup like structure is formed by the lobes. A single dentigerous ridge in the inner surface and minute denticles in a single line make up the median lobe of each lateral lip [Figure 3]. Prominent cuticular protruberance was present on the external surface of the cuticle of the latero-ventral lip known as labial papilla. These characteristic features of lips may be helpful in the taxonomic studies.

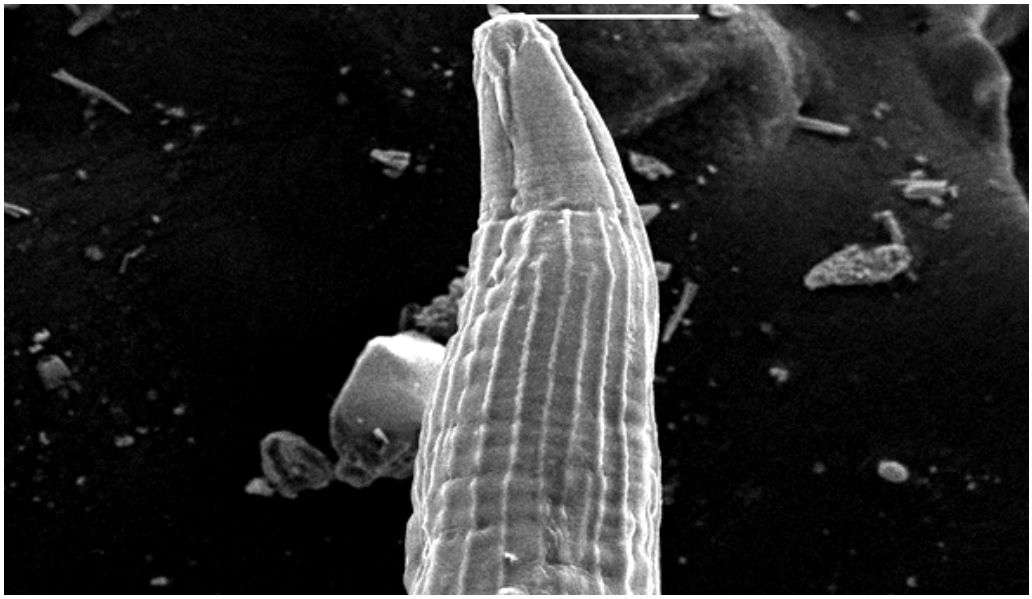

Fig 1. Scanning electron micrographs of the anterior end of an adult *A. galli*

Fig 2. Three large trilobed lips surrounded the mouth opening and smooth cuticular plate covered the inner surface of each lip and the outer surface is covered with cuticle.

A series of continuous transverse annulations occurs in cuticle with diverse striations from cephalic to posterior region of

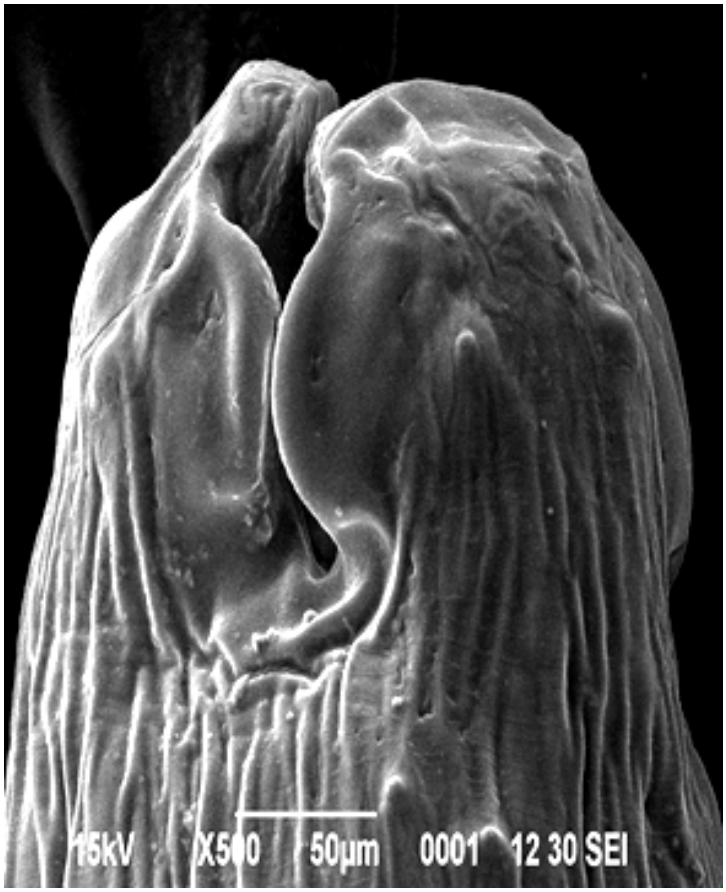

Fig 3. Enlarged view of lips showing the rim that bearsa series of denticles, and the papilla which seems eye-like oval structure.

the body [Figure 4]. Parallel concentric rings of fine transverse grooves of striations occur around the cylindrical body. A segmented appearance is given by deep transverse grooves (annulations) present at a regular interval of the body [Figure 4]. These rings are discrete grooves (ridges in reality) which are necessary for the flexibility and growth of the body. It also projects a segmented appearance to body.

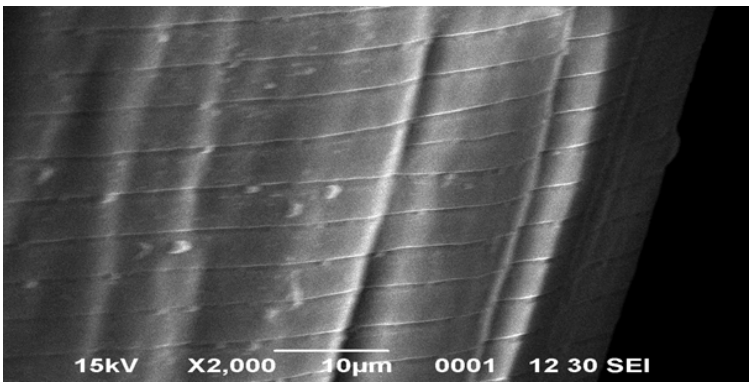

Fig 4. Middle portion of an adult *A. galli* body with fine corrugated cuticle arranged in a series of unique transverse striations named annulations, which forms the continuous ring around the body.

3.1 *Ascaridia galli* female

Sexual dimorphism in *Ascaridia galli* is as follows, females have a blunt and rounded posterior end and pre cloacal sucker and ventrally coiled tail in males. Posterior region of females have a single large anal opening just before the tip of tail with a pair of papillae just near to its tip. Female measuring about 40–66 mm in length and 0.31–0.58 mm in width at the anterior end and 1–1.63 mm at the level of vulva. Vulva is situated a little posterior to the middle of the body. Distance of vulva from the anterior end varies from 19 to 35 mm. Distance of anus from the tip of tail varies from 0.57 to 0.73 mm. Tail is straight which measures about 0.73–0.97 mm in length with blunt end.[Figures 5, 6 and 7]. Comparatively elaborated and more complex posterior end was present in male.

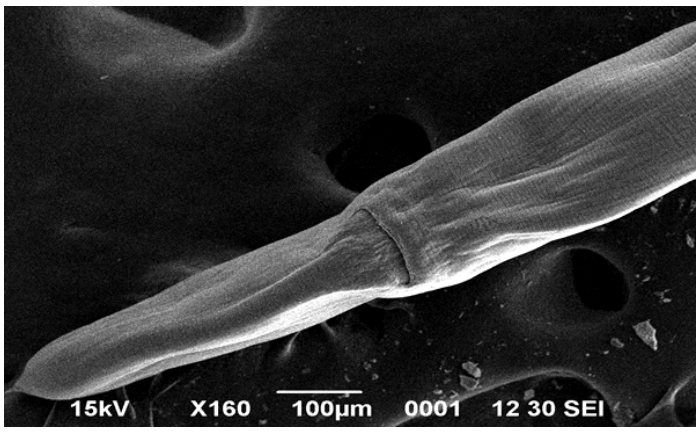

Fig 5. Scanning electron micrographs of an adult *A. galli* female with blunt and rounded posterior end and pre cloacal sucker.

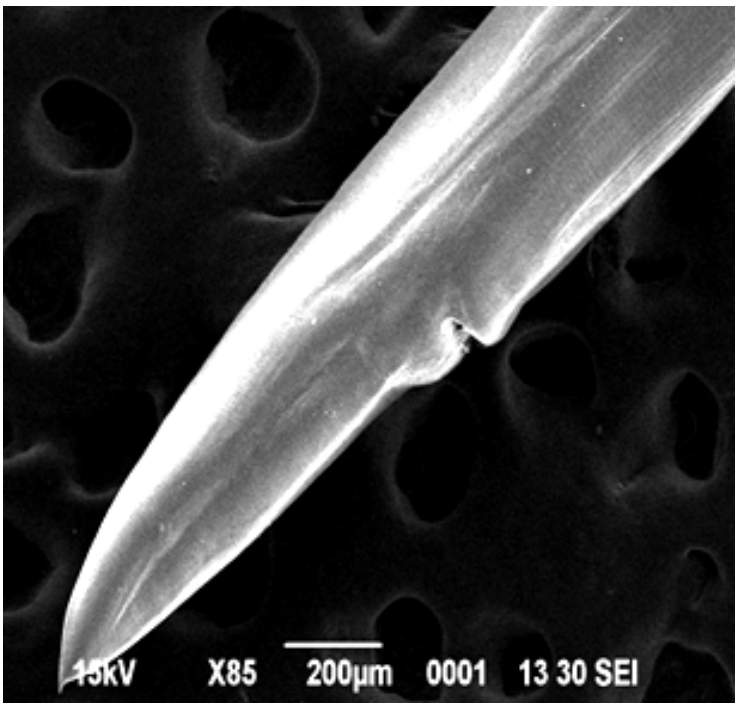

Fig 6. Single large anal opening of female *A. galli* just before the tip of tail.

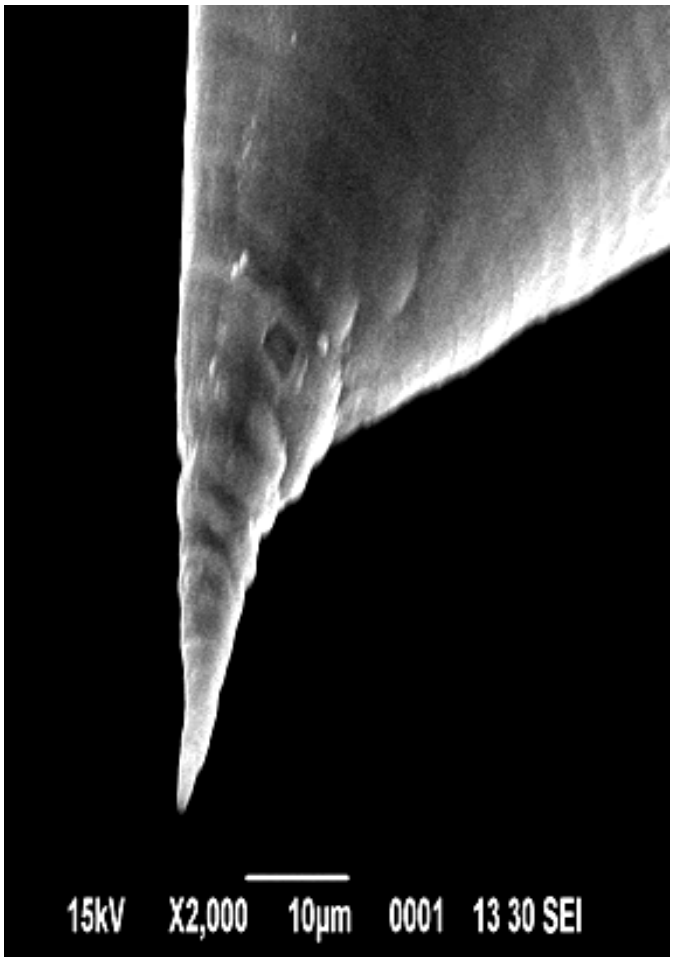

Fig 7. The end region of tail cuticle is without annuli

3.2 *Ascaridia galli* male

Males are smaller than females and more slender, measuring about 40–47 mm in length and 0.40–1.20 mm maximum in breadth. Ventral part of body composed of thickened cuticular bosses, spicula without alae, tail length is 0.49 to 0.87 mm. Two major apertures were present in male, anus located at the posterior end, and anterior to it a precloacal sucker is present [Figures 9 and 10]. Minute bulges are present on either side of preclocal and clocal opening called caudal papillae [Figures 9 and 10]. Posterior to the pre cloacal sucker cuticular vesicles and cloacal papillae are present. Sclerotized ring support the precloacal sucker and this sucker helps in the attachment during copulation [Figure 10]. Precloacal sucker is oval shaped measuring about 0.2 to 0.29 mm in length and 0.15–0.19 mm in diameter, located at the distance of 0.25–0.57 mm in front of cloaca. Present observation shows that the sucker rim is well developed and spherical ridge forms a sclerotized ring and also an oval protrusion at the centre. Distance of cloaca from the tip of tail is 0.6-0.77 mm. Ten pairs of caudal papillae are present in the male in the following four groups (i) pre anal three pairs (ii) adanal one pair (iii) postanal three pairs (iv) subterminal three pairs. Spicules are similar almost equal in size, measuring 1.65-1.9 mm in length. The distal ends of spicules normally blunt with slight notch [Figures 8 and 9]. Extreme terminal tip was pointed and slightly expanded at the base. Expanded region of caudal alae is the lateral longitudinal region around the anus, significantly extended on both sides. On the ventral side of the tail region numerous bulges or caudal papillae or phasmids were seen on the either side of the anus. These caudal papillae functions as sensory organs of the male tail. Circular protrusions were formed by the anus with a central anal opening.

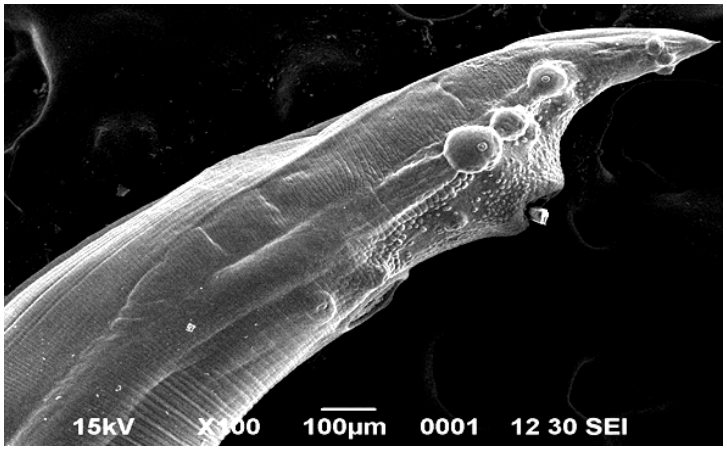

Fig 8. Scanning electron micrographs of an adult *A. galli* male with a fine pointed tail, anus and caudal papillae. Caudal papillae emerge on either side in the form of small knobe like structure.

Fig 9. *A. galli* male with a ring-like structure precloacal sucker which helps in grasping during copulation. Anal region expanded both side to form a flap-like structure caudal alae. The anal opening is surrounded with numerous small blisters like structure

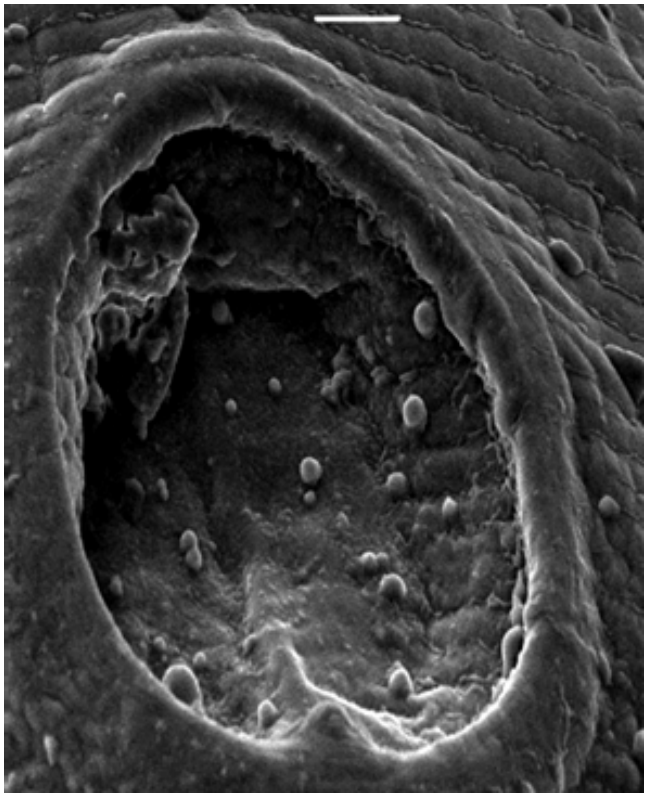

Fig 10. Enlarged view of preanal sucker bounded by adouble-walled sclerotized rim

4 Discussion

In *A. galli*, position of cephalic papillae is also an important feature. Occurrence of four papillae, wherein two occur on the dorsal lip, and other one on the both side of latero ventral lips[(35)]. In the Ascaridian species presence of three lips is one of the characteristic features, but all lips are not similar to each other. All lips are trilobed and consist of a broad median lobe edged at each side by two small lateral lobes. Major structure of the lip is composed by the median lobe, while the small extensions at the base is formed by the lateral lobes. Contrary in other species like *A. hermaphrodita* and *A. platyceri* these three lobes are almost equal in shape and size. Minute teeth or denticle in the inner surface of the medial lobe of each lip also is a characteristic feature of some species of *A. galli* like *A. australis*, *A. hermaphrodita* and *A. platyceri*[(36)]. According to Ashton 1996, outer surface of lips has three paired cephalic papillae and two amphids. Major chemosensory organ of nematodes is amphids which helps in host finding and also in controlling the development[(37)]. Subannuli in *A. galli* is first reported and explaind by the reference[(38)]. During embryonic development of *A. galli* from larvae to adult, circular or transverse annuli divide into subannuuli. Such unique cuticular organization in *A. suum* seems to be the characteristic feature of ascarids[(39,40)]. In the present and previous studies presence of the longitudinal ridge appears to form differentiation between the dorsal and ventral side which is one of the well-known observations in *A. galli*[(41,42)]. Hassanain et al., 2009 also exposed this ridge in *A. galli* by SEM, and failed to detect it as a different structure and uncertainly explained it as a median centroid[(38)].

Females are longer with straight and blunt tail end, while males are shorter than females with curved and elaborated tail end[(26)]. Kung 1949 stated that spicules and papillae at the posterior end are the recognizable characteristic features between different species[(43)]. According to Cheng 1986 sexual dimorphism among females include thick shelled eggs, oviparous, vulva near middle of the body. Different members of the phylum nematode, show a stark similarity in the exoskeleton of cuticle. Basal lamina at the interior and epicuticle to the exterior is also recognised in many cases. It covers the digestive and reproductive systems as well as body surface[(44)]. Cuticle is well criss-crossed and is made up of carbohydrates, soluble and insoluble proteins like cuticulins, collagens and lipids[(45,46)]. The cuticle is a primary target site of anthelmintic drugs[(47)].

Males have ten pairs of caudal papillae which are in different order, i.e., subterminal on ventral surface of caudal end, pre-anal, post-anal; **preanals three pairs** – first pair anterior to pre-anal sucker, second close to the first pair and at level of pre-anal sucker;

sub-terminals three pairs, first smallest of all anal pairs, present near to second pair, second and third pairs comparatively more prominent surrounded by small cuticle raised structures giving them rosette- like appearance and second pair occur laterally and third one lying ventrally on the extreme tail region was according to the references [17,21]. Well developed and equal spicules are occurred, which are enclosed in spicular sheath, and protruding out at anal opening [17,21]. Hassanain et al., 2009 stated that cervical area of the worm have small papillae. The posterior region of *A. gali* is furnished with more complex structures, distended tail with poorly developed caudal alae on either side. Preclocal sucker and normal protrusion occurs on the ventral surface, situated near the cloacal opening and bounded by poor circular rim [38]. Thickened cuticular bosses at the ventral part of rear end of body with cuticular ornamentation, spicula without alae, tail length of 0.45 to 0.80 mm, i.e. 0.96 to 1.14% of total body length, spicula length of 0.65 to 2.40 mm; and for female: tail length of 0.40 to 1.54 mm, i.e. 0.61 to 1.88% of total body length [48]. The entire generic characteristic shown by the *A. galli* as follows: generally lateral cuticular flanges present in Ascaridiinae; interlabia absent in lips; club-shaped oesophagus present without the posterior bulb. Male: a chitinous rim in the precloacal sucker; spicules are equal or subequal in shape, caudal alae narrow, relatively larger papillae; no gubernaculums was seen [13].

A. galli parasites reduced weight gain, egg production, welfare, and immunity as well as blockade and damage of the intestinal tract in hens when high worm burdens are present but no major changes were seen in blood variables or behavioural activities [49]. High *A. galli* worm burden lowers stored energy reserves such as liver lipids in laying hens as compared to uninfected hens [50]. These energy reserves are used by the infected hens to maintain the production at the time of infection. According to some studies, neither artificial nor natural infection of *A. galli* was found to influence external and internal egg quality, regardless of infection intensity [50,51].

5 Conclusion

The present study of an ultra-structure of gastrointestinal parasite *A. galli* in desi fowls, suggests the taxonomical status, ways and means to formulate the appropriate strategies as one of the control measures for getting the maximum benefit by rearing of backyard chickens in rural areas.

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Prevalence of Gastrointestinal Helminth Parasites in *Gallus gallus domesticus* in Lucknow, U. P, India

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Abstract Poultry industry occupies an important position in the provision of animal protein and plays a vital role in the national economy. Helminthiasis caused by helminth parasites is one of the most common infections affecting health of poultry and indirectly leading to great economic loss among small livestock holders. The prevalence of gastrointestinal helminth parasites in *Gallus gallus domesticus* was studied from January 2017 to December 2019 in the parasitology laboratory of Zoology Department, Babasaheb Bhimrao Ambedkar University, Lucknow, India. In this study, a total of 557 domestic fowls were examined to identify the different types of gastrointestinal helminth infections. During regular examination of helminth parasites, the highest prevalence was observed for *Ascaridia galli* (41.7%), followed by *Cotugnia diagnopora* (17.6%), *Raillietina tetragona* (11%), *Heterakis gallinarum* (7.4%) and *Raillietina cesticillus* (6.64%). In the monsoon season, prevalence was found to be higher than the summer and winter. Females were found to be more infected than the males. There was no trematode infection detected during the study period. The gross pathological lesions were observed in case of *Heterakis gallinarum* infection. The results of this study suggest that both nematodes and cestodes are prevalent in domestic fowls in the studied area.

Keywords Helminths, Gastrointestinal, Domestic Fowl, Monsoon, Gross Pathology, Prevalence

1. Introduction

The chicken *Gallus gallus domesticus* is believed to have descended from the wild Indian and South East Asian red jungle fowl [1]. Birds are an important part of the ecosystem as they play vital role in ecological, medicinal, nutritional and economical point of view. Poultry farming is the process of raising domesticated birds such as chickens and ducks for the purpose of producing meat or egg for food. India has 498 million poultry population with an average growth rate of 8–10% per annum. India ranks third in egg production and sixth in broiler meat production [2].

Poultry farming developed enormously in recent years and has become one of the most demanding forms of animal husbandry activities. Though the impact of parasitic diseases in farm birds, reared on cage systems have diminished due to modernization in poultry farming and bio security measures, farm birds maintained on deep litter system and backyard free ranging birds still remain susceptible to parasitic infection via litter droppings and scavenging habits. The domestic chicken feeds on a wide range of food substances. This range from grains, fruits to insects which may harbour infective stages of parasites thereby predisposing them to parasites particularly gastro-intestinal parasites [3,4]. These parasitic infections may cause considerable damage and great economic loss to the poultry industry due to malnutrition, decreased feed conversion ratio, weight loss, lowered egg production and death in young birds. Improved poultry management practices are responsible for the reduction in incidence of

parasitic infections.

Helminths constitute the most important group of gastrointestinal parasites of fowl both in a number of species and extent of damage they cause; the main genera of nematode responsible for infection in fowl include *Capillaria*, *Heterakis*, and *Ascaridia* [5] additionally, cestodes of significant importance are of the two genera *Railletina* and *Hymenolepis*. The trematodes infection is not very common in domestic fowl. In villages free range management system is used to raise domestic fowl with little or no supplementary feeding and without any veterinary care thereby exposing them to parasitic infection [6]. Parasitism ranks high among factors that serve as a threat to chickens, the presence of a few parasites does not usually cause a problem; however, a large number can have a devastating effect on growth, egg production, and overall health. The Helminths are the most important group of parasites that affect the chickens both in terms of number and extent of damage caused to the gastrointestinal tract in the chickens.

So, keeping in view the importance of these parasites in chickens, this study undertaken to find out the prevalence of gastrointestinal helminth parasites of the chickens (*Gallus gallus domesticus)* especially in the Lucknow, Uttar Pradesh, India, so that treatment strategy can be made accordingly and to provide guidelines in adopting the preventive measures to control the parasitic infection.

2. Materials and Methods

2.1. Study Area

The study was conducted in and around Lucknow, stands at an elevation of approximately 123 meters (404 ft) above sea level. Lucknow district covers an area of 2,528 square kilometres (976 sq mi) [7,8]. Bounded on the east by Barabanki, on the west by Unnao, on the south by Raebareli and in the north by Sitapur, Lucknow sits on the northwestern shore of the Gomti River. This city has a humid subtropical climate with cool, dry winters from mid-November to February and dry, hot summers with thunderstorms from late March to June. The rainy season is from July to September when the city gets an average rainfall of 896.2 millimetres (35.28 inches) from the south-west monsoon winds, and occasionally frontal rainfall will occur in January. In winter the maximum temperature is around 25 ℃ (77 ℉) and the minimum is in the range of 3 ℃ (37 ℉) to 7 ℃ (45 ℉) [9].

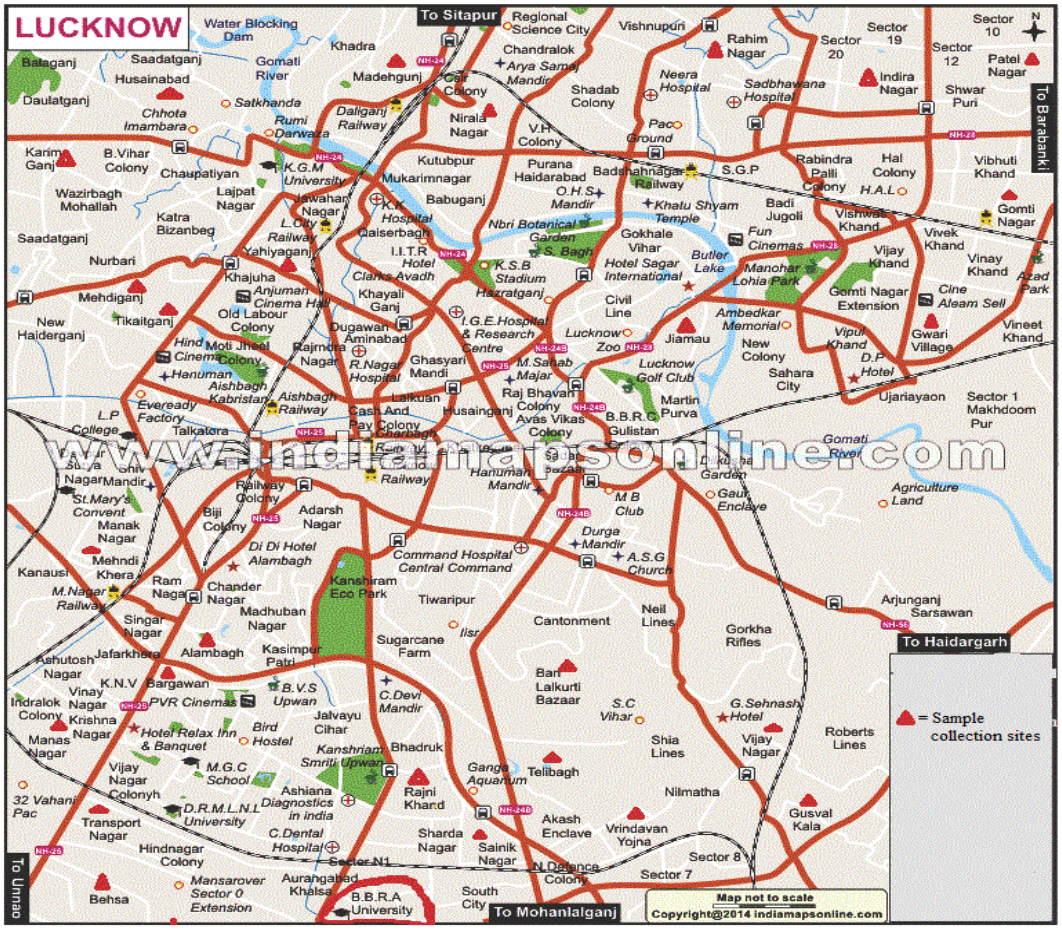


Figure 1. Map of study area showing different sample collection sites [10]

2.2. Study Population

Study population includes 557 domestic chickens managed under unorganized backyard systems. The age of the studied birds was determined through information collected from owners. Growers were 12-24 weeks old and adult aged 32 weeks were collected during study period. Chickens that were old enough to fend for themselves called as growers but had not started reproducing, while adult included cocks that were mating and hens that had at least one clutch of chicks.

2.3. Study Period

Study was conducted during January 2017 to December 2019 in and around the Lucknow to determine the prevalence of gastrointestinal helminth parasites of domestic chicken.

2.4. Parasitological Examination

During the present study, the domestic fowls were collected from January 2017 to December 2019 from different regions of study sites. The hosts were then brought to the parasitology laboratory of Department of Zoology, Babasaheb Bhimrao Ambedkar University Lucknow for the parasitic examination. For the collection of endoparasites the gastro intestinal tracts of *Gallus gallus domesticus* were dissected thoroughly to investigate the presence of parasites, according to the procedure as described by Fowler [11].

2.5. Preservation

Nematodes were collected from the gastrointestinal tract of host with the help of forceps, washed in saline water and killed in hot 70% alcohol, and stored in the glycerine alcohol solution and thick parasites kept in lectophenol. Cestodes were also collected from the same host and preserved in Carnoy's fluid for the identification. Morphology of cestodes was studied by preparing a permanent slide according to the methods as described by Cable [12]. Parasites were observed under light bright field microscope (10X, 40X, 100X), and photographs were taken by Evos XL imaging microscope. Parasites were identified according to the keys and description given by Soulsby [13]. The prevalence of Helminthiasis was recorded as per formulae described by Margolis et al. [14].

2.6. Definitions

The ecological terms used in this study are-

$$\text{Prevalence} = \frac{\text{Total number of hosts infected}}{\text{Total number of hosts examined}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{Total number of Parasites}}{\text{Total number of host infection}}$$

$$\text{Relative Density or Abundance} = \frac{\text{Total number of Parasites}}{\text{Total number of hosts examined}}$$

2.7. Data Analysis

The most common measurements of parasite population levels in hosts are prevalence, mean intensity and mean abundance [15]. Prevalence refers to the percentage of organisms infected by a particular species of parasite. Mean intensity is the number of parasites of a given species per infected host. Mean abundance refers to the number of parasites of a given species per host examined, infected and uninfected. The nomenclature used to define ecological parameters is in consistency with that of Margolis et al. [14]. The information obtained from laboratory test and observation was entered on the IBM SPSS version 20. Chi-square ($\chi 2$) test was used to analyse the sample data. Chi-square test was used to assess whether there is a statistically significant difference in gastrointestinal parasitic infection between season, gender and age. A statistically significant association between variables was considered to exist if the calculated p-value is less than 0.05 with 95% confidence level.

3. Results

3.1. Prevalence of Helminth Infection

During the present study, different helminth parasites belonging to two classes; cestoda and nematodes were observed. A total of 557 specimens of fowls were examined during the present study, which revealed 45.96% (256/557) of infection by helminths in the study area (Table1). Different types of helminth parasites were recovered during the study, including two nematodes *Ascaridia galli* (41.7%) *and Heterakis gallinarum* (7.4%) and three cestodes i.e., *Cotugnia diagnopora* (17.6%), *Raillietina tetragona* (11%), *Raillietina cesticillus* (6.64%).

3.2. Seasonal Prevalence

The study showed that the prevalence of parasites in fowl was throughout the year, but the prevalence varied from season to season. The highest prevalence was observed during monsoon 65.40% (121/185) and summer 50.53% (94/186) and least in winter 22.04% (41/186). During summer 186 fowls were examined, out of which 90 (48.4%), 16 (8.6%) and 29 (15.6%), 20 (10.8%), 12 (6.5%) were found to be infected with *Ascaridia galli, Heterakis gallinarum, Cotugnia diagnopora, Raillietina tetragona Raillietina cesticillus,* respectively. Similarly, during monsoon out of 185 specimens examined, 102 (55.1%), 20

(10.8%), 45 (24.3%), 26 (14.1%) and 18 (9.7%) were infected with *Ascaridia galli, Heterakis gallinarum, Cotugnia diagnopora, Raillietina tetragona Raillietina cesticillus,* respectively. However, a lowest prevalence of these helminth parasites was observed during the winter. Out of 186 specimens examined 40 (21.5 %); 5 (2.7 %), 24 (12.9%), 15 (8.1%) and 7 (3.8%) were infected with *Ascaridia galli, Heterakis gallinarum, Cotugnia diagnopora, Raillietina tetragona Raillietina cesticillus,* respectively (Table 2 and Graph 1). *Ascaridia galli* was more prevalent in both single and multiple type of infection (Table 3). Thus, the order of prevalence in the study area was monsoon >summer >winter. The Mean Intensity (MI) of the parasite was recorded maximum for *Raillietina* spp. At the same time Relative Abundance (RA) was highest for *Ascaridia galli.*

3.3. Age-wise Prevalence

Domestic fowls of different age groups were examined. Out of 557 specimens, 268 were growing age (12-24 weeks) fowl and of which, 119 (44.4%) were infected with helminth parasites, similarly in the 289 adult fowl (32 weeks) specimens, 137 (47.40%) were infected with helminth parasites. Results clearly indicate that, there is no significant age resistance shown by the hosts against helminthic infection. Thus, the hosts of any age group may be exposed to helminthic infections with a slight resistance shown by the growing age fowl because they are kept inside the houses (Table-4).

3.4. Gender-wise Prevalence

Out of 557 specimens of *Gallus gallus domesticus* examined during the present study, 270 were females and 287 were males. A prevalence of 50.37% (136/270) was found in females and in males, 41.81% (120/287) respectively was observed during the study period. The results show that there is no marked but a slight resistance shown by males as compared to females (Table-5).

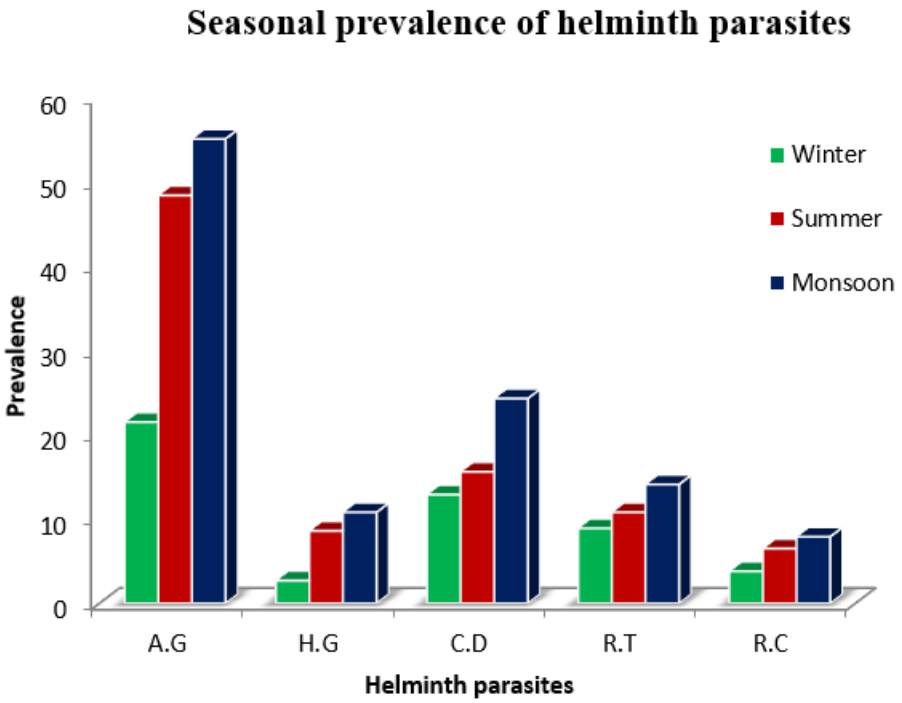
Table 1. Overall prevalence of gastro intestinal helminths in *Gallus gallus domesticus*

Total no of hosts examined	No of infected hosts	Prevalence of infection
557	256	45.96%

Table 2. Season wise prevalence of gastrointestinal helminth parasites in *Gallus gallus domesticus*

Season	Total no. of Intestines	Infected intestines	Non Infected intestines	Prevalence (%)	No. infected intestines with particular parasitic spp. (% prevalence)				
					Nematodes		Cestodes		
					A.G	H.G	C.D	R.T	R.C
Winter ()	186	41	145	22.04	40 (21.5)	5 (2.7)	24 (12.9)	15 (8.9)	7 (3.8)
Summer	186	94	92	50.53	90 (48.4)	16 (8.6)	29 (15.6)	20 (10.8)	12 (6.5)
Monsoon	185	121	64	65.05	102 (55.1)	20 (10.8)	45 (24.3)	26 (14.1)	18 (9.7)
Total	557	256	301	45.96	232 (41.7)	41 (7.3)	98 (17.6)	61 (11)	37 (6.64)
χ^2 P				72.57 0.001	48.37 0.001	9.605 0.008	9.117 0.010	3.423 0.181	5.340 0.069

(A.G-*Ascaridia galli*, **H.G-** *Heterakis gallinarum*, **C.D**- *Cotugnia diagnopora*, **R.T**- *Raillietina tetragona* **R.C**- *Raillietina cesticillus***)**



Graph-1. Seasonal prevalence of gastrointestinal helminth parasites in *Gallus gallus domesticus*

Table 3. Single and multiple species infection of helminth parasites in the gastrointestinal tract of *Gallus gallus domesticus*

	No of hosts examined	Parasitic species	No of infected intestines	Prevalence (%)
Single type	557	*Ascaridia galli*	143	25.67
		Heterakis gallinarum	40	7.1
		Cotugnia diagnopora	9	1.6
		Raillietina tetragona	6	1.0
		Raillietina cesticillus	3	0.5
Multiple type		*Ascaridia galli* + *Cotugnia diagnopora* + *Raillietina tetragona*	55	9.8
		Ascaridia galli + *Cotugnia diagnopora* + *Raillietina cesticillus*	34	6.1

Table 4. Age wise prevalence of gastrointestinal helminths infection in *Gallus gallus domesticus*

Age	Total no. of Intestine	Non infected intestines	Infected intestines	Prevalence (%)
Grower	268	149	119	44.4
Adult	289	152	137	47.40
Total	557	301	256	45.96 χ^2= 0.505 P= 0.478 P> 0.05

Table 5. Gender wise prevalence of gastrointestinal helminths infection in *Gallus gallus domesticus*

Gender	Total no of Intestine	Non infected intestines	Infected intestines	Prevalence (%)
Female	270	134	136	50.37
Male	287	167	120	41.81
Total	557	301	256	45.96 χ^2= 4.10 P= .043 P< .05

4. Discussion

During the study period overall prevalence of infection was found to be 45.96%, more or less similar to the prevalence (37.6%) as reported by Agbolade et al. [16]. Among all identified nematode parasites *A. galli* (57.31%) was the highest prevalent parasite, similar perevalence of *A. galli* is also reported by Puttalaksshamamma et al. [17]; Katoch et al. [18]: Sreedevi et al. [19]. Although mortality from *A. galli* is not significant, may lead to death of infected birds due to the obstruction of intestinal lumen Sreedevi et al. [19]. The prevalence of *H. gallinarum* (7.4%) was lower as compared to *A. galli* (41.7%) infection, but *H. gallinarum* play an important role as a carrier of protozoan parasite, *Histomonas meleagridis* which cause fatal disease in birds. But in Goromonzi District in Zimbabwe [20] and Bhubaneswar [21] regions *H. gallinarum* was the common nematode identified with 64.62% and 52.94% of infection, respectively. Whereas in Greneda [22] and Bangalore regions [17] *Raillietina tetragona* was the common

parasite than the other cestode parasites. Trematode parasites were not detected during the study period. It might be due to lack of favourable environment for the perpetuation of the vectors of trematodes. Similar finding in desi fowl were also observed earlier [23-25,17,18].

The present study shows that, single type infections of nematode were more prevalent than multiple types of infections with the cestodes. Contrary, multiple type infections with helminths in domestic fowl was observed by various researchers in the references [26-30].

Overall age-wise prevalence of endoparasites during the study period was 44.4% and 47.40% in growers and adults, respectively. No significant relationship was found between the prevalence of infection among chicks and adults ($P>0.05$). Significant differences were observed by Paul et al. [31] and Momin et al. [32] in Bangladesh and the infection was highly prevalent in adults. High prevalence of endoparasites in adult birds could be due to their gregariousness as compared to chicks, therefore, exposing them to more intermediate hosts than the former. Moreover, chicks were kept inside to protect them from predators. Contrary Dar et al. [33] and Hembram et al. [34] observed more prevalence in chicks and Molla et al. [35] observed more prevalence in growers than the adult birds.

The gender-wise prevalence of endoparasites in study area was 48.14% and 40.288% in female and male birds respectively. Females were more susceptible to endoparasites than males with a statistical significance. The results are contrary with other researchers where no statistical difference was reported [34,36,31] in backyard poultry. This could be due to the voracious feeding habits of female birds especially during egg production, then that of males which are largely selective in nature [34,36,31]. Contrary Radfar et al. [37]; Dar et al. [33]; Momin et al. [32] and Sheikh et al. [38] reported more prevalence of parasitic infections in males than female birds.

Overall seasonal prevalence of endoparasites during study period in Monsoon, summer and winter seasons was 65.05%, 50.53% and 22.04% respectively. Though the prevalence was high during rainy season, significant ($P<0.05$) relationship between the season and prevalence of endoparasites was observed in the present study. The environmental conditions of the study region are hot and humid which are favorable for development and survival of preparasitic stages of parasites and for insects, which in turn act as vectors for helminths leading to increased availability of infective stages for backyard poultry [39], especially during the process of searching the feed. Climatic conditions mainly temperature and humidity may alter the population dynamics of the parasites, resulting in variations in the prevalence and intensity of helminthic infections [23]. Significant relationship between the seasons and prevalence of gastrointestinal parasites was observed during rainy season being more favorable for the prevalence of parasites by Dube et al. [39] and Sreedevi et al. [19] in rural area of Zimbabwe and Gannavaram (Andhra Pradesh) respectively. The present findings are also in agreement with Mungube et.al [25]; Alam et al. [40] and Hembram et al. [34] who reported higher prevalence of infection during rainy season in semi-arid zone of Eastern Kenya, Bangladesh and Odisha, India respectively. Contrary, Hange et al. [41]; Solanki et al. [42] and Rehman et al. [36] reported highest prevalence of helminth infection in winter season compared to summer and rainy seasons. High prevalence of endoparasites during summer season than winter and rainy seasons in free ranging birds was reported by Paul et al. [31]; Naphade et al. [43] and Sheikh et al. [44]. The high prevalence rate of gastrointestinal parasitism in desi fowl in the present study could be attributed to the fact that the desi fowl were free ranging and had free access to infective stages in the environment and to their respective intermediate hosts like beetles, earth worms, ants etc. in search of feed as they act as intermediate hosts for helminth parasites. In the present study, nematodes *viz*., *Ascaridia galli* (41.7%) *and Heterakis gallinarum* (7.4%) and three cestodes i.e., *Cotugnia diagnopora* (17.6%), *Raillietina tetragona* (11%), *Raillietina sp.* (6.64%) were observed. In case of ascaridiosis the lumen of the intestine was filled with thick white pasty mucus, intestinal blockage due to numerous *Ascaridia galli* worms of varying sizes, thickening of intestinal wall with velvety appearance of mucosa and enteritis was noticed and the findings were in accordance with the reports of Salam [46] and Bsrat et al. [45] and increased goblet cell activity was clearly evident. However, Bsrat et al. [45] also observed diffuse haemorrhages on mucosal layer, mucoid frothy intestinal fluid, ulceration and mild enteritis with foci at different areas of intestine. In heterakiosis, the caecum revealed thickening of caecal mucosa with small slender worms in the lumen causing nodular typhlitis similar to the reports of Rabbi et al. [24]. Microscopically cross sections of parasites in the lumen along with cellular debris, infiltration of lymphocytes, heterophils and macrophages were also found [46]. The lesions recorded were similar to the observations made by Salam a [46], Salam b [47] in backyard poultry.

Chemical control of helminth parasites is simple, low-priced and can be used both therapeutically and prophylactically against helminths. Helminth parasites treated with chemicals have several drawbacks like weakening of natural immunity and presence of drug residues in food products and in environment [48,49]. Chemical anthelmintics (piperazine, albendazole, levamisole, Ivermectin, benzimidazoles and fenbendazole) can stimulate resistance, so there is need of alternative ways to control helminths [50]. There are several medicinal plants which have anthelmintic activity and slow rate of resistance. Medicinal plants which show *in vitro* anthelmintic activity include *Anacardium occidentale, Allium sativum*, *Tribulus terrestris*, *Bassia latifolia, Piper betle, Morinda citrifolia L.I, Cassia occidentalis L.* and *Aloe secundiflora*, whereas *in vivo* studies include the

usage of *Psorelia corylifolia*, *Piper betle*, *Pilostigma thonningi*, *Caesalpinia crista*, *Ocimum gratissimum* and *Anacardium occidentale,* [50]. In UK nematode parasites were treated with chenopodium oil from many years, obtained from *Chenopodiumam brosioides*. As well as, male fern *Dryopteris filix-mas* and *Artemisia spp.* plants have tendency against cestodes such as *Moniezia spp.* and nematodes, such as *Ascaridia spp*. [51]. There are several medicinal plants have good anthelmintic potential in poultry and may be a good alternative of synthetic drugs, and their use will not cause drug resistance in pathogen populations and drug residues in poultry meat.

5. Conclusions

Gastrointestinal helminth parasites were studied in domestic fowls. Only 2 genuses of nematodes and 3 genuses of cestodes were identified but trematodes were not detected during present study. Pathologically gross lesion was observed so; further studies should be conducted to know the pathology and such gastrointestinal helminth parasites. This study on prevalence of gastrointestinal parasites in desi fowls suggests ways and means to formulate the appropriate strategies as one of the control measures to get the maximum benefit by rearing of backyard poultry in rural areas. Proper anthelmintic drugs in proper dose and hygienic environment can minimize the risk of helminth infection. Economic losses per year should be estimated to explain the better control program caused by these helminth parasites.

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