Occurrence of *Oesophagostomum* species in slaughtered sheep in area of Hyderabad, Telangana State

Rajeshwari Gaddam¹ · G. S. Sreenivasa Murthy¹ · Sudhakar Kommu¹

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Abstract A study was conducted in Chengicherla slaughter house of Hyderabad to find the occurrence of *Oesophagostomum* worms, 594 *oesophagostomum* positive intestines were examined. Among these, three species of *oesophagostomum* worms were recovered viz., *Oesophagostomum columbianum*, *Oesophagostomum venulosum* and *Oesophagostomum asperum* were identified by light microscopy as per the keys provided by Soulsby (Helminths, arthropods and protozoa of domesticated animals. Bailliere Tindall, London, 1982) and Singh (Veterinary helminthology. ICAR, Delhi, 2003).

Keywords Light microscopy · FAAL staining · *Oesophagostomum columbianum* · *Oesophagostomum venulosum* · *Oesophagostomum asperum*

Introduction

Parasitic infections in sheep are the major limiting factors of productivity. Of them *Oesophagostomosis* is one of the most prevalent and pathogenic gastrointestinal nematode infections of livestock in India (Gupta et al. 1987). *Oesophagostomum* worms are known as nodular/knotty worms of livestock (Levine 1978; Anderson 2000; Nwosu

et al. 2011), as several species form nodules in the wall of intestine (Soulsby 1982), thus affecting the large intestine causing nodular worm disease and often resulting in vast economic losses due to condemnation of intestines. Among those *Oesophagostomum columbianum* is common and causes widely prevalent parasitosis with significant economic importance in small ruminants (Olivares et al. 2000). Other common species involved in ovine *Oesophagostomosis* are *Oesophagostomum venulosum* and *Oesophagostomum asperum* [classified as per keys provided by Soulsby (1982) and Singh (2003)].

Materials and methods

Collection of worms

The affected intestines with cheesy yellowish nodules on caecum and colon (Fig. 1) were tied at both ends and were brought to laboratory in an ice box, dissected longitudinally and the worms embedded in mucosa are separated and washed to remove the host material and pooled into a petri dish containing PBS (pH 7.2) and are classified to respective species based on microscopic examination and are further subjected to clearing and staining.

Microscopic examination

The worms were initially fixed in hot glacial acetic acid to straighten and flatten the worms, dehydrated in 70% alcohol for 30 min, worms were further subjected to dehydration in ascending grades of alcohol i.e., in 80% alcohol for 30 min, 90% alcohol for 20 min, absolute alcohol for 15 min, followed by clearing in lactophenol for 24 h. Cleared anterior and posterior ends of the worm were

✉ Rajeshwari Gaddam
rajeshwari.g04@gmail.com
G. S. Sreenivasa Murthy
gssmurthy26@rediffmail.com
Sudhakar Kommu
vety0756@gmail.com

¹ Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad 30, India

Fig. 1 Photograph showing knotty nodules on caecum (on large intestine) and small intestine. N_l nodules on large intestine, N_s nodules of small intestine

mounted on a clean glass slide using lactophenol and observed under light microscope at various magnifications.

Formalin alcohol azocarmine and lactophenol (FAAL) staining

Freshly collected worms were washed five times in PBS to remove dirt and as such live worms were kept in the FAAL stain (Conc. Formalin-5 ml, Alcohol/Rectified Spirit-20 ml, Lactophenol-75 ml, Azocarmine G-5 mg) for overnight staining at 27 °C and examined by mounting in the FAAL stain (Sabu et al. 2012).

Results and discussion

The morphology of the anterior proximity as mouth cone, the number and the shape of the elements of corona radiata have been employed as primary taxonomic characteristics in identification of various oesophagostomum species (Yadav and Tandon 1992). The worms are white milky coloured, show bent hook like structure anteriorly and posteriorly females show copulatory cement with tapering tails (Khanmohammadi et al. 2013), enlarged bursa in males (Fig. 2).

Oesophagostomum columbianum

The anterior part of parasite had a well de-marketed large cylindrical and sub globular mouth collar. Behind the mouth collar cuticle formed a cervical vesicle which was limited posteriorly by a cervical groove. Excretory canal open on the lateral aspect of cervical groove and behind the cervical groove starts the lateral cervical alae which were

Fig. 2 Photograph showing adult male and female worms of *Oesophagostomum*. Female (f) with copulatory cement (cc), male (m) with bursa (b)

well developed producing a marked dorsal curvature of the anterior part of the body. The anterior end of cervical alae were pierced by cervical papillae, seen as projections (Fig. 3) are similar to descriptions of Soulsby (1982). The buccal capsule was surrounded by external corona radiate (ECR) consisting of 20 elements or leaf like structures and the internal corona radiata (ICR) containing 2 small elements for each ECR has 40 elements (Fig. 4), are similar to findings of Soulsby (1982) and also with the observations of SEM studies of Yadav and Tandon (1992).

Male present with well developed bell shaped bursa with two large lateral lobes and not very distinct dorsal lobe consisting of bursal rays—ventral ray cleft, medio-lateral and postero-lateral rays of lateral rays were fused proximally well separated from latero ventral ray, externo-dorsals arising from common trunk with dorsal, dorsal was divided into two divergent terminal branches, each of which giving a short lateral twig. Pair of pre-bursal papillae were present. Spicules were equal, alate with blunt tips (Fig. 5) which were similar to findings of Soulsby (1982) and Singh (2003). In females tail was tapering with slightly prominent vulva, opening anterior to anus (seen as transverse slit between tail tip and vulva) and coupling cement was observed at vulva of copulated females. Vagina was very short; transverse (Fig. 6) leading into kidney shaped Par-ejectrix were in similar to the findings of Soulsby (1982).

Fig. 3 Photomicrograph showing anterior end of *O. columbianum* $\times 100$. cg cervical groove, cv cervical vesicle, cp cervical papillae, mc mouth capsule, ca lateral cervical alae, o oesophagus

Fig. 4 Photomicrograph showing anterior end of *O. columbianum* $\times 450$. cg cervical groove, cv cervical vesicle, icr internal corona radiata, ecr external corona radiata, mc mouth capsule

Fig. 5 Photomicrograph showing posterior end of male *O. columbianum* (formalin, alcohol, azocarmine and lactophenol) FAAL stained $\times 100$. d dorsal ray, ed externodorsal ray, ml mediolateral, pl posteriolateral, ll lateral lobe, dl dorsal lobe, lv lateroventral, s spicules, v ventral ray system

Fig. 6 Photomicrograph showing posterior end of female *O. columbianum* $\times 100$. a anus, v vagina, t tail

Oesophagostomum venulosum

The anterior end of parasite showed a well de-marketed mouth collar. Behind the mouth collar cuticle formed a cervical vesicle which was limited posteriorly by cervical groove. Excretory canal open on lateral aspect of cervical groove, there were no lateral cervical alae present, surrounding the buccal capsule 18 elements in ECR and inner corona radiata showed two small elements for each ECR (Fig. 7) which are in harmonious with findings of SEM studies conducted by Khanmohammadi et al. (2013),

Fig. 7 Photomicrograph showing the anterior end of *O. venulosum* $\times 100$. *cg* cervical groove, *cv* cervical vesicle, *cp* cervical papillae, *icr* internal corona radiata, *ecr* external corona radiata, *mc* mouth capsule, *o* oesophagus

Lapage (2000). Male revealed large bell shaped structure of bursa with two lateral lobes with bursal rays projecting on to the bursal surface. Medio lateral and posterior lateral rays of bursa were closely applied to each other and were of somewhat divergent from the antero-lateral ray are in coincidence with findings of Singh (2003). The externodorsal arising high up on the main stem of the dorsal ray and got bifurcated in its posterior two-fifth. Pre bursal papillae were seen anterior to the bursa lobes and spicules are long, slender and tubular (Fig. 8). The tail of the female parasite was finely pointed, the anus was seen as a fissure. The vulva of the females was covered with sexual cement or coupling cement (Fig. 9) are coherent with findings of SEM studies conducted by Khanmohammadi et al. (2013).

Oesophagostomum asperum

Oesophagostomum asperum had 12 elements in ECR surrounding the buccal capsule. The cephalic vesicle at the anterior region was well developed and highly inflated and ridged forming into 3–4 tiers (Fig. 10) are in confirmation with descriptions of Soulsby (1982), SEM studies conducted by Yadav and Tandon (1992) and Singh (2003).

In males bursa was well developed, with rays: ventral ray cleft, medio-lateral and postero-laterals were fused proximally, exteno-dorsals arising from the common trunk with dorsal, dorsal was divided into two divergent branches, each of which gives off a short lateral branch. Spicules were paired, tubular, alate and are equal (Fig. 11). In

Fig. 8 Photomicrograph showing the posterior end of male *O. venulosum* $\times 100$. *s* spicules, *d* dorsal ray, *ed* externodorsal ray, *pb* prebursal papillae, *v* ventral rays, *al* antero-lateral ray, *ml + pl* closely applied medio-lateral and postero-lateral rays

Fig. 9 Photomicrograph showing the posterior end of female *O. venulosum* $\times 100$. *v* vagina, *a* anus, *t* tail

females tail tapered abruptly. Anus was rather prominent. Vulva was situated at a short distance from posterior end of the body. Vagina was long, opening into relatively anteriorly placed ovejector (Fig. 12) are in coincidence with the descriptions of Singh (2003).

Conclusions

Species of *Oesophagostomum* can be differentiated by using light microscopy and staining protocols as *O. columbianum* having 20 ECR and well developed cervical alae, *O. venulosum* shows 18 ECR with no cervical alae and *O. asperum* shows anterior tier system with 12 ECR which makes them to differentiate among them. Species

Fig. 10 Photomicrograph showing the anterior end of *O. asperum* $\times 100$. *cg* cervical groove, *cv* cervical vesicle, *ecr* external corona radiata, *ec* excretory canal, *mc* mouth capsule, *o* oesophagus

Fig. 11 Photomicrograph showing the posterior end of male *O. asperum*. FAAL stained $\times 100$. *s* spicules, *d* dorsal ray, *ed* externo-dorsal ray, *v* ventral rays, *al* antero lateral ray, *ml + pl* proximally fused medio-lateral and postero-laterals

differentiation can be done in females by the use of distance between tail to anus and vagina which requires more effort in measuring in *O. columbianum* and *O. venulosum* but it can be easily notified in *O. asperum* due to presence of an abrupt constriction in front of posterior extremity unlike in *O. columbianum* and *O. venulosum* and also anus and vulva were placed very close to each other. In females

Fig. 12 Photomicrograph showing the posterior end of female *O. asperum* $\times 100$. *v* vagina, *a* anus, *t* tail, *p* pars ejectrix

in which vulva was covered with copulatory cement indicates sexually matured and copulated females.

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