Gross, histopathology and molecular diagnosis of oesophagostomosis in sheep

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Abstract A total of 120 tissue samples (Rumen, reticulum, omasum, abomasum, small intestine and large intestine) were collected from slaughter houses in Chennai, Kanchipuram and Tiruvallur districts and five samples from necropsy room at Department of Veterinary Pathology, Madras Veterinary College, Chennai, Tamil Nadu. Overall prevalence of GI parasites was 52.00%. Among the positive samples, single infection was found to be more (53.84%) than mixed infection (46.15%). Out of which, oesophagostomosis was 17.00% and found as single infection. In oesophagostomosis, significant gross lesions observed in the small and large intestine were multiple small to large, round to irregular, hard, fibrotic, raised nodules. The wall of the large intestine was greatly thickened and oedematous and the nodules were seen projecting into the lumen and mucosal surface was intact. On histopathological examination, ileum of sheep showed parasitic nodules with central baso-eosinophilic necrotic area, larval stages in the necrotic area, surrounded by layers of inflammatory cells and finally encapsulated in a thick fibrous connective tissue capsule. Immunohistochemistry (IHC) with Vimentin showed intense cytoplasmic positive signals DAB Brown. PCR was carried out targeting ITS2 regions of the *O. columbianum* and *O. venulosum*. DNA

isolated from both the faecal eggs and adult worms showed good amplification.

Keywords Oesophagostomosis · Parasitic nodules · Histopathology · Large intestine · Larval stages · IHC · Vimentin · PCR

Introduction

Parasitic diseases pose a major threat in the growth and development of animal health all over the world (Mahfooz et al. 2008; Saeed et al. 2010). The productivity of animals is affected due to GI parasitic infections in terms of lowered production and mortality (Sykes 1994; Waller 1999). Sheep are mostly infected with *Teladorsagia*, *Trichostrongylus*, *Haemonchus*, *Nematodirus*, *Cooperia*, *Chabertia* and/ or *Oesophagostomum* and are responsible for substantial economic losses due to the diseases, treatment cost and deworming cost (Zajac and Conboy 2006; O'Connor et al. 2006; Sackett and Holmes 2006). Among GI parasites, *Oesophagostomum columbianum* and *O. venulosum* occurs in small ruminants and responsible for anemia, protein-losing enteropathy, hypoproteinaemia and death (Chiejina 1987). *Oesophagostomum columbianum* causes knotty gut or pimply gut in sheep (Nwosu et al. 2007; Mbaya et al. 2009) which results in partial or total condemnation of intestines (Ahmed et al. 1994; Nwosu et al. 1996). In India, no information available on gross, histopathology and molecular diagnosis except the prevalence of GI parasites in sheep (Soundararajan 2001; Khajuria and Kapoor 2003; Swarnkar et al. 2010). This paper reports gross, histopathology and molecular diagnosis of oesophagostomosis in sheep.

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Fig. 1 **a** Sheep-Colon-Oesophagostomosis—Multiple, small to large, round to irregular, hard, fibrotic, raised nodules. **b** Sheep-Oesophagostomosis- greatly thickened and oedematous caecum. **c** Sheep-Ileum-HP-Oesophagostomosis-Central eosinophilic necrotic area with development larval stages H&E, $\times 40$. **d** Sheep-Ileum-HP-Oesophagostomosis-Central eosinophilic necrotic area with development larval stages H&E, $\times 40$

Materials and methods

Collection of tissue samples

A total of 120 tissue samples (Rumen, reticulum, omasum, abomasum, small intestine and large intestine) in Chennai, Kanchipuram and Tiruvallur districts, respectively were collected from each individual animal after slaughtering and five samples collected from necropsy room at Department of Veterinary Pathology, Madras Veterinary College, Chennai, Tamil Nadu from each individual animal during the time of necropsy. Small intestine like duodenum, jejunum and ileum were collected having length approximately 60 cm. large intestine like caecum (whole), colon and rectum were collected.

Gross and histopathology

Samples were collected in container having 10% formal saline and were kept for 24 h for proper fixation. The fixed samples were dehydrated in ascending grades of alcohol, hardened, cleared, embedded in paraffin and sectioned at

5 μ m thicknesses for light microscopy and stained with haematoxylin and eosin (H&E) following standard histopathological procedures (Stevens and Wilson 1996).

Immunohistochemistry

Tissue sections taken in 3-amino propyltriethoxysilane (Sigma-Aldrich, USA) coated slides were pretreated with proteolytic enzyme (protease) for 15 min prior to immunostaining. Further, incubated with 1:50 dilution of monoclonal rabbit antivimentin (Vimentin (EP21), Pathn Situ, USA) antibody to detect the extent of mesenchymal tissue of mesodermal origin in the nodules and visualized using horseradish peroxidase labeled anti-rabbit antibody and diaminobenzidine as chromogen. Tissues were counterstained with Harry's hematoxylin.

Isolation of strongyle eggs

The strongyle eggs from faecal samples were isolated using saturated salt solution as per the method of Mochizuki et al. (2006).

Fig. 2 **a** Sheep-Ileum-HP-Oesophagostomosis-Nodule with worms (arrows) H&E, $\times 40$. **b** Sheep-Ileum-HP-Oesophagostomosis-Cross section of worm showing eosinophilic platymyarian muscle cells (arrow), intestine and outer cuticle H&E, $\times 400$. **c** Sheep-Ileum-HP-Oesophagostomosis-Cross section of worm with oesophagus (arrow) H&E, $\times 100$. **d** Sheep-Ileum-HP-Oesophagostomosis-Cross section of worm (arrow) H&E, $\times 400$

DNA extraction

The genomic DNA from the strongyle eggs were extracted by following the stool mini kit protocol using QIAamp stool Mini Kit (Qiagen, USA) and the genomic DNA from the adult parasites were extracted by following the tissue protocol using QIAamp DNA Mini Kit (Qiagen, USA). The extracted DNA samples were stored at -20°C until they were analyzed.

Amplification by PCR

The ITS2 region of rDNA sequence of *O. columbianum* and *O. venulosum* were amplified using species specific primers viz., OCspF (5'-CAT TGC AAC ATG CAC TAT GG-3') and OCspR (5'-ACA GTT GTC ATA CAG GCC CC-3') to amplify the partial ITS-2 for *O. columbianum* egg and pair of primers OVspF (5'-CGT GTG TGT GAT CCT CGT TC-3') and OVspR (5'-CGG TTG TCT CAT TTC ACA GG-3') to amplify partial ITS-2 for *O. venulosum* egg in 20 μL reaction volumes using 10 μL EmeraldAmp GT PCR Master Mix (Takara, Japan), 2 μL DNA

template, 0.5 μL (10 pmol/ μL) forward primer and 0.5 μL (10 pmol/ μL) reverse primer and 7 μL nuclease free water (NFW) (Bandyopadhyay et al. 2009). DNA for both was amplified with an initial denaturation step of 10 min at 94°C , 35 cycles at 94°C for 1 min, annealing temperature of 50°C for 1 min and 72°C for 1.5 min and a final elongation step for 10 min at 72°C . The expected product size of amplicons was 113 and 182 bp for *O. columbianum* and *O. venulosum* respectively.

Results and discussion

In this study, the overall prevalence of GI parasites was 52.00%. Among the positive samples, single infection was found more (53.84%) than the mixed infection (46.15%). Out of which, oesophagostomosis was 17.00% as single infection similar to the report of Soudararajan (2001) in sheep at the Nilgiris hills of Tamil Nadu.

Gross lesions in the small and large intestine of sheep due to oesophagostomosis showed many small to large, round to irregular, hard, fibrotic, raised nodules of $> 5\text{ mm}$

Fig. 3 **a** Sheep-Ileum-HP-Oesophagostomosis-Heavy infiltration of eosinophils, plasma cells and lymphocytes H&E, $\times 400$. **b** Sheep-Ileum-HP-Oesophagostomosis-Nodule-Central caseous eosinophilic necrotic areas H&E, $\times 20$. **c** Sheep-Ileum-HP-Oesophagostomosis-Central necrotic area surrounded by inflammatory cells, a multinucleated giant cell H&E, $\times 40$. **d** Sheep-Ileum-HP-Oesophagostomosis-Multinucleated giant cells (arrow) H&E, $\times 400$

diameter. The wall of the large intestine was greatly thickened and oedematous. Nodular masses are scattered throughout the large intestine. The intestinal mucosa was showing congestion of the mucosal surface around the nodules (Fig. 1a, b). These gross lesions observed in this study are in accordance to that of Shelton and Griffit (1967).

The ileum of sheep infected with oesophagostomosis showed parasitic nodules with central baso-eosinophilic necrotic area, larval stages in the necrotic area, surrounded by layers of inflammatory cells and finally encapsulated in a thick fibrous connective tissue capsule (Fig. 1c, d). The cross section of worms showed eosinophilic platymyarian muscle cells, intestine and outer cuticle, oesophagus, excretory duct and large basophilic triangular uterus with severe infiltration of eosinophils, plasma cells and lymphocytes; a few inflammatory cells infiltrated even into the glands (Fig. 2a–d). These findings are in agreement with Shelton and Griffit (1967).

Multinucleated giant cells were found in the parasitic nodules. The nuclei were multiple and scattered within cytoplasm or on one side or a row or cluster.

Multinucleated giant cell was arranged as a row in the necrotic area (Fig. 3a–d). Parasitic nodule was stained with vimentin, a special stain for the connective tissue showed presence of golden brown cytoplasm with basophilic spindle shaped fibroblast nucleus (Fig. 4a). The central necrosed area was calcified and was basophilic.

Ileum of sheep showed presence of multiple parasitic eggs embedded in the mucosal layer which were having thin eosinophilic egg shell membrane and basophilic embryonic material (Fig. 4b). Muscular degeneration was observed in the tissue having parasitic nodule (Fig. 4c). Caecum of sheep showed necrosis of villi with extensive haemorrhages in the submucosal layer, the extravasated RBCs pushed the submucosal layer upwards with congestion and infiltration of lymphocytes (Fig. 4d). These lesions are in accordance to that of Shelton and Griffit (1967).

All the samples tested by PCR for *O. columbianum* and *O. venulosum* were found positive and amplified targeted ITS2 region with the product size of 113 and 182 bp respectively (Fig. 5). These results are in accordance to that of Bandyopadhyay et al. (2009).

Fig. 4 **a** Sheep-colon-Immunohistochemistry (IHC)-Vimentin-Oesophagostomosis-Intense cytoplasmic positive signals DAB brown, $\times 100$. **b** Sheep-Ileum-HP-Oesophagostomosis-Parasitic eggs embedded in the mucosal layer, H&E, $\times 400$. **c** Sheep-Ileum-HP-Oesophagostomosis-Muscular degeneration H&E, $\times 100$. **d** Goat-Caecum-HP-Extensive haemorrhage in the submucosal layer H&E, $\times 40$

Fig. 5 **a** Agarose gel electrophoresis of the PCR products obtained from *O. columbianum* and *O. venulosum* amplified using species specific ITS2 primer. L-DNA marker (100 bp), 1,2-Egg and worm of *O. columbianum* and 3-Egg of *O. venulosum*

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Author's contribution AC. Satish: Conduct of research work for his M.V.Sc thesis. Literature collection, typing and manuscript preparation; K. Nagarajan: Chairman for guidance and assistance in research, correction and submission of manuscript; C. Balachandran: Advisory member for guidance and assistance in research, histopathological examination and correction of manuscript; C. Soundararajan: Advisory member for guidance and assistance in research, identification of parasites and correction of manuscript; R. Legadevi: Assisted in molecular work.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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