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Ultrastructure of *Dirofilaria repens* of dog

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Abstract
Dirofilaria repens is a filarial nematode which cause subcutaneous dirofilariasis. The study presented the detailed ultrastructural features of *D. repens*. The nematode present in a nodular mass in the tunica vaginalis of a dog was studied for light and electron microscopic features. For transmission electron microscopy (TEM), nematode was fixed in glutaradehyde and resin blocks were prepared. Ultrathin sections (70 nm) were stained with uranyl acetate and lead citrate and observed under Transmission electron microscope. Transmission electron microscopic studies revealed that the body wall of the parasite is a well-developed multilayered cuticle. The intestine was lined by single layer of thin columnar epithelial cells which are connected by tight junctional complex. The uterine wall composed of muscle fibers surrounded by basal lamina and cuboidal epithelium lining the lamina. Microfilarial larvae were unsheated with distinct trilaminated body wall and contain nucleus and intracytoplasmic organelles mitochondria and rough endoplasmic reticulum. This study will provide additional information on the structures of *Dirofilaria*.

Keywords: *Dirofilaria repens*, dog, transmission electron microscopy

1. Introduction
Dirofilariasis is a parasitic disease of dogs caused by *Dirofilaria* species which are transmitted by the intermediate hosts and vector, mosquitoes. In India, dogs are primarily affected by two main filarial parasites: *Dirofilaria immitis* (heartworm) in the right ventricle of the heart and *D. (Nochtiella) repens* Railliet *et* Henry, 1911 in the subcutaneous tissue [1]. Dogs, cats, and wild carnivores are final hosts of *D. repens* [2]. This parasitosis is widely distributed in Africa [3], Asia [4] and southern Europe [5, 6].
Till date very few transmission electron microscopic studies on *D. repens* is reported. Thus, we studied the ultra-structural features of *D. repens* which was present in a nodular mass in a dog.

2. Material and Methods
The nematodes which were present in a nodular mass in the tunica vaginalis of a dog were collected for light and electron microscopy. The nematodes structure was studied microscopically [7]. For transmission electron microscopy (TEM), nematode was fixed in 2.5% glutaradehyde at 4 ^{0}C and processed as described earlier [8]. After several washing, the 1mm^3 transversely cut parasites were post fixed in 1% osmium tetraoxide for 1 hour at 4 ^{0}C followed by dehydrated at various grade of acetone (30-100% acetone) for 30 min each at 4 ^{0}C and clearing with 2 changes of toluene for 30min at room temperature and further processed and embedded in pure epoxy resin to make blocks. Ultrathin sections (70 nm) were cut and stained with uranyl acetate and lead citrate. The sections were visualized under Transmission electron microscope (Morgagni 268D, Fei Electron Optics) at SAIF-AIIMS, New Delhi.

3. Results
Microscopically, the parasites were identified as *Dirofilaria repens* as described earlier [7] (Fig. 1). The outermost layer of the parasites composed of thick multilayered cuticular ridges, followed by transverse smooth muscles striations. The cuticle protruded into each lateral chord which extended into the body cavity and were divided into sublaterals. Gravid uterus and intestine were also present in the body cavity.
Transmission electron microscopic (TEM) studies revealed that the body wall of the parasite is a well-developed multilayered cuticle.

Beneath it is the hypodermis and extends towards the body through its hypodermal cords (Fig. 2A). Hypodermis varies in thickness where the lateral areas are thicker than the region of the muscle band. The somatic muscle consists of two parts: a contractile part, myofibrillar and a non-contractile part, sarcolemic bulb (Fig. 2B). This myofibrillar part consists of thick, myosin and thin, actin myofilaments, presence of glycogen and mitochondria in the interfibrillar areas (Fig. 2C). They are inter-laminated obliquely at regular intervals by interfibrillar structures. Sarcolemic bulb consists of glycogen granules, ribosome and mitochondria. The interfibrillar structures are penetrated by sarcotubules which contain fibrous elements and dense bodies. Numerous mitochondria are present in sarcotubular membrane (Fig. 2D).

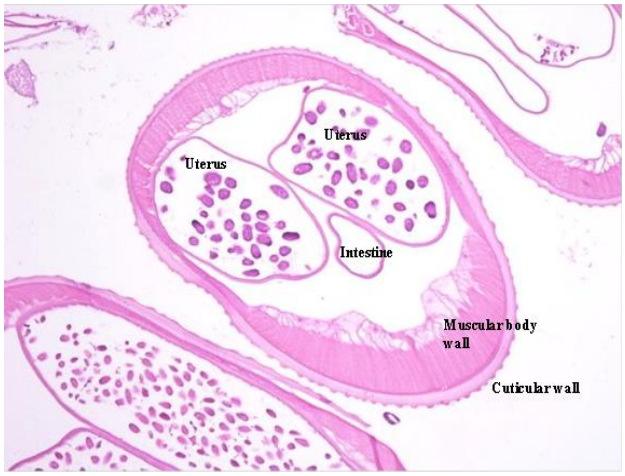

Fig 1: Photomicrograph of a transverse section of *Dirofilaria repens* showing the multilayered muscular wall with ridged cuticle. The body cavity showed cross section of the uterus and intestine. (H E, 40X)

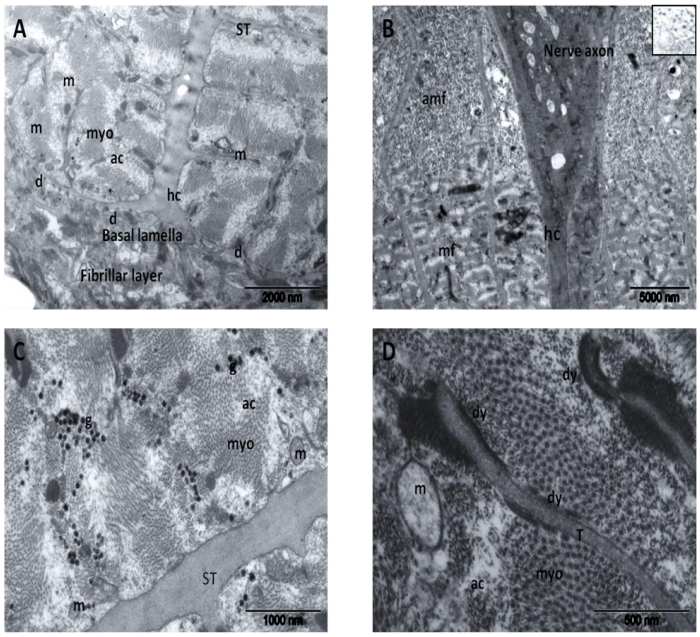

Fig 2: A-D: Transmission Electron Microscopic features of the body wall: A. Well-developed multilayered cuticle composed of fibrillar layer and basal lamella. It is followed by the hypodermis which extends stowards the body through its hypodermal cords (hc). The hypodermis is attached to the basal lamella by tight desmosomes (d) at alternating distance. Underneath are the somatic muscle cells. B. The somatic muscle consists of two parts: a contractile part, myofibrillar (mf) and a non-contractile part, sarcolemic bulb (amf). This myofibrillar part consists of thick, myosin (myo) and thin, actin (ac) myofilaments, presence of glycogen (g) and mitochondria (m) in the interfibrillar areas. Sarcolemic bulb consists of glycogen granules, ribosome and mitochondria (inset). The hypodermal cord (hc) extends upto the non-contractile part and showed multivessiculated body (MVB) forming nerve axon. C. The interfibriller structures are penetrated by sarcotubules (ST) and contain fibrous elements, dense bodies often associated with sarcotubular membrane, numerous mitochondria (m) and glycogen (g) particles. D. In the myofirillar part, dyads (dy) are formed by cisternal structures along the T-tubules (T).

Further, TEM showed that the intestine was lined by single layer of thin columnar epithelial cells which are connected by tight junctional complex (Fig. 3A). Epithelial cell is divided into an apical, central and basal part. The apical cell surface is modified into irregularly arranged microvilli with dense central core (Fig. 3B). The central part of the epithelia contains a large nucleus and a few organelles like Golgi complexes, ribosomes, mitochondria. Lipid and pigment granules were also seen. The basal plasma membranes are deeply invaginated to form labyrinth of cisternal membrane in the basal part of the epithelia (Fig. 3C).

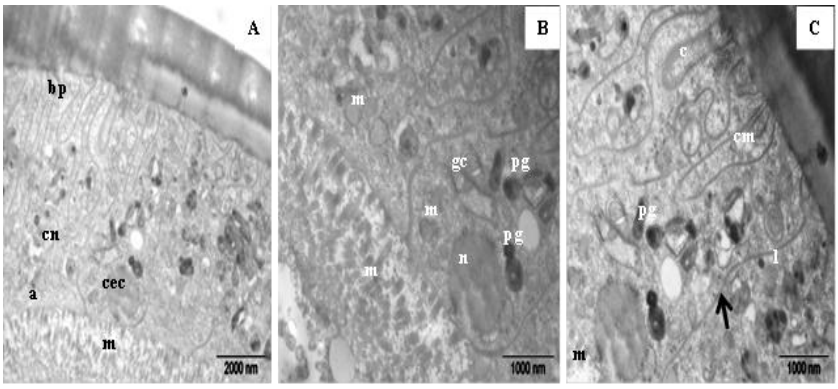

Fig 3: A-C: Transmission Electron Microscopic features of the Intestine: A. The intestine is lined by single layer of thin columnar epithelial cells (cec) which are connected by tight junctional complex (tj). Epithelial cell is divided into an apical (ap), central (cn) and basal part (bp). B. The apical cell surface is modified into irregularly arranged microvilli (mv) with dense central core. The central part of the epithelia contains nucleus (n) and a few organelles like Golgi complexes (gc), ribosomes (r), mitochondria (m). Lipid (l) and pigment granules (pg) are also seen. C. The basal plasma membranes are deeply invaginated to form labyrinth of cisternal membrane (cm) in the basal part of the epithelia. Epithelial cells have terminal bars (arrow) at the lateral aspects.

Ultrastructural features of the uterine wall composed of muscle fibers surrounded by basal lamina and cuboidal epithelium lining the lamina (Fig. 4A). Each epithelial cell has a large euchromatic nucleus with one distinct nucleolus (Fig. 4B). The cytoplasm comprises an extensive system of rough endoplasmic reticulum and mitochondria. The basal part of the cell has basal infoldings. Uterine epithelial cells lumina were lined by electron dense granular material and the uterine lumen was packed with numerous microfilariae. Microfilarial larvae were unsheated with distinct trilaminated body wall and contain nuclear column cells and intracytoplasmic organelles mitochondria and rough endoplasmic reticulum (Fig. 4C).

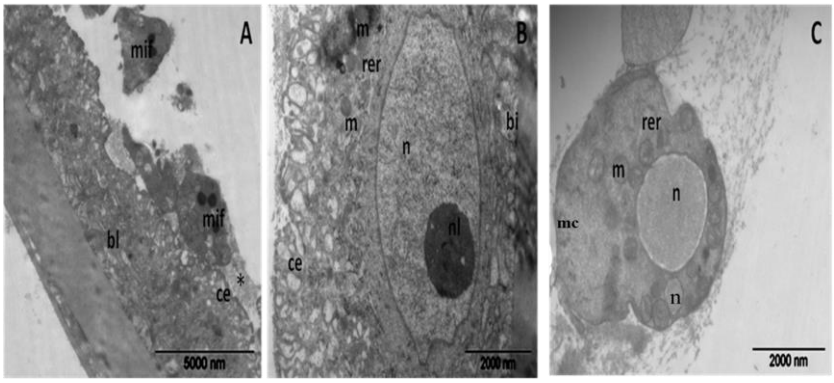

Fig 4: A-C: TEM, Uterus: A. The uterine wall composed of muscle fibers surrounded by basal lamina (bl) and cuboidal epithelium (ce) lining the lamina. Uterine epithelial cells lumina are lined by electron dense granular material (*) and the uterine lumen was packed with numerous microfilariae (mif). B. Each epithelial cell has a large euchromatic nucleus (n) with one distinct nucleolus (nl). The cytoplasm comprises an extensive system of rough endoplasmic reticulum (rer) and mitochondria (m). The basal part of the cell has basal infoldings (bi). C. Microfilarial larvae were unsheated and have distinct trilaminated body wall and contain nuclear column cells (n) and intracytoplasmic organelles mitochondria (m) and rough endoplasmic reticulum (rer) and muscles cells (mc).

4. Discussion

The occurrence of dirofilariasis in domestic dogs differs by state and geographical area. Generally, the infection is predominant in dogs where vaccination is not routinely recommended. However there are limited reports of this infection from India. *D. immitis* are geographically limited to North-Eastern parts of India while *D. repens* to Southern part of India, with an overlapping area centrally [9]. In a large number of animals, subcutaneous dirofilariasis appears non-symptomatic (healthy carriers) [10]. The morphology of the worm provides a much better degree of accuracy for diagnosis than any other serological and molecular technique.

Transmission electron microscopic (TEM) studies of *D. repens* is scarce. We report *D. repens* infestation in a dog with special reference to TEM studies of the parasite. Ultrastructure of *D. repens* is similar to those described for *D. immitis* except for the smooth cuticle and lack significant longitudinal ridges [11]. Microfilarae were unsheathed, with blunt head and a tapering tail [12]. The cuticle of the nematodes is multilayered and is regarded as an extra-cellular structure [13]. The somatic muscle of *D. repens* is similar to that of *D. immitis* [14] and *Ascaris lumbricoides* [15] and is termed as obliquely striated since it conform neither striated nor smooth category. The myofibrils of *D. repens* composed of thick and thin filaments. In nematodes two broad morphological patterns exist; the banded and obliquely striated and the non-banded category. Glycogen granules and mitochondria which were present in the interfibrillar areas and sarcolemic bulb may act as a source of energy for the muscle contraction. The dyads of body muscle cells are probably sites between terminal cisternae of the sarcoplasmic reticulum and the plasma membrane or inter cisternal membrane. Dyads help to trigger signals from the plasma membrane for the release of calcium ions required for contraction of the muscles [16].

The intestine is largely responsible for food digestion and assimilation as well as the synthesis and storage of

macromolecules. In *D. repens* it is lined by monolayer of thin columnar epithelial cells or enterocyte which are connected by tight apical junctional complex. Discrete electron dense regions can be detected just beneath the apical, lumenal surface of the intestinal cells, joining each cell to its neighbor [17]. The apical cell surface is modified into irregularly arranged microvilli which is similar to that of *D. immitis* [18] except for the presence of dense central core in microvilli in this case. Microvilli are encased by a fuzzy layer of glycoproteins which protect the microvillar surfaces from mechanical injury or from pathogenic attack and act as filter allowing only digestion products to reach the absorptive surfaces and a scaffold for presenting digestive enzymes to the contents of the intestinal lumen [19]. Sphaerocrystals or pigmented granules are described in the enterocytes of *D. immitis* [18] and many other nematodes. These granules are chemically highly complex aggregate of peroxidated lipid and protein which is formed by the action of free-radicals and lipid peroxides. These gut granules also served as markers of intestinal differentiation and is shown to be lysosome-related organelles in *Caenorhabditis elegans* [20, 21]. Microfilarial features are similar to that described for *D. immitis* [22]. Talluri and his co-workers [23] studied the comparative ultrastructure of *Dirofilaria repens* (Nematoda: Filarioidea) development in susceptible and refractory strains of *Aedes aegypti*. They observed simple trilaminated cuticular structure.

5. Conclusion

Thus the present study describes detailed ultrastructural features of *D. repens.* This may provide additional knowledge in the field of ultrastructure of *D. repens*.

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7. References

1. Railliet A, Henry A. Researches sur les ascarides des carnivores. Compted Rendus des Séances de Société Biologique de Paris. 1911; 70:12-16
2. Megat Abd Rani PA, Irwin PJ, Gatne M, Coleman GT , McInnes LM, Traub RJ. A survey of canine filarial diseases of veterinary and public health significance in India. Parasites & Vectors, 2010, 3-30.
3. Kamalu BP. Canine filariasis caused by Dirofilaria repens in southeastern Nigeria. Veterinary Parasitology. 1991; 40:335-38
4. Harrus S, Harmelin A, Rodrig S, Favia G. Dirofilaria repens infection in a dog in Israel. The American Journal of Tropical Medicine and Hygiene. 1999; 61:639-41
5. Aranda C, Panyella O, Eritja R, Castella J. Canine filariasis: importance and transmission in the Baix Llobregat area, Barcelona (Spain).Veterinary Parasitology. 1998; 77:267-75
6. Vakalis NC, Himonas CA. Human and canine dirofilariasis in Greece. Parassitologia. 1997; 39:389-91
7. Leishangthem GD, Choudhury A, Singh ND, Bhosale S. Subcutaneous Dirofilaria repens infestation in non-descript canines. Journal of Parasitic Disease. 2016; 40(2):558-561
8. Leishangthem GD, Mabalirajan U, Singh VP, Agrawal A, Ghosh B, Dinda AK. Ultrastructural changes of airway in murine models of allergy and diet-induced metabolic syndrome. ISRN Allergy. 2013 doi: 10.1155/2013/261297
9. Megat Abd Rani PA, Irwin PJ, Gatne M, Coleman GT, Traub RJ. Canine vector-borne diseases in India: a review of the literature and identification of existing knowledge gaps. Parasites & Vectors. 2010; 3:28
10. Tarello W. Cutaneous lesions in dogs with Dirofilaria (Nochtiella) repens infestation and concurrent tick-borne transmitted diseases. Veterinary Dermatology. 2002; 13:267-74.
11. Gutierrez Y. Diagnostic Pathology of Parasitic Infections with Clinical Correlations. 2nd Edition. New York: Oxford University Press, 2000.
12. Ananda KJ, D'Souza PE, Jagannath MS. Methods for identification of microfilaria of *Dirofilaria repens* and *Dipetalonema reconditum.* Journal of Veterinary Parasitology. 2006; 20:45-47.
13. Jenkins T. Electron microscope observation of the body wall of *T. suis* Schrank 1788. (Nematoda: Trichuroidea): the cuticle and bacillary band. Zeitschrift Fur Parasitenkunde. 1969; 32:374-388
14. Lee CC, Miller JH. Fine structure of *Dirofilaria immitis* body wall musculature. Experimental Parasitology. 1967; 20:334-44
15. Rosenbluth J. Ultrastructural organization of obliquely striated muscle fibers in *Ascaris lumbricoides*. J Cell Biol 1965; 25:495.
16. Rosenbluth J. Ultrastructure of dyads in muscle fibres of *Ascaris lumbricoides*. The Journal of Cell Biology. 1969; 42:817-25
17. Legouis R, Gansmuller A, Sookhareea S, Bosher JM, Baillie DL, Labouesse M. LET-413 is a basolateral protein required for the assembly of adherens junctions in *Caenorhabditis elegans*. Nature Cell Biology. 2000; 2:415-22.
18. McGhee J.D. The *C. elegans* intestine, Worm Book, ed. The *C. elegans* Research Community, WormBook. 2007; doi/10.1895/wormbook.1.133.1, http://www.wormbook.org.
19. Clokey GV, Jacobson LA. The autofluorescent "lipofuscin granules" in the intestinal cells of *Caenorhabditis elegans* are secondary lysosomes. Mechanisms of Ageing and Development. 1986; 35(1):79-94.
20. Hermann GJ, Schroeder LK, Hieb CA, Kershner AM, Rabbitts BM, Fonarev P *et al* Genetic Analysis of Lysosomal Trafficking in *Caenorhabditis elegans.* Molecular Biology of the Cell. 2005; 16:3273-3288.
21. Lee CC, Miller JH. Fine structure of the intestinal epithelium of the *Dirofilaria immitis* and changes occurring after vermicidal treatment with caparsolate sodium. Journal of Parasitology. 1969; 55(5):1035-45
22. Kozek WJ. Ultrastructure of the microfilaria of *Dirofilaria immitis*. Journal of Parasitology. 1971; 57:1052-1067.
23. Talluri MV, Bigliardi E, Cancrini G. Comparative ultrastructural study of *Dirofilaria repens* (Nematoda: Filarioidea) development in susceptible and refractory strains of *Aedes aegypti*. Italian Journal of Zoology. 1993. 60(4):377-383.