Ultrastructural studies of three species of *Oesophagostomum* (nematoda) by scanning electron microscopy

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Abstract The surface structure of three predominant intestinal helminth species of *Oesophagostomum* i.e., *Oesophagostomum columbianum*, *Oesophagostomum venulosum* and *Oesophagostomum asperum* were studied with the help of scanning electron microscopy. *Oesophagostomum columbianum* has hook like bent structure anteriorly with well developed lateral cervical alae that are interrupted at several intervals. It has external corona radiata (ECR) and internal corona radiata (ICR), ECR comprises of 21 elements and ICR comprises two small elements to each element of ECR. Posterior end of male *O. columbianum* has bursa with well developed genital cone. On the other hand *O. venulosum* showed presence of 18 elements in ECR with each element containing two small elements internally constituting ICR. *Oesophagostomum asperum* had three tier arrangement of the cephalic vesicle with 12 elements in ECR with each element containing two small elements in constituting ICR. Vagina of matured females is covered with copulatory cement.

Keywords *Oesophagostomum columbianum* · *O. venulosum* · *O. asperum* · External corona radiata (ECR) · Internal corona radiata (ICR) · Scanning electron microscopy (SEM)

Introduction

Parasitic infections in sheep are the major limiting factors of production. Of them oesophagostomosis is one of the prevalent and pathogenic gastrointestinal nematode infections of livestock in India (Gupta et al. 1987; Singh et al. 1997). There are three species of *Oesophagostomum* infecting sheep of India, viz, *O. columbianum*, *O. venulosum* and *O. asperum* (Soulsby 1982 and Singh 2003). Among these *O. columbianum* is common prevalent parasite with significant economic importance in small ruminants (Olivares et al. 2000). The utilization of SEM has enabled parasitologists to provide more information on morphology of parasites (Gibbons 1986). In the present study scanning electron microscopic features of common oesophagostomes of sheep are elucidated.

Materials and methods

The intestines of sheep were brought to the laboratory in an ice box by tying both ends of the intestines from Chengicherla slaughter house, Hyderabad, Telangana. Later these intestines with nodules were dissected longitudinally and faecal contents were evacuated. The worms embedded in mucosa are separated and washed in Phosphate buffered saline (PBS of pH 7.2) to remove host material and morphological characterization was done with light microscopy as per keys provided by Soulsby 1982 and Singh 2003.

After morphological identification, the anterior and posterior ends of the worms were cut and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4 °C and post fixed in 2% osmium tetroxide for 4 h. Later they were dehydrated in graded ethyl alcohol and

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dried to critical point drying, mounted over the stubs with double carbon conductivity tape, and coated with a thin layer of gold, further studied by using SEM-MODEL: JOEL-JSM 5600.

Results and discussion

Oesophagostomum columbianum

The anterior end of *O. columbianum* had a well demarcated mouth collar. Behind the mouth collar the cuticle was inflated to form a cervical vesicle which was limited posteriorly by a cervical groove. Behind the cervical groove originates the lateral cervical alae which are well developed and are interrupted at several intervals. These alae may provide a degree of longitudinal stiffening as per studies of Yadav and Tandon (1992). Anterior end of these cervical alae are pierced by papillae called as cervical papillae (Fig. 1).

Mouth collar had an oral aperture anteriorly and a series of depressions at the rim of its posterior margin. The oral aperture was surrounded by leaf like structures constituting corona radiata i.e., ECR having 21 elements and the ICR containing two small elements for each element of ECR. Cuticle surrounding the corona radiata had 8 concentric rings or corrugations. Surrounding the corrugations it has a sensory depression/pit called amphid, and elongations called cephalic papillae which are four in number (Figs. 2, 3).

Male revealed bell shaped structure of bursa with two lateral lobes; the central part of inner surface of bursa was porous having a genital cone (Fig. 4). In female, the tail ends in a fine point with anus seen as fissure. The presence

Fig. 1 SEM photograph of anterior end of *O. columbianum*. ×100. *ca* cervical alae, *cg* cervical groove, *cv* cervical vesicle, *mc* mouth capsule/mouth collar

Fig. 2 SEM photograph of mouth capsule of *O. columbianum*. ×1500. *a* amphid, *cp* cephalic papillae, *d* depressions, *ecr* leaves of external corona radiata

Fig. 3 SEM photograph showing leaves of corona radiata of *O. columbianum*. ×4500. *ecr* leaves of external corona radiata, *icr* leaves of internal corona radiata

Fig. 4 SEM photograph showing the inner aspect of bursa of male *O. columbianum*. ×1000. *Gc* genital cone, *p* porous area

of coupling cement around vulva made it difficult to study the ultrastructure of vagina (Fig. 5).

Oesophagostomum venulosum

The anterior end of parasite showed well demarked mouth collar with an oral aperture and rim of its posterior margin had a series of depressions. Behind the mouth collar, cuticle was slightly inflated to form cervical vesicle which was limited posteriorly by cervical groove. Excretory canal open on lateral aspect of cervical groove, and there were no lateral cervical alae (Fig. 6).

The mouth collar surrounding the oral aperture showed 18 elements in ECR with protuberances on it seen on

higher magnification and ICR showed 2 small elements for each element of ECR. Surrounding the corona radiata there was a depression/pit called amphid which was sensory in function, and four elongations called cephalic papillae (Fig. 7).

Post end of *O. venulosum* male revealed large bell shaped structure of bursa with two lateral lobes with bursal rays projecting on to the bursal surface. Spicules are slender and show curved sword shaped structure under higher magnifications (Fig. 8). The tail of the female parasite was finely pointed. In the female parasite, the anus was seen as a fissure. The vulva of the females was covered with coupling cement which made it difficult to study the ultrastructure of vagina (Fig. 9).

Fig. 5 SEM photograph showing the vagina of female *O. columbianum*. $\times 90$. *a* anus, *cc* copulatory/sexual cement

Fig. 6 SEM photograph showing the anterior end of *O. venulosum*. $\times 350$. *a* amphid, *cg* cervical groove, *cv* cervical vesicle, *d* depressions, *ep* opening of excretory canal (excretory pore), *ecr* leaf of external corona radiata, *icr* leaf of internal corona radiata

Fig. 7 SEM photograph showing mouth capsule of *O. venulosum*. $\times 900$. *a* amphid, *cp* cephalic papillae, *d* depressions, *ecr* leaf of external corona radiata, *icr* leaf of internal corona radiata, *mc* mouth capsule/ mouth collar

Fig. 8 SEM photograph showing the bursa of male *O. venulosum*. $\times 350$. *br* bursal rays pointing on to surface, *s* spicules

Fig. 9 SEM photograph showing the vagina of female *O. venulosum*. $\times 120$. *a* anus, *cc* copulatory/sexual cement

Fig. 10 SEM photograph showing the anterior end of *O. asperum* (3 tier arrangement). $\times 500$. *cv* cervical vesicle

Oesophagostomum asperum

Oesophagostomum asperum anterior region revealed 3 tier arrangement of cuticle (Fig. 10). Mouth capsule surrounding the oral aperture shows 3 concentric rings and cephalic papillae. There are 12 elements in ECR and posterior rim of mouth capsule shows depressions (Fig. 11).

Male bursa had bursal rays projected as beaded structures on to the surface of bursa, there were pre bursal papillae projecting seen anterior to the bursal lobes (Fig. 12) and females had an abrupt constriction in front of posterior extremity. Anus and vulva were placed very close to each other; vulva was covered with copulatory cement (Fig. 13).

SEM helps in detail surface structural study of a parasite helping in better understanding of morphology. SEM studies of *O. columbianum*, revealed very well developed

Fig. 11 SEM photograph showing the mouth capsule of *O. asperum*. $\times 1000$. *cp* cephalic papillae, *d* depressions, *ecr* leaf of external corona radiata

Fig. 12 SEM photograph showing the bursa of male *O. asperum*. $\times 500$. *br* bursal rays pointing on to surface, *pbp* prebursal papillae, *s* spicules

lateral alae which were interrupted at intervals. Oral opening of mouth capsule was surrounded by eight concentric rings, externally had four cephalic papillae and a pair of amphids. Male revealed bell shaped structure of bursa with two lateral lobes that were similar to findings of Yadav and Tandon (1992). Presence of 21 leaf shaped elements in ECR at the margin of buccal cavity is in coincidence with the microscopic studies of Singh (2003) and Soulsby (1982). The central part of inner surface of bursa was porous, showed the presence of genital cone which is in agreement with the descriptions of Duggal and Kaur (2006).

Oesophagostomum venulosum showed presence of 18 elements in ECR and inner corona radiata showed two small elements for each element of ECR. The male parasite had a large bursa and the spicules were long, tubular,

Fig. 13 SEM photograph showing the vagina of female *O. asperum*. $\times 250$. a anus, cc copulatory/sexual cement

slender and had a curved sword shaped structure under higher magnifications were in confirmation with SEM studies of Khanmohammadi et al. (2013) and are also in confirmation with microscopic studies of Singh (2003).

Oesophagostomum asperum revealed 3 concentric rings around oral aperture showing presence of 12 elements in ECR. The cuticle of the anterior region was ridged giving three tier appearance. Male bursa showed the presence of bursal rays as beaded projections on the surface of bursa and females showed an abrupt constriction in front of posterior extremity were in confirmation with the SEM studies of Yadav and Tandon (1992).

Conclusions

In this study presence of 21 leaflets in ECR and well developed cervical alae in *Oesophagostomum columbianum*, 18 leaflets in ECR with no cervical alae in *O. venulosum* and 12 leaflets in ECR of *O. asperum* makes them to differentiate among these species.

The buccal capsule of the parasite was well elucidated revealing the presence of depressions at posterior rim of

buccal capsule, number of leaflets of ECR and ICR, cephalic papillae and pits as amphids. Leaflets of ECR (have shown protruberances under higher magnification) were closed in some SEM photographs and open in others indicating movements of them helping in closing and opening of buccal cavity. In *O. asperum* three tier system of arrangement of cuticle at anterior end was well elucidated. In female's study of structure of vulva was not possible as vulva was covered with copulatory cement (in sexually matured and copulated).

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