PRELIMINARY MORPHOLOGICAL AND HISTOCHEMICAL
STUDIES ON OESOPHAGOSTOMUM COLUMBIANUM
(CURTICE, 1890) STOSSICH, 1899
I. (The Tegument and the Musculature)

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT
FOR THE DEGREE OF
MASTER OF PHILOSOPHY
IN
ZOOLOGY (Parasitology)

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(India) 1981
Certified that the dissertation entitled

"Preliminary Morphological and Histochemical studies on *Oesophagostomum columbianum* (Curtice, 1890; Stossich, 1899.1) (The Integument and the Musculature)" towards the partial fulfilment of the requirement of the degree of Master of Philosophy in Zoology (Parasitology) comprises the work done by Mr. Jamaluddin under my supervision. The dissertation is suitable for the purpose and I permit Mr. Jamaluddin to submit it for the same.

( HISAMUDDIN FAROOQUI )
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Jamal ud Din

(JAMAL UDDIN)
**INTRODUCTION**

*Oesophagostomum columbianum* (Curtice, 1890) has been chosen for the present studies because of its high incidence in ovine and caprine hosts as well as its patho-biological importance. This parasite much considerably effects the health of live-stocks and presumably causes the serious intestinal disorders which is manifested by the formation of nodular patches in the infested caecum.

Although the incidence of *O. columbianum* in India is quite high, still a countrywide epidemiological data is wanting for the economical losses to the sheep and goat industry in India. The occurrence of this parasite in the large instenstine of the host is usually concurrent with *Haemonchus contortus*, *Trichuris ovis*, and *Bunostomum trigonoccephalum*. Among these, *Trichuris ovis* is relatively more abundant than other nematodes.

Despite a high infection of *O. columbianum* in the agrarian tropics and sub-tropics, surprisingly little work seems to have been done on the morphology and physiology of this parasite. However, the work done so far is based on its ecology. The remarkable contribution regarding this is that of Agarwal (1966). Sometimes the incidence is recorded as high as 100% with a maximum worm burden of 400 parasites per host (Table-I). Ershov (1960) has also reported such a high infection rate in the Amur region of USSR in spring and peak summer, a condition similar to what has been
observed at Aligarh during the past few years.

*Cesophagostomum columbianum* has direct life cycle pattern as worked out by Veglia, 1923. According to her, eggs are released in the lumen of the host's intestine and are voided along with the faeces. In the environment the larvae hatch out within 10 - 17 hours at 25 - 27°C. These larvae become infective on the 7th - 8th day followed by two molts. The infection is acquired by swallowing the contaminated food and water. When the larvae reach the intestine they penetrate into the intestine and become encysted therein. The third molt occurs within the encysted forms resulting in the 4th stage larvae. After the penetration they leave the mucosa on the 6th - 8th day and enter the intestinal lumen. There they follow the 4th molt after acquiring growth on 32nd day and transform into mature males and females. Dash (1923) has further elaborated the studies on the life history of *C. columbianum* under Indian biotopic conditions.

The larval forms of *C. columbianum* are considered to be highly pathogenic while they penetrate the intestinal mucosa of the host where they have been found to form cysts in quite good number causing the "Nodular disease" which may also become more serious due to secondary bacterial infections (Dhar & Singh, 1968). The disease probably causes serious physiological changes which in turn results retarded health and low productivity of the adult host (Tripathi, 1968). Host mortality has already been reported in case of severe
infection even after the worms are removed from the heavily infected hosts (Monnig, 1937).

The present studies deals with the preliminary morphological and histochemical observations on the body wall of *O. columbianum*.
## TABLE-I

Incidence And Maximum Worm Burden of *Oesophagostomum columbiaum* at Aligarh (Based on Weekly random sampling).

<table>
<thead>
<tr>
<th>Month/Year</th>
<th>Total host examined</th>
<th>Total Nos. of positive hosts</th>
<th>% of infection</th>
<th>Maximum Worm burden/infected host</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>Sheep</td>
<td>Goat</td>
<td>Sheep</td>
<td>Goat</td>
</tr>
<tr>
<td>Jan.</td>
<td>80</td>
<td>120</td>
<td>48</td>
<td>18</td>
</tr>
<tr>
<td>Feb.</td>
<td>102</td>
<td>200</td>
<td>71</td>
<td>20</td>
</tr>
<tr>
<td>March</td>
<td>90</td>
<td>130</td>
<td>58</td>
<td>6</td>
</tr>
<tr>
<td>April</td>
<td>70</td>
<td>95</td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>May</td>
<td>75</td>
<td>105</td>
<td>72</td>
<td>8</td>
</tr>
<tr>
<td>June</td>
<td>40</td>
<td>81</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>July</td>
<td>39</td>
<td>70</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>August</td>
<td>25</td>
<td>70</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Sept.</td>
<td>60</td>
<td>180</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>October</td>
<td>96</td>
<td>200</td>
<td>67</td>
<td>20</td>
</tr>
<tr>
<td>November</td>
<td>90</td>
<td>200</td>
<td>72</td>
<td>18</td>
</tr>
<tr>
<td>December</td>
<td>100</td>
<td>250</td>
<td>93</td>
<td>37</td>
</tr>
<tr>
<td>January</td>
<td></td>
<td></td>
<td>76</td>
<td>10</td>
</tr>
<tr>
<td>1979</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>95</td>
<td>125</td>
<td>81</td>
<td>15</td>
</tr>
<tr>
<td>March</td>
<td>80</td>
<td>130</td>
<td>56</td>
<td>11</td>
</tr>
<tr>
<td>April</td>
<td>60</td>
<td>95</td>
<td>48</td>
<td>10</td>
</tr>
<tr>
<td>May</td>
<td>85</td>
<td>100</td>
<td>51</td>
<td>5</td>
</tr>
<tr>
<td>June</td>
<td>58</td>
<td>80</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>July</td>
<td>45</td>
<td>80</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>August</td>
<td>35</td>
<td>75</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Sept.</td>
<td>59</td>
<td>96</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>October</td>
<td>85</td>
<td>150</td>
<td>68</td>
<td>8</td>
</tr>
<tr>
<td>November</td>
<td>92</td>
<td>190</td>
<td>78</td>
<td>17</td>
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<td>December</td>
<td>75</td>
<td>200</td>
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<td>January</td>
<td>95</td>
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<td>85</td>
<td>9</td>
</tr>
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<td>1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>110</td>
<td>195</td>
<td>86</td>
<td>19</td>
</tr>
<tr>
<td>March</td>
<td>83</td>
<td>120</td>
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<td>18</td>
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<tr>
<td>April</td>
<td>60</td>
<td>88</td>
<td>51</td>
<td>43</td>
</tr>
<tr>
<td>May</td>
<td>59</td>
<td>107</td>
<td>38</td>
<td>3</td>
</tr>
</tbody>
</table>
MATERIAL AND METHODS

Live specimens of *Cestophagostomum columbianum* were obtained from the caecum of sheep and goats slaughtered at Aligarh abattoir. The parasites were brought to the Laboratory in physiological saline in thermos container. The specimens were then washed repeatedly with the saline and sorted out. For routine morphological preparations and histochemical tests the material was fixed in various fixatives. The specimens were cut into small pieces for effective fixation and penetration of the fixatives. Paraffin processed material was cut at 4 - 6 mm on rotary microtome. The fixatives used were Carnoy's fluid, 10% neutral formalin, Bouin's fixatives and 70% Alcohol.

The *to to* preparations were glycerine processed and mounted in glycerine jelly or temporary mounts in lactophenol were used for gross morphological studies. The stains for morphological preparations used were Haematoxylin and Eosin, Haidenhain Azan and Mallory's triple stains.

For histochemical studies various tests were performed which revealed localization of certain basic constituents. These have been furnished in Table-II. The techniques followed are those which have been cited in Lillie (1965), The BDH Manual (1972), and Pearse (1960). Fortepan (35 mm rapid film) ASA 160 was used for photomicrography.
### TABLE II

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Substance Localized</th>
<th>Fixative used</th>
<th>Technique employed</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Polysaccharides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>Bouin's</td>
<td>Best's Carmine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycogen &amp; Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mucopolysaccharides</td>
<td></td>
<td>amylase</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Acid mucopolysaccharides</td>
<td>Carnoy's Alcian blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Proteins</td>
<td></td>
<td>Mercury bromophenol blue</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Lipids</td>
<td>10% formaline (neutral)</td>
<td>Suden Black B</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Bound Lipids</td>
<td></td>
<td>Acetone Sudan Black B</td>
<td></td>
</tr>
</tbody>
</table>
HISTORICAL REVIEW

Oesophagostomum columbianum Curtice was first described by Curtice in 1890 under the genus Oesophagostoma. Oesophagostoma was later emended to Oesophagostomum by Stossich (1899) with the change in the name of the genus the name of the species was also changed as O. columbianum. Stewart (1898) designated it as a type species of the genus Hypostomum and sub-genus Proteracrum by Railliet and Henry (1913). Later on, subsequent authors reviewed the genus Oesophagostomum and the genus Hypostomum and the sub-genus Proteracrum were regarded as its synonyms. It has now been firmly established as distinct species and has been designated as Oesophagostomum columbianum Curtice (1890).

Besides the type species, the other ones included in the genus Oesophagostomum are:

- O. dentatum (Rudolphi, 1803) Molin 1861, O. quadrispinulatum (Marcone, 1901) Allicata, 1935, O. maplestonei (Schwartz, 1931),
- O. venulosum (Rudolphi, 1809) Railliet, 1885
- O. asperum (Railliet and Henry, 1913, O. indicum Maplestone, 1931),

These have been comprehensively reviewed catalogued by Baylis (1936).
The life cycle of *O. columbianum* has been elucidated by Veglia (1923) and lately by Dash (1973) several contributions have been made on its control and epidemiology. Although a little work seems to have been done so far on its morphology and histochemistry, more valuable contributions have been made on the larval development of this parasite by Agarwal (1961), Das (1967, 1968), Singh & Chhabra (1965) & Chhabra & Singh (1967), Shankar (1970), on Immunization by Dhar & Singh (1968, 1970), Dobson (1974) and on general to ecological as well as Epidemiological factors (Misra, 1972, Tripathi, 1969). The investigations of Das (1967, 1968) and Gupta (1976) on in vitro cultivation on various stages of this parasite of considerable significance and may be regarded as an effective model for such studies on other parasitic and pathogenic nematodes of livestock. However, the references available on these aspects are relatively few, the notable among these being those of Ohmori (1975) on the arrangements of somatic muscles. Beyond this, certain preliminary physio-biochemical investigations have also been made on the infectivity of larval stages of *O. columbianum* (Knight, 1972). Bremner et al. (1973) who reported secretion of acetyl cholin esterase.
THE INTEGUMENT

The generalised pattern of the integument of Nematode comprises the cuticula, sub-suticula and the musculature.

The Cuticle: The nematode cuticle generally comprises three basic regions named as cortical layer, the matrix and the fibre-layer, which are further divisible into finer layers. Much is now known about the complexity of the nematode cuticle and up to some extent of its composition. The early investigations on this structure have been reviewed by Chitwood and Chitwood (1950) along with their own findings. It was the first comprehensive review by these investigators who presumably considered it as a triple layered structure which may be divisible into various strata. Some aspects of the composition of these layers have also been discussed. This aspect has also been consequently taken into account by Hobson (1948), Fairbairn (1957; 1960), Anya (1965) Lee (1966). At ultra-structure level also, several workers have recently been investigated and reviewed the structure and elucidated the composition of this structure. Notable among them are Inglis (1964), and Lee (1972).

In *O. columbiaorum* the integument comprises the following layers:
1. External cortical layer
2. Internal cortical layer
3. Fibrillar layer
4. Matrix
5. External fibre layer
6. Middle fibre layer
7. Internal fibre layer
8. Basal membrane

The outer cortical layer measures about 4 μm in thickness and is denser in appearance than the inner cortical layer and the matrix (Plate-I). This layer is PAS positive but non liable to diastase digestion that is glycogen deficient in nature (Plate V, 1,2). This is also highly protenaceous (Plate-IV, 2). The fat moiety is also found in the form of fat droplets (Plate-VI, 2). The outer most surface of this layer is composed of acid mucopolysaccharides which gives a positive reaction with Alcian blue (Plate-VI, 1). The inner cortical layer, measuring about 6 μm in thickness which gives a lighter appearance in routine stained morphological preparations but almost similar moieties are present as described above for outer cortical layer except the acid mucopolysaccharides. These two layers are followed by a fibrillar layer, measuring about 4 μm in thickness.
This is sandwiched in between the inner cortical layer and the matrix. It appears like a connective tissue and a network of fibrils. This layer stains blue with Heidenhain's Azan like the generalised connective tissue. This is slightly PAS positive but non liable to diestase digestion. The fat moiety is present in this layer in sufficient amount in the form of bound lipids only (Plate-VII, 1,2). Next to the fibrillar layer lies the matrix which appears somewhat homogeneous in nature. This layer measures about 7 um in thickness. It is highly proteaceous in nature as it gives a dense reaction with Mercurobromophenol blue (Plate-IV, 2). The fat moiety is absent in this layer. However it shows slightly positive reaction with PAS comparatively than the cortical layers (PlateIV, 1).

Next to the matrix are three fibre layers closely situated each other each measuring about 2 um in thickness. These are composed of fine fibres. The nature of these layers are denser appearing on the inner sides. However the thickness of the entire cuticle greatly varies in different body regions as it appears thicker in the middle body region while comparatively thin at the anterior and posterior regions of the worm. The orifices of the body like the vulva, rectal opening and cloaca are also lined by cortical layers only. In both the sexes the cuticle also varies in thickness. It is thicker in males than in the females. The variation in thickness
in different regions of males and females are given below (Table-III).

<table>
<thead>
<tr>
<th>Body regions</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior end</td>
<td>8 um</td>
<td>10 um</td>
</tr>
<tr>
<td>Oesophageal region</td>
<td>15 um</td>
<td>15 um</td>
</tr>
<tr>
<td>Middle region</td>
<td>22 um</td>
<td>20 um</td>
</tr>
<tr>
<td>The vulva</td>
<td>-</td>
<td>10 um</td>
</tr>
<tr>
<td>Posterior region of the body</td>
<td>15 um</td>
<td>12 um</td>
</tr>
</tbody>
</table>

The inference could be drawn from the above data that there happens to be a gradual increase in thickness of the cuticle from anterior to the posterior region of the body. Regarding the nature and composition of the cuticle, various views have been given by different authors viz. Grube (1850), who considered the cuticle as chitin which is homologous to the arthropod chitin, whereas Flury (1912) regarded it as Keratin in *Ascaris*. 
Later on Magath (1919) termed it as cornein. According to Mueller (1929) the cuticle of the nematode is a secretion product. However, entirely different view was given by Chitwood (1930). According to him the various layers of the cuticle of nematodes are formed as a result of protoplasmic condensation by the undifferentiated cells during their development. The most plausible idea regarding this fact accepted till now, however, is that Chitwood's hypothesis but the matter still prevails a great confusion. For ascertaining the nature and composition of the structure a lot of morphological and histochemical studies on the developmental stages are still wanting for reaching any firm conclusion.

*The sub-cuticula*

A middle layer in between the somatic muscles and cuticula constitute the sub-cuticula or the hypodermics. Stewart (1906) named it as epidermis. Controversies are also there about its origin. According to Hamann (1891) it is of ectodermal in origin, while zur Strassen (1904) regarded it as mesodermal in origin.

The sub-cuticula unlike those of other nematodes is a syncytial layer. In *O. columbianum* it measures
about 5 μm in thickness. It comprises a fine protoplasmic sheath including some granular inclusions therein, which gives it a denser appearance than the cuticula (Plate-I). This layer is not present throughout the entire body regions as it is absent in extreme anterior and posterior regions as a result of which the underlying muscles are directly attached to the basement membrane of the cuticle in these regions. The thickness also varies in different body regions from 3 μm - 6 μm. This layer bulges inside at four places forming two lateral, one ventral and one dorsal ridges thereby leaving four sectors of pseudocoelom for muscle attachments. As a matter of fact it is known as a syncytial layer, but on the contrary cell boundaries or nuclei were not observed in the sub-cuticula of *O. columbianum* during present investigation.

As regards its various moieties this layer shows certain PAS positive dense substance in it which are nonglycogen in nature (Plate-V, 1, 2), and highly protenaceous (Plate-IV, 2). The fat moiety in this region is present only in the form of certain bound lipids in lesser amount (Plate-VII, 1, 2). This layer is probably a transitional one, in between the coelom and the cuticle, via muscular layer. The probable
function of this layer which the present author tends to conceive is to transport the materials either from cuticle to pseudocelom and vice versa. This has to be ascertained through bio-chemical and histochemical studies in future.

**The musculature**

There are two general types of musculature present in the nematodes. 1. Somatic musculature and ii. the specialized muscle. The musculature underlying the sub-cuticula is somatic musculature. The specialized muscles are generally the modifications of the somatic muscles at various places. The somatic muscle closely lie underneath the sub-cuticula. This musculature is fundamentally very important feature of the nematodes for the classification as has already been emphasized by Schneider (1860) who also proposed the terms plewtymarian and coelomyarian to these muscles. The term platytmarian is employed to those in which the fibrillar portion is situated at the base of the muscle cell and the muscle cell is flat along with the body cavity. The term Coelomyarian indicates the elongated form of the muscle cells with the fibrillar portion situated in between the muscle cells and exhibiting a group at distal end. Also considering the number of the muscle cells present in each sector, the terms polymyarian and mero-myarian also were suggested for
many cells or fewer cells in a sector respectively.

In the case of *O. columbianum* the muscles are of platymyarian and meromyarian types. Each cell averaging 8x12 um in size with a basal flat fibrillar portion and a flat contractile portion (Plate-I). This muscle layer shows the presence of ample glycogen and moderate portain moiety (Plate-IV, 2). However, the lipids are absent but some bound lipids are present (Plate-VII, 2).

The glycogen is presumably present to supply energy for neuromuscular activities which is a need based substance in such parasites exhibiting very mobile and active way of living as also experienced in physiological saline in vitro keep on moving for hours and hours which reveals itself the need of a quantum of energy. The extensive neuromuscular activities presumably be governed by neurosecretions as recently been reported by Brenner et al (1973) who reported the secretion of acetylcholinesterase in such parasite.

The results of histochemical investigations performed on *O. columbianum* has been furnished in Table-IV.
TABLE-IV

Results of various histochemical tests performed on the integument of Q. columbianum

<table>
<thead>
<tr>
<th>Tests Performed</th>
<th>Substance localized</th>
<th>External cortical layer</th>
<th>Internal Fibrillar layer</th>
<th>Matrix layers</th>
<th>Fibre Basement</th>
<th>Sub-cuticula</th>
<th>Muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>glycogen</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>PAS after diastase digestion</td>
<td>-do-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Bests' Carmine</td>
<td>-do-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bests' Carmine after diastase digestion</td>
<td>-do-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mercury bromophenol blue</td>
<td>Proteins</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Alcian blue</td>
<td>Acid mucopoly saccharides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sudan black B</td>
<td>Lipids</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetone Sudan black B</td>
<td>Bound lipids</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

+++ = Intensely stained
** = Moderately stained
+ = Slightly stained
- = Negative
REFERENCES


* References not seen in original.
LEGENDS TO PLATES

BL = Basement layer
C = Cuticula
EC = External cortical layer
FL = Fibrillar layer
IC = Internal cortical layer
IFL = Inner fibre layer
MFL = Middle fibre layer
ML = Muscle layer
MT = Matrix
OFL = Outer fibre layer
SC = Sub-Cuticula
PLATE-I

Diagram showing integument of *O. columbiae*.

*Camera-lucida sketch x 2000.*
Fig. 1. Anterior region of male *Q. columbiaeum* from to to mount x100.

Fig. 2. Posterior region of male *Q. columbiaeum* from to to mount x 100.
PLATE-IV

Fig. 1  Transverse section of *Q. columbiae*um through integument stained with Heidenhains Azan x400.

Fig. 2  L.S. of *Q. columbiae*um through integument stained with Mercurobromophenol blue x400.
PLATE-V

Fig. 1 Transverse section of *Q. columbiaeum* through integument, PAS x 400.

Fig. 2 L.S. of *Q. columbiaeum* through integument, Bests' carmine x 400.
PLATE-V
PLATE-VI

Fig. 1  L.S. of *Q. columbianum* through integument, Alcianblue x 400.

Fig. 2  L.S. of *Q. columbianum* through integument, Sudan black B x 400.
Fig. 1  L.S. of *O. columbiaeum* through integument, Acetone Sudan black B x 400.

Fig. 2  L.S. of *O. columbiaeum* through integument, Acetone Sudan black B x 400.