INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

OPEN ACCESS

Received: 12-04-2020

Accepted: 23-04-2020

Published: 18-06-2020

Editor: Dr. Natarajan Gajendran

Citation: Jaiswal K, Mishra S, Bee A (2020) **Scanning electron microscopy of *A scaridia galli* in *Gallus gallus domesticus* in Lucknow, U.P, India.** Indian Journal of Science and Technology 13(19): 1944-1954. https://doi.org/10.17485/IJST/v13i19.102

***Corresponding author**.
Anjum Bee
Research Scholar, Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, 226025, India. Tel.: 07905696218
anjumkhalid49@gmail.com

Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

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

Kamal Jaiswal[1], Suman Mishra[2], Anjum Bee[3]*

1 Professor, Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, 226025, India
2 Assistant Professor, Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, 226025, India
3 Research Scholar, Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, 226025, India. Tel.: 07905696218

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.

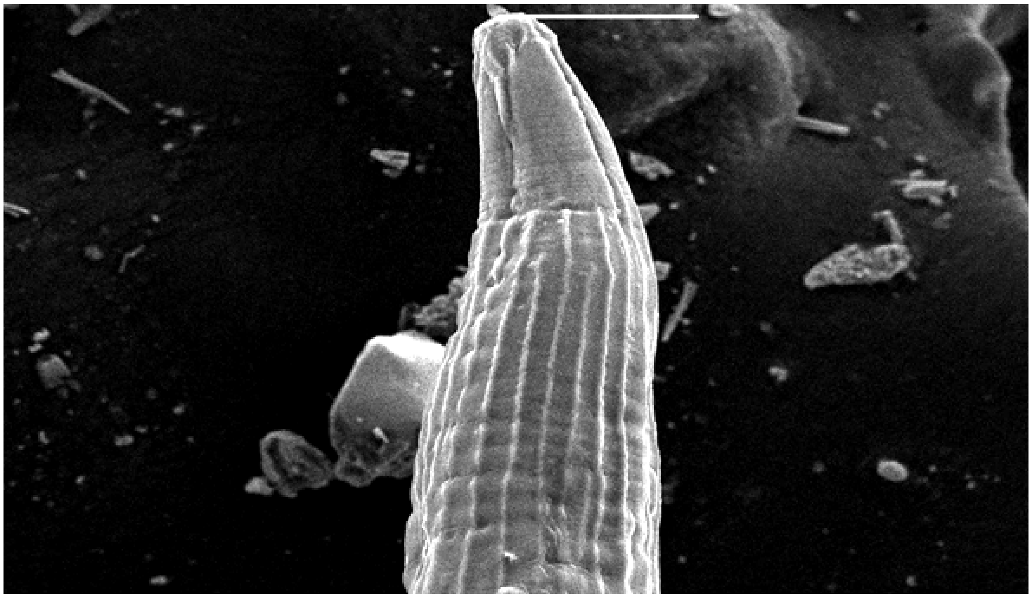

Fig 1. Scanning electron micrographs of the anterior end of an adult *A. galli*

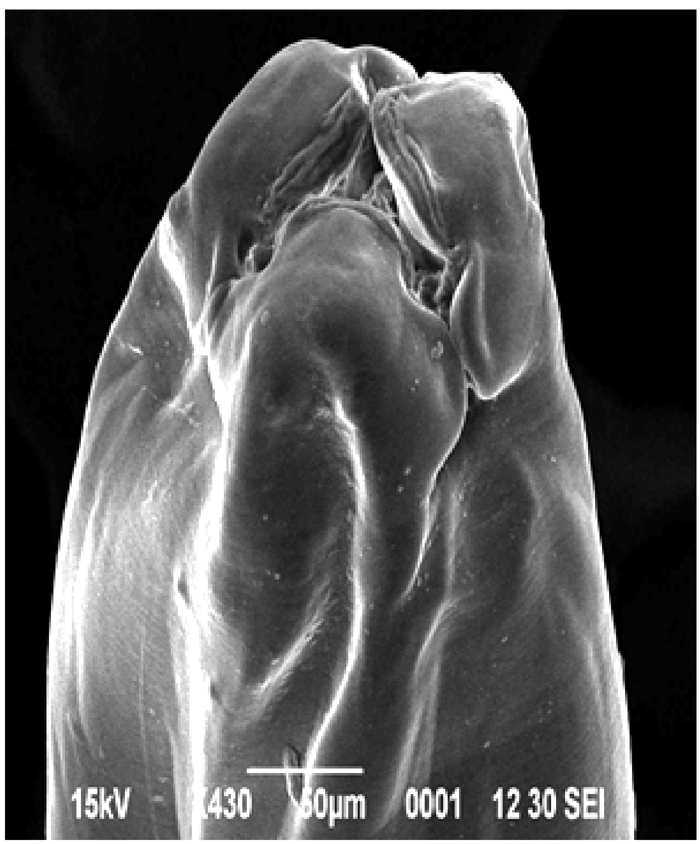

Fig 2. Three large trilobed lips surrounded the mouth opening and smooth cuticular plate covered the inner surface of each lip and the outer surface is covered with cuticle.

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

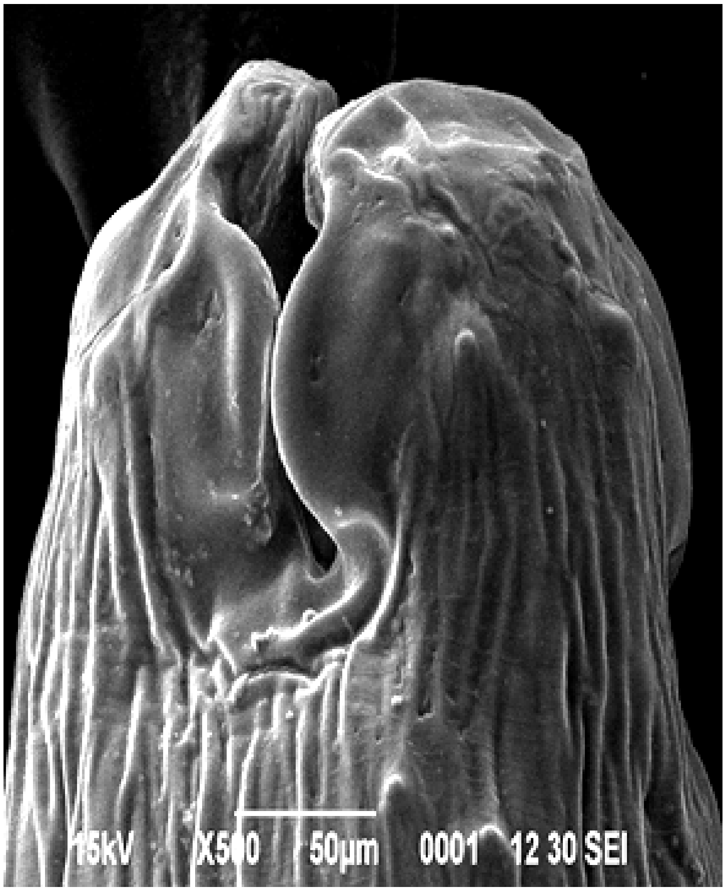

Fig 3. Enlarged view of lips showing the rim that bearsa series of denticles, and the papilla which seems eye-like oval structure.

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.

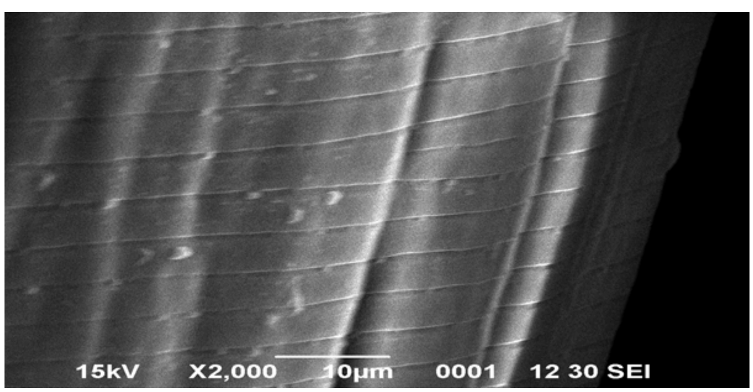

Fig 4. Middle portion of an adult *A. galli* body with fine corrugated cuticle arranged in a series of unique transverse striations named annulations, which forms the continuous ring around the body.

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.

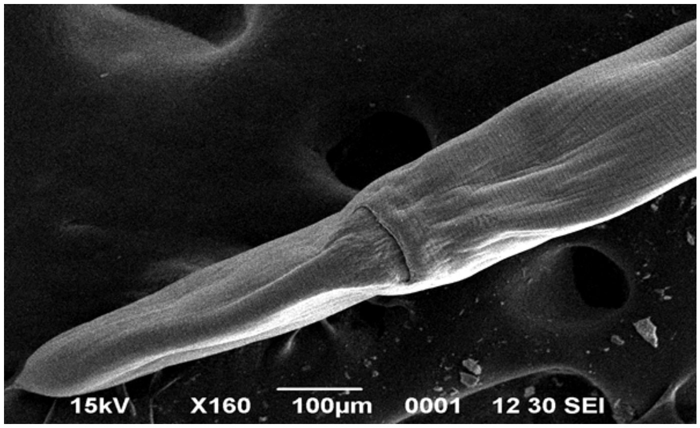

Fig 5. Scanning electron micrographs of an adult *A. galli* female with blunt and rounded posterior end and pre cloacal sucker.

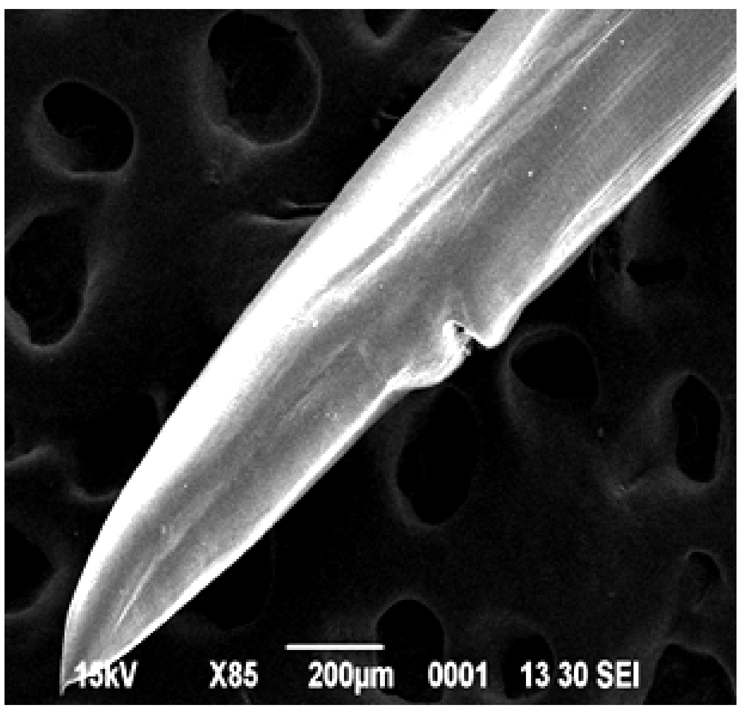

Fig 6. Single large anal opening of female *A. galli* just before the tip of tail.

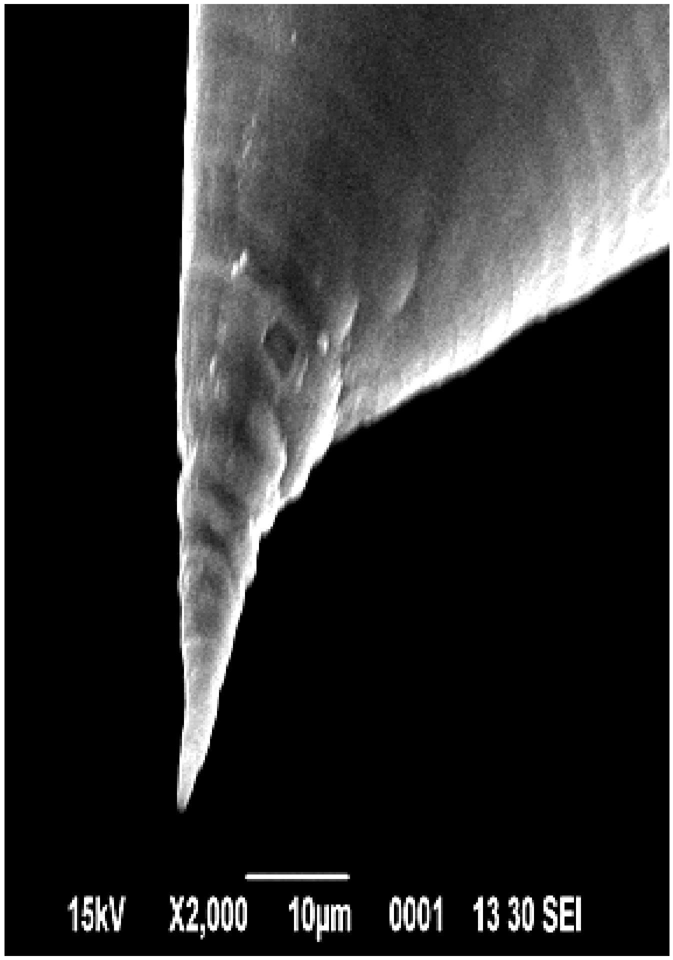

Fig 7. The end region of tail cuticle is without annuli

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.

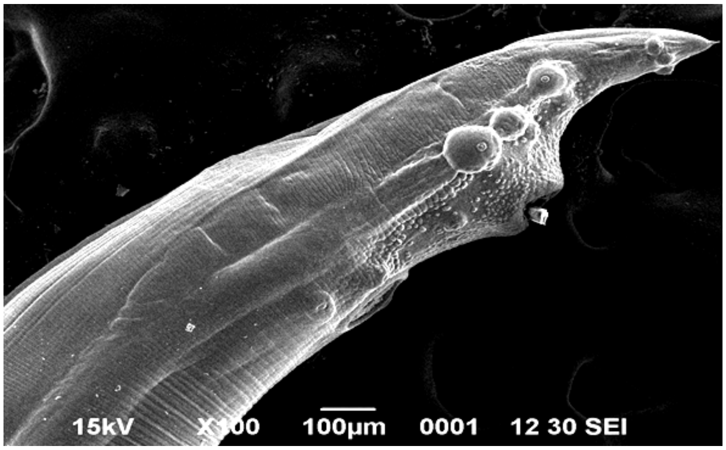

Fig 8. Scanning electron micrographs of an adult *A. galli* male with a fine pointed tail, anus and caudal papillae. Caudal papillae emerge on either side in the form of small knobe like structure.

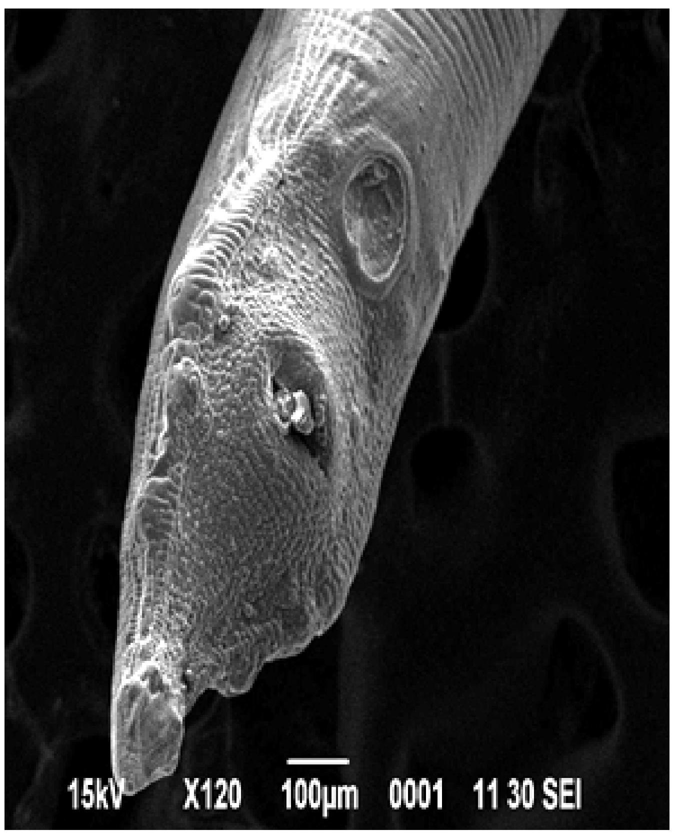

Fig 9. *A. galli* male with a ring-like structure precloacal sucker which helps in grasping during copulation. Anal region expanded both side to form a flap-like structure caudal alae. The anal opening is surrounded with numerous small blisters like structure

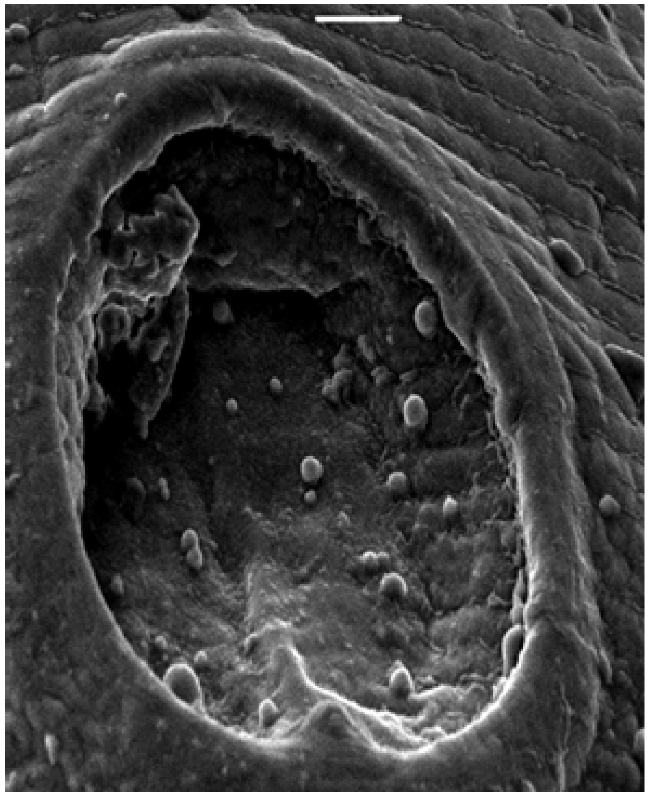

Fig 10. Enlarged view of preanal sucker bounded by adouble-walled sclerotized rim

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.

Acknowledgment

Authors acknowledge to Honourable Vice-Chancellor Prof. Sanjay Singh and other authorities of the University for providing necessary infrastructural facilities to carry out the research work.

References

1) Permin A, Ranvig H. Genetic resistance to Ascaridia galli infections in chickens. *Veterinary Parasitology*. 2001;102(1-2):101–111. doi:10.1016/s0304-4017(01)00525-8.
2) U.S. Department of Agriculture, Economic Research Service (ERS). In: and others, editor. Red Meat and Poultry Yearbooks. U.S. Department of Agriculture, Economic Research Service (ERS). 2011;p. 1997–2010.
3) Thompson DP, Geary TG. Helminth surfaces: structural, molecular and functional properties. In: Marr JJ, Nilsen T, Komuniecki R, editors. In: Molecular Medical Parasitology. London, UK. Academic Press. 2003;p. 297–338.
4) Mehta R, Nambiar RG, Food and Agriculture Organization of the United Nations (FAO) Animal Production and Health Proceedings. The poultry industry in India. In: Thieme O, Pilling D, editors. Poultry in the 21stCentury: Avian Influenza and Beyond. 2007;p. 29–30.
5) Oniye SJ, Audu PA, Adebate DA, Kwaghe BB, Ajanusi OJ. Survey of Helminth Parasites of laughing dove (Strepto peliasenegalensis. *African journal of natural sciences*. 2001;4:65–66.
6) Frantovo D. Some parasitic nematodes (neumatoda) of birds (aves) in the Czech Republic. *Acta Societatis Zoological Bohemicae*. 2000;16(1):13–28.
7) Butcher GD, Hogsette JA, Jacobs RD, et al. Nematode Parasites of Poultry (and where to find them). *Animal Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences*. 1997. Available from: https://www.scribd.com/document/109402600/Cap-Ilaria.
8) Schmidt GD, Roberts LS, et al. Foundations of Parasitology. Dubuque, Iowa. Wm. C. Brown Publishers. 1996;p. 355–465.
9) Grewal PS, Ehlers RU, Shapiro-Ilan D. Nematodes as Biocontrol Agents. 2005.
10) Croll NA. The Organization of Nematodes. 1976;p. 123–182.
11) Lee DL. The Physiology of Nematodes. San Francisco. W.H. Freeman and Company. 1965.
12) Croll N, Matthews BE. Biology of Nematodes. Blackie & Son Ltd. 1977;p. 1–152.
13) Yamaguti S, Systema, Helminthum. The nematodes of vertebrates. New York and London. Interscience Publishers. 1961;p. 1261–1261.
14) Blaxter ML, Page AP, Rudin W, Maizels RM. Nematode surface coats: Actively evading immunity. *Parasitology Today*. 1992;8(7):243–247. doi:10.1016/0169-4758(92)90126-m.
15) Soulsby EJL. Helminths, arthropods and protozoa of domesticated animals. London. Tindall and Cassell Bailliere. 1968.
16) Ackert JE. The Morphology and Life History of the Fowl Nematode Ascaridia lineata (Schneider). *Parasitology*. 1931;23(3):360–379. doi:10.1017/s0031182000013731.
17) Permin A, Hansen JW. The Epidemiology, Diagnosis and Control of Poultry Parasites: An FAO Handbook. . Rome, Italy. Food and Agriculture Organization of the United Nations. 2003;p. 24–29.

18) Permin A, Bojesen M, Nansen P, Bisgaard M, Frandsen F, Pearman M, et al. Ascaridia galli populations in chickens following single infections with different dose levels. *Parasitology Research*. 1997;83(6):614–617. Available from: https://dx.doi.org/10.1007/s00436050306. doi:10.1007/s004360050306.
19) Ponnundurai G, Chellappa DJ. Prevalence of helminth parasites of chicken in different systems of management. *Journal of Veterinary Parasitology*. 2001;15:73–74.
20) Ramadan H, Znada NA. Morphology and Life History of Ascaridia galli in the Domestic Fowl that are Raised in Jeddah. *Journal of King Abdulaziz University-Science*. 1992;4(1):87–99. doi:10.4197/sci.4-1.9.
21) Ramadan HH, Znada NYA. Some pathological and biochemical studies on experimental ascaridiasis in chickens. *Nahrung*. 1991;35(1):71–84. doi:10.1002/food.19910350120.
22) Permin A, Christensen JP, Bisgaard M. Consequences of concurrent Ascaridia galli and Escherichia coliinfections in chickens. *Acta Veterinaria Scandinavica*. 2006;47(1):43–54. doi:10.1186/1751-0147-47-43.
23) Permin A, Bisgaard M, Frandsen F, Pearman M, Nansen P, Kold J. The prevalence ofgastrointestinal helminths in different poultryproduction systems. *British Poultry Science*. 1999;40:439–443. doi:10.1080/00071669987179.
24) Ackert JE, Herrick CA. Effects of the Nematode Ascaridia lineata (Schneider) on Growing Chickens. *The Journal of Parasitology*. 1928;15(1):1–15. doi:10.2307/3271596.
25) Griffiths HJ. A Handbook of Veterinary Parasitology: Domestic Animals of North America. Minneapolis, Minnesota, USA. University of Minnesota Press. 1978;p. 46–47.
26) Ackert JE. The large roundworm of chickens. *Veterinary Medicine*. 1940;35:106–108.
27) Permin A, Ranvig H. Genetic resistance to Ascaridia galli infections in chickens. *Veterinary Parasitology*. 2001;102(1-2):101–111. doi:10.1016/s0304-4017(01)00525-8.
28) Vassilev I, Ossikovski E, Bozhkov S, Kambourov P, Bankov I, Roupova L, et al. On the pathogenesis of ascaridiosis in fowl. *Bulletin of the Central Helminthological Laboratory*. 1973;16:43–58.
29) Gauly M, Duss C, Erhardt G. Influence of Ascaridia galli infections and anthelmintic treatments on the behaviour and social ranks of laying hens (Gallus gallus domesticus). *Veterinary Parasitology*. 2007;146(3-4):271–280. doi:10.1016/j.vetpar.2007.03.005.
30) Gary DB, Richard DM. Intestinal parasites in backyard chicken flock 1 In: Series of Veterinary Medicine- Large animal clinical sciences. vol. 76. 2012.
31) Census 2011. Lucknow District Population Census 2011, Uttar Pradesh literacy sex ratio and density. 2011.
32) . . Available from: https://timesofindia.indiatimes.com/archive/year-2012.
33) Cable RM. An Illustrated Laboratory Manual of Parasitology. Minneapolis; Minnesota, USA. Burges Publishing Co. 1958;p. 156.
34) Ptaszyńska AA, Borsuk G, Mułenko W, Demetraki-Paleolog J. Differentiation of Nosema apis and Nosema ceranae spores under Scanning Electron Microscopy (SEM). *Journal of Apicultural Research*. 2014;53(5):537–544. doi:10.3896/ibra.1.53.5.02.
35) Ashour AA. Scanning electron microscopy of Ascaridia galli (Schrank, 1788), Freeborn, 1923 and A. columbae (Linstow , 1903). *Journal of the Egyptian Society of Parasitology*. 1994;24:349–355. Available from: https://europepmc.org/article/med/8077754.
36) Hodova I, Barus V, Tukac V. Note on morphology of two nematode species Ascaridia hermaphrodita and Ascaridia platyceri (Nematoda): scanning electron microscope study. *Helminthologia*. 2008;45:109–113. doi:10.2478/s11687-008-0021-4.
37) Ashton FT, Schad GA. Amphids in strongyloides stercoralis and other parasitic nematodes. *Parasitology Today*. 1996;12(5):187–194. doi:10.1016/0169-4758(96)10012-0.
38) Hassanain MA, Rahman EHA, Khalil FAM. New Scanning Electron Microscopy Look of Ascaridia galli (Schrank, 1788) Adult Worm and its Biological Control. *Research Journal of Parasitology*. 2009;4(4):94–104. doi:10.3923/jp.2009.94.104.
39) Fagerholm HP, Nansen P, Roepstorff A, Frandsen F, Eriksen L. Differentiation of cuticular structures during the grow th of the third-stage larva of Ascarissuum (Nematoda, Ascaridoidea) after emerging from the egg. *Journal of Parasitology*. 2000;86(3):421–427. doi:10.1645/0022-3395(2000)086[0421:docsdt]2.0.co;2.
40) Fagerholm HP, Nansen P, Roepstorff A, Frandsen F, Eriksen L. Growth and Structural Features of the Adult Stage of Ascaris suum (Nematoda, Ascaridoidea) from Experimentally Infected Domestic Pigs. *The Journal of Parasitology*. 1998;84(2):269–269. doi:10.2307/3284481.
41) Lalchhandama K, Roy B, Dutta BK. Anthelmintic activity ofAcacia oxyphyllastem bark againstAscaridia galli. *Pharmaceutical Biology*. 2009;47(7):578–583. doi:10.1080/13880200902902463.
42) Lalchhandama K. Pharmacology of Some Traditional Anthelmintic Plants: Biochemical and Microscopic Studies. Saarbrücken, Germany. LAP Lambert Academic Publishing. 2010;p. 35–38.
43) Kung CC. Notes on some Avian Species of Ascaridia. *Journal of Helminthology*. 1949;23(3-4):95–106. doi:10.1017/s0022149x00032442.
44) Cheng TC, Parasitology. Division of Hardcourt Brace & Company. San Diego, California, USA. Academic Press. 1986.
45) Page AP. The nematode cuticle: synthesis, modification and mutants. In: Kennedy M, Harnett W, editors. Parasitic Nematodes: Molecular Biology. 2001;p. 167–193.
46) Lee DL. The structure and composition of the helminth cuticle. *Advances in Parasitology*. 1966;4:187–254. doi:10.1016/s0065-308x(08)60450-9.
47) Alvarez LI, Mottier ML, Lanusse CE. Drug transfer into target helminth parasites. *Trends in Parasitology*. 2007;23(3):97–104. doi:10.1016/j.pt.2007.01.003.
48) Kajerova V, Barus V, Literak I. Nematodes from the genus Ascaridia parasitizing psittaciformbirds: a review and determination key. *Veterinarni medicina-Czech*. 2004;49:217–223. doi:10.17221/5698-VETMED.
49) Sharma N, Hunt PW, Hine BC, Ruhnke I. *Te impacts of Ascaridia galli on performance, health, and immune responses of laying hens: new insights into an old problem. Poultry Science*. 2019;98(12):6517–6526. doi:10.3382/ps/pez422.
50) Sharma N, Hunt PW, Hine BC, Sharma NK, Chung A, Ruhnke I, et al. Performance, egg quality and liver lipid reserves of free-range laying hens naturally infected with *Ascaridia galli*. *Poultry Science*. 2018;97(6). doi:10.1017/S0022149X00032442.
51) Sharma N, Hunt PW, Hine BC, Sharma NK, Iqbal Z, Normant C, et al. The effect of an artificial *Ascaridia galli* infection on egg production, immune response and liver lipid reserves of free-range laying hens. *Poultry Science*. 2018;97(2):494–502. doi:10.3382/ps/pex347.