

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

**SUMMARY OF THESIS**

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**SUMMARY-**

Poultry play a very important role for mankind through food supply income and employment generation but a range of different parasitic infections are re-emerging with increased poultry production system. Parasites are an organism that resides in or on another host and gets benefit from that organism. Worms are internal parasites that affect poultry. Low level of infection does not cause any harm as compared to heavy infection. Clinical signs of parasitic infection include unthriftiness, retarded growth and low feed conversion, reduced egg production and death in severe cases. Additionally, parasites can worsen an existing disease condition or flock can be more susceptible to diseases.

Helminths (Nematodes and Cestodes) are important gastrointestinal parasites of commercial poultry. Nematodes (roundworms) are most common species in number and in economic impact. Present studies show that poultry maintained under free-range conditions may be heavily parasitized. *Ascaridia galli* is one of the most common roundworm of poultry. Normally, nematodes are morphologically different and have separate sexes. Roundworms that affect domestic fowl includes large roundworms (Ascarids sp.), and cecal worms (*Heterakis gallinarum*). Large roundworms are most damaging worm of backyard flocks. They can be easily seen with the naked eye. Roundworms have a direct life cycle; in which, worm eggs are passed in the faeces of infected birds and pass directly to birds that consume contaminated feed, water, or feces. As well as, worm eggs may be taken up by snails, slugs, earthworms, grasshoppers, beetles, cockroaches, earwigs, and other insects. Known as intermediate hosts, these insects carry the eggs and when eaten by a bird pass the eggs to the bird.

Cecal worms (*Heterakis gallinarum*) are commonly found in ceca (two blind pouches at the junction of the small and large intestines) of domestic fowl. Though cecal worms are not too much devastating for chickens, but they can act as a carrier for *Histomonas meleagridis*, a species of protozoan parasite that causes histomoniasis (blackhead).

A lot of work has been done on the GI nematode parasites of domestic fowl in different cities of India but in Lucknow it is inadequate hence there is an urgent need of detailed investigation. Therefore, it is required to study the prevalence of nematodes in domestic fowl and identifying them morphologically and by molecular tools. Therefore, the present thesis has been designed to investigate nematodes morphologically and by molecular tools in *Gallus gallus domesticus*.

The first chapter of the thesis gave description on nematodes, its origin, morphology, life cycle, and occurrence of nematodes in *Gallus gallus domesticus* along with the aim and objective relevant to the theme of the proposed topic.

The second chapter includes the review of literature highlighting parasite identification, prevalence, morphological description, molecular characterization, and impact of helminths infection on their hosts, as well as nematode parasites interaction with their hosts considering the aims and objective of the proposed thesis.

The third chapter revealed prevalence of helminth parasites during the study period from January 2017 to December, 2019, in different area of Lucknow, Uttar Pradesh state of India. The study was carried out in three seasons; summer, winter and rainy season. During the study, different helminth parasites belonging to two classes; cestoda and nematoda 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. 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%). 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). Month-wise prevalence of various gastrointestinal parasites revealed that the highest incidence rate of these parasites was recorded in the month of July (72.34%) followed by August (68.88%) whereas the lowest was reported in the month of January (15.780%) followed by December (19.94%). 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 of July (MI= 45.00) and high relative abundance in the month of July (RA= 32.55).

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), *Heterakis gallinarum* (0.98). Growing age fowls were 44.4% infected, similarly adult fowls were 47.40% infected with helminth parasites. A prevalence of 50.37% (136/270) was found in females and in males, 41.81% (120/287) respectively.

In fourth chapter ultra-structure was identified by using light and scanning electron microscope. *A. galli* has three trilobed lips surround the mouth, anchored with each other and having the smooth cuticle. Two types of lips are found, one is mid-dorsal which is broadly elliptical and two latero-ventral oval lips. The esophagus is club shaped. Females have a blunt and rounded posterior end and males have pre cloacal sucker and ventrally coiled tail. 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. Tail is straight with a caudal spine. Eggs are large, oval in shape and covered by thick albuminous shells that are more resistant to desiccation and preserve for a long time in the environment. Males are smaller than females and more slender measuring about 40–47 mm in length and 0.40–1.20 mm in maximum breadth. Preanal sucker is oval shaped with chitinous rim. 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.

*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. Body length of female is 7.1–9.79 mm and 0.31–0.41 mm in width. Males measure 4.73–6.72 mm in length and 0.28–0.36 mm in diameter. 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 left spicule is significantly longer than the right, and has a tapered sharp end. The right short spicule is larger, has wing-like protrusions, its distal end is partly coarse, gradually tapered to a pointy hook, and the gubernaculum is absent. 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. The preanal sucker is easily seen, round, well-developed, surrounded by a chitinized ring.

The fifth chapter confirmed the species *Ascaridia galli*, *Heterakis gallinaum* of nematode by molecular applications (PCR). SDS based PCI method was followed for DNA extraction of these samples. DNA Quantification was done by using spectrophotometer reading O.D 260/280 nm. Reported primers have been used for Polymerase Chain Reaction. Sequences of 1000 bps, 1001 bps were generated in *Ascaridia galli* and *Heterakis gallinaum* respectively. 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. 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* (MN158368) (98.57%). 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 sixth chapter deals with the significance and future prospects of the study. Present study on nematodes focussed on prevalence and the significance of light microscopy findings for studying morphology and life cycle of GI nematode

parasites and a three dimensional comprehensible ultrastructure of nematode parasites by scanning electron microscope along with molecular study that seems essential for identifying and characterising nematodes.