A STUDY ON THE STRUCTURE AND DISTRIBUTION OF RIDGES IN THE CUTICLE OF *HAEMONCHUS CONTORTUS* (RUDOLPHI, 1803) COBB, 1898

Jatinderpal Singh

Department of Zoology, Baring Union Christian College, Batala-143505, Punjab (India)

E mail Id: doctorjatinder@yahoo.com

ABSTRACT

The pathogenic nematode, *Haemonchus contortus* selected for the present study inhabits the abomasums of sheep and goat. The longitudinal cuticular ridges found in both the sexes of this nematode, pertaining to the anterior region of the body, are supported by tough resistant triangular struts which form a sort of exoskeleton to help the worm in its locomotory movements. These struts arise from the well developed median layer of the cuticle and the less developed basal layer passes beneath them. The presence of struts beneath the longitudinal ridges of the cuticle provides enough strength to the worm to manipulate its way, between the mucus coated folds of the host, to reach the gastric mucosa for its blood sucking activity. The structure, chemical composition and pattern of surface cuticular ridges (Synlophe) were studied. The cuticular struts were found to be positive for general carbohydrates, acid mucopolysaccharides and –NH_2 group proteins.

Number of Figures: 6 **Number of References:** 37

INTRODUCTION

Externally the cuticle of animal-parasitic nematodes revealed a variety of structures which were reviewed by Chitwood and Chitwood (1950). These include much prevalent transverse markings, longitudinal ridges and alae. According to Cheng (1964), the cuticle of parasitic nematodes is generally smooth and the various structures such as spines, bristles, warts, punctuations, papillae, striations and ridges may be present. The arrangement and position of such structures is of taxonomic importance. Longitudinal cuticular ridges represent the raised areas running along the body. They were found to be more specific in Trichostrongylids and were discussed by Lee (1965) in adult *Nippostrongylus brasiliensis* where their number reaches to fourteen. Struts supported by fine fibres of collagen and lying in the fluid layer extend into the longitudinal ridges of the cuticle. The pattern of the longitudinal ridges was discussed by a number of workers: Lee (1965), Lichtenfels (1974), Lichtenfels (1977), Lichtenfels and Pilitt (1983a, 1983b), Lee and Nicholls (1983), Martin and Lee (1983), Lichtenfels *et al* (1986), Nembo *et al* (1993), Lichtenfels *et al* (1994) and Rahman and Hamid (2007). Durette- Dessett and Cabart (1994) determined that the distribution and distances of the cuticular ridges follow a common function in ostertagiinae nematodes and discussed the possibility of the potential use of this phenomenon a taxonomic criterion. Litchenfels *et al* (1994) while giving new morphological characters for identifying individual adult specimens of *Haemonchus* spp. described the characteristics of surface cuticular ridges.

Haemonchus contortus is a blood-sucking nematode occurring in the abomasums of sheep and goat. It has been ranked as the most important parasite of small ruminants in all the tropical and temperate areas of the world (Sood, 2006). Acute anaemia, low packed cell volume (PCV), diarrhea, dehydration, peripheral and internal fluid accumulation, edema (bottle jaw), weak and listless behaviour are common signs of its infestation. It is singly the most important of all the gastrointestinal nematodes that constrain the survival and productivity of sheep and goat owned by rural and poor farmers in the developing world (Qadir *et al* (2010). The histomorphology and histochemistry of various organ-systems of *Haemonchus contortus* has been studied by Singh and Johal (1997), Singh (2000), Singh and Johal (2001a, 2001b, 2001c and 2004). The present research paper describes the structure and distribution of cuicular ridges in *Haemonchus contortus*, which can fill the hitherto existing gaps in information regarding this aspect.

MATERIALS AND METHODS

The nematode *Haemonchus contortus* was extracted from the abomasum portion of stomach of sheep (*Ovis aries*). In order to remove debris, the nematode worms were washed in 0.85% NaCl solution. For histomorphological and histochemical studies, the worms were fixed in alcoholic Bouin's fixative and Carnoy's fixative, dehydrated in a graded series of alcohol, cleared in methyl benzoate and embedded in paraffin wax. The sections were cut at 7µm in transverse and longitudinal planes by using rotary microtome. The serial sections arranged on slides were stained with haematoxylin and eosin. For the histochemical localization of carbohydrates, glycogen, acid mucopolysaccarides, proteins and lipids the following staining methods were used.

General carbohydrates were studied by Periodic acid Schiff's staining technique (McManus, 1948). Glycogen was detected histochemically by Best's carmine staining (Best, 1906) and acid mucopolysaccharides by Alcian blue (Steedman, 1950). Nucleic acids were detected by Gallocyanin chromalum (Einarson, 1951) and Methyl green pyronin Y (Kurnick, 1955) techniques. For the localization of proteins, Mercuric bromophenol blue staining (Bonhag, 1955) and Ninhydrin Schiff's staining (Yasuma and Ichikawa, 1953) were used. The histochemical presence of lipids was detected by Sudan black B staining (McManus, 1946) and Oil red O in isopropanol (Lillie and Ashburn, 1943). The slides were examined under the microscope and photo micrographed.

RESULTS AND DISCUSSION

In *Haemonchus contortus* a cuticular modification in the form of longitudinal ridges, running antero-posteriorly along the longitudinal axis of the body, is present. The ridges are more prominent in the anterior 1/3rd of the body, progressively diminish in size in the mid region and are nearly absent in the posterior region. In a transverse section, these appear like cuticular elevations strengthened by inner hard spine like cores or struts having the shape of an elongated triangle with a round base. The total number of these ridges counted from a transverse section is 26-32 and the distance between the two successive ridges is 26µm. In certain transverse sections, the ridges were found to be protruding out more than others. The struts arise from the well developed median layer. The less developed basal layer of cuticle passes beneath them. The inner and outer cortical layers merge at the tips of the struts. (Fig. 1).

The struts present beneath the cuticular ridges are positive for general carbohydrates, glycogen, acid mucopolysaccharides and $-NH_2$ group proteins as evidenced by Periodic acid Schiff's staining, Best's carmine staining, Alcian blue staining and Ninhydrin Schiff staining respectively. These were found to be devoid of lipids. (Figs. 2, 3, 4, 5 and 6).

Harris and Crofton (1957) and Wisse and Daems (1968) have described that the cuticle is a multilayered structure functioning both as a barrier to undesirable elements in their surrounding environment and also as a flexible skeleton.

Haemonchus contortus inhabiting the abomasum portion of stomach of sheep and goat is always exposed to a highly acidic environment with a pH of approximately 3.0 (Smyth, 1996) and is also subjected to the powerful muscular movements of the stomach which may dislodge it from its niche. It is the ridged, tough and resistant cuticle of the parasite which helps it to survive under such conditions.

Lee (1965) has reported the presence of fourteen longitudinal ridges extending from the cephalic area to the posterior end in *Nippostrongylus brasiliensis*. Internally, these ridges have been found to be supported by moderately electron dense struts embedded in the cortex and matrix layers. Lee (1965) has further stated that the hard cuticular ridges of *N. brasiliensis* are used to damage the intestinal mucosa of the host and thus predisposing it for the action of the histolytic enzymes released by the nematode, while feeding. Coop *et al.* (1973) too, observed some local abrasions of the mucosa lying in the vicinity of attached *Nematodirus battus*, irrespective of the fact that the parasite does not possess any biting or rasping apparatus and the injury must be due to the action of longitudinal ridges. Based on their studies on *Nippostrongylus*, Lee and Atkinson (1976) attribute another function to the cuticular ridges i.e. providing a mechanical support to the parasite during its three dimensional movement in-between the mucus covered villi.

In *Nematodirus battus*, a sexual dimorphism has been seen as far as the length of the longitudinal ridges is concerned. These have been found to be extending on the entire length of the male and only to the anterior half of the female (Martin and Lee, 1983). However, the above mentioned authors have not assessed the value of longitudinal ridges as a taxonomic feature. On the other hand, Maggenti (1981) has described that the total number and spacing of the longitudinal ridges vary according to the nematode species. Later, Durette-Dessett and Cabart (1994) have studied the distribution of the cuticular ridges along with some other characters in six of the genera of Ostertagiinae and found these to be genus specific. They have further suggested that more comparative evaluation of inter-ridge pattern is required to assess their value as a taxonomic criterion. The distribution of surface cuticular ridges or synlophe has been used by Lichtenfels *et al* (1986) to distinguish the populations of *H. contortus* and *H. placei*.

Rahman and Hamid (2007) noticed the differences in the number of cuticular ridges in nematodes recovered from different host species.

In *Haemonchus contortus*, the longitudinal ridges found in both the sexes are more prominent in the anterior 1/3rd of the body and nearly absent in the posterior 2/3rd suggesting their functional significance pertains only to the anterior region of the body. Structurally, the ridges are covered by a cuticular sheath enclosing a spine like core or strurt, which appears to form a sort of exoskeleton to provide mechanical strength to the worm for its locomotory movements especially in reaching to the abomasal mucosa. As *H. contortus* is provided with a well developed buccal lancet for puncturing the mucosa (Singh and Johal, 2001c), so their involvement in inflicting some injury is not perceived. On the other hand, these may be helping to displace the mucus covered abomasal folds, thus facilitating the worm in its penetrating movements. Since the ridges are similar in male and female *Haemonchus*, so no sexual dimorphism is apprehended. In certain transverse sections the cuticular ridges are found to be protruding out more than others, which ascertains their locomotory function.

Lee (1965) has reported that the struts beneath the longitudinal ridges of the cuticle of *Nippostrongylus brasiliensis* contain protein without any tanning or keratinization and are surrounded by a layer of material of unknown composition. He has further accounted the absence of lipids and acid mucopolysaccharides in these supporting structures. On the contrary, in the present study on *Haemonchus contortus*, the struts are found to be positive for – NH_2 bound proteins, general carbohydrates and glycogen suggesting that these structures are of metabolic importance and the presence of mucopolysaccharides accounts for their resistant nature. The lipids are absent here too.

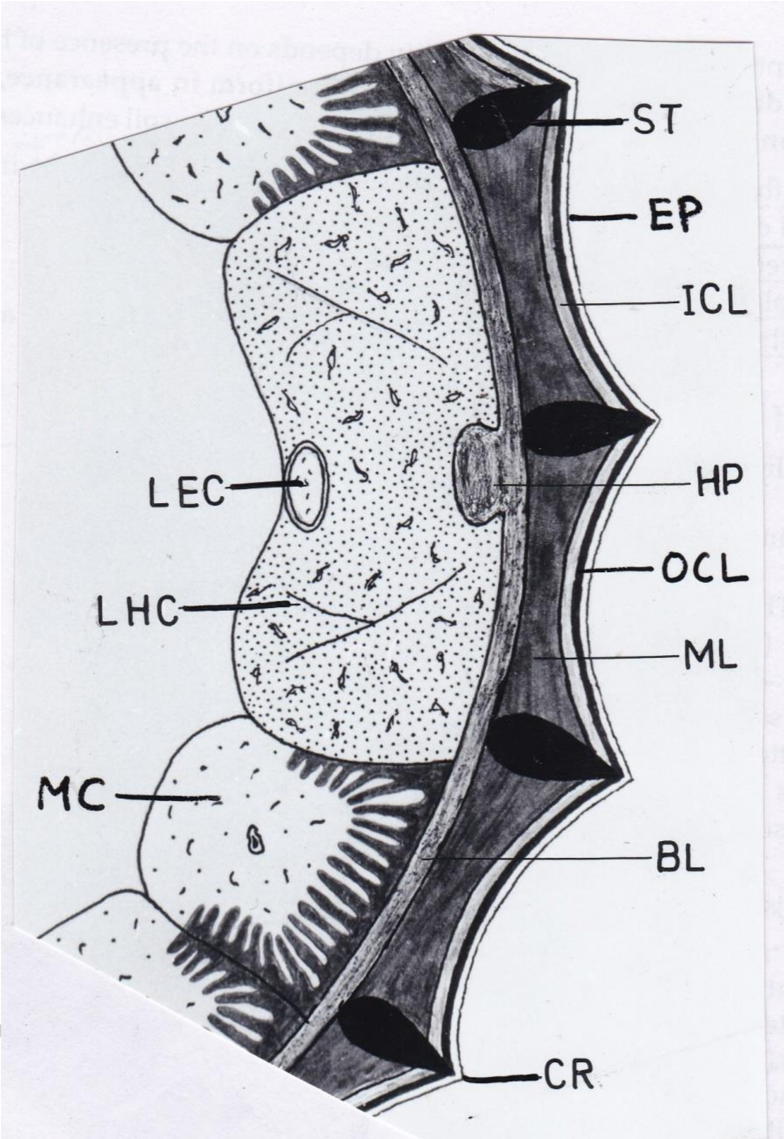

Figure 1. A diagrammatic representation (reconstructed with the help of transverse and longitudinal sections) of the body wall of *Haemonchus contortus* showing the cuticular ridges (CR), struts (ST), epicuticle (EP), outer cortical layer (OCL), inner cortical layer (ICL), median layer (ML) and basal layer (BL) of the cuticle, lateral hypodermal cord (LHC), lateral excretory canal (LEC), hypodermal pore (HP) and muscle cell (MC).

Figure 2. Transverse Section of *H. contortus* showing concentration of carbohydrate in the epicuticle (EP) and struts (ST) of the body wall, basal lamina (BAO) and luminal border of oesophagus. (Periodic acid Schiff's staining)

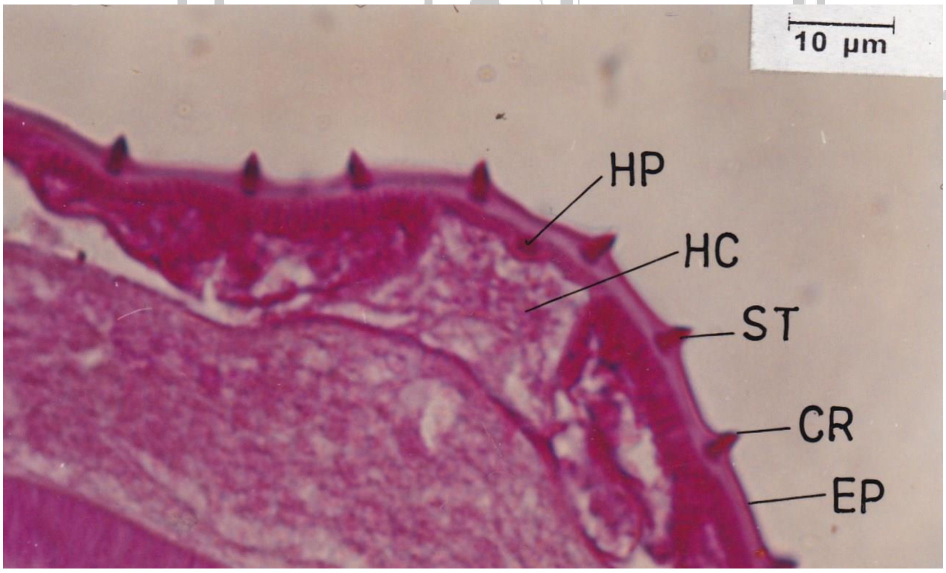

Figure 3. A portion of T. S. of *H. contortus* showing concentration of glycogen in the epicuticle (EP), cuticular ridges (CR), struts (ST), hypodermal pore (HP) and hypodermal cord (HC) of the body wall. (Best's carmine staining).

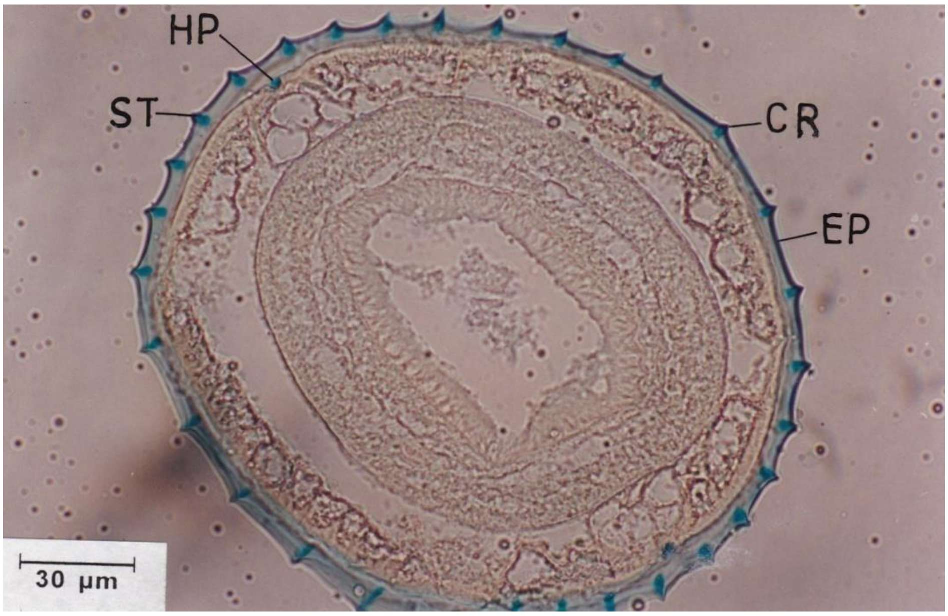

Figure 4. Transverse Section of *H. contortus* showing concentration of acid mucopolysaccharides in the epicuticle (EP), cuticular ridges (CR), struts (ST) and hypodermal pore (HP) of the body wall. (Alcian Blue staining)

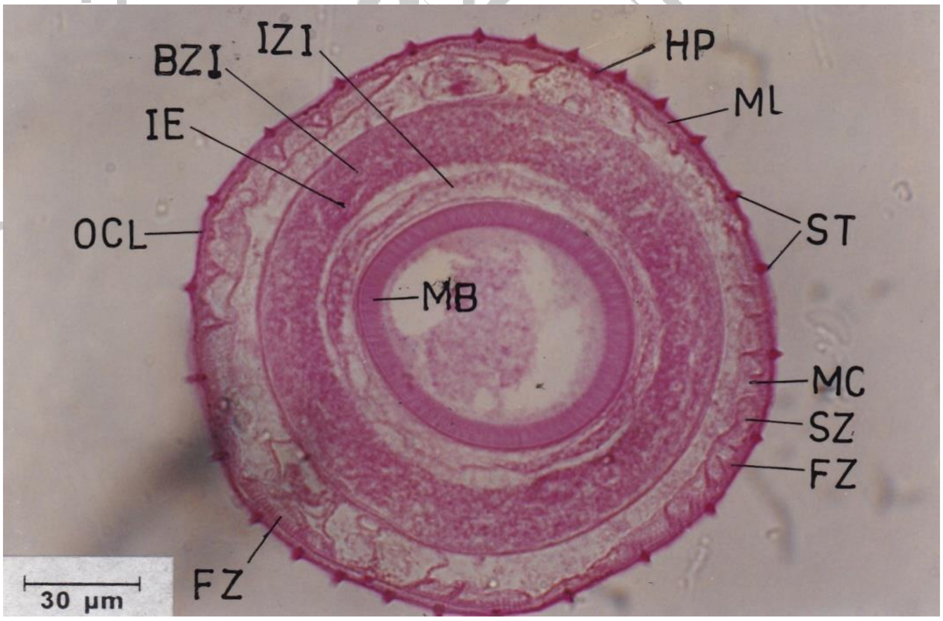

Figure 5. Transverse Section of *H. contortus* showing concentration of proteins in the struts (ST), hypodermal pore (HP), outer cortical layer (OCL) of the cuticle, fibrillar zone (FZ) and sarcoplasmic zone (SZ) of muscle cell (MC), intestinal epithelium (IE), microvillar border (MB), basal zone (BZI) and inner zone (IZI) of intestinal epithelium. (Ninhydrin Schiff's staining)

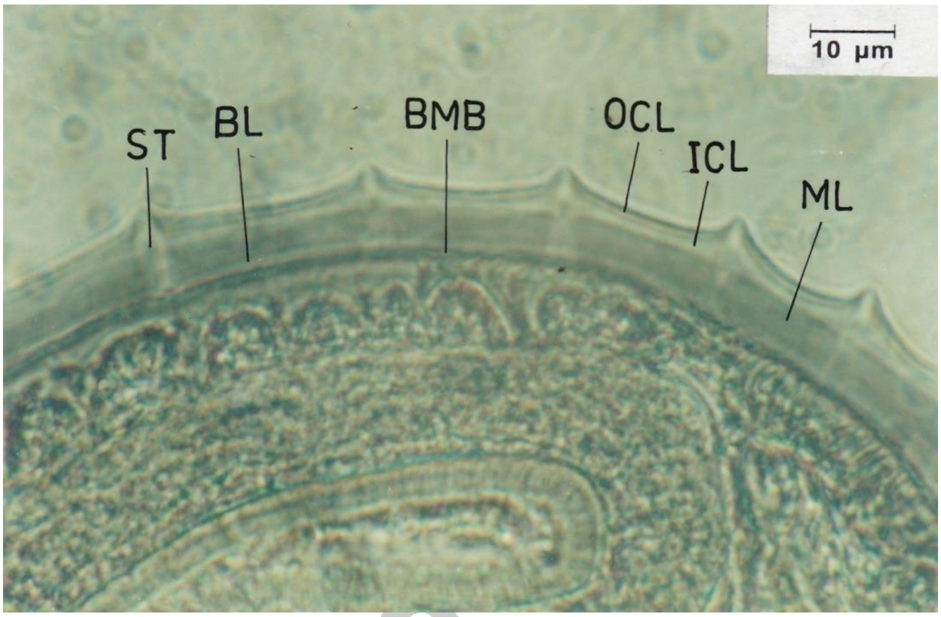

Figure 6. A portion of T. S. of *H. contortus* revealing distribution of lipid in the outer cortical layer (OCL), inner cortical layer (ICL), median layer (ML), basal layer (BL), basement membrane (BMB) and negatively staining for struts (ST) of the cuticular ridges. (Sudan Black B staining).

ACKNOWLEDGEMENTS

The author is thankful to Prof. (Dr.) Manjit Johal for her guidance and constant support for this research work. The author is also indebted to the Professor and Head, Department of Zoology, Punjabi University, Patiala and Principal, Baring Union Christian College, Batala for providing necessary facilities to carry out the research.

REFERENCES

Best, F., *Z. Wiss. Mirk.* **23**: 319. Cited from : *Histochemistry, Theoretical and Applied*. A.G. E. Pearse (ed.), J and Churchill, London, 1906.

Bonhag, P. F., *J. Morphol.* **96**: 381. Cited from : *The Structure of Nematodes*, A.F. Bird (1971), Academic Press, New York and London, 1955.

Cheng, T. C., *The biology of animal parasites*. W.B. Saunders Company, Philadelphia and London. Pp. 390-472, 1964.

Chitwood, B. G. and Chitwood, M. B., *An Introduction to Nematology*. University Park Press, Baltimore, Maryland, 334pp., 1950.

Coop, R.L., Angus, K.W. and Mapes, C. J., The effect of large doses of *Nematodirus battus* on the histology and biochemistry of the small intestine of lambs. *International Journal of Parasitology*, 3 : 349-361, 1973

Durette-Dessett, M. C. and Cabart, J., The distances between cuticular ridges follow a Gausserian function in Ostertagiine nematodes: the potential use of this phenomenon as a taxonomic criterion. *Syst. Parasitol*. **29** (1): 13-22, 1994.

Einarson, L., *Acta Pathol. Microbiol. Scand.*, 28: 82. Cited from: *The Structure of Nematodes*, A. F. Bird (1971), Academic Press, New York and London, 1951.

Harris, J. E. and Crofton, H. D., Structure and function in the nematodes: internal pressure and cuticular structure in *Ascaris*. *J. Expt. Biol*. **34**: 116-130, 1957.

Kurnick NB, Stain technology, **30**: 213-230. Cited from: *Histological and Histochemical methods*, J. A. Kiernan (ed.), Pergamon Press (1981), Oxford, 1955.

Lee, D. L., The cuticle of adult *Nippostrongylus brasiliensis*. *Parasitology*, **55**: 173-181, 1965.

Lee, D. L. and Atkinson, H. J., *Physiology of nematodes*. 2nd edition, Macmillan Press Ltd., London. 215 pp., 1976.

Lee, D.L. and Nicholls, C.D., The use of plasma etching to reveal the internal structure of *Nippostrongylus brasiliensis* (Nematoda). Parasitology, **86**: 477-480, 1983.

Lichtenfels, J.R., Number and distribution of ridges in the cuticle of *Nippostrongylus brasiliensis* (Travassos, 1914) (Nematoda: Heligmosomatoidea). *Journal of Parasitology*, **60**: 285-288, 1974.

Lichtenfels, J.R., Differences in the cuticular ridges among *Cooperia* spp. of North American ruminants with an illustrated key to species. *Proceedings of the Helminthological Society of Washington*, **44**: 111-119, 1977.

Lichtenfels, J.R and Pilitt, P.A., Cuticular ridge patterns of *Nematodirus* (Nematoda: Trichostrongyloidea) parasitic in domestic ruminants of North America, with a key to species. *Proceedings of the Helminthological Society of Washington*, **50**: 261-274, 1983a.

Lichtenfels, J.R. and Pilitt, P.A., Cuticular ridge patterns of *Nematodirella* (Nematoda: Trichostrongyloidea) of North American ruminants, with a key to species. *Systematic Parasitology*, **5**: 271-285, 1983b.

Lichtenfels, J.R., Pilitt, P.A. and Le Jambre, L.F., Cuticular ridge patterns of *Haemonchus contortus* and *Haemonchus placei* (Nematoda: Trichostrongyloidea). *Proceedings of the Helminthological Society of Washington*, **53**: 94-101, 1986.

Lichtenfels, J.R., Pilitt, P.A. and Hoberg, E. P., New morphological characters for identifying individual specimens of *Haemonchus spp.* (Nematoda: Trichostrongyloidea) and a key to species in ruminants of North America. *Journal of Parasitology*, **80**: 107-119, 1994.

Lillie R. D. and Ashburn I. L., Supersaturated solution of fat stains in dilute isopropanol for demonstration of acute fatty degeneration not shown by Herztieimer technique. *Arch. Pathol*., **36**: 432, 1943.

Maggenti, A. R., *General Nematology*, Springer Series in Microbiology. Springer Verlag, New York, Heidelberg and Berlin, 372 pp, 1981.

Martin, J. and Lee, D.L., *Nematodirus battus*: Structure of the body wall of the adult. *Parasitology*, **86**: 481-487, 1983.

McManus, J. F. A., Cited from: *The Structure of Nematodes*, A. F. Bird, Academic Press, New York and London, 1948.

McManus, J. P. A., In: *Histochemistry: Theoretical and Applied*. A.G. Pearse (ed.), J.A. Churchill Ltd., London, 1946.

Nembo, B., Goudey-Perriere, F., Gayral, P. and Perriere, C., Microtopography of *Nippostrongylus brasiliensis* (Nematoda: Heligmosomatidae) : Parasitic larval stages and adults.

Journal of Parasitology, **217** (3): 273-285, 1993.

Qadir, S, Dixit, A. K. and Dixit, P., Use of medicinal plants to control *Haemonchus contortus* infection in small ruminants. *Veterinary World*, **3** (11): 515-518, 2010.

Rahman, W.A. and Hamid, S.A., Morphological characterization of *Haemonchus contortus* in goats (*Capra hircus*) and sheep (*Ovis aries*) in Penang, Malaysia. *Tropical biomedicine*, **24** (1): 23-27, 2007.

Singh, J., Histomorphological and histochemical studies of some organ-systems and *in vitro* effect of neem leaf extract on *Haemonchus contortus* (Rudolphi,1803). Ph.D. Thesis, Punjabi University, Patiala, 2000.

Singh, J. and Johal, M., A study on spermatogenesis in a nematode, *Haemonchus contortus. Trends in Life Sciences*. **12** (2): 81-86, 1997.

Singh, J. and Johal, M., Structure of the excretory system of adult *Haemonchus contortus* (Nematoda). *Current Nematology*, **12** (1, 2):69-72, 2001a.

Singh, J. and Johal, M., Structural variations in the genital epithelium of male *Haemonchus contortus* (Nematoda). *Bionature*, **21** (2): 77-83, 2001b.

Singh, J. and Johal, M.,Observations on the foregut (stomodaeum) of *Haemonchus contortus* (Rud., 1803). *Uttar Pradesh Journal of Zoology*, **21** (2):139-145, 2001c.

Singh, J. and Johal, M., Histological study on the intestine of *Haemonchus contortus* (Rud., 1803). *Journal of Parasitology and Applied Animal Biology*, **13** (1, 2):13-24, 2004

Smyth, J. D., *Animal Parasitology*. Cambridge University Press, Cambridge, New York and Melbourne, 549 pp, 1996.

Sood, M. L., Histochemical, biochemical and immunological studies in *Haemonchus contortus* (Nematoda: Trichostrongyloidea)-an Indian Perspective. *Journal of Parasitic Diseases*, **30** (1): 4-15, 2006.

Steedman, H. F., Alcian blue 8 G.S.: a new stain for mucin. *Quart. J. Micro.Sci*., 91: 477, 1950.

Wisse, E. and Daems, W. T. *J. Ultrastruct Res*., **24**:210, 1968.

Yasuma, A. and Itchikawa, T., J. Lab. Clin. Med., **41**: 296, *Histochemical Techniques*, Bancroft, J.D. (1975), 1953.