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ULTRASTRUCTURAL FEATURES OF THE DIGESTIVE SYSTEM OF THE ADULT HELMINTH *Heterakis gallinarum* SCHRANK, 1788 (NEMATODA: HETERAKIDAE)

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Abstract. Species of the Heterakidae family are similar in appearance. These signs cause difficulties in determining the systematic position of the species. For this purpose, the digestive organs of adult *H. gallinarum* nematode were studied at the ultrastructural levels by light and electron microscopic methods. The obtained results were compared with the structures of other species of the family. The digestive organs of adult *H. gallinarum* nematode are divided into 3 parts - stomodeum, intestine and proctodeum. The esophagus in turn is divided into procorpus, metacorpus and bulb. Its wall consists of basement membrane, muscle cells and cuticle. In the bulb, the muscle cells are large and relatively large in number. The intestine is divided into ventricular, middle and prerectal areas. It consists of a thick basement membrane, a single-layered epithelium with microvillas. Epithelial cells are divided into 5 areas that differ from each other according to their structure. The smooth membranes of the epithelial cells create a barrier connection with each other. Sphincter cells are noted at the end of the intestine. After that part, the rectum was found in females and the cloaca in males nematodes. The lumen of both is covered with cuticle. The ultrastructural features of the digestive organs of the nematode *H. gallinarum* are consistent with the main systematic features of the family.

Keywords: *Nematode, H. gallinarum, digestive organs, ultrastructure, TEM.*

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1. Introduction

The class of nematodes has a wide species composition and the morphological structures of their digestive organs are also diverse. Those signs, in turn, play an important role in determining the systematic positions of helminths. In this regard, since most of the species included in the Heterakidae family are similar in appearance, their determination causes certain difficulties. Among the helminths included in the above-mentioned family, there is some information on the study of the digestive organs of *H.*

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dispar, H. spumosa and *H. gallinarum* with the help of light and electron microscopic methods (Lee, 1975; Wright & Hui, 1976; Zmoray & Guttekova, 1978; Bogoyavlenskiy *et al*., 1982; Bird & Bird, 1991; Mehlhorn & Harder, 1997; Rzayev, 2023a). From the sources presented, only studies on the complete ultrastructural characteristics of the digestive organs of the nematode *H. dispar* were conducted (Rzayev, 2023a). In other species, the digestive organs were either studied only by histological method or the entire digestive system was not covered (Bogoyavlenskiy *et al*., 1982; Nasirov *et al.*, 2008). Three species of nematodes (*H. gallinarum*, *H. dispar* and *H. altaica*) belonging to the Heterakidae family were found out of 27 species of helminths recorded in domestic waterfowl in the territory of the Republic of Azerbaijan (Rzayev, 2013; 2021a; 2021b; 2023c; Rzayev *et al.*, 2021a; 2021b; Seyibeyli & Rzayev, 2018). We have conducted studies on the ultrastructure of some of the parasites found in birds, fish and other invertebrates in the territory of our country (Nasirov *et al*., 2020; 2024; Seyidbeyli *et al*., 2020; Rzayev *et al*., 2020; 2022; 2023; 2024). Taking into account the above, we aimed to study the ultrastructural features of the digestive organs of the nematode *H. gallinarum* with the help of light and electron microscope methods, which is spread in poultry farms in the country, has a wide range of hosts and belongs to the Heterakidae family.

2. Material and methods

Eleven years old domestic geese (*Anser anser* dom.) previously identified as infected with helminths *H. gallinarum* were obtained from the city of Ganja and brought to the Laboratory of Parasitology of the Institute of Zoology and examined by method full parasitological dissection (Dubinina, 1971). *H. gallinarum* nematodes were collected from the cecum of the birds. Fixed preparations of helminths were studied under a stereomicroscope MBS-9 and a light microscope Primo Star (Carl Zeiss, Germany). For the identification of the species, Ryzhikov's (1967) key guide was used. In order to study the ultrastructure of the helminths, the collected adult nematodes were divided into several parts and immediately were fixed in a solution consisting of 2.5% glutaraldehyde, 2% paraformaldehyde, 4% surcosa, 0.1% picric acid prepared in 0.1 M phosphate buffer (pH 7.4). For further research, the fixed material was brought to the Electron Microscopy laboratory (Azerbaijan Medical University). After keeping the samples in that fixer for one day, they were postfixed in 1% osmium tetraoxide solution prepared in phosphate buffer (pH 7.4) for two hours. Araldite-Epon blocks were prepared from the material using general methods adopted in electron microscopy (Kuo, 2014; Yushin *et al*., 2021). Semi-thin (1–2 μm) sections taken from the blocks on a Leica EM UC7 (Leica, USA) ultramicrotome, stained with methylene blue, azur II and basic fucsin (D'Amico, 2005; Morikawa *et al.,* 2018), were viewed under a Primo Star (Zeiss, Germany) microscope. Images of the necessary sections were taken with a digital camera EOS D650 (Canon, Japan). Ultrathin sections (50–70 nm) of the blocks, double-stained with uranyl acetate and lead citrate, were examined under the Transmission Electron Microscope JEM-1400 (JEOL, Japan) at a voltage of 80–120 kV. The morphometric analysis of the electronograms was carried out in TIF format via a computer program (TEM Imaging Platform) developed by Olympus Soft Imaging Solutions GmbH (Germany) (Agayeva *et al.,* 2020). Data analysis was carried out with different parameters (Min, Max, mean ± SD).

3. Results and discussion

During the study of the nematode *H. gallinarum* by electron microscopic method, it was found that the internal organs of the helminth (digestive and reproductive) are located in the saclike pseudocoelomic cavity. It was determined that the thickness of the basal membrane (Bmp), which forms the wall of the pseudocoelomic body cavity, which contains fluid in addition to the organs, is 0.044-0.087 μm (0.065±0.004 μm). The wall of the pseudocoelomic cavity of the nematode *H. gallinarum* is presented in an electronogram obtained through an electron microscope (Figure 1A).

During the light and electron microscopic examination of the nematode H. gallinarum, it was found that the digestive system is divided into three main parts. In the front part of the body there is stomodeum – buccal cavity (stoma), pharynx, esophagus, then intestine and finally proctodeum - posterior part of intestine. Thise organs are composed of different differentiated cells. The first part of the digestive organs of *H. gallinarum* nematode begins with 3 lips of cuticular origin. The lumen of the buccal cavity and the pharynx covered with cuticle. The helminth's esophagus is divided into three parts, as in other species of the family: anterior - the procorpus, which has a cylindrical structure; metacorpus and basal section - bulb. The esophagus is surrounded by a basement membrane. Lateral and radial muscle cells are observed inwards. The lumen is finished with a cuticle. In addition to the structures mentioned in the esophagus, there are glandular cells which drains open to the lumen, processes of nerve cells and esophagal valves with cellular structures to prevent food from returning. In the cytoplasm of lateral and radial muscle cells, there are a large number of mitochondria, glycogen, fibrils, ribosomes, etc. organelles are observed. The cuticle found at the lumen of the esophagus is divided into cortical, homogenous and basal layers. In the bulb part, the muscle cells are large in size as well as in number. The cytoplasm of muscle cells is rich in fibrils. Glandular cells are noted in the front and back of the bulb and the lumen is also covered with cuticle. Between the esophagus and the intestines is a valve consisting of several cells of epithelial origin.

The intestine of the nematode *H. gallinarum* is of endodermal origin and is tube-shaped, as in other representatives of the family. The intestine of the helminth is divided into anterior (ventricular), middle and posterior (prerectal) parts. The general image of the intestine of the studied nematode under a light microscope is presented in figure 1B, and its diameter is 39.22-78.51 μm (65.52±3.92 μm). An electron microscopic view of the intestinal wall is given in figure 1C. It has been found that the intestine of the nematode is covered externally by a thick basement membrane (Bm) (Figure 1D). Its thickness is 0.23-0.28 μm (0.25±0.005 μm). Under the basement membrane is a single-layer epithelial cells (10.4-14.5 μm (12.3±0.35 μm)) rich in cytoplasmic organelles. The epithelial cell, in turn, is divided into several structurally different parts. Microvilli (Mv) are noted in the part of the intestine lumen and their length is 2.1-2.3 μm (2.2±0.016 μm) (Figure 1C). The intestinal wall of *H. gallinarum* nematode studied by electron microscopic method was determined to be ultrastructurally composed of 5 parts: basement membrane (Bm), reticular area (Re), main or plasmatic part (Pla), terminal or fibrillar area (Fib), part with microvillas (Mv) (Figure 1C). The named fields are structurally different. Figure 1D shows the basement membrane (Bm) and reticular (Re) area of the intestinal wall. The basement membrane was found to be thicker than other parts of the helminth. In the reticular area, three-layer membranous structures with protrusions of the basement membrane, which are not observed in other parts of the

intestinal wall, are noted (Figure 1D). They cover almost the entire reticular area and extend to the border of the plasmatic area.

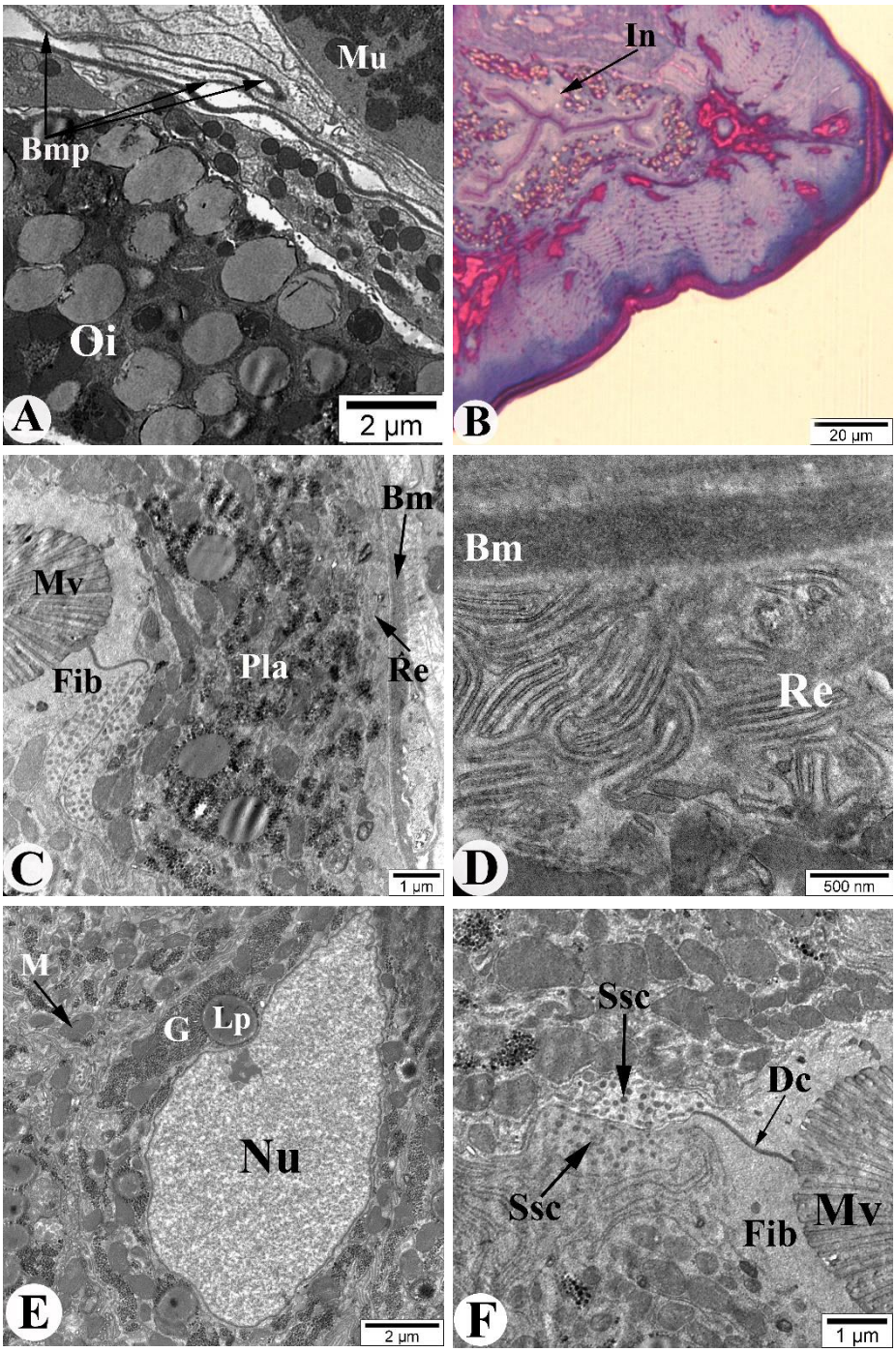

Figure 1. Light and electron microscopic images of the digestive organs of the nematode *H. gallinarum*. A- the wall of the pseudocoelomic cavity, B- intestine, C - layers of the intestinal wall, D - basement membrane and reticular area, E - plasmatic part, F - areas with fibrillar and microvillas. Designations: Oi - oviduct, Mu - muscle cell, Bmp - basement membrane of pseudocoelomic cavity, In - intestine, Bm - basement membrane, Re - reticular part, Pla - plasmatic part, Fib - fibrillar part, Mv - microvillas, M - mitochondria, G - glycogen, Lp - fat droplets, Nu - nucleus, Ssc - smooth septate junction, Dc - dense connections. (B) - semi-thin section (1 µm), Morikawa et al. (2018) one-step staining, (A, C-F) - ultrathin sections (50-70 nm), stain: uranyl acetate and Pb citrate

They create a network by multiplying the area of the plasmolemma and are believed to be involved in metabolism, transporting substances into the body cavity of the helminth. The next and largest area is the plasmatic part. Here are the nucleus (nucleolus inside), cytoskeleton elements, granular endoplasmic reticulum, non-granular endoplasmic reticulum, electron-dense granules, holji complex, lipid granules, glycogen, ribosome, mitochondria, various lysosomal bodies, vacuole, basal bodies, membrane-like vesicles, etc. organelles are located (Figure 1E). The nucleus in the center is either oval or round in shape. Its diameter is 5.0-6.0 μm (5.7±0.12 μm). The nucleus is clearly visible and observed near the nuclear envelope. Mitochondria, glycogen, ribosomes, lipid granules and endoplasmic reticulum are predominant organelles in the plasmatic part. In Figure 1F, fibrillar (Fib) and microvillas (Mv) areas are presented in sequence. Microvillas are associated with fibrils in the terminal part of the epithelial cell. Microvillas are covered with glycocalyx. That glycocalyx protects the microvillas from physical and toxic effects. The fibrillar or terminal part consists only of small fibrils. Dense connections (Dc) are observed between the epithelial cells (mainly in the fibrillar area) that make up the intestinal wall of the nematode *H. gallinarum* (Figure 1F). In addition to the above, the smooth membranes of the epithelial cells of the intestinal wall are also found to have a barrier connection (Ssc-smooth septate junction) with each other (Figure 1F). The sphincter (muscle) cells are located at the end of the intestine of the helminth *H. gallinarum* and open into the rectum in females and into the cloaca in males. The part of the wall of the rectum and the cloaca in the lumen area covered with cuticle.

The digestive and reproductive organs of the investigated nematodes, including the parasites belonging to the Heterakidae family and whose ultrastructure was studied, are located inside the pseudocoelomic cavity (Rzayev, 2023a; 2023b). As a result of the ultrastructural study of the wall of the pseudocoelomic cavity of *Caenorhabditis elegans*, a free-living nematode, it was found that it cannot be attributed to cells of mesodermal, endodermal or ectodermal origin (Bird & Bird, 1991). From the parasitic worms, there is some information about the structure of the pseudocoelomic cavity of the nematode *Ascaris lumbricoides* (Harris & Crofton, 1957). Although the ultrastructure of the digestive and reproductive organs of the species included in some families (Capillariidae) has been studied, no information has been given about the pseudocoelomic cavity (Nasirov, 1996). There is detailed information about the pseudocoelomic cavity of the species included in the Heterakidae family and whose ultrastructure has been studied (*H. spumosa* and *H. dispar*) (Mehlhorn & Harder, 1997; Rzayev, 2023a). The wall of the pseudocoelomic cavity of the nematode *H. gallinarum* belonging to the genus Heterakis, whose ultrastructure was studied by us, was determined to consist of a basement membrane (Figure 1A). The above-mentioned signs are also reflected in the works of other scientists (Bird & Bird, 1991). The digestive organs of most nematodes consist of 3 parts, provided that the basic principles are preserved. However, in species belonging to different families, the digestive organs differ somewhat morphologically (Bird & Bird, 1991). One of the characteristic morphological features of the representatives of the Heterakidae family studied by us is that the rear part of the esophagus consists of a bulb. In literature sources, there is only partial information about the helminths *H. spumosa, H. dispar* and *H. gallinarum* from the species included in the mentioned family (Mehlhorn & Harder, 1997; Bogoyavlenskiy *et al*., 1982; Nasirov *et al.*, 2008; Rzayev, 2023a). A number of sources have been found about the structure of the digestive organs of the *H. gallinarum*

nematode (histological and to a lesser extent, electron microscopic) (Lee, 1975; Zmoray & Gutteková, 1978; Bogoyavlenskiy *et al*., 1982; Nasirov *et al.*, 2008). As a result of the analysis of the above-mentioned literature, it was found that the digestive organs of nematodes consist of stomodeum, intestine and proctodeum parts (Bird & Bird, 1991; Mehlhorn & Harder, 1997; Bogoyavlenskiy *et al*., 1982; Nasirov *et al.*, 2008). It was determined that the digestive organs of the nematode *H. gallinarum*, whose ultrastructure was studied by us, also consist of 3 parts. In the literature, it is shown that the esophagus of the helminths *H. gallinarum* and *H. dispar* is divided into several parts (procorpus, metacorpus - the anterior part is thin, the posterior part is bulb) (Bogoyavlenskiy *et al*., 1982; Rzayev, 2023a). The esophagus of the nematode studied by us consists of the mentioned parts. Among the species included in the family, only the ultrastructural characteristics and morphometric indicators of the cuticle located in the lumen of the esophagus wall of the nematode *H. dispar* were studied. It was found that the same cuticle is composed of 3 different layers (Rzayev, 2023a). The same structures were observed in *Leptonemella juliae* (Nematoda, Adenophorea), a representative of other families (Hoschitz *et al*., 2001). Cuticle layers were also found in *H. gallinarum* nematode. Depending on the location of the cuticle, the secretion of cuticle is also different. Thus, while the cuticle that covers the helminth from the outside takes its development from the hypodermis, the cuticle in the lumen part of the wall of the oral cavity, pharynx and genital organs develops from epithelial cells and the cuticle located in the lumen of the esophagus develops from both epithelial and muscle cells (Altun & Hall, 2009). In the bulb part of *H. dispar* nematode, large muscle cells rich in fibers and 3 glandular cells were found (Rzayev, 2023a). Those structures were also observed in the nematode *H. gallinarum.*

Among the parasitic and free-living nematodes, the species whose intestines have been studied at the ultrastructural level are the following: *Strongylus equinus, Ascaris suum, A. lumbricoides, C. elegans, Parascaris equorum, Oxyuris equei, Ancylostoma caninum* (Bogoyavlenskiy *et al*., 1982; McGhee, 2007). There is some information about the structure of the intestines of *H. gallinarum, H. spumosa* and *H. dispar* species from the parasites of the Heterakidae family (Bogoyavlenskiy *et al*., 1982; Mehlhorn & Harder, 1997; Rzayev, 2023a). The analysis of the mentioned literature shows that the intestine is tubular and consists of a basement membrane, a single-layered epithelial cells that ends with microvillas. The number of epithelial cells in the cross-section of the intestinal wall varies depending on the species. The presence of a thin layer of smooth muscle in the intestinal wall is available only in the helminth *H. spumosa* (Mehlhorn & Harder, 1997). In our studies, no muscular layer was detected in the intestinal wall of the nematode *H. gallinarum*. In the *H. dispar* nematode, smooth septate junctions were found at the border of intestinal epithelial cells (Rzayev, 2023a). Those smooth septate junctions were also observed in *C. elegans* species from other families (Herndon *et al.*, 2018). The mentioned structures were also found in the helminth *H. gallinarum*, whose ultrastructure was studied by us (Figure 1F).

The last of the digestive organs of H. gallinarum is the posterior part of the intestine, the ultrastructure of which is different in male and female individuals. At the same time, both individuals have sphincter muscles or intestinal valves (intestinal rectal valve) between the intestine and its posterior part (Bird & Bird, 1991; Lee, 1975). Sphincter muscles have also been noted in the nematode *H. gallinarum.* Covering the lumen of the posterior part of the intestine with cuticle was found in nematodes *H. gallinarum* and *H. dispar*. According to some scientists, those structures are considered

as a continuation of the cuticle that covers the body wall of the parasitic worm (Bogoyavlenskiy *et al*., 1982). Thus, the ultrastructure of the digestive organs of *H. gallinarum* nematode was studied separately with the help of light and electron microscopic methods and a comparative analysis was given with the structures of other species of the family. The structure of the digestive organs of the *H. gallinarum* nematode is compatible with the main systematic features of the family.

4. Conclusion

As a result of the ultrastructural study of the digestive organs of *H. gallinarum* nematode, it was found that they are divided into 3 parts - stomodeum, intestine and proctodeum, located in the pseudocoelomic cavity of the helminth. The esophagus, in turn, is divided into procorpus, metacorpus and basal parts and its wall consists of basement membrane, lateral and radial muscle cells and cuticle. In the bulb, the muscle cells are large and relatively large in number. The lumen of the bulb is also covered with cuticle. The intestine developed from the endoderm and is divided into ventricular, middle and prerectal areas. It consists of a thick basement membrane, a single-layered epithelium with microvillas. Epithelial cells are divided into 5 various parts that differ from each other according to their structure. The smooth membranes of the epithelial cells create a barrier connection (smooth septate junction) with each other. The intestine is ended with sphincter cells and opens into the rectum in females and into the cloaca in males. The lumen of both is covered with cuticle.

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