Investigating the presence of *Oesophagostomum* species in slaughtered goats in the central Nepal

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ABSTRACT

Oesophagostomum infects various hosts like caprines, ovines, porcines, rodents, primates, and humans. The aim of the current study was to identify different species of Oesophagostomum in the intestines of sacrificed goats during festival seasons in the central part of Nepal. From ten slaughtered male goats, O. asperum and O. venulosum were identified by light microscopy and line drawing methods accompanied by the taxonomic keys. These methods were used to completely describe the presence of two Oesophagostomum species for the first time in Nepalese goats and are crucial in the laboratory with the limited facilities.

Keywords: *Copulatory bursa, Oesophagostomum asperum, Oesophagostomum venulosum, taxonomy.*

INTRODUCTION

The genus *Oesophagostomum* (nodular worm) (Strongylida: Chabertiidae) represents a dominant nematode parasite of suids, ruminants, primates, African rodents and humans (Stewart & Gasbarre, 1989; Thornton, 1924). Few species of *Oesophagostomum* like three species like *O. bifurcum, O. stephanostomum*, and *O. aculeatum* can infect humans (Polderman and Blotkamp 1995; Chabaud and Lariviere 1958; Skrjabin et al. 1961; Blotkamp et al. 1993). The freshly laid eggs of *Oesophagostomum* are passed into the feces, hatch into L1 and then into L3 in the environment in one week. When hosts ingest L3 along with soil or grasses, they develop into adult worms (https://web.stanford.edu/group/parasites/ParaSites2002/oesophagostomiasis/epi.html, Retrieved February 12, 2021). This parasite is the underlying cause of clinical and veterinary oesophagostomiasis characterized by weight loss, persistent diarrhea, and granuloma formation, and caseous lesions or abscesses in the intestines of variety of hosts including man and animals (Goldberg, 1952; Stewart & Gasbarre, 1989). Because the parasite can be transmitted between vertebrates and humans, it is critically important as veterinary and public health all over the world.

In Nepal, coprologic studies have been conducted to find out the prevalence rates of strongyles, however, it is difficult to identify the species of *Oesophagostomum* from other species of strongyles. It is very difficult to identify the species by microscopy only because eggs are morphologically similar to false hookworm (*Ternidens deminutus*), hookworms and other strongyles like *Trichostrongylus*, *Bunostomum*. Few laboratories use coproculture of L3 larvae or histopathology of gastrointestinal tract to isolate adult worms. In these situations, morphological

keys will be supportive in complete identification of these species when molecular studies like polymerase chain reaction (PCR) and Scanning Electron Microscopy (SEM) are lacking (Gaddam et al., 2017a). These techniques are both expensive and time consuming for developing countries like Nepal. Therefore, we aimed to identify *Oesophagostomum* species up to species level in goats using morphological keys. Although *O. columbianum,* the small ruminant nodular worm (*O. asperum*), and the large bowel worm (*O. venulosum*) are predominantly reported in goats and sheep (Goldberg, 1952; Yu et al., 2012; Zhao et al., 2013), we have recorded only two species, (*O. asperum* and *O. venulosum*) in goats in the central part of Nepal.

MATERIALS AND METHODS

Sample collection, processing, and examination

During the festive seasons of Hindus, 10 sacrificed goats were purposively sampled in the Chitwan area that lies in the central southern part of the country. The stomach and intestines of those goats were carefully collected, and they were dissected longitudinally. The worms embedded in mucosa were separated and collected in the saline solution containing 0.9% sodium chloride. Some of the worms were put in 70% ethanol. Then, they were brought to the laboratory in Kathmandu.

The nematodes (milky colored with bent hook-like structure anteriorly and posteriorly) were washed several times with saline solution (0.9%). Then, they were placed in watch glass containing lactophenol (HIMEDIA, India) for 5 minutes. The nematodes were put in a slide containing glycerine. Canada balsam (Fizmerk, India) was used to tighten the coverslip. In another context, the nematodes preserved at 70% alcohol were washed in normal saline (0.9%). Nematodes were also stained by Giemsa's stain (Fisher Scientific) and Lugol's Iodine (Fisher Scientific) differently and slides were prepared. Finally, all the specimens were observed under light microscope (Optika Microscopes Italy, B-383PLi) at a total magnification of X40, X100, and X400 and images (1920 pixels x 1020 pixels) were taken using SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright (C) 2013-2014. The live images were drawn at the tracing paper over the screen of a laptop. Then, *O. venulosum* and *O. asperum* were confirmed by the keys published by various authors (Gaddam, Murthy, & Kommu, 2017a; Khanmohammadi, Halajian, & Ganji, 2013; Yadav & Tandon, 1992). These keys were mainly based on the presence of the mouth cone, the number and the shape of the elements of corona radiata, the pattern of the cervical vesicle, and position of vagina and anus in the female, and the structure of spicules on the male.

RESULTS AND DISCUSSION

In this study, out of 10 goats, only two were found to be infected with *Oesophagostomum* spp. Both goats had multiple infestations of *O. asperum* and *O. venulosum*. Morphometric measurements of different structures of males and females *O. asperum* and *O. venulosum* were recorded (Table 1).

Table 1: Characteristic features and measurements (mm) of female and male *Oesophagostomum aspersum* and *Oesophagostomum venulosum*. The measurements are expressed in minimum to maximum and average values are put in the parenthesis.

Characteristic features	***O. asperum***		***O. venulosum***	
	Female measurements (mm) (N=2)	**Male measurements (mm) (N=4)**	**Female measurements (mm) (N=3)**	**Male measurements (mm) (N=3)**
Body length	8.4-8.7 (8.55)	8.49-9.12 (8.79)	12.48-17.78 (14.88)	11.35-13.1 (12.23)
Max width body	0.38-0.40 (0.39)	0.42-0.45 (0.43)	0.42-0.6 (0.50)	0.40-0.49 (0.45)
Esophagus length	0.74-0.78 (0.76)	0.76-0.88 (0.81)	0.83-0.94 (0.87)	0.80-0.90 (0.85)
Max esophagus width	0.12-0.12 (0.12)	0.13-0.15 (0.14)	0.10-0.13 (0.12)	0.11-0.12 (0.12)
Width at end of esophagus	0.31-0.32 (0.31)	0.24-0.28 (0.26)	0.31-0.42 (0.36)	0.24-0.27 (0.26)
Width at start of esophagus	0.22-0.23 (0.22)	0.15-0.16 (0.16)	0.06-0.17 (0.12)	0.10-0.15 (0.13)
Tail length	0.08-0.09 (0.09)	-	0.30-0.43 (0.35)	-
Tail tip to vulval part	0.13-0.16 (0.15)	-	0.70-0.80 (0.76)	-
Vulva width	-	-	0.02-0.03 (0.03)	-
Vagina length	-	-	0.05-0.06 (0.05)	-
Rectal length	-	-	0.20-0.48 (0.29)	0.15-0.20 (0.18)
Length of spicules	-	1.21-1.35 (1.29)	-	0.60-1.2 (0.88)
Average length of rays	-	0.51-0.56 (0.54)	-	0.18-0.30 (0.23)

The anterior end of both male and female *O. asperum* was characterized by the presence of a salient mouth collar or mouth capsule (MC) formed by the cuticle layer (Figure 1-3). The microscopic study showed that the MC was characterized by external corona radiata (ECR) with a total of 12 elements although we could draw only six of them on one surface (Figure 2). In spite of 24 internal corona radiata (ICR), we observed 12 of them on one side of the capsule. There was a cervical groove (CG), a ventrally prominent structure, extended behind the lateral line. Cuticle upper to the CG was widening to form the cephalic vesicle (CV) which was well developed, highly swollen, and was ridged producing three to four tiers (Gaddam et al., 2017a; Yadav & Tandon, 1992). However, these ridges changed into fine annular striations or lateral alae posteriorly, even after esophagus (Figure 1). In male *O. asperum*, bursa was well developed with rays. There were 12 bursal rays of various sizes located inside the flap-like bursal pouch. However, their endings were provided with sharp hook-like structures. There were paired long, tubular, alate, and equal thread-like spicules. The spicules were curved at their tips and were provided with a small rounded knob (Figure 4-5). In female *O. asperum*, the vulva and vagina were located at a short distance from the posterior end of the body. Microscopically, anus and vulva lie quite close to one another as revealed by using the scanning electron microscope (SEM) by others (Yadav & Tandon, 1992).

The reproductive pores consisted of an upper labium and a lower labium that had some longitudinal stripes (Yang, 1996). Tail tapered sharply (Figure 6-7).

In summary, a total of 12 ECR, the highly developed cervical vesicles, the paired, tubular, alate, and equal thread-like spicules with a rounded knob at the tips, the vulva and the long vagina located closely from the posterior part of the body, and the closely placed anus and vulva are the characteristic features of *O. asperum*. In contrast, 18 ECR, less developed CVs, a large bell-shaped bursa with muscular bursal rays, prebursal papillae anterior to the bursal lobe, the slender and tubular spicules, and the short, transverse, and kidney-shaped female openings are the main taxonomic keys of *O. venulosum*.

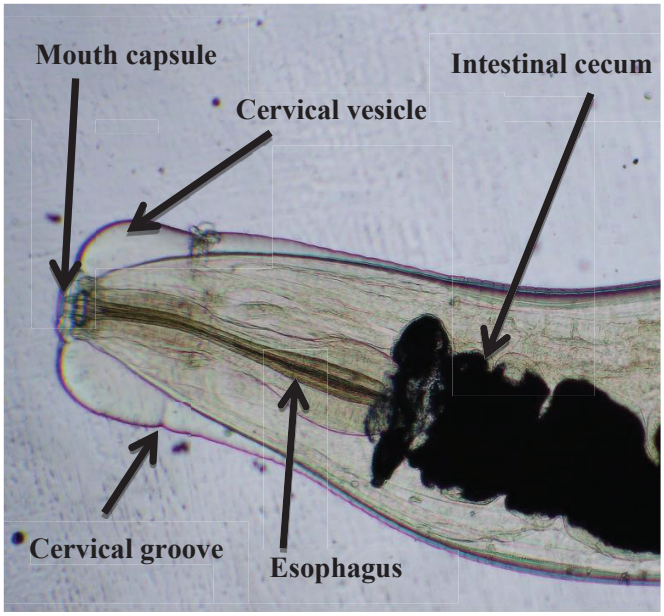

Figure 1: Photomicrograph showing the anterior end of *O. asperum* (X40).

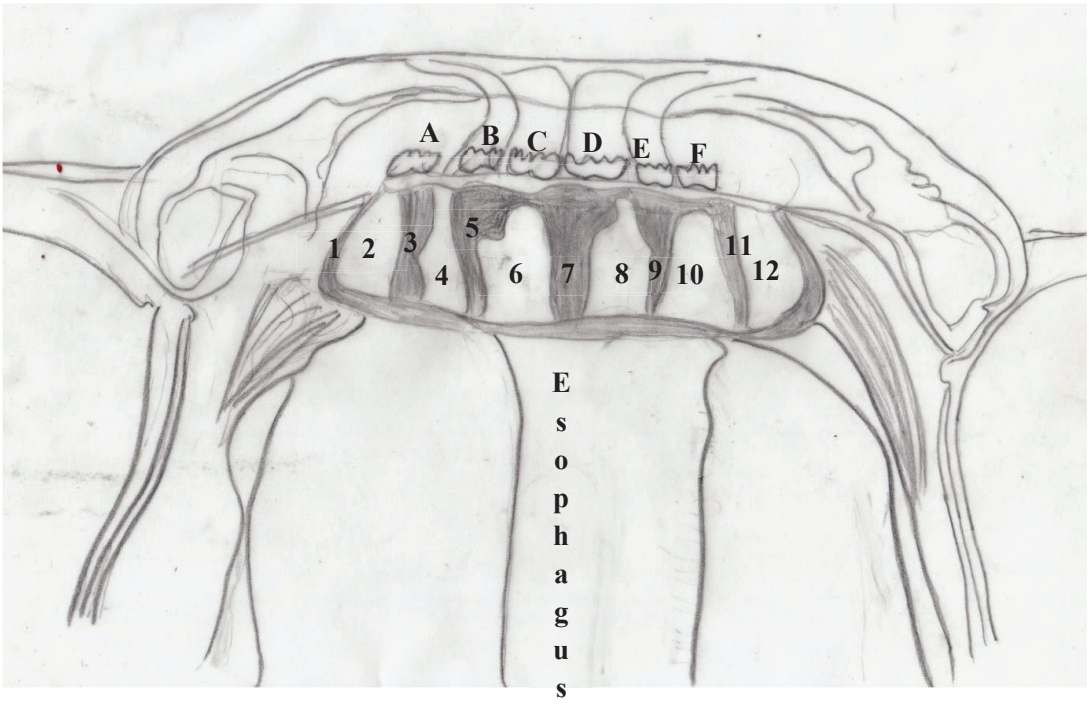

Figure 2: Anterior end of *O. asperum* (X400). 1 to 12 represents internal corona radiata on ventral surface. A to F represents external corona radiata.

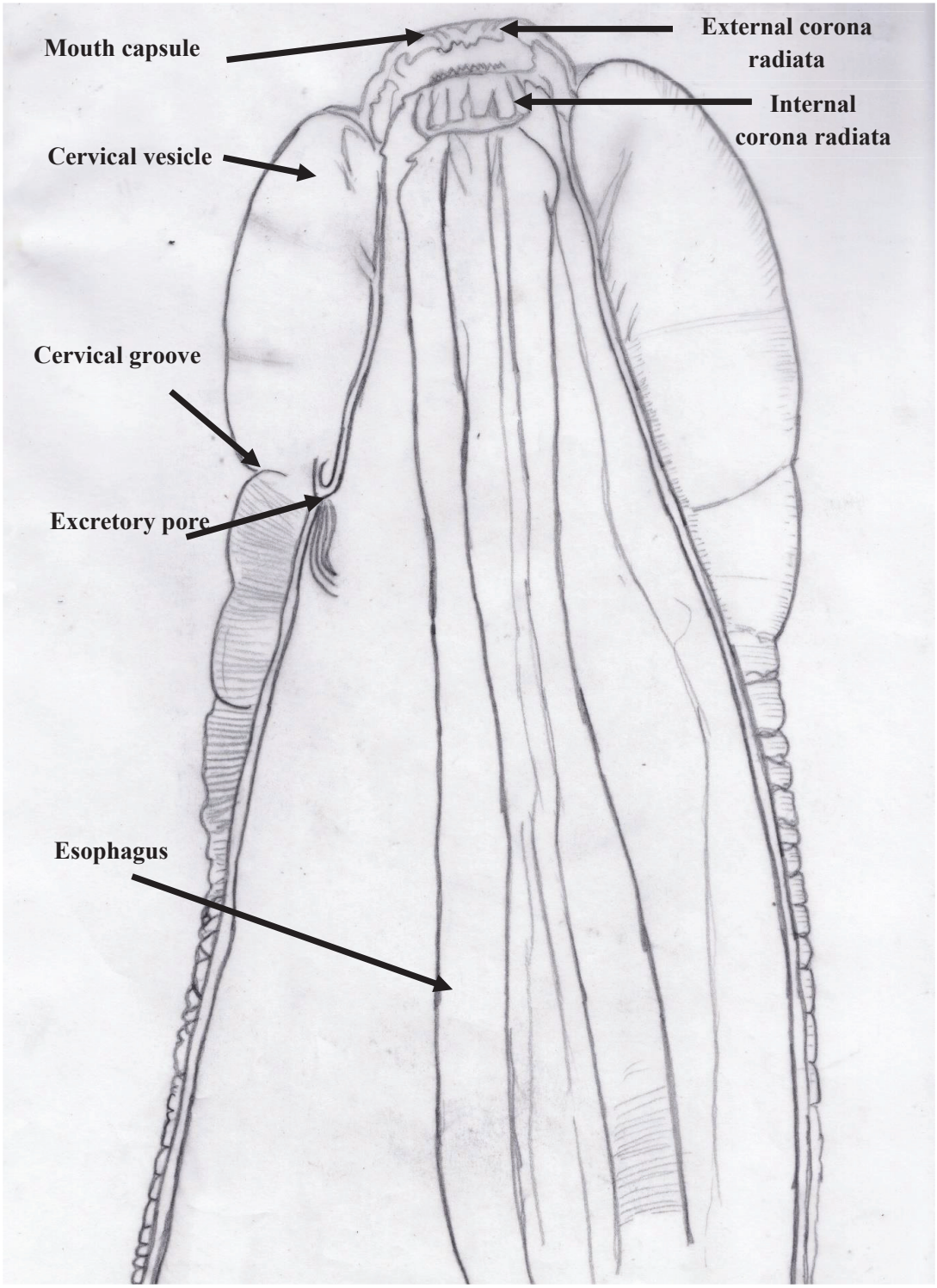

Figure 3: Anterior end of *O. asperum* (X100)

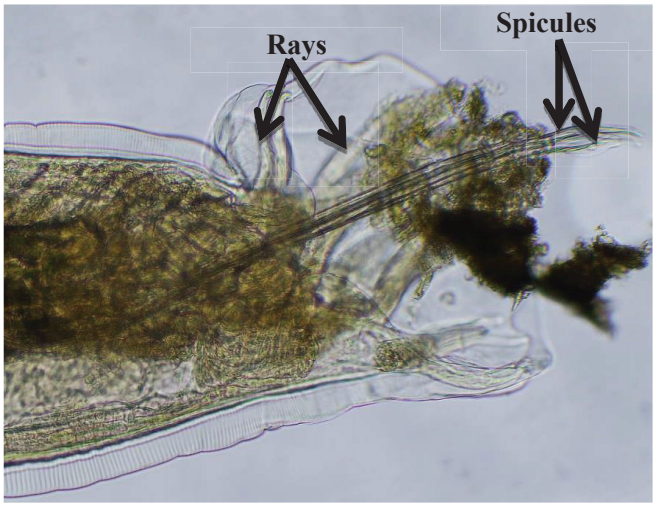

Figure 4: Photomicrograph showing the posterior end of male *O. asperum* (X100)

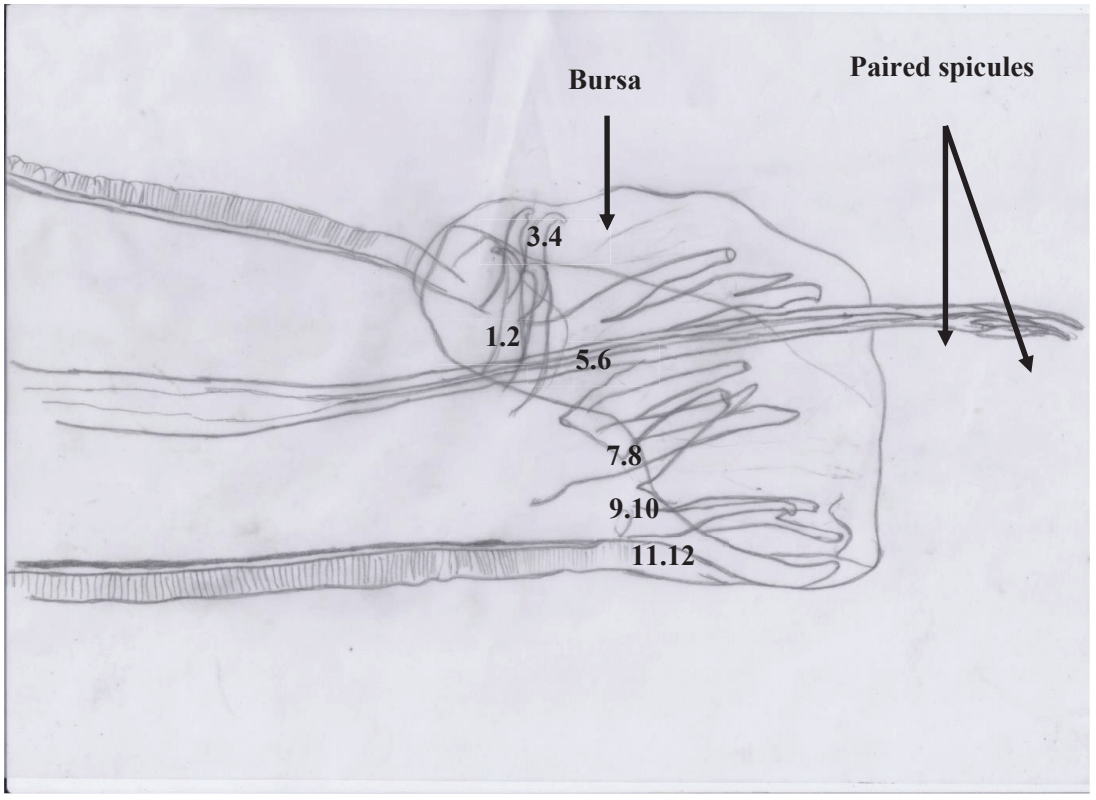

Figure 5: Posterior end of male *O. asperum* (X100). This part includes bursa copulatrix with a paired spicule and 12 rays. Each pair has been represented by point in between two rays

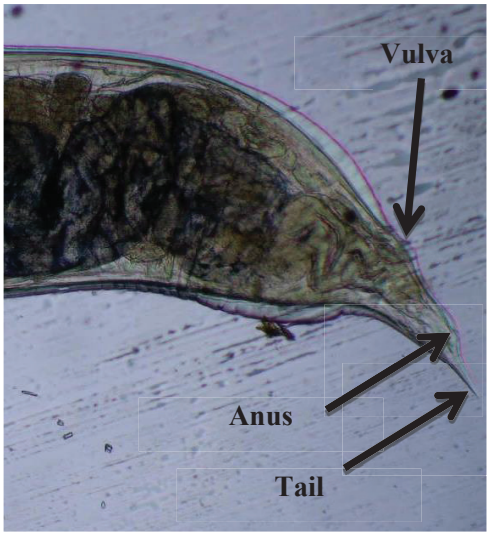

Figure 6: Photomicrograph showing the posterior end of female *O. asperum* (X40).

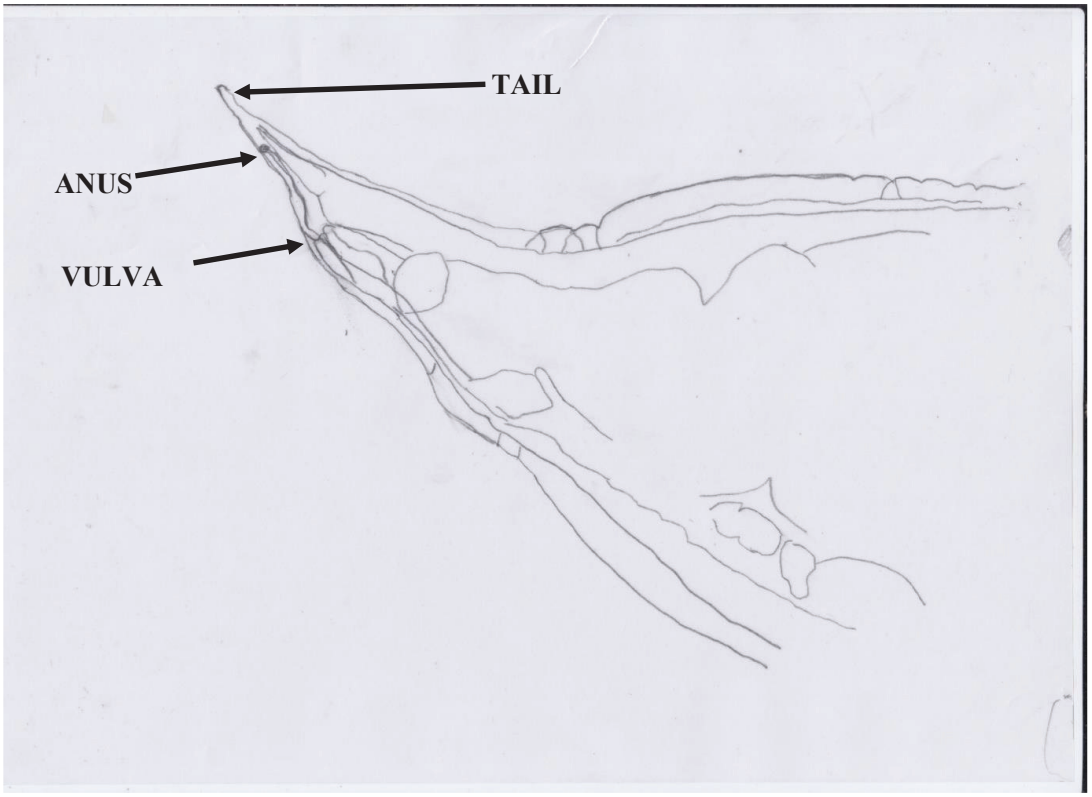

Figure 7: Posterior end of female *O. asperum* (X100). This part includes vulva with long flaps, anus, and tail

Both male and female *O. venulosum* had a well-developed mouth collar at the anterior end. The MC seemed to be a cone-like structure isolated from the body. MC contained 18 elements within the ECR although we observed only nine elements, whereas 36 elements within the ICR although only 18 of them on one surface of the collar. Cervical vesicle ranged from behind the mouth collar to CG; however, it was less developed compared to that in *O. asperum* (Gaddam et al., 2017a; Gaddam, Murthy, & Kommu, 2017b; Khanmohammadi et al., 2013). Lateral cervical alae were lacking as similar to other studies (Khanmohammadi et al., 2013) (Figure 8, 9). Male *O. venulosum* showed a large bell-shaped structure of bursa with two lateral lobes with bursal rays as described previously (Gaddam et al., 2017a). The bursal rays are muscular. Prebursal papillae were observed anterior to the bursal lobes. Spicules were slender and tubular (Figure 10, 11). In female, the vagina was very short, transverse, and kidney-shaped as described previously (Khanmohammadi et al., 2013). Its anus looks like a fissure. The tail is a finely pointed (Gaddam et al., 2017a, 2017b) (Figure 12, 13).

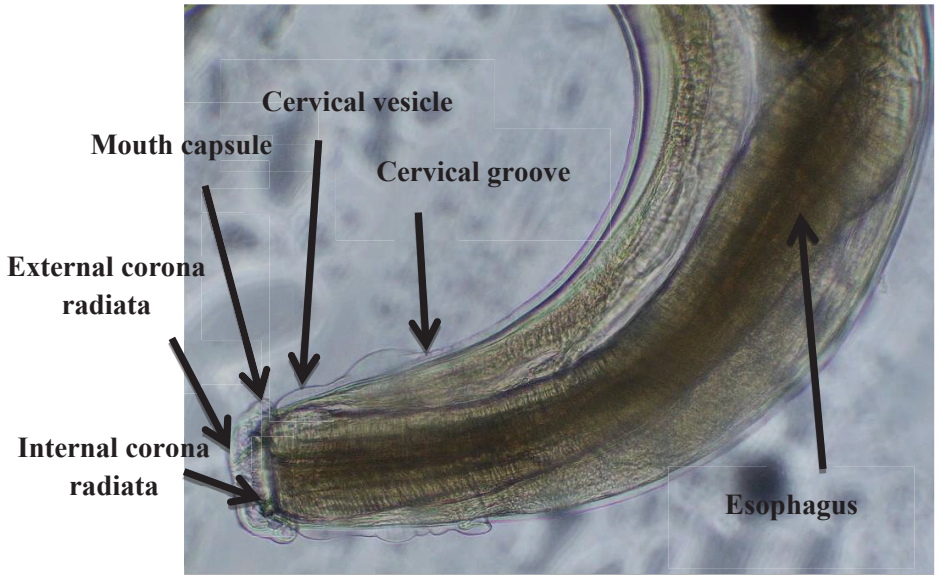

Figure 8: Photomicrograph showing the anterior end of *O. venulosum* (X100)

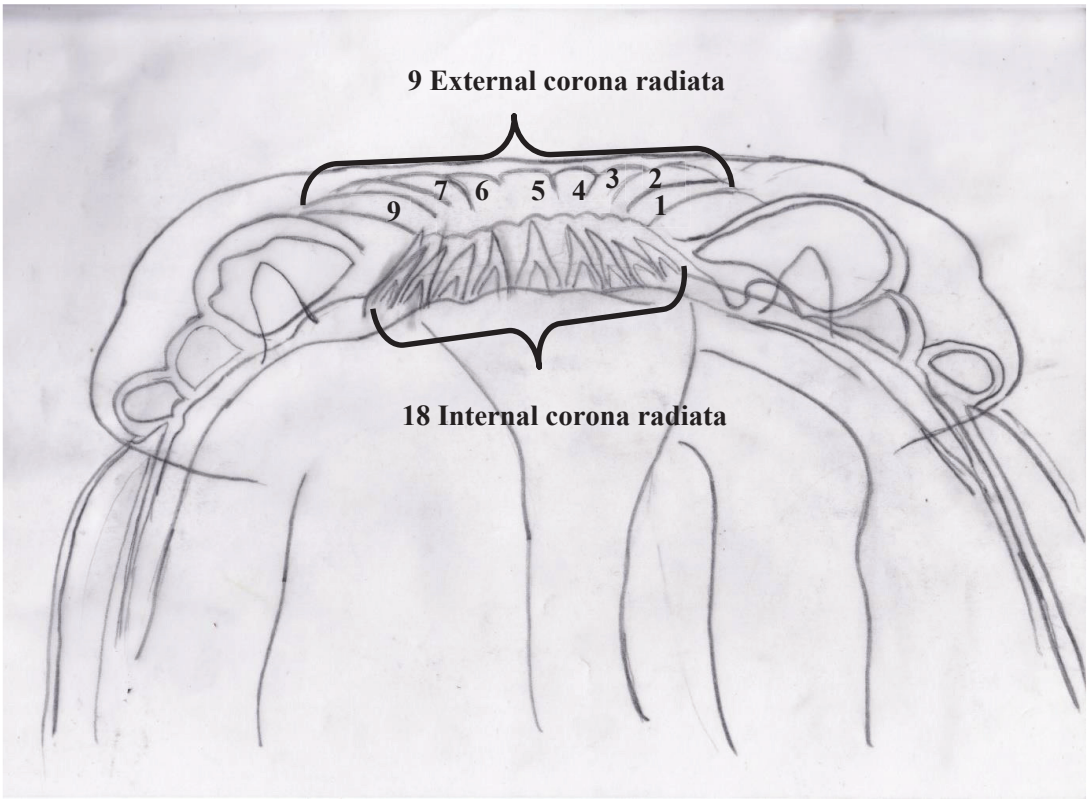

Figure 9: Anterior end of *O. venulosum* (X400). One surface shows a total of 18 internal corona radiata and nine external corona radiata.

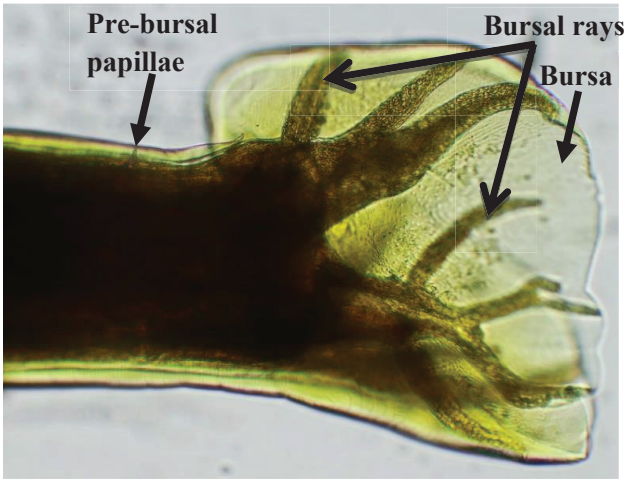

Figure 10: Photomicrograph showing the posterior end of male *O. venulosum* after processing in Lugol's Iodine (X100)

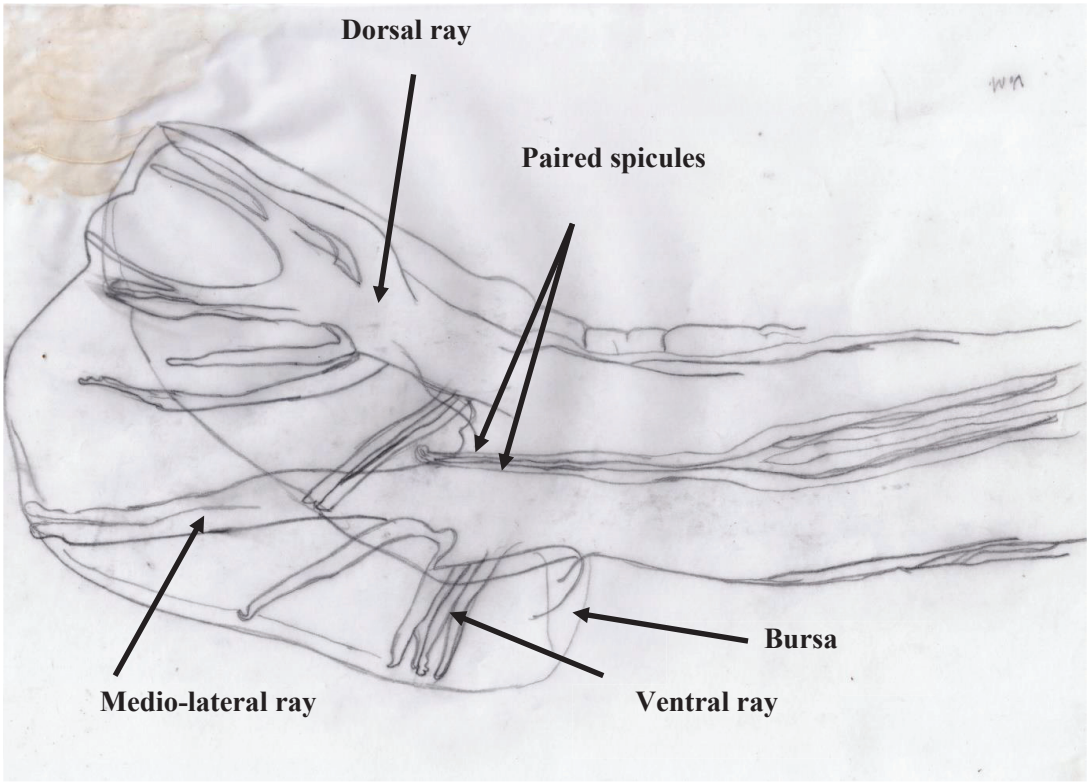

Figure 11: Posterior end of male *Oesophagostomum venulosum* (X100). This part includes bursa provided with bursal rays and spicules.

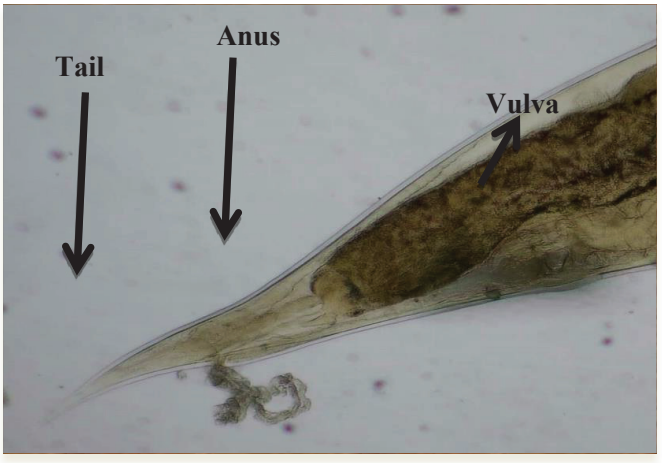

Figure 12: Photomicrograph showing the posterior end of female *O. venulosum* (X40).

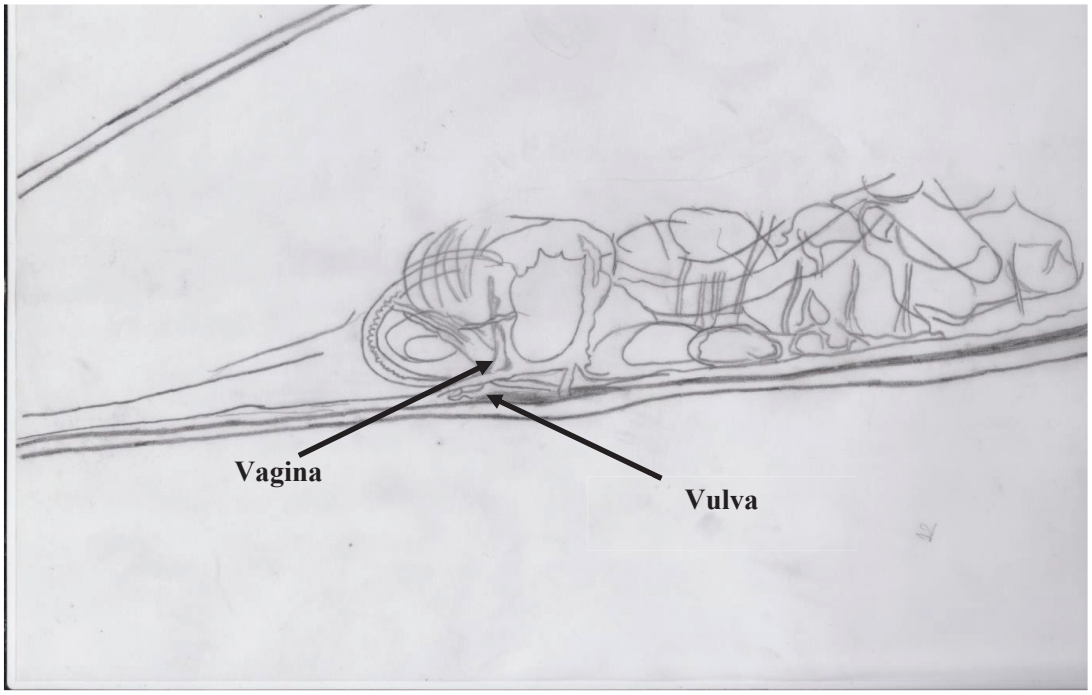

Figure 13: Posterior end of female *O. venulosum* (X100). This part includes vulva, vagina, ovary, and uterus.

CONCLUSION

In conclusions, this is the first study using light microscopes, line-drawings of camera lucida-like systems, and morphologic keys for identifying *O. asperum* and *O. venulosum* in the goats of Nepal. These materials and methodologies are crucial in taxonomy of nematodes in the low resource settings. However, the SEM data and molecular methods like PCR would be more confirmatory compared to these techniques. Therefore, further studies related to nematodes should be conducted using more advanced tools and techniques to confirm them in goats and other domestic animals.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ANIMAL RIGHTS

The authors declare that the study was conducted on naturally-infected goats which had been sacrificed for religious purposes in the central part of Nepal. No experimental infection was established during this research work.

Abbreviations: CG: Cervical Groove; CV: Cephalic Vesicle; DR: Dorsal Ray; ECR: External Corona Radiata; ICR: Internal Corona Radiata; MC: Mouth Capsule; SEM: Scanning Electron Microscope

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