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On the structure of *Ascaridia galli*, the roundworm of domestic fowl

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ABSTRACT

Ascaridia galli Schrank is by far the most notorious nematode parasite of birds including poultry fowls in terms of prevalence and pathogenicity. In spite of its immense impact on poultry industry and wildlife management, information on its detail morphological and anatomical structures is scanty. Scanning electron microscopy revealed that the extreme anterior cephalic region is a triangular mouth consisting of three prominent lips. Each lip is lined with fine teeth on the internal rim, and externally studded with eye-like sensory papillae. The body cuticle constituted series of striations called annulations. Annuli are transverse concentric rings and were divided further into parallel subannuli. Female had a simple straight tail with a ventrally located anal opening. The male posterior was curved and pointed, and relatively elaborate having a precloacal sucker in addition to the anus. These posterior openings were surrounded on both sides by a row of minute protrusions called caudal papillae and the lateral caudal alae. The precloacal sucker was surrounded by a sclerotized ring. Light microscopy showed that the cuticle was multilayered and continuous with the hypodermis, which in turn was supported with a thick musculature composed of fibrillar contractile and granular noncontractile protoplasmic portions. The body space, pseudocoel, contained digestive tract and reproductive organs such as testis, vas deferens and seminal vesicle in male, and ovaries, oviducts and uteri in female. The seminal vesicle housed spermatozoa, and the uteri, fertilized eggs. The eggs were elliptical, covered with chitinous shell that enclosed the embryo.

Key words: Anus; *Ascaridia galli*; cuticle; egg; light microscopy; muscle; nematode; reproductive organs; papillae; scanning electron microscopy.

INTRODUCTION

Poultry husbandry is by far the most common livestock sector throughout the world. Poultry meat and eggs are undoubtedly the most affordable sources in terms of high nutritive diet and universally consumed foodstuff. Rapid growth in consumer demand for livestock products in the developing countries is being met by corresponding growth in poultry meat and egg production.[1] In India alone poultry meat and egg production has been the fastest growing agricultural or livestock industry, with an average growth of 8% per annum and an estimated annual turnover of US $7,500 million.[2] There has been an increased global demand and improved systems of poultry farming due to an ever increasing number of human population. But with

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increased poultry management, a range of different parasitic infections are re-emerging, which pose serious hindrance to successful economic output.

Poultry production is relentlessly impeded by parasitic infections, of which the most persistent and most devastating is that of helminth parasites. Of the helminth parasites, *Ascaridia galli* (Schrank, 1788) Freeborn, 1923 (synonyms *A. lineata* Schneider, 1866; *A. perspicillus* Rudolphi, 1803) is inordinately the most pernicious and most prevalent among roundworms that cause debilitating health problems in poultry chickens and, resulting in poor performance, thereby, hamper poultry productivity.[3,4] Thus, ascaridiosis remains the major conundrum and cause of economic losses in poultry free-range and floor production systems.[5,6]

A. galli is an intestinal helminth parasite belonging to the group of ascarid worms (phylum Nematoda; Class Secernentia; order Ascaridida; family Ascaridiidae). Members of the genus *Ascaridia* Dujardin, 1845, are essentially parasitic in birds,[7] and most prevalent in fowl, particularly in chicken and turkey, and also in geese, guinea fowl and a number of wild birds; the principal host manifestly being the chicken.[3,8] *A. galli* is the most ubiquitous and pathogenic species,[9] and is the most significant nematode of poultry in India in terms of intensity and impact.[10] This worm is the major cause behind unremitting economic losses in poultry sector by causing reductions in growth rate and weight loss of fowl,[11,12] and induces damage to the intestinal mucosa, leading to blood loss,[13] partial or complete obstruction of the intestine, and increased mortality due to secondary infections.[14,15]

A. galli is the largest nematode in poultry. Heavily infected birds may show droopiness of wings, bleaching of the head, emaciation and diarrhea. Diarrhea may be accompanied by anemia and intestinal obstruction in very heavy infections.[16] The primary damage reduced efficiency of feed utilization, but death has been observed in severe infections. Reduced egg production and weight loss are common symptoms in broiler chickens. Young birds are most susceptible, and heavier breeds seem more resistant than the lighter breeds such as leghorns and white minorcas.[17]

Infection can spread very fast because of the nematode's direct life cycle and the environmental resistance of its eggs favour infections under poultry management systems.[18] Toxins of *A. galli* adversely influence enzyme systems in the intestinal mucosa and interfere with the normal absorption of nutrients in the intestine.[19] Ascaridosis results in significant behavioural changes as the infected birds showed a higher food intake and lower locomotion activity during the prepatent and patent periods.[20] Due to these factors the backyard (traditional) poultry production system is often characterized by low input, low output and periodic destruction of large proportion of the flock due to disease outbreaks. As a result, economic losses and animal welfare are both important issues.

In spite of its widespread occurrence known for centuries, notwithstanding its profound impact on poultry industry, the nematode has not received much attention in connection with comprehensive biology. There has been no meticulous description of the morphology of the roundworm for a long time, except Ashaur (1994),[21] who gave a sketchy remark on the general topography using scanning electron microscopy. Recent investigations on the study of anthelmintic effects on the nematode have brought about the finer details of the morphology[22,23] and the anatomy.[24,25]

MATERIALS AND METHODS

Collection of roundworms

Fresh sentient roundworms, *A. galli*, were collected from the small intestines upon necropsy of the native-bred domestic fowl, *Gallus domesticus* Linnaues, 1758 (family Phasianidae), which were procured from the local chicken vendor at Aizawl, India. The nematode was identified at the Department of Zoology, North-Eastern Hill University, Shillong, India,

as described elsewhere.[23-25] The fresh worms were collected in and thoroughly washed with 9% neutral phosphate-buffered saline (PBS). The most robust specimens of the lot were chosen for microscopic processing.

Chemicals and reagents

All the chemicals and reagents used were standard analytical grades, obtained either from Merck or S.D. Fine Chemicals Limited, Mumbai, India, except alcohol, which was supplied by Bengal Chemicals, Kolkata, India.

Scanning electron microscopy (SEM)

Some of the roundworms were fixed in 4% neutral phosphate-buffered formaldehyde at 4ºC at least for 24 h. After post fixation in 1% buffered osmium tetraoxide for 1 h, the worms were washed with PBS. Subsequent dehydration was carried out through ascending concentration of acetone up to pure acetone. Following the standardized scanning electron microscopic protocols developed by Dey *et al.*[26] and Roy and Tandon,[27] specific for helminth parasites, the specimens were treated with tetramethylsilane for 10 minutes and then allowed to dry at room temperature (~25ºC). The dried materials were placed on metal stubs and sputter-coated with gold in a fine-coat ion sputter, JFC-1100 (JEOL). The gold-coated specimens were observed under scanning electron microscope (LEO 435 VP) at an electron accelerating voltage of 20 kV.

Photomicrography using light microscope

Some roundworms were fixed in aqueous Bouin's fluid overnight. The fixative was completely removed under running tap water, and the specimens were dehydrated through grades of alcohol up to pure ethanol. After cutting them into small pieces, they were treated with a mixture of xylene and clove oil until clear transparent specimens were obtained, and then cleared in pure xylene. The specimens were then treated with a series of mixtures of paraffin and xylene in proportional ratios towards pure paraffin. After complete infiltration with liquid paraffin and molten wax at ~58ºC and embedding in solid paraffin, they were solidified under room temperature, and trimmed into rectangular blocks. Sections were cut at 8-10 µm thickness using Erma Japan type rotary microtome (Biocraft & Scientific Industries, India). The sections were stretched on an automated hot plate and fixed on albumin coated glass slides, and then deparaffinized using pure xylene. Finally they were completely dehydrated through grades of alcohol, stained with eosin and haematoxylin, and mounted with cover slip using DPX mountant. Photomicrographs were prepared from Zeiss image analyzer HBO 50.

RESULTS

SEM observations of the morphology

A. galli had an elongated, cylindrical, and somewhat semi-transparent body, yellow whitish in colour, and tapering towards both ends. The whole body was enclosed in a tough proteinaceous covering called cuticle. Triangular mouth opened at the extreme anterior end (Fig. 1A). The dorso-ventral margin appeared to be marked with a continuous ridge along the longitudinal axis of the body. There were three prominent denticulate lips surrounding the mouth, and having smooth cuticle and appeared anchored to one another (Fig. 1B). The lips were of two types, a broadly elliptical one is mid-dorsal and two oval ones were latero-ventral lips. Each lip is not globular but it has a wide base with gentle tapering towards apex. These lips are mechanical organs for ingesting food materials. Each lip can be further divided into three distinct lobes, one median at the center, and two 1atera1 lobes at the sides. Together they form a cup-like structure. The inner surface of median lobe of each 1atera1 lip was possessing single dentigerous ridge, which was composed of a single line of minute denticles (Fig. 1C). Prominent cuticular protuberance named

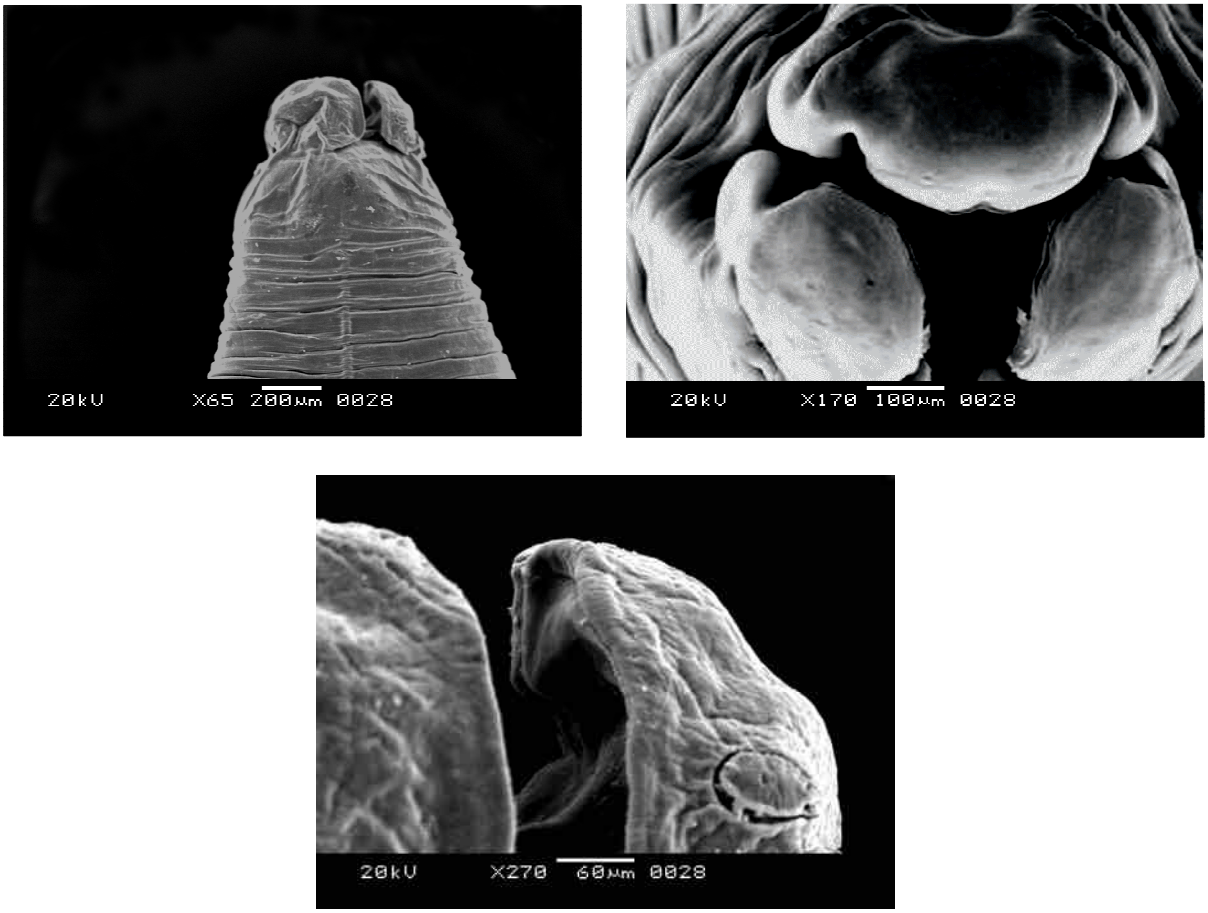

Figure 1. Scanning electron micrographs of an adult *A. galli* towards the anterior end. A. The cylindrical body tapers at the anterior end into a conspicuous cephalic terminal, the mouth, which is entirely composed of lid-like structures called lips. The entire body covering is the cuticle and is marked transverse striations and a middle longitudinal ridge, except at the mouth region. B. A frontal view of the anterior tip showing three separate lips enclosing a cavity, which serves as the opening for the digestive system; the lips can be distinguished into two types, one at the top is elliptical and is relatively broad, is the mid-dorsal lip, two at the bottom are oval and are smaller, are the ventro-lateral lips. Each lip has three distinct regions, a broad median lobe at the centre, flanked on either side by small lateral lobes. C. A single lip enlarged to show the rim that bears a series of spikes called denticles, and a distinct eye-like oval structure that is the papilla.

labial papilla was seen situated on external surface of the cuticle of the latero-ventral lip. The surface fine topography of the body showed a smooth cuticle characterized by a series of continuous transverse annulations with distinct striations from the cephalic region to the posterior end of the body (Fig. 2A). Striations are fine transverse grooves in the form of parallel concentric rings running completely around the cylindrical body. However, they appeared to converge or interrupted at points at the longitudinal ridge. Annulations are deep transverse grooves occurring at regular intervals giving the body a segmented appearance. Annulations were finer divided into small subannuli (Fig. 2B).

Sexual dimorphism in ascarids is morpho-

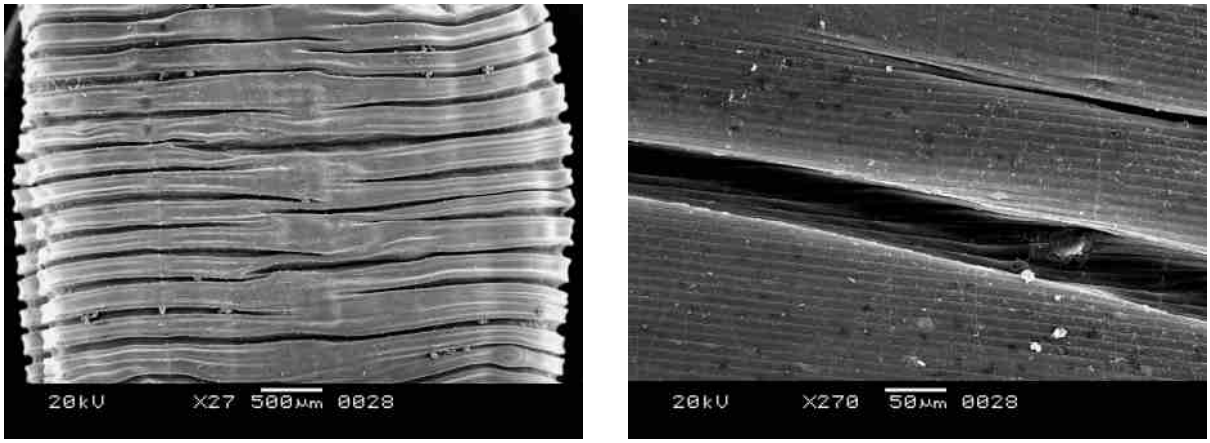

Figure 2. Scanning electron micrographs of an adult *A. galli* towards the middle portion of the body. A. The cuticle is finely corrugated giving rise to a series of unique transverse striations called annulations, those that form continuous ring around the body are annuli, and those that constitute interrupted smaller grooves are subannuli. B. A closer view of the cuticular surface reveals finer division of annulations into concentric parallel rings, the subannuli, that run throughout the entire length of the body except at the extreme ends.

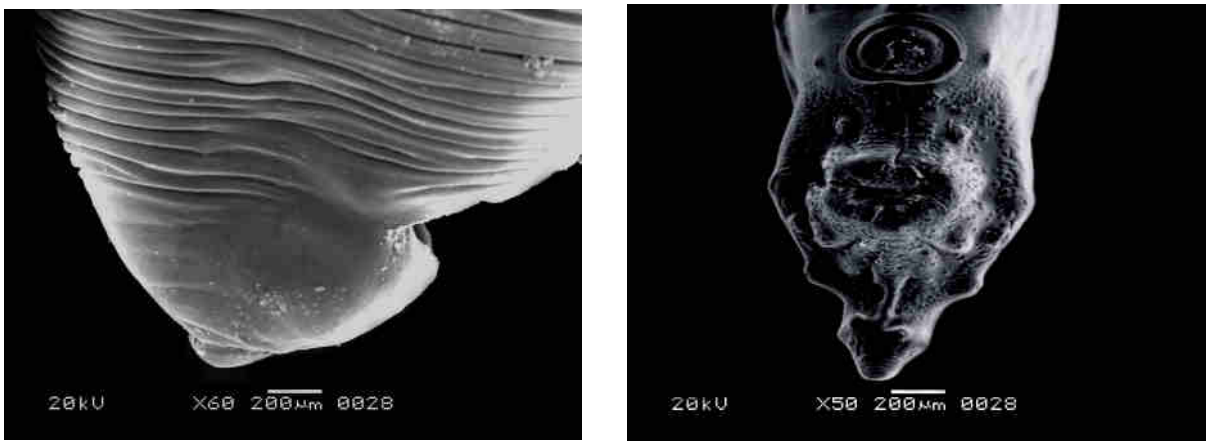

Figure 3. Scanning electron micrographs of adult *A. galli* towards the posterior portion of the body. A. Female has a characteristic straight and blunt tail end; an anal opening can be seen at the right hand side. The cuticle is without annuli at the end region. There are no structural paraphernalia. B. The male is characterized by an elaborate, finely pointed tail with two particularly distinct ring-like structures; towards the extreme terminal is the anus and anterior to it is the precloacal sucker, which serves as the holdfast during copulation. The preanal sucker is bounded by a distinct screrotized rim, which is double-walled. Small protuberances on either side are caudal papillae or phasmids. Both sides of the anal region are expanded to form flap-like caudal alae. The anal opening is at the centre of the anal protrusion, which is surrounded by numerous, undefined blebs.

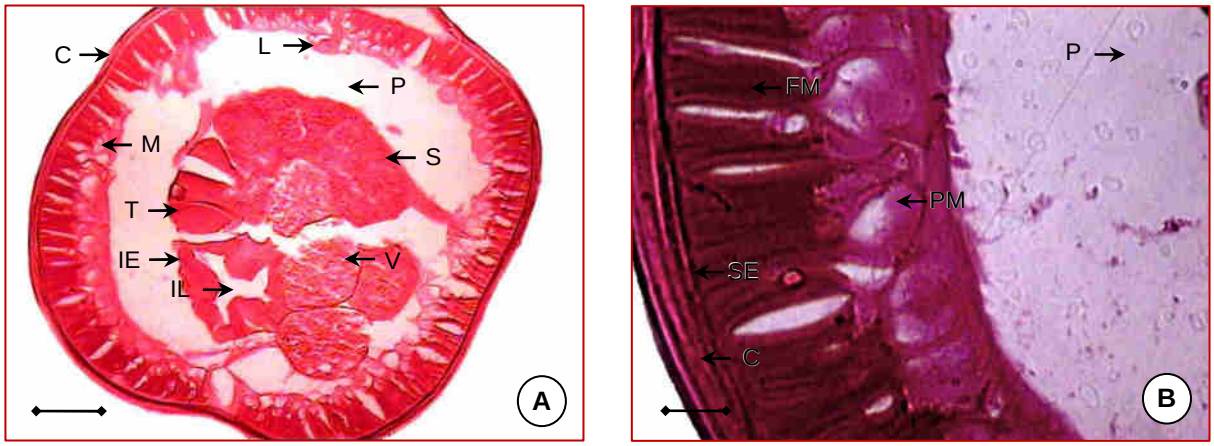

Figure 4. Photomicrographs of histological sections of a male *A. galli* through the middle portion of the body. A. The cylindrical body is entirely surrounded by a thick proteinaceous cuticle (C), which is attached with a conspicuous layer of muscles (M); the muscle layer encloses a body cavity called pseudocoel (P) in which are present the intestine, testis (T), seminal vesicle (S), and vas deferens (V); the intestine is composed of the epithelial layer (IE) and the lumen (IL); numerous spermatozoa can be seen inside the seminal vesicle (S); a prominent lateral longitudinal excretory canal (L) is at the top [x 50, bar = 500 µm]. B. Magnification of the body layer showing the cuticle (C) made up of several layers of proteinaceous syncytial epdermis (SE), and the somatic muscle layer is clearly divided into fibrillar (FM) and proplasmic (PM) components [x 200, bar = 50 µm].

logically characterized by ventrally coiled tail with precloacal sucker in males, and a blunt and rounded posterior end in females. The posterior portion of female *A. galli* also possessed a single large anal opening just before the extreme end and possessing pair of papillae just near to its tip. The tail end was rather straight and blunted (Fig. 3A). The posterior end of male was comparatively elaborate and more complex. There were two prominent apertures present, anus towards the extreme end and precloacal or preanal sucker immediately anterior to it (Fig. 3B). The eextrem terminal tip was finely pointed and a slight expansion at the base. The precloacal sucker was supported by a sclerotized ring and functions as an aid to attachment during copulation. The lateral longitudinal region around the anus was considerably outstretched on both sides to form an expanded region named caudal alae. Several small dome-shaped bulges of the cuticle were found on the ventral side of the tail region on either side of the anus and are called caudal papillae or phasmids. These caudal papillae are the sensory organs of the male tail. The anus was, in contrast to the precloacal sucker, not bounded by any structure, but instead formed a circular protrusion with a centrally located anal opening.

Observations of histological structure

Under light microscopy, histology of *A. galli* revealed that the body is cylindrical with supported by the circular cuticle. The cuticle appeared as a complex layer of proteinaceous rings forming a hard and thick unit, and is composed of several discrete concentric layers running continuous around the body (Fig. 4A,B). Below the cuticle was a syncytial epidermis, or sometimes referred to as hypodermis, giving rise to a complex layer of longitudinal muscles towards the outside and a meshwork of connective tissues towards the inner side. The muscular layer comprises two distinct portions: a fibrillar, contractile muscular portion, which runs lengthwise against the epidermis, and a granular non-contractile protoplasmic portion projecting towards the centre of the body. The middle por-

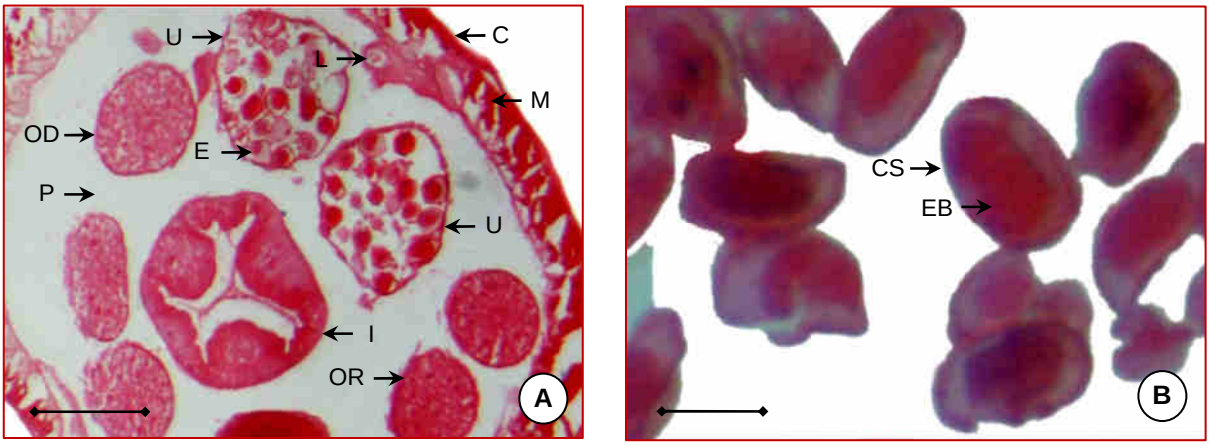

Figure 5. Photomicrographs of histological sections of a female *A. galli* through the middle portion of the body. A. The cuticle (C) and muscular layer (M) constitute the body wall, and surround the pseudocoel (P), which contains a prominent gut (I), two uteri (U) are very conspicuous containing numerous eggs (E), circular ovary (OR) is visible at the bottom, a distinct oviduct (OD) is on the top right [x 80, bar = 500 µm]. B. A portion of the uterus magnified to show the eggs. Each egg is elliptical or roughly polyhedral in shape and encapsulated by a smooth transparent chitinous shell (CS) that encloses the embryo (EB), the light/whitish portion indicates the yolk [x 200, bar = 50 µm].

tion of the body was occupied by a large cavity called the pseudocoel. It is not considered a true body cavity being not of mesodermal origin. A large gut was visible lying centrally in the pseudocoel, and run throughout the length of the body. The gut was essentially made up of single -layered epithelial cells arranged circularly to form a visible triradiate lumen at the centre. The epithelial cells facing the pseudocoel constituted the luminal surface, which was provided with microvilli projected from the individual epithelial cells, thus, forming the intestinal brush border. The lateral longitudinal excretory canal was observed as a small projection from the edge of the protoplasmc portion of the muscle into the psudocoel.

Other than these common structures, male and female nematodes are endowed with different reproductive organs. In male (Fig. 4A), the gut was surrounded by large circular seminal vesicles, vas deferens and the testis, which comprises the male reproductive organs. The seminal vesicle was profuse with the male gamete, spermatozoa, in the form of small granular bodies. In female (Fig. 5A), the gut was surrounded by two ovaries, two uteri and oviducts. Numerous egg cells or oogonia were clustered inside each of the uterus, the walls of which are comparatively thicker than the ovaries and oviduct (Fig. 5B). Each egg was oval or nearly polyhedral in shape, about 80x50 µm in size, completely enclosed in a tough protective shell, known to be made up of chitin, and inside was the yolk and embryo.

DISCUSSION

A. galli possesses all the salient features of the ascarid nematodes. The body is cylindrical, highly extended, covered with a collagenous cuticle, anterior end with distinct lips, and the posterior with anus. In addition marked sexual differences between male and female can be easily diagnosed by the relatively longer females with straight blunt tail end; whereas males are comparatively shorter with an elaborate, curved tail end.[17] The posterior components in *Ascaridia*, especially those of papillae

and spicules, are the identifying characters between different species.[28] The distinguishing characters of *A galli* are, for male: ventral part of rear end of body with cuticular ornamentation composed of thickened cuticular bosses, 10 pairs of caudal papillae, spicula without alae, length of tail 0.48 to 0.85 mm, i.e. 0.94 to 1.12% of total body length, length of spicula 0.65 to 2.40 mm; and for female: length of tail 0.40 to 1.54 mm, i.e. 0.61 to 1.88% of total body length.[29]

A. galli morphological features also reflect all the characters of the generic diagnosis[7,30] as follows: Ascaridiinae with cuticular lateral flanges generally present; lips without interlabia; oesophagus club-shaped, without posterior bulb. Male: precloacal sucker with chitinous rim; caudal alae narrow, papillae relativelly large; spicules equal or subequal; gubernaculum absent. Female: uteri opposed; vulva near middle of the body; oviparous; eggs thick shelled.

The cuticle is a resilient exoskeleton that exhibits a stark similarity in different members of the phylum Nematoda. A typical cuticle is composed of three basic layers, namely the cortex (exocuticle), the matrix (mesocuticle) and the fiber layers (endocuticle), and each with finer layers. In many instances the epicuticle to the exterior and basal lamina to the interior are also identified. It not only covers the body surface, but also lines the digestive and reproductive systems.[31] Once thought of as an acellular, nonliving entity, it is now known to be a specialized cellular complex, with high metabolic activity, particularly as a center of protein synthesis (collagen fibrogenesis).[32] It is chemically composed of highly criss-crossed, soluble and insoluble proteins like collagens and cuticulins, lipids, and carbohydrates.[33,34] The components of the cuticular layers are synthesized and secreted from the hypodermis. The cuticle-hypodermis complex constitutes a single functional unit that perform protective and selective absorption functions,[35] thus a primary target site of anthelmintic drugs.[36]

The cuticle though apparently smooth is actually in the form of fine circumferential transverse rings throughout the body, except at the extreme anterior and posterior ends, when observed under scanning electron microscope. These rings give the body a somewhat segmented appearance, but they are in reality discrete grooves and ridges essential for the growth and flexibility of the body. These striations are called annulations. The subannuli in *A. galli* were first observed and described recently by Hassanain *et al*.[22] There are circular or transverse annuli which were split into subannuuli during embryonic development of *A. galli* larvae to adult and also it's lengthening in size to adult due to the cuticular development of worm. Such unique cuticular organization appears to be characteristic of ascarids, as was noted in *A. suum*.[37,38]

One notable observation in *A. galli* in the present and previous studies[23,24] is the presence of a continuous longitudinal ridge that appeared to form a demarcation between the dorsal and ventral faces. Closer examination showed that the transverse annuli are not continuous rings but broken at this longitudinal ridge. It has been described in detail in *Caenorhabditis elegans*, the most well studied nematode, that the annuli of the dorsal and ventral regions are separated by the alae, and on stages without alae, the dorsal and ventral regions (and annuli) meet with an obvious discontinuity above the lateral seam cells.[39] Although lateral alae are so far reported only in *A. hermaphrodita* and *A. platyceri* among the species of *Ascaridia*,[30] it is tempting to speculate that the observed ridge in *A galli* is nothing but a rudimentary alae, or at least a vestigial one. Scrupulous examination of the SEM of Hassanain *et al.* also clearly revealed this ridge in *A. galli*, and dubiously described it as median centrid, but failed to notice it as a distinct structure.[22]

The underlying muscular layer is relatively thick and well-defined. The unusual division of muscle into the contractile (striated) and non-contractile (nonstriated) portions is due to unique arrangement and distribution of the muscle cells. All of the muscle cells are spindle-

shaped and longitudinally oriented. The muscle fibres, or myofibrils, are entirely located in the contractile region containing the nucleus, mitochondria, and stored glycogen granules and lipids. The muscular layer is attached to the cuticle fibers running from the each myofibril, passing through the basal lamina.[31]

The presence of three labial lips is one of the diagnostic features among the species of *Ascaridia*, but by no means are the lips similar. In *A. galli*, each lip is trilobed comprising a broad median lobe flanked at each side by two small accessory lateral lobes. The median lobe in fact constitutes the major structure of the lip, while the lateral lobes form small extensions at the base. In contrast the three lobes are much more equal in shape and size in other species like *A. hermaphrodita* and *A. platyceri*. Further a flat reinforcement of the inner surface of the medial lobe of each lip with denticles or minute teeth in *A. galli* is also characteristic of only a few species including *A. australis*, *A. hermaphrodita* and *A. platyceri*.[30] These features of the lips may provide important taxonomic tools.

The distribution of cephalic papillae is also specific in *A. galli.* There are exactly four, one on each of the latero-ventral lips and two on the dorsal lip.[21] Three paired cephalic papillae and two amphids are found on the outer surface of the lips. The amphidial surface is provided with several pores. Amphids are the main chemosensory organs of nematodes and are accrued to play important roles in host finding behaviour and the control of development.[40] The neurons found in the amphidial channel are responsible for a wide range of sensory functions such as detecting aqueous chemoattractants, repellants and volatile odorants; thermoreception, and chemotaxis during larval development to recognize suitable hosts.[41] Small papillae were also seen scattered on the cervical area of the worm.[22] In some sites, two successive transverse annuli might be fused at a short distance from lateral toward the middle of the cuticle with subsequent increasing the subannuli numbers and the width.

Unlike the female, the male *A. galli* is endowed with complex structures towards the posterior extremity. The tail region was slightly distended forming poorly developed caudal alae on either side. On the ventral surface of the alar regions were normal anal protrusion and the preclocal sucker, which was situated a short distance anterior to the cloacal opening. The cloacal opening was situated on the top of the anal mound. Again it was recently described that the preclocal sucker had an ill bounded circular rim.[22] But present observation convincingly shows that the sucker rim is well structured, demarcated with an apparent spherical ridge, forming a sclerotized ring, followed by a distinct depression, and at the center is an oval protrusion.

On either side of the clocal and preclocal opening are a series of minute bulges, the caudal papillae. The cloacal papillae were arranged in two groups, the precloacal and postcloacal ones, characteristics of ascarids.[42] The precloacals papillae were arranged in the two sided-laterals of the worm. The postcloacals papillae were arranged in one group. Caudal papillae are described to be ten pairs, arranged in distinct groups, i.e., pre-anal, anal, post-anal and subterminal on ventral surface of caudal end; pre-anals three pairs – first pair anterior to pre-anal sucker, second at level of pre-anal sucker and close to the first pair; sub-terminals three pairs, first smallest of all anal pairs, lying close to second pair, second and third pairs comparatively more prominent surrounded by small cuticular raised structures giving them rosette-like appearance, lying laterally and ventrally respectively, on extreme tail region body. Spicules are well developed, equal, covered in spicular sheath, and protruding out at anal opening.[8,12]

Although the digestive system is complete consisting of mouth, buccal capsule, esophagus or pharynx, intestine, rectum and anus. The majority of the composition is represented by a long straight tube of the gut or intestine extending from the anterior to the posterior end. The intestine is composed of epithelial cells arranged in one cell layer thick, directly connecting the anterior opening to the anus, and the

internal wall is usually lined with microvilli for increased absorption of nutrients.[34] The intestinal epithelium consists of large epithelial cells resting on a thick collagenous basal lamina, and the brush border is provided with microvilli.[43]

Among the species of *Ascaridia*, eggs are essentially formed in the ovaries, eggs arising in the anterior ovary pass into the posterior uterus; similarly, eggs from the posterior ovary pass into the anterior uterus and the mature polyhedral eggs are transported and housed in the uterine chambers, which are fully lined with tough muscle layer. In young females eggs may be fertilised at any place in the uteri; in adults, fertilisation occurs near the distal ends of the uteri.[3] A fertilization membrane is formed soon after the penetration of the sperm, and develops into a hard chitinous shell. A second membrane, often called vitelline membrane is secreted by the embryo toward the inner surface of the egg shell, and is composed of esterified glycosides.[31,44] The fully developed eggs of *A. galli* are elliptical or almost polyhedral in shape with smooth shells and measure 73-92 by 45-57 µm.

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