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Original Article

Microscopic Study of Mechanoreceptors and Chemoreceptors of Anterior and Posterior Ends of *Toxocara Canis* Using Scanning Electron Microscopy and Light Microscope

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Abstract

The present study investigated the fine structure of amphids and phasmids, cuticle, muscles, and digestive tracts of *Toxocara canis* using optical and electron microscopy, hematoxylin-eosin (H&E) staining, and other specific stains. A number of 38 adult *T.canis* worms were obtained from the animal shelter of Urmia, and their small intestines were fixated in acidified formal alcohol and 10% formalin solutions. The anterior and posterior parts of male and female *T.canis* worms were prepared and cut at a thickness of 4-5 µm according to the conventional method in the histological laboratory. The samples were then stained using H&E and specific periodic acid-Schiff, Masson's trichrome, and Orcein staining. The structure of amphid (anterior), phasmid (posterior), cuticle, muscles, and digestive tracts of male and female worms were studied under light microscopy. Basal, intermediate, cortex, and cuticle surface coating of the parasite were visible. Alae were also observed as the thickenings in the cuticle. The muscle layer structure consists of non-branched cylindrical cells. The intestinal tract is composed of cuticular cogs, the esophagus is of filamentous-muscular structure, and the intestine is made of columnar epithelial tissue with microvilli and glycocalyx. The amphid structure consisted of cuticular protrusions with penetrations of the cephalic framework into their inner layers. Phasmid structure also includes protrusions in the cuticle and invagination of sensory neurons. It was concluded that for the most part, the histological structure of the cuticle can be studied by optical microscopy. The muscle structure in this parasite was very similar to the skeletal muscle in mammals. Furthermore, the epithelial structure of the intestine in this parasite was largely similar to the intestinal epithelium in mammals. Finally, regarding the amphid and phasmid structures, it was observed that they were protrusions covered by cuticles where neural, filamentous, and muscular structures were the core of these protrusions.

Keywords: Histology, Light Microscope, Papillae, Receptors, *Toxocara canis*

Étude Microscopique des Mécanorécepteurs et des Chimiorécepteurs des Extrémités Antérieure et Postérieure de *Toxocara Canis* à l'aide de la Microscopie Electronique à Balayage et du Microscope Optique

Résumé: La présente étude a étudié la structure fine des amphides et des phasmides, de la cuticule, des muscles et du tube digestif de *Toxocara Canis* en utilisant la microscopie optique et électronique, la coloration à l'hématoxyline et à l'éosine (H&E) et d'autres colorations spécifiques. Un certain nombre de 38 vers adultes de *T. Canis* ont été obtenus du refuge pour animaux d'Urmia, et leurs petits intestins ont été fixés dans de l'alcool formel acidifié et des solutions à base de formol à 10%. Les parties antérieure et postérieure des vers mâles et femelles de *T. canis* ont été préparées et coupées à une épaisseur de 4-5µm selon la méthode conventionnelle au

laboratoire d'histologie. Les échantillons ont ensuite été colorés en utilisant H&E et une coloration spécifique de PAS (Periodic Acid Schiff), la coloration au trichrome de Masson et la coloration à l'orcéine. La structure de l'amphide (antérieur), du phasmide (postérieur), de la cuticule, des muscles et du tube digestif des vers mâles et femelles a été étudiée sous microscopie optique. Les revêtements basaux, intermédiaires, corticaux et cuticulaires du parasite étaient visibles. Des alaes ont également été observés comme des épaississements dans la cuticule. La structure de la couche musculaire est constituée de cellules cylindriques non ramifiées. Le tractus intestinal est composé de cogs cuticulaires, l'œsophage est de structure filamento-musculaire et l'intestin est constitué de tissu épithélial cylindrique avec des microvillosités et du glycocalyx. La structure amphide était constituée de saillies cuticulaires avec des pénétrations du cadre céphalique dans leurs couches internes. La structure phasmide comprend également des saillies dans la cuticule et l'invagination des neurones sensoriels. Il a été conclu que pour la plupart, la structure histologique de la cuticule peut être étudiée par microscopie optique. La structure musculaire de ce parasite était très similaire à celle du muscle squelettique chez les mammifères. De plus, la structure épithéliale de l'intestin chez ce parasite était largement similaire à l'épithélium intestinal chez les mammifères. Enfin, en ce qui concerne les structures amphides et phasmides, il a été observé qu'il s'agissait de protubérances couvertes de cuticules où les structures neurales, filamenteuses et musculaires étaient au cœur de ces protubérances.

Mots-clés: Histologie, Microscope Optique, Papilles, Récepteurs, *Toxocara canis*

1. Introduction

Nematodes are considered a threat to human and animal life due to their high diversity and prevalence. On the other hand, the coexistence of humans and dogs is responsible for common diseases among them (Baker, 2002). The role of dogs as a definitive host for a number of parasites common between humans and animals has been extensively studied as a worldwide public health issue for many years (Shalaby et al., 2010). Humans also exacerbate the risk of disease transmission to or from semi-domestic dogs in rural areas or societies with poor socio-economic conditions lacking veterinary attention and awareness of common diseases (Zhou et al., 2008).

Worms get nourishment from sucking blood (*Ancylostoma*), ingestion of degraded tissues and blood by worms immersed in the mucus (*Trichuris*), feeding on the contents of the intestine (*Ascaris*), or ingestion of body fluids (*fillers*). The cuticle, which is the outer covering of the nematode, is resistant to digestive secretion. In nematodes, there are usually four papillae in different sizes and shapes with sensory function. There is also a pair of cervical papillae located at a certain distance from the anterior end of the parasite, depending on the genus and species of nematode Cervical papillae are of different types: thorn-like, minuscule, and nonexistent. Some papillae are very small and not observable in some nematodes. Larger papillae can be observed in bumps of sizes ranging from a needle-like appendage to a complex structure with posterior toothed portions. They are needle-like projections or complicated structures with dentigerous posterior. The location, shape, and size of these papillae are used in the investigation of taxonomic characteristics. These organs act as mechanical receptors which help nematodes to cross small pore spaces (McLaren, 1976; Naem, 2007).

Amphids which are observed in various shapes and sizes are located in anterior part of nematodes. Some amphids act completely as chemical receptors, while some serve as optical receptors in coordination with a gland. Phasmids are found in the posterior part of male and female nematodes. They are involved in the assessment of the received stimuli and help the worm to establish and relocate to a favorable environment (McLaren, 1976). A microscopic investigation of adult worms revealed that the worm is coated by a non-cellular cuticle containing various thick layers.

The cuticle is produced by an underlying layer that is predominantly a smooth hypodermis (epidermis). The nuclei of the hypodermis are found in four cords which are formed by hypodermal thickening. Dorsal and ventral cords are encapsulated by longitudinal axons,

whereas the lateral cord runs along the secretory canal and is covered by a series of apparently branched tubes. Somatic cells have contractile swellings, and there is a translucent nucleus within the cytoplasmic region of cells (Shalaby et al., 2009).

Toxocariasis is caused by *T.canis* in canids and *Toxocara cati* in the felids. Nematodes are pervasive parasites with a complex life cycle. The apparent differences among the species have been investigated using optical and electron microscopy. The species of *T.canis* can be detected by light microscopy mostly based on the size and shape of the lips. The difference between the visual aspects of the lips and the shape of the dentigerous ridges are also examined by scanning electron microscope (SEM)(Minciullo et al., 2018). The anterior end of the *T.canis* is bent in the ventral direction and has a large monotone caudal ala.

The SEM revealed worms with three well-defined lips, each with dentigerous ridges, and two small holes or invaginations bordering the outer margin of the lips. Dents are triangular in lateral view but blunt and wide when viewed in other directions. A report has demonstrated that the dorsal lip has two large papillae, while each ventral and lateral lip has large papillae and a small papilla. Moreover, a large amphid pair can be observed near the dorsal lip, whereas there are a large amphid and a small one on the lateral and ventral lips (Shalaby et al., 2009; Shalaby et al., 2018). The anterior is lance-shaped and contains three swollen lips. The inner and outer rings, as well as the papillae, are easily visible.

The length of the male worm is 4-10 cm, it has a caudal ala, and a digitiform appendage can be seen at its posterior end. *T.canis* adult worms have been detected in the small intestine of dogs, red foxes, gray and Egyptian-Mongolian wolves, and jackals from across the globe. The occurrence of the disease is more common among younger animals. Adult worms feed on nutrients in epithelial cell shedding. In ascariasis induced by adult worms, the clinical signs are only observed in abnormal locations, such as the bile duct. The affected animals exhibit visible signs of depression and anorexia, while anemia is detected in chronic cases. The present study aimed to assess the structure of chemical receptors of the *T.canis* parasite (amphid and phasmid) and some of its internal organs using hematoxylin-eosin (H&E) staining and specific staining for mammalian and avian tissues.

2. Material and Methods

In the present study, 38 adult *T.canis* worms were obtained from the animal shelter of Urmia, West Azerbaijan, Iran. To this end, newly received pups were treated by mebendazole 1g per 50 kg for five days (Tolide Darouhai Dami Iran Co., Tehran, Iran), followed by a single administration of praziquantel 50 mg per kg (Tolide Darouhai Dami, Tehran, Iran) and separated before sending for specimen preparation.

The collected worms were washed in water and drained with filter paper before fixation in 10% buffered formalin and acidified formal alcohol (AFA). Due to the small size of chemoreceptors (amphid and phasmids) in the *T.canis* parasite, the SEM was initially used to observe the exact structure and location of these receptors. For optical microscopy study of amphids and phasmids in male and female *T.canis* worms, the worms collected from the feces of dogs were fixed in 10% formalin after being washed in water and draining. The anterior and posterior parts of male and female *T.canis* worms were then prepared according to the conventional method in a histological laboratory and incised into 4-5 μm sizes. Thereafter, these sections were stained with H&E, periodic acid-Schiff (PAS), Masson's trichromem, and orcein staining. The structure of amphid (anterior end) and phasmid (posterior end), cuticle, muscles, and intestinal tract of male and female worms were studied under the light microscope.

The PAS staining was employed to examine carbohydrate aggregation and intensity, orcein staining was used to investigate the elastic structure, and Masson's trichrome staining was utilized to identify

collagen strands in collagenous tissues. The stains used in this study were all the same stains used for mammalian and avian tissues to determine the similarities or differences between the structures of this parasite and those of mammals and birds (Hesari et al., 2015).

3. Results

3.1. Cuticle's Histology

The primary examination of body covering in *T.canis* parasites using PAS staining and light microscopy revealed that the outer covering of the worm consists of a cuticle supported by somatic tissue. This cuticle reacted positively to PAS and was highly stainable. The H&E staining of the longitudinal slices of *T.canis* parasite demonstrated that the cuticle structure was almost similar throughout the body. The basal layer, as the deepest layer of the cuticle, the median layer which is relatively thick, a thin acidophilic layer above the median which was recognized as the cortical layer, and a thick surface coating as the outermost layer were all visible and distinguishable. The outer first shed cuticle and the inner second shed cuticle were not distinguishable from other layers. The wrinkles on the cuticle surface of the worm are due to the infolding of different layers of the cuticle, and they do not form a special structure detectable by light microscopy. The thickness of the cortical layer and the surface coating layer were reduced at the infolding sites of the cuticle (Figure 1, parts A and B).

The collagenous structures of the basal membrane of the cuticle were easily stained by Masson's Trichrome staining. Positive Masson's Trichrome streaks and bands (blue) were also evident in the thickness and length of the cuticle (Figure 1, parts C and D). The orcein staining of elastic fibers clearly displayed these streaks in outer layers, especially in cortical and surface coating layers (Figure 1, part E). Finally, the histological examination of the ala showed an apparent thickening in the cuticle along the whole body of the ala. The muscle structure underneath the ala was changed, and the subcuticle structure at the location of the ala was seemingly differentiated, creating a special structure. Histological sections and H&E staining on the ala illustrated that ala is mostly created by thickening of the median layer which is bounded by basal layer and surface layers of cuticle. There was no somatic layer in the ala structure (Figure 1, part F).

3.2. Histology of Muscles

Histological examination of the muscle layer in *T.canis* revealed that this tissue was mainly in the subcuticle region in direct contact with the cuticle. This layer was not observed in the distal parts of the coelom cavity. This muscle layer is composed of cylindrical, non-branching, somatic cells with multiple cornered nuclei. The striated muscle tissue observed in the cytoplasm of mammalian muscle cells is not detectable in this worm. The use of specific staining revealed that the muscle cells in this worm are not grouped, lack muscle support structures, such as epimysium, perimysium, and endomysium, and form a uniform muscle structure (Figure 2 part A, B, C, and D).

3.3. Histology of Intestinal Organs

Histological examination of the dentigerous ridges as the apparatus for attachment to the host in this parasite revealed that the dents were visible as sharp cuticular protrusions in a row on both sides of each lip (Figure 3, part A). Histological study of the structure of the esophagus as the second recipient of nutrient intake indicated that the different esophageal sections had the same histological structure, except the variation in the lumen diameter. The esophagus of this parasite is formed as a tube with two adjacent lips located in the interior of the corpus (the anterior part of the esophagus). These two lips have filamentous-muscular structures, demonstrating parasite-specific histological features when stained with H&E. The difference in the inner diameter of the esophagus has led to various names, such as isthmus and bulb for different sections (Figure 3, part B).

Examination of the intestinal structure of *T.canis* revealed that the intestinal wall in this worm has a fairly uniform structure along the intestine. It consists of a basal membrane, a simple elongated columnar

epithelium, as well as microvilli and glycocalyx, on its surface covering the entire inner surface of the intestine. The nucleus of the epithelial cells which is located at the base of the cell is usually round and well-defined. The nucleus of some simple columnar epithelial cells may be in rows above other nuclei (Figure 3 part C). Microvilli and glycocalyx were clearly visible in the PAS staining of the basal membrane. Furthermore, the PAS staining of the cytoplasm of simple columnar epithelium of the intestine displayed relatively large fragments of carbohydrate structures (Figure 3, part D). Finally, the intestinal tract is connected to the outside by a conduit at the far end of the worm. Throughout the respiratory tract up to the anal canal, the intestinal epithelial canal is identically covered by simple ciliated columnar epithelium (Figure 3, parts E and F).

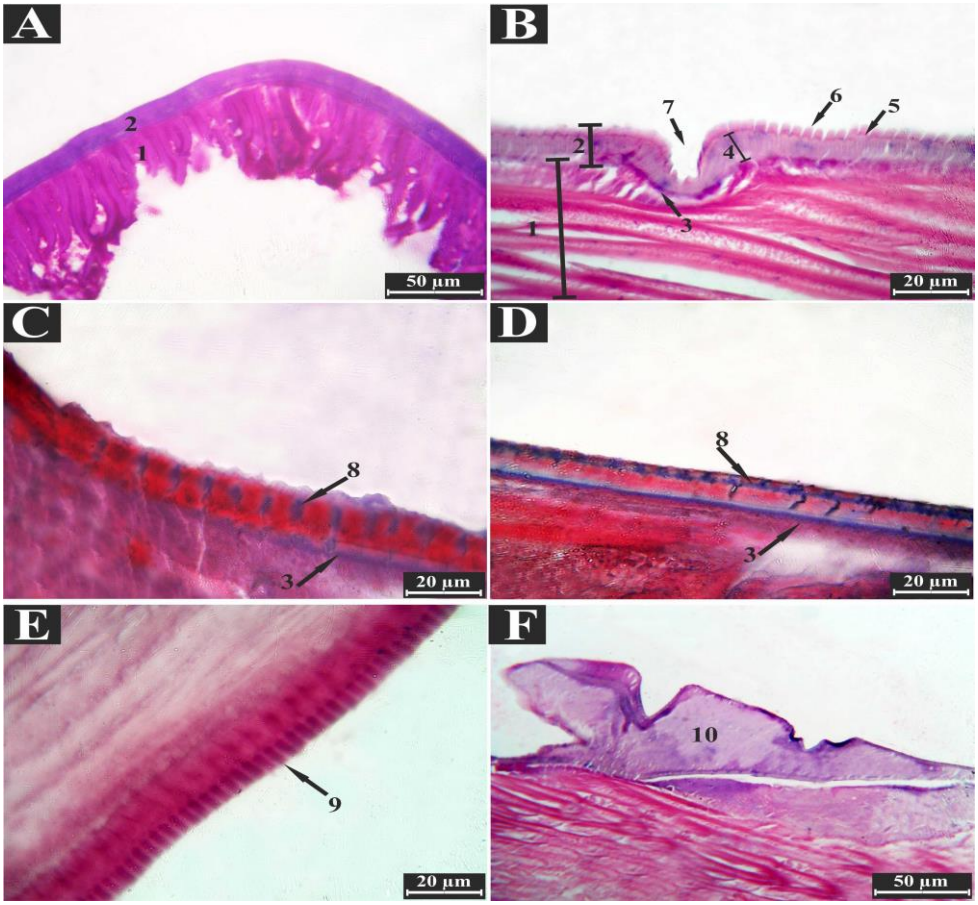

Figure 1. Histological section of cuticle and ala structures in female *T.canis* parasite
A: Transverse section of *T.canis* with PAS staining, magnification ×100. The body of parasite is wrapped in a cuticle which is supported by a muscle layer. Cuticle has reacted favorably to PAS. 1: muscle layer 2: cuticles.
B: Longitudinal section of T.*canis* with H&E staining, magnification ×400. 1: muscle layer / 2: cuticle / 3: basal layer / 4: median layer / 5: cortical layer / 6: invagination of cuticle.
C: Transverse section of *T.canis* with Masson's trichrome staining, magnification ×400. 3: basal layer / 8: collagenous strands are present along the whole thickness of cuticle layer
D: Transverse section of *T. canis* with Masson's trichrome staining, magnification ×400. 3: basal layer / 8: collagenous strands on the upper layers of cuticle which are spread along the cuticle forming an integral stripe.
E: Transverse section of *T. canis* with orcein staining, magnification ×400. 9: elastin fiber strands which are visible on the exterior surface of cuticle.
F: Longitudinal section of ala in *T.canis* with H&E staining, magnification ×100. 10: ala is formed by thickening of median layer of cuticle and the muscle layer does not penetrate it.

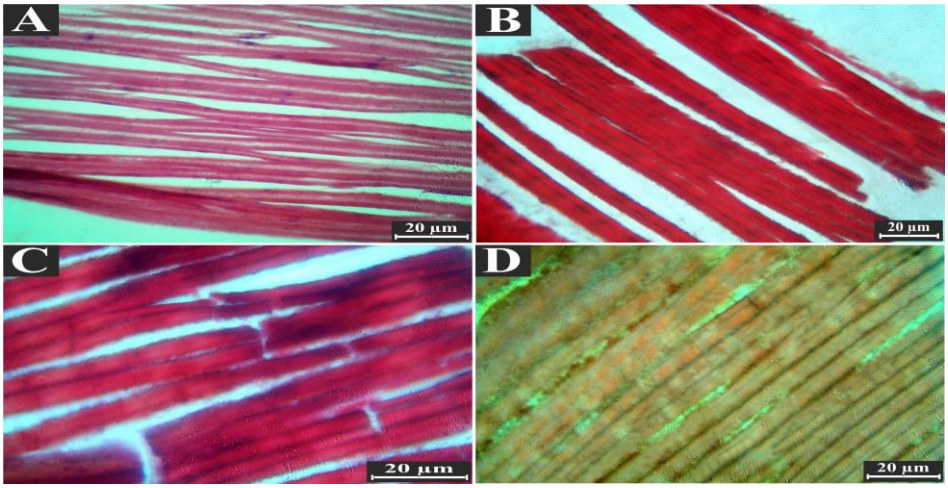

Figure 2: Histological section of muscle layer in female *T.canis* parasite
A, B: Longitudinal section of muscle layer stained by H&E, magnification ×400. Somatic cells are completely cylindrical, non-branching with multiple cornered nucleoli.
C: Longitudinal section of muscle layer stained with Masson's trichrome, magnification ×400. In this staining, no collagenous tissue was found between the muscle cells. The muscle layer in this worm does not form muscle groups and acts as a uniform muscle layer.
D: longitudinal section of muscle layer stained with Orcein, magnification ×400. The elastic tissue between muscle cells is mostly hardly detectable.

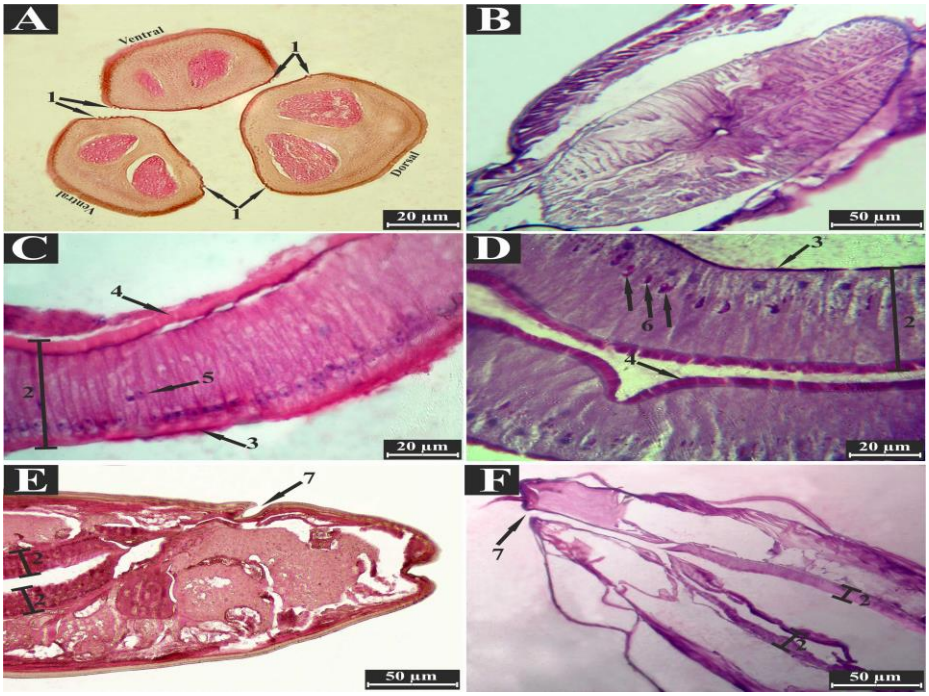

Figure 3: Histological section of different regions of intestinal tract in male and female *T.canis* parasite.
A: transverse section of lips using H&E staining, magnification ×400. 1: positioning and morphology of denticles.
B: transverse section of corpus esophagi using H&E staining, magnification ×100. The esophageal interior of this parasite encompasses a pair of lips with filamentous-muscular structure.
C: transverse section of intestinal wall with H&E staining, magnification ×400. 2: simple ciliated columnar epithelial tissue / 3: basal layer / 4: cilia with glycocalyx / 5: nucleoli of some simple columnar cells are located higher relative to other cells.
D: longitudinal section of intestinal wall with PAS staining, magnificati
on ×400. 3: basal layer / 4: cilia with glycocalyx 6: relatively large carbohydrate bodies absorbed by the absorbing columnar cells.
E: longitudinal section of posterior end of female *T.canis* with orcein staining, magnification ×100. 2: simple ciliated columnar epithelial tissue / 7: anal tube.
F: longitudinal section of posterior end of male *T.canis* with H&E staining, magnification ×100. 2: simple ciliated columnar epithelial tissue / 7: anal tube.

3.4. Histology of Physical and Chemical Receptors

The SEM imaging was used to identify and prepare serial sections of the lips and the posterior end of *T.canis* to study the chemical and physical receptors in this worm. The lips were covered with cuticles from the sides. Inside each lip, there is a framework or secretory network containing the nerve fibers and filamentous muscles specific to the parasite. At the apex of the dorsal lip, there is a pair of large double papillae, while large double papillae, small papillae, and an amphid are seen at the site of each ventral lip. This amphid is engorged by cuticular tissue, and the cephalic framework is visible in the subcuticular region. The location of the amphid was detectable using SEM imaging and with the help of papillae positions. Amphids were measured as 3-4 µm; moreover, large and small papillae were 7-10 and 6-8 µm, respectively (Figure 4 part A, B, C, and D).

Phasmid structure was also investigated using SEM and serial sectioning methods. The phasmid location was initially verified after observing post cloacal papillary structures and then studied under an optical microscope. Regarding the histological structure of the phasmids, similar to the amphids, they are surrounded by a cuticle which is engorged at the location of the phasmid. Muscular and nerve tissues are detectable inside this swelling. Phasmids were measured as 2-3 µm in size (Figure 5 part A, B, C, D and E).

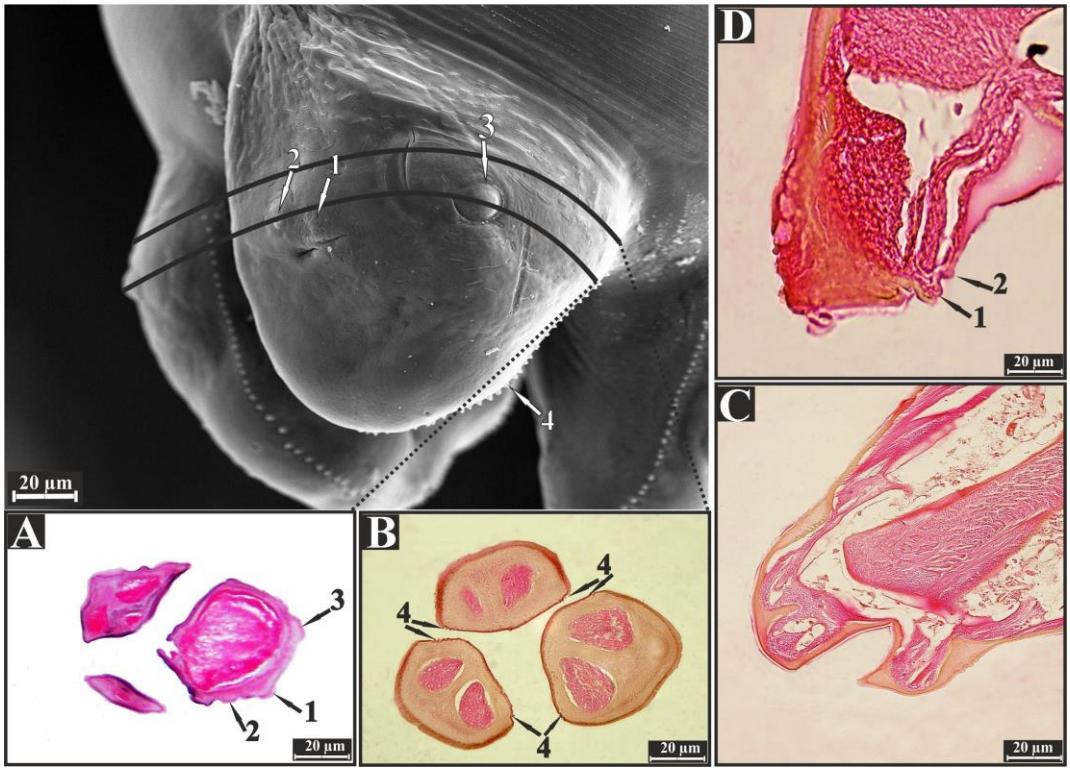

Figure 4. A, B, C and D scanning electron microscopy and light microscopy of lip in male *T.canis* using H&E tissue staining Magnification: ×400. 1: small papilla on ventral lip / 2: amphid / 3: large double papilla / 4: dentigerous ridges.

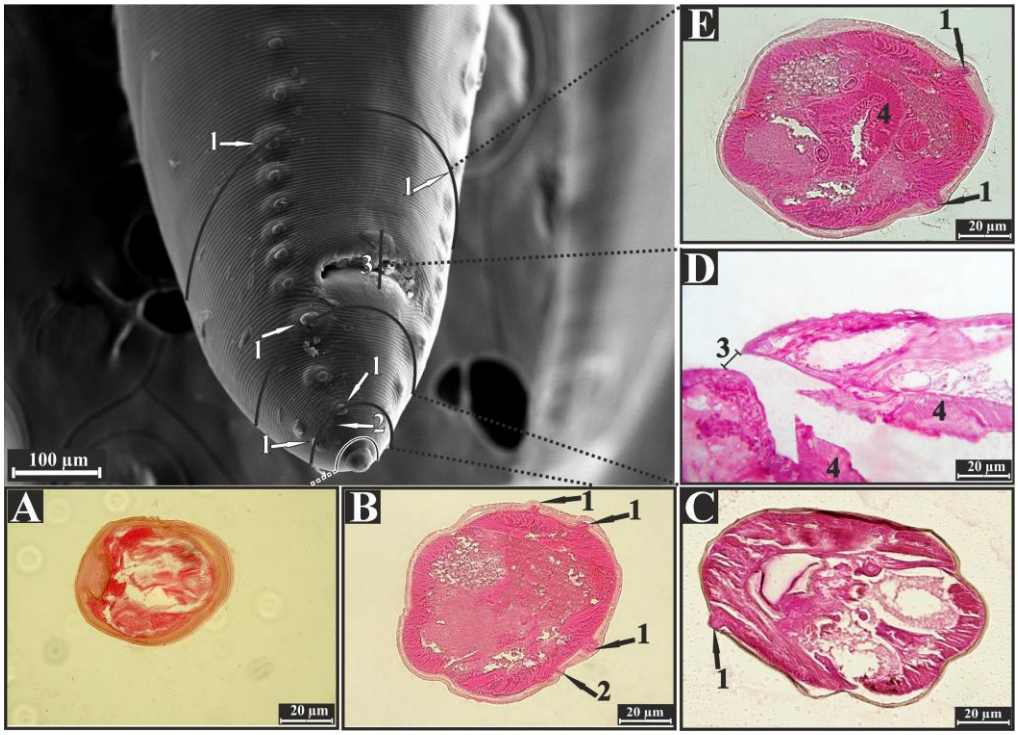

Figure 5. A, B, C, D and E scanning electron microscopy and light microscopy of tail in male *T.canis* using H&E tissue staining Magnification: ×400. 1: papillae / 2: phasmid / 3: cloaca / 4: intestine.

4. Discussion

Although numerous studies have been conducted on *T.canis*, there is not sufficient information available about this parasite, especially considering the chemical receptors. In the current study, *T.canis* specimens collected in Urmia were initially fixed in the appropriate solutions before being sent for SEM imaging to determine the exact location of the chemoreceptors. Subsequently, for the first time, the structure of these receptors was evaluated using specific staining and light microscopy. The histological capabilities of optical microscopy in the investigation of cuticular structure, muscular tissue, and intestine were evaluated.

Previous reports pointed to morphological similarities between *Ascaris lumbricoides* and *T.canis* in the sense that the anterior end of the ala, a large dorsal lip, two ventral lips, and ordered denticles are apparent in their inner sections. The external ring of papillae in *A.lumbricoides* was surrounded by four large papillae, two of which are dorsolateral located on the dorsal lip and one ventrolateral located on each ventral lip (Shalaby et al., 2010). According to Shalaby et al. (2010), the anterior end of *T. canis* is lance-shaped with three prominent lips, and the inner and outer rings of papillae and amphids were clearly visible. Denticles were also reported in a single row on the inner labial surface with relatively equal sizes. Ventral and dorsal papillae are different from those of *A.lumbricoides*, while cuticular striations in the vicinity of alae were more packed and less distinct (Shalaby et al., 2010). The findings of the present study were in agreement with previous reports regarding the morphology of the *Toxocaridae* family of parasites.

Little is known about the sensory organs and their histological structure in nematodes. In general, the

sense organ is composed of one or more ciliated dendritic processes, a sensory neuron, and two supporting cells, namely a socket and a sheath cell (Fagerholm et al., 2004). Amphids in nematodes are of different shapes and sizes: some of which are low-performance chemical receptors and some light receptors accompanied by their associated flushing glands (McLaren, 1976). Dendritic processes are situated inside the phasmid canal, indicating that phasmids have a chemosensory function similar to amphids (Fagerholm et al., 2004). The results of the current study confirmed the circular shape of the amphid and its central canal using optical and electron microscopy.

The investigation of dendrites in amphids of *Strongyloides stercoralis* and *Caenorhabditis elegans* demonstrated no signs of striated rootlets in the mechanocillia (Perkins et al., 1986). The reported amphids of *Physaloptera rara* also had a single pore (Naem and Asadi, 2013). The number of amphid pores can also be different among the members of a family in order. In this regard, there is a single large pore in *Spinitectus beaveri*, *Spirocerca lupi*, and three species of *Habronema* (Naem, 2007; Gorgani et al., 2013). Nonetheless, double-pored amphids have been reported in some other species of *Spinitectus* (Gorgani et al., 2013). Phasmids are involved in assessing the intensity of stimuli to the worm and helping them survive in an appropriate environment (Okulewicz et al., 2012).

Fagerholm et al. (2004) investigated the position of phasmids on the male *Hysterothylacium auctum* and reported that a pair of them is positioned laterally and equidistant from the rostrocaudal axis of the body and at 20 μm from the tip of the tail. In female worms, the phasmids were located symmetrically on the ventral to the lateral line. Phasmid's papilla in males was cone-shaped with a diameter of 5 μm with a central 1 μm knob (Fagerholm et al., 2004). In the current study, phasmid in *T.canis* had a size of 3-5 μm and was situated at 50-70 μm from the tip of the tail. Phasmids in male and female worms were situated symmetrically and had a central pore. These findings were verified with both light and electron microscopy.

A previous study hypothesized that phasmid position may be defined genetically, and it could mutate during the first larval division stage and blast cell stage in different groups of nematodes (Fitch, 1997). Only in some nematode specimens, amphids and phasmids are clearly visible under the light microscope and are reliably discernible from the particles adhering to the cuticle. Optical microscope examination of male *Hemicycliophora* and *Criconematidae* revealed no signs of phasmids or similar structure on the tail. Nonetheless, phasmid-like structures were identified in the mid-tail region of *Sphaeronema* under light microscopy but only with much difficulty (Moravec and Justine, 2014).

Amphid structure in *Acrobeles complexus*, a microbial feeding nematode, has been investigated. In these amphids, 13 sensory neurons were identified, 12 of which enter a sensory channel formed by a proximal sheath cell and a distal socket cell. The length of amphid in this parasite which is measured as the distance between the opening of the sensory channel and where the sensory neurites enter the socket cell is reported as 15 μm. At the entry point of sensory channels, multiple lamellar projections of the sheath cell wrap the sensory dendrite (Bumbarger et al., 2009).

Male *Baylisascaris procyonis* has a relatively long and narrowing tail, and a knob is usually present at the distal end of the tail. Cephalic papillae are on both sides and irregularly spaced, while there is a prominent double papilla on the outer margin of the anterior section of anus. There are five pairs of papillae in male worms, among which the first pair is double in size, compared to other pairs. One papillae pair is located near the anus, while the other four (caudal) pairs are situated near the distal end of the tail. The fourth pair of these papillae is curved and located next to the fourth pair of phasmids which have an open orifice at their center (Snyder and Fitzgerald, 1985). We also viewed the narrowing of the tail and posterior knob of male

T.canis. Six pairs of papillae were placed in irregular distances post-cloaca where the second pair was twice in size, compared to others. Phasmids were located adjacent to the fourth and fifth pairs.

Anterior papillae are studied more thoroughly in *T.canis*, in comparison with posterior papillae. There is a pair of amphids, as well as a large and a small papilla, at the sub-ventral lip. The number and rows of papillae in *A. lumbricoides* and A*scaris suum* are the same as *B.procynis*; nonetheless, phasmids in some species of *B.procynis* are located after the third pair of papillae, while they are situated posterior to the fourth pair in other species. The number and arrangement of papillae in *Parascaris equorum*, which is an equine roundworm, are similar to *B.procynis* except that in the former, the phasmids are located on the left side and near the third pair of papillae. Papillae in *Toxascaris leonina* are clearly different from the species of *B.procynis* (Snyder and Fitzgerald, 1985; Falcone et al., 2001; Xue et al., 2014; Shalaby et al., 2018).

The prominent papillae on the lips of *ascaris* are more likely to function as mechanoreceptors. These cephalic papillae may also have dual functions where the central swelling on the papillae acts as a mechanical receptor, while the lateral welling serves as chemical receptors. The cause of the difference in the number of papillae in *Ascaris* is not yet understood (Snyder and Fitzgerald, 1985).

The scanning electron microscopy of papillae on the lips of *B.procyonis* shows similarities to *T.leonina* and *A.lumbricoides* in terms of shape and morphology; however, it is different from *Toxocara* species (Snyder and Fitzgerald, 1985). Regarding the overall number of denticles on three lips in the present study, 100-128 denticles were counted on each lip of *T.canis*. This number of *B.procynis* is reported to be 650, 730 on *A.lumbricoides* and 210 on *T.leonina* (Snyder and Fitzgerald, 1985). The denticles on *T.leonina* are small, and their number was reported as 70 on each lip by Snyder and Fitzgerald (1985), while Xue et al. (2014) reported their number as 65-90 on each lip. This discrepancy in various reports can be ascribed to differences in hosts, the severity of infection, and geographical location (Xue et al., 2014).

The presence of papillae at the posterior end of *Ascaris* species has been verified in *A.equorum* using light and electron microscopy (Owen and Slocombe, 1985; Okulewicz et al., 2012). Lim (2008) has reported 25 pairs of pre-cloacal and 5 pairs of post-cloacal papillae in *T.leonina*. Nonetheless, Xue et al. reported these values as 23-30 and 4, respectively (2014). In the present study, two small circular apertures were observed on each lip of *T.canis*. Moreover, the presence of pre-and post-cloacal papillae was verified using light and electron microscopy.

In the same context, Xue et al. (2014) conducted a study on the morphological characteristics of *T.leonina*. They reported morphological differences between previous studies and theirs, including differences in the size, width, and length of cephalic ala and the number of denticles per lip. There are no reports on the number of denticles in *T. canis* to date for reference purposes.

In their study, Shalaby et al. (2009) found that adult *T.canis* worms were coated with an acellular cuticle with several thick layers produced by a substrate layer that is mostly smooth hypodermis (epidermis). The cuticle is essential for protective, nutritional, and shape-preserving purposes; moreover, it is an antagonist for somatic cells. The nuclei were only found in the four cords produced by hypodermic cells. Dorsal and ventral cords are longitudinally filled with axons, whereas lateral cords are located along the excretory channels and are surrounded by a series of seemingly branching ductules. Muscle cells had contractile and cytoplasmic parts, and a clear nucleus with nucleoli was visible within their cytoplasmic region (Shalaby et al., 2009). The results of the present study were in line with previous reports regarding the structure of muscle tissue.

Another study on the histological structure of the cuticle indicated that the cuticle in the *T.canis* parasite is composed of six layers, including basal, median, cortical, surface coat layer, as well as the outer first shed cuticle and the inner second shed cuticle

(Bruňaská et al., 1995). In the present study, basal, median, cortical, and surface coat layers were identified; however, the outer first shed cuticle and inner second shed cuticle layers were not distinguishable from the adjacent layers.

The thickness of cortical and surface coat layers was reduced at the site of cuticle infolding. The histological structure of ala was also attributed to the thickening of a median layer of the cuticle. The use of Masson's trichrome and orcein staining demonstrated that the elastin and collagen of cuticles were similar to those of mammalian and avian. These results have not been reported in other studies so far.

Regarding the structure of the intestinal tract (teeth, esophagus, and intestine), the results of the present study confirmed the similarities between the intestinal tract of this parasite and the epithelial structure of intestines in mammals and avians. This tract was composed of a layer of simple columnar cells covered by microvilli and glycocalyx in the absence of goblet cells. The basal layer of epithelium had a collagen-carbohydrate structure, and it seems that carbohydrate is absorbed in large portions in the intestine of the parasite.

It can be concluded that the basic structure of chemical receptors and their positioning can be studied using light microscopy. The light microscope is also useful in the investigation of other structures and organs of *T.canis* and can be utilized in these studies. This is also true for the investigation of the cuticle, muscle tissue, and intestinal tract. The results of the current study regarding the similarities of collagen and elastin structures of the cuticle, as well as the epithelium of the intestine in this parasite, could lead to the investigation of new strategies in the development of novel drugs and effective anti-parasitic agents which do not damage the host cells.

Authors' Contribution

B. Gh. examined the dogs, removed the worms, performed all parasitological, histological process using stereo and light microscope, and drafting the manuscript. S. N. designed the project, planning the work, transferred the samples to McMaster University, Canada, prepared the samples for SEM study, edited SEM micrographs, and drafting the manuscript. A. A. F. participated in designing the study, planning the work, commented on LM micrographs, and drafting the manuscript. All authors reviewed and approved the manuscript.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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