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STRUCTURE OF THE BUCCAL LANCET OF *HAEMONCHUS CONTORTUS* (NEMATODA)

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ABSTRACT

The structure of the buccal lancet of the adult *Haemonchus contortus*, a blood sucking parasite of sheep, was studied. Results are based on the serial sections (longitudinal and transverse) and whole mount stained specimens studied under light microscope. The buccal lancet is a dorsoventrally flattened, cuticular structure arising from the cuticular lining of buccal cavity. The lateral edges of the lancet are slightly rounded. Anteriorly, it is attached to the dorsal lip and posteriorly it makes its continuity with the dorsal oesophageal wall. Two sets of longitudinal muscle fibres control the back and forth movements of the buccal lancet and dorsal oesophageal gland duct traverses the whole length of the lancet to open at its tip. The topographical feature of the dorsal gland opening, present at the tip of the lancet, associates it with the blood sucking activity of the parasite.

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INTRODUCTION

Chitwood and Chitwood (1950) and de Coninck (1965) reported the various modifications existing in the structure of buccal capsule and lips of nematodes. Bird (1971) suggested that the variability of stoma reflects the different modes of feeding in different nematode species. In parasitic nematodes and predatory forms, a buccal spear was reported which is used to pierce the prey and suck out the contents as in *Dorylaimus* and *Seinura* (Linford and Oliveira, 1937 and Jenkins and Taylor, 1967), whereas the presence of a stylet is a regular feature of plant parasitic nematodes (Coomans and de Coninck, 1963: Wright, 1965 and Lopez-Abella *et al*, 1967).

In blood feeding strongylids, the large buccal capsule is modified to form teeth, jaws or lancets to pierce and penetrate the mucosa and reach the capillaries. The studies on the buccal capsule especially the dorsal gutter and teeth were made by light microscopy on *Strongylus edentates* (Imminck, 1924) and *Strongylus equinus* (Dvoinos, 1975). By using transmission electron microcsopy on *Strongylus vulgaris*, Mobark and Ryan (1998) observed that the globular buccal cavity in a longitudinal section contained two ear- shaped dorsal teeth and a dorsal gutter (dorsal oesophageal gland duct) which reached the anterior end of the capsule. On the other hand Weise (1977) made both light and electon microscopic studied on the dorsal buccal lancet of *Haemonchus contortus* and stated that the opening of the dorsal oesophageal gland duct was located at the anteroventral surface of the lancet. Guimaraes and Caldeira (1997) too, observed the dorsal buccal lancet having an oval orifice on the ventral surface in *Haemonchus simile*. Dvoinos *et al* (1994) studied the buccal capsule of *Coronocyclus ulambajari* and found the presence of large tongue like dorsal cone protruding from the floor of the buccal capsule overlying the large dorsal oesophageal teeth. The duct of the dorsal oesophageal gland opened at the tip of this dorsal cone. In *Bunostomum phlebotomum*, by using scanning electron microscopy, Leite (1992) too, ascertained the presence of a single deep dorsal cone along with two ventro-lateral lancets.

Haemochus contortus (Rudolphi, 1803) Cobb, 1898, selected for the present study is a serious nematode parasite of sheep (*Ovis aries*) and goat (*Capra hircus*) of cosmopolitan distribution. It causes severe anaemia resulting in weight loss, poor milk yield and wool production. Medium infection causes sheep to loose condition and heavy infection may result into death. Thousands of worms may occur in a single ruminant stomach and it has been estimated that 4000 worms suck about 63 cm^3 of blood per day (Smyth, 1996). Baker *et al* (1959) have estimated that a single worm causes an average daily loss of 0.08 ml of blood. Keeping in view its wide distribution and economic importance, *Haemonchus contortus* has been selected for the present study. Previously, the microscopic structure of various organ-systems of *Haemonchus contortus* was studied by Singh & Johal (1997), Singh (2000), Singh & Johal (2001a, 2001 b and 2001c) and Singh & Johal (2004). The present study pertains to the structure of the buccal lancet of this highly pathogenic nematode.

MATERIALS AND METHODS

Collection of parasites:

Naturally infected stomachs of sheep (*Ovis aries*)) were collected from the abattoirs from different abattoirs in Batala, Punjab, India. Abomasum portion of stomachs were taken to the laboratory, cut open along greater curvature and searched for adult male and female *Haemonchus contortus*. Motile active worms were collected in petri dishes containing normal saline.

Preservation of parasites:

The male and female worms were preserved in 70% alcohol. Higher concentration of alcohol was avoided which may result in structural artifects due to shrinkage. The morphological and morphometric study requires minimum body surface distortions and shrinkage. Some samples were also preserved in glycerin and 70% alcohol in the ratio of 1:3.

Microscopic studies:

After preservation, the worms were cleared in lactophenol. For studying some of the external and internal characters of the parasites the fresh specimens were stained in 1% methylene blue at 60 degree centigrade for 18 hours. The specimens were differentiated in 80% alcohol and cleared in lactophenol. The parasites were finally mounted in Kaiser's glycerin jelly or lactophenol.

For histological 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 5 µm in longitudinal planes by using rotary microtome. The serial sections arranged on albuminised slides were stained. The slides were examined under the microscope and photo micrographed and few camera lucida diagrams were also drawn.

RESULTS AND DISCUSSION

The buccal lancet of *Haemonchus contortus* is a specialized, dorsoventrally flattened, cuticular structure. It is about 12 to 15 µm long and 3 to 4 µm wide and at about 9 µm anterior to the base, it bends dorsally thus giving a slightly curved appearance. It is positioned in the dorsal region of the buccal cavity with its ventral and inner surface lying in continuity with the cuticular lining of the oesophagus and on its outer dorsal margin, it is attached with the dorsal lip through an intercuticular junction (Figs. 1 and 2)

The buccal lancet consists of three parts: (1) The anterior sharp part (2) The middle ampullary part and (3) The posterior basal part. The anterior sharp part of buccal lancet has an oval orifice at its tip. The ampullary part has an ampulla formed by the swelling of the dorsal oesophageal gland duct. The lateral edges of the lancet in this part are slightly rounded. The basal part of the buccal lancet is continuous with the cuticular lining of the oesophagus. The cuticular lining of the oesophagus is continuous with that of the basal part of the buccal lancet and only a thickening indicates the demarcation of the later from the former. The outer margin of the lancet in this part is not smooth but forms a lobed projection at a distance of about 5 µm from the posterior end. Due to this lobed projection the lancet appears V-shaped (Figs. 3, 4, 5 and 6).

Two sets of longitudinal muscle fibre are attached with the buccal lancet. One group of muscle fibres is seen between the dorsal lip and lobed projection of the lancet. The second group of muscle fibres is seen at the base of the lancet. The lancet protrudes out as a result of the contraction of the longitudinal muscle fibres of the first group and relaxation of basal musle fibres of the second group. The lancet moves back into the buccal cavity when the muscle fibres attached between dorsal lip and lobed projection become relaxed accompanied by the contraction of the basal muscle fibres (Figs. 4, 5 and 6)

The dorsal oesophageal gland duct traverses through the centre of the lancet and when full of secretion become swollen to form an ampulla near its opening to outside (Fig. 6). The diameter of the dorsal oesophageal gland duct is 0.7 µm.

The lumen of the buccal cavity increases when the buccal lancet moves

out to pierce the abomasal mucosa of the host. The coordinated activity of the longitudinal muscle fibres attached to the dorsal and basal portions of the lancet facilitate its back and forth movements to puncture the abomasal mucosa. The opening of the dorsal oesophageal gland duct at the tip of the lancet suggests that some secretion or anticoagulant is secreted by the dorsal gland which helps in blood sucking activity of the parasite. The presence of an ampulla formed by the dilation of dorsal oesophageal gland duct, just near the tip of the lancet helps to store the secretion before its passage into the injured capillaries of the host's abomasal tissue.

According to Veglia (1915) the buccal lancet of *Haemonchus contortus* possesses a pointed tip, a bilobular base and is of curved appearance. Anteriorly it shows two thorn shaped edges, of which the anteriormost is sharper than the posterior one. But in the present study it was found that anteriormost end of buccal lancet appears to have thorn shaped edges due to the presence of the opening of oesophageal gland duct in the middle of the distal tip of the lancet. Weise (1977) while making light and scanning electron microscopic studies on the buccal lancet of *Haemonchus contortus* found it to be compressed antero-ventrally and having a rounded anterior tip and devoid of any bilobular base. Veglia (1915) described that the base of the lancet is joined to the mouth capsule by a movable joint allowing the movement of the lancet in dorsoventral configuration.

In the present study, however, the base of the lancet is narrow and lies in continuity with the cuticular lining of oesophageal wall. It dilates to form a lobe like projection on its dorsal margin at a distance of 9 µm from the posterior end it. It is to the top shelf of this lobe that the muscle fibres extending from the cuticular junction lying between the dorsal lip and the lancet are attached. These muscle fibres probably help the back and forth movement of the lancet. With the contraction of these muscle fibres the lancet protrudes out to puncture and anchor to the mucosal tissue of the host gut and with the relaxation of these muscles the lancet is brought back in position.

Weise (1977) suggested the presence of the longitudinal muscle fibres connected to the posterior end of the posterior part of the lancet control its anterior and posterior movements. Such longitudinal muscle fibres performing the similar function are also seen at the proximal end of the lancet in the present study. They counteract the action of anterior group of muscle fibres extending between the dorsal lip and the lobed projection of the lancet to bring the lancet back in position.

Veglia (1915) observed that the opening of the dorsal oesophageal gland duct is situated at the base of the lancet in its articulating socket. But Weise (1977) found that the opening of the dorsal oesophageal gland to be located on the antero-ventral surface of the lancet.

In the present study the dorsal oesophageal gland duct traversing the whole length of the lancet and opening at the anterior end is observed. But before opening it dilates to form an ampulla for storing the secretion produced by the dorsal gland before it is passed out in the injured capillaries of the host abomasum. The presence of such an ampulla is also reported in the dorsal oesophageal duct of *Strongylus vulgaris* by Mobarak and Ryan (1998).

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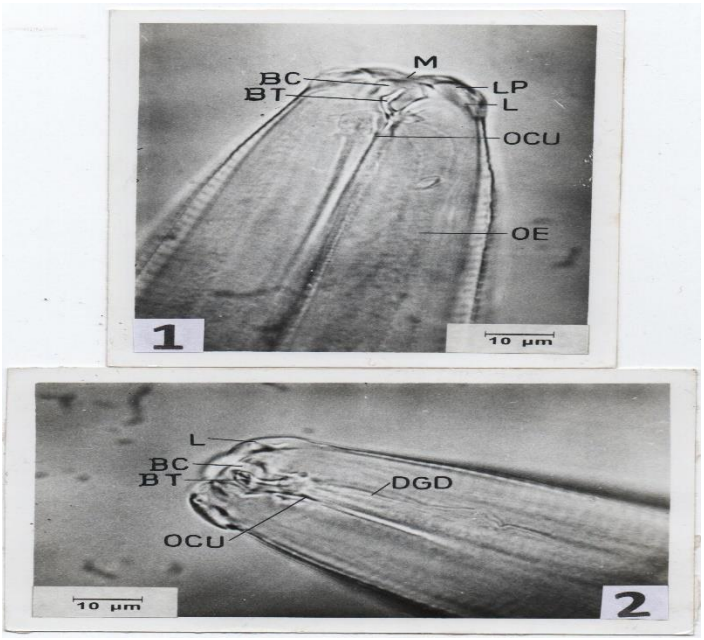

Fig. 1: Anterior region of *H. contortus* showing mouth (M), lip (L) bearing lip papilla (LP), buccal lancet (BT), buccal cavity (BC), oesophageal cuticular lining (OCU) and oesophagus (OE). **(**Stained with methylene blue, mounted in lactophenol**).Fig. 2:** Anterior end of *H. contortus* revealing the presence of buccal lancet (BT), buccal cavity (BC), lip (L), oesophageal cuticular lining (OCU), dorsal oesohageal gland duct (DGD). **(**Stained with methylene blue, mounted in lactophenol)

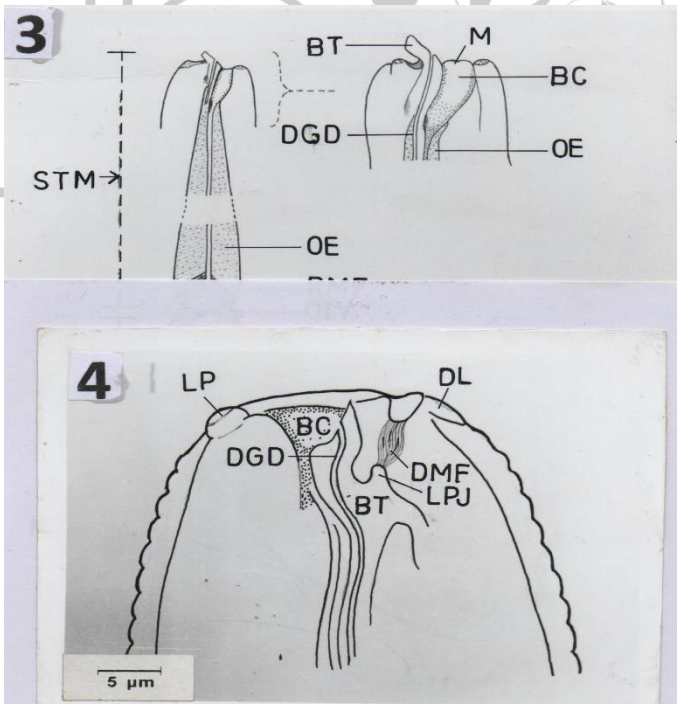

Fig. 3: A camera lucida drawing of stomodael region (STM) showing mouth (M), buccal lancet (BT), buccal cavity (BC), oesophagus (OE), dorsal oesophageal gland duct (DGD).**Fig. 4:** A camera lucida drawing showing buccal lancet (BT), buccal cavity (BC), lip palilla (LP), dorsal lip (DL), lobed projection (LPJ), muscle fibres annexing between the dorsal lip (DL) and lobed projection of the lancet (DMF), dorsal oesophageal gland duct.

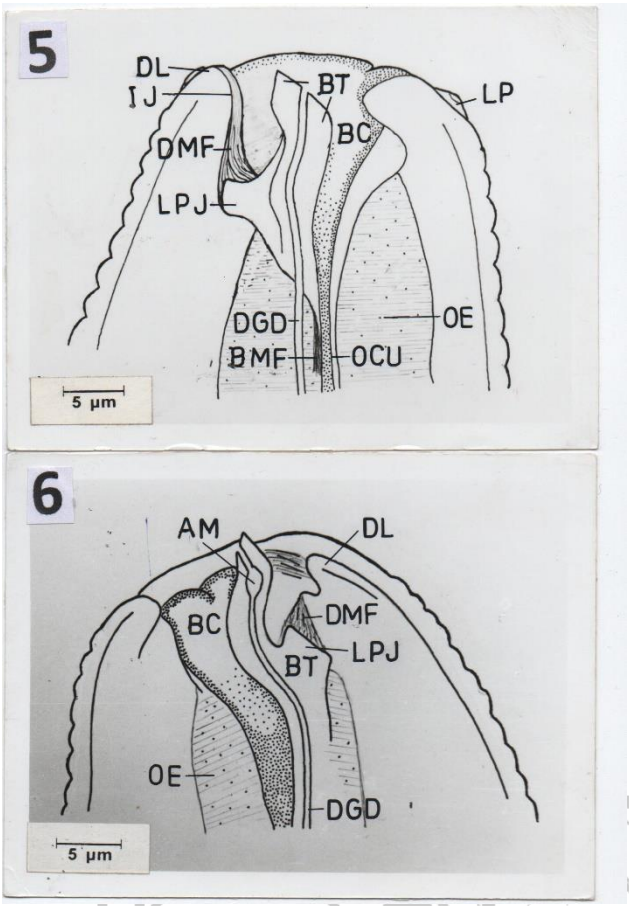

Fig. 5: A camera lucida drawing of buccal lancet (BT), lip papilla (LP), dorsal lip (DL), intercuticular junction (IJ), buccal cavity (BC), oesophageal cuticular lining (OCU), lobed projection (LPJ), muscle fibres connecting the dorsal lip and lobed projection of the lancet (DMF), basal longitudinal muscle fibres (BMF), oesophagus (OE) and dorsal oesophageal gland duct (DGD).

Fig. 6: A camera lucida drawing of buccal lancet (BT), dorsal lip (DL), buccal cavity (BC), oesophageal cuticular lining (OCU), lobed projection (LPJ), muscle fibres connecting the dorsal lip and lobed projection of the lancet (DMF), basal longitudinal muscle fibres (BMF), oesophagus (OE), dorsal oesophageal gland duct (DGD), and ampulla (AM).

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