

Gastrointestinal cooperiasis: A mini review on an incidence of unusual malady of *Cooperia pectinata* (Ransom, 1907) in a calf from Tamil Nadu

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Received: 17.2.26; Accepted: 13.3.26

ABSTRACT

A review on cooperiasis in cattle caused by *Cooperia pectinata* was described based on the authentic episode of the malady in a calf. A four-month-old Jersey cross-bred calf was presented for necropsy with the clinical history of inappetence, anaemia, diarrhea, weakness and death. Systematic post-mortem examination was conducted. Externally, carcass was thin with blanched mucous membranes. Internally, edematous fluid accumulated in pericardial sac, thoracic and abdominal cavities. Proximal small intestine revealed scattered nodules. Lumen of the distal abomasum and duodenum contained pale viscous contents and numerous pale yellowish-brown, hairy nematodes. Histologically, small intestine revealed acute catarrhal enteritis. Mucosal villi were blunt, fused and showed scattered areas of epithelial necrosis. Muco-serosal nodules revealed necrotic cell debris, throngs of neutrophils, eosinophils and few macrophages encircling the dead and degenerating nematodes. Nodules were thought to be hypobiosis of parasitic larval (L4&L5) stages. Muco-serosal nodules uphold the penetrating nature of the parasite and explained the host cell-mediated immune response. Morphologically, the parasites were identified as *Cooperia pectinata*. Adult male was identified with its morphology of spicules, structure and arrangement of bursal rays. Adult female was identified with its morphology of vagina and ovijector. The penetrating nature of the *C. pectinata* was responsible of intestinal pathology which in turn caused acute malady characterized by anaemia, diarrhea, loss of body weight gain, emaciation and death. The paper elaborates the pathology and pathogenesis of *C. pectinata* in cattle and records the incidence in southern India *at-long-last* documented evidence after 1940 from Madras state.

Keywords: Calf, *Cooperia pectinata*, Enteritis, Morphology, Pathology, Review

INTRODUCTION

Gastrointestinal nematodiasis is an economically significant parasitism in ruminants¹. Among them, trichostrongylidae parasites are weighty and genus *Cooperia* spp. is reported to cause substantial reduction in production in domestic ruminants^{2,3}. Among 19 valid species of *Cooperia* in ruminants, four species such as *C. oncophora*, *C. pectinata*, *C. punctata* and *C. spatulata* are reported to occur in cattle^{4,5}. All four are lumen dwelling nematodes of small intestine. These parasites have pre-parasitic, free-living (L1-L3) and parasitic larval stages (L4-L5) and mature adult stage in its life cycle⁶. *C. pectinata* and *C. punctata* are found to be more pathogenic as its developmental larval stages penetrates deeper into the intestinal mucosa and cause destruction of mucosal epithelial cells, formation of muco-serosal nodules and catarrhal enteritis^{7,8}. Species of *Cooperia* are differentiated by its morphology of spicules in male and vulva of females^{5,9}. Advances in molecular diagnostic techniques are though unambiguous, traditional morphological features are equivalently prompt in identifying species of *Cooperia* in ruminants as a host-specific parasite^{4,5}. *C. pectinata* are found to occur in tropical and subtropical countries¹⁰. Though the occurrence is rare in India, prevalence is documented by copromorphology from Northern India^{1,11,12} howbeit, it is hardly ever reported in Southern India more specifically from Tamil Nadu¹³. Hence, this paper documents the first descriptive report on occurrence, morphology and

How to cite this article : Prasath, N.B., Selvaraj, J., Saravanan, M., Velusamy, R. and Latchumikanthan, A. 2026. Gastrointestinal cooperiasis: A mini review on an incidence of unusual malady of *Cooperia pectinata* (Ransom, 1907) in a calf from Tamil Nadu. . Indian J. Vet. Pathol., 50(3) : 204-213.

pathology of *Cooperia pectinata* in a calf from Southern India (District Thanjavur, Tamil Nadu). Further, the paper reviews the morphology, incidence, pathology and treatment of *C. pectinata* in cattle based on the earlier documents and discusses them in context to the present report in calves.

MATERIALS AND METHODS

A four-month-old Jersey cross-bred calf was presented to Veterinary Clinical Complex, Veterinary College and Research Institute (VCRI), Orathanadu, Thanjavur, Tamil Nadu, India with the history of weakness, inappetence and hypothermia. Animal had cream-like diarrhea since one week. Animal was dull and showed pale conjunctiva indicating anaemia on clinical examination. First-line of treatment was initiated for anaemia and diarrhea. Animal collapsed and died on the immediate next day in spite of treatment regimen. The carcass was presented to the Department of Veterinary Pathology, VCRI, Orathanadu for necropsy. Systematic postmortem examination was carried out. Representative tissue samples were collected in 10% neutral buffered formalin for histopathology. Tissues were processed as per standard paraffin embedding technique and tissue sections of 3-5 μ m thickness were prepared using manual rotatory microtome (Leica) and were subjected to Haematoxylin-Eosin (H&E) staining protocol¹⁴. The duplicate sections were subjected to Alcian blue-PAS staining¹⁵. Blood smears were prepared from peripheral blood and heart blood for cytological examination. Blood smears were air-dried and stained with Leishman-Giemsa cocktail stain. Worms collected from abomasum and small intestine were preserved in normal saline for

morphological identification. Worms were cleared in lactophenol and morphological parameters were studied under zoom stereomicroscope (Nikon) and measured with a calibrated micrometer eyepiece (10x).

RESULTS

Externally, carcass was thin (Fig. 1a) and visible mucous membranes were pale (Fig. 1b). Internally, subcutaneous adipose tissue was meagre. Prescapular lymph nodes were slightly enlarged (Fig. 1b). Pericardial sac contained about 50 ml pale-yellowish, thin and clear transudate (Fig. 2a). Epicardial fat was gelatinized. Epicardial vessels were accentuated and engorged with blood. Both the lungs were pale, slightly enlarged and edematous. Liver was slightly enlarged with swollen hepatic lymph nodes. Gall bladder was distended with about 200 ml greenish, thin bile. Abomasal serosa was severely congested (Fig. 2b) and mucosa was slightly congested and on the surface of it showed few brownish, tiny, thread-like live round worms (5-14 mm length). Mesentery was severely congested at its attached border. Mesenteric lymph nodes were slightly enlarged and oedematous (Fig. 2c). The serosa of the small intestine revealed many cream-coloured nodules of 5-10 mm diameter scattered in duodenum and proximal jejunum (Fig. 3a&b). Small intestinal lumen contained grayish

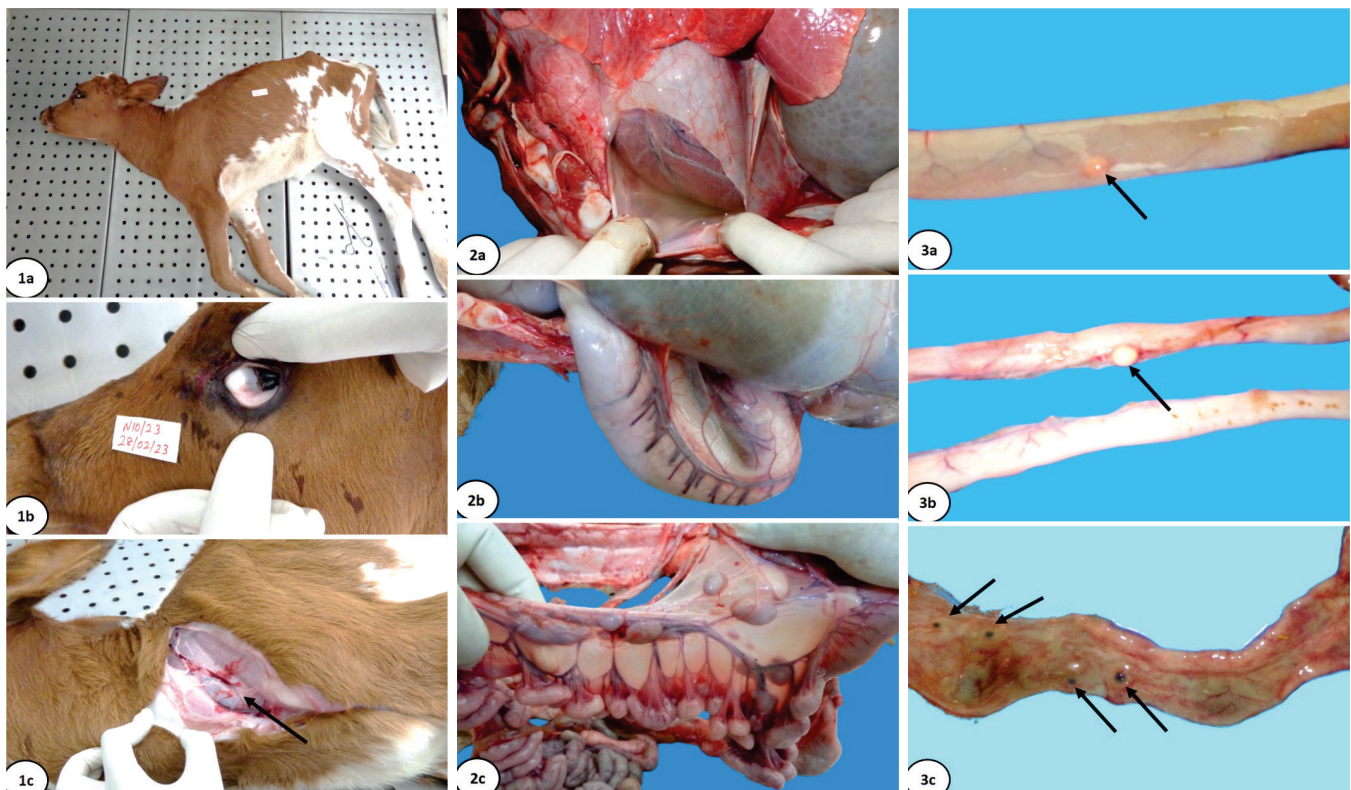
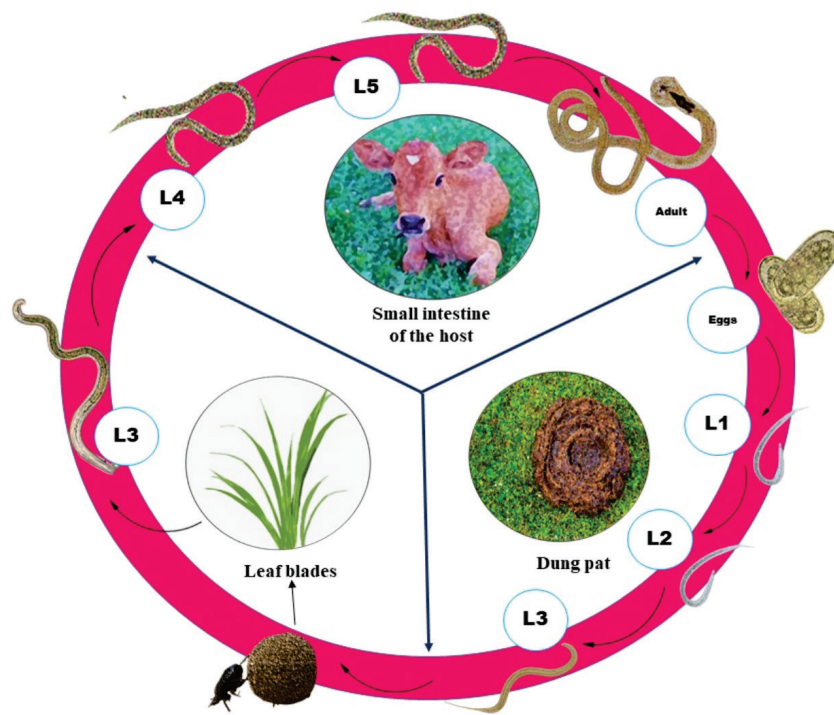


Fig. 1a-c. Thin emaciated carcass – Jersey-cross calf; Pale conjunctiva; Enlarged prescapular lymph node; **Fig. 2 a-c.** Hydropericardium; congestion of serosa of abomasum; Enlarged mesenteric lymph nodes; **Fig. 3 a-c.** Grayish-white coloured nodule in the serosa of duodenum (3a) and jejunum (3b); Blackish-brown nodules in the mucosa of the small intestine (3c).



Life cycle of *Cooperia pectinata*. Adult female lays fertilized eggs in intestine which are excreted in dung. Egg hatch into L1 and transform by two moults into L2 and infective L3 in dung pat. Dung beetles disseminate eggs/infective L3 to distant places. L3 is double-sheathed infective stage, it moves to leaf blades and hibernate until ingested by cattle. Once it reaches in small intestine L3 exsheaths and moult to transform into L4 and L5. L5 matures into adult dimorphic *Cooperia pectinata* as male and female worms. Occasionally, under unfavourable environment inside the host, L4/L5 undergoes hypobiosis. Host cell-mediated immune response either disintegrate the hypobiotic larva or else it develop into adult worm once favourable conditions exist in intestine

white pasty contents smearing the mucosal surface. Mucosa was slightly congested. Mucosa of duodenum, jejunum and ileum showed multiple blackish brown slightly firm nodules of 1-5 mm

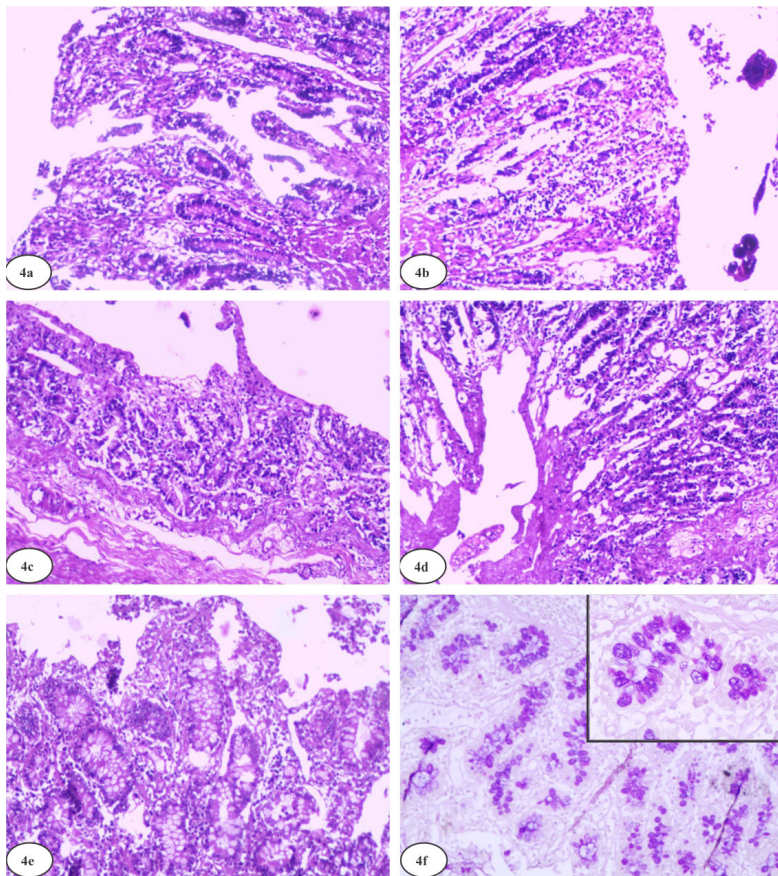


Fig. 4a&b. Widening of villi with inflammatory cell infiltration – Small intestine H&E x100; **Fig. 4c&d.** Atrophy and fusion of mucosal villi – Small intestine H&E x100; **Fig. 4e.** Goblet cell hyperplasia – Small intestine H&E x100; **Fig. 4f.** Goblet cell hyperplasia – Small intestine PAS stain x100 (inset – 400x).

diameter (Fig. 3c). Luminal surface showed multiple, brownish, tiny, thread-like live round worms (5-14 mm length). Skeletal muscles were pale and slightly dry. Meningeal vessels were slightly congested.

Microscopically, duodenum and jejunum revealed multiple cut-sections of nematodes within the lumen. Mucosa showed subacute enteritis characterized by widening of villi with infiltration of neutrophils, eosinophils, lymphocytes and a few plasma cells (Fig. 4a&b). Mucosa also showed blunting (Fig. 4c), fusion and atrophy of villi (Fig. 4d). The small intestinal mucosa showed increased goblet cell activity (Fig. 4e). Hyperplasia of goblet cells in the mucosa under combined alcian blue-PAS technique revealed magenta coloured glycoproteins (Fig. 4f) suggestive of neural mucins. Nodular portions of serosa showed necrotic debris with cut section of nematode (Fig. 5a). Nodular portions of mucosa revealed throngs of eosinophils and a few neutrophils surrounding the nematode. Necrotic debris composed of dead cells, neutrophils, lymphocytes, plasma cells, globular leucocytes, macrophages and scattered eosinophils surrounding the disintegrated nematode section (Fig. 5b). Microscopically, the cut-section of nematode within the intestinal lumen showed cuticle with struts and around ten large cuticular

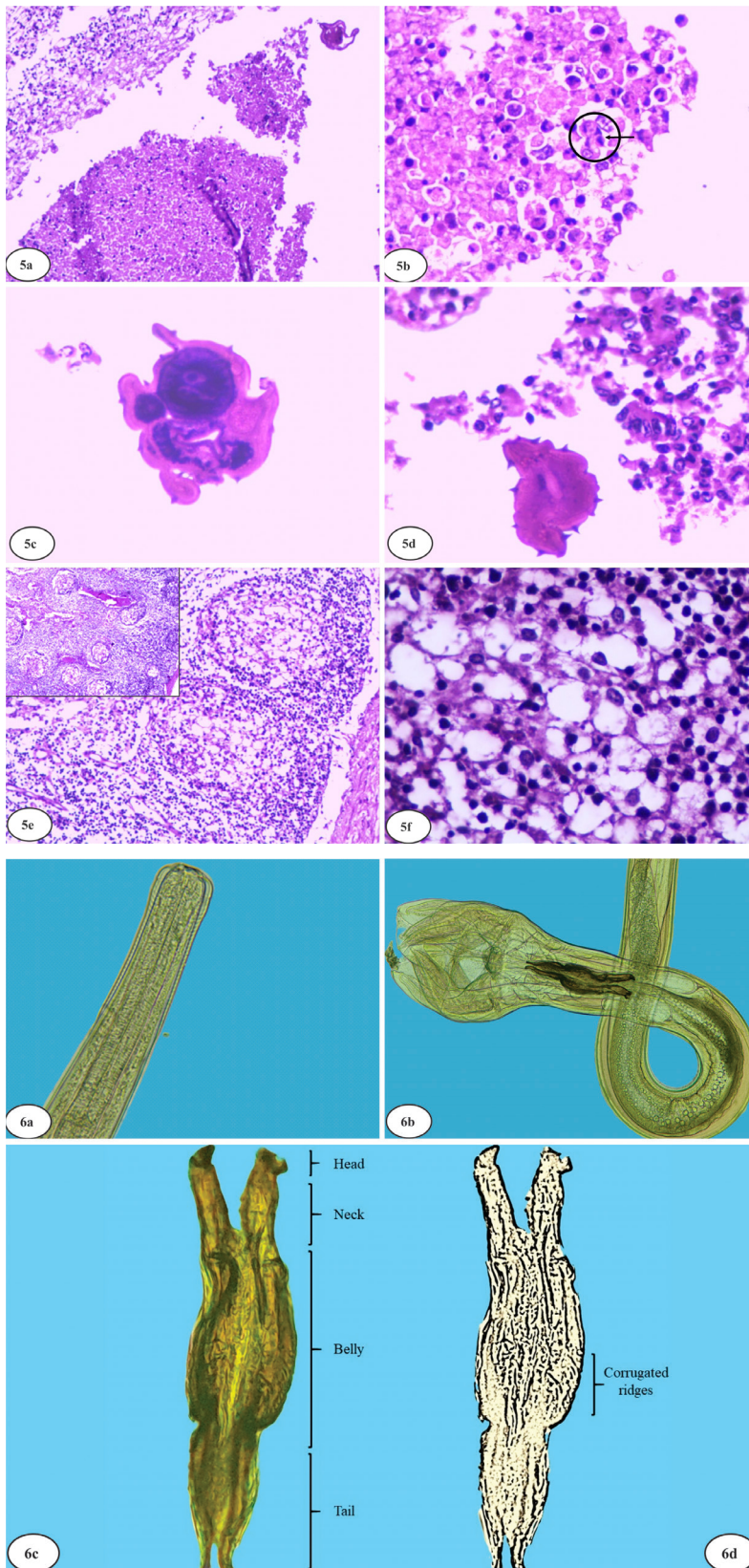


Fig. 6a. Cephalic end of male *Cooperia pectinata* x1000; **Fig. 6b.** Tail end of male *Cooperia pectinata* x1000; **Fig. 6c.** (Right): Bukly spicule of male *Cooperia pectinata* x1000; **Fig. 6d.** (Left): Sketch of edited original picture to show spicule of male *Cooperia pectinata* with well-demarcated corrugated ridges in the belly portion x1000.

Fig. 5a. Necrosis with cut section of nematode – Serosal nodule – Small intestine H&E x100; **Fig. 5b.** Necrotic debris with throng of inflammatory cells around disintegrated nematode – Serosal nodule – Small intestine H&E x400; **Fig. 5c.** Cut-section of nematode within the intestinal lumen showing cuticle with struts and internal oesophagus H&E x400; **Fig. 5d.** Cuticular ridges showing prominent ten ridges H&E x400; **Fig. 5e.** Moderate lymphoid depletion and lymphocytolysis in prescapular and mesenteric lymph nodes H&E x100 (Inset H&E x40); **Fig. 5f.** Lymphocytolysis in mesenteric lymph node H&E x400.

ridges and internal oesophagus (Fig. 5c). Cuticular ridges were obvious in H&E section showing prominent five dorsal, five ventral and inconspicuous lateral ridges (Fig. 5d). Mesenteric lymph nodes revealed moderate lymphocytolysis whereas severe lymphoid depletion was observed in the peripheral lymph nodes (Fig. 5e&f) and hepatic lymph nodes. Liver revealed moderate vacuolar degeneration of hepatocytes.

Nematodes collected from abomasum and duodenum were identified based on the following morphological features. Head had cephalic inconspicuous cuticular swelling (Fig. 6a). Body had longitudinal cuticular striations (ridges). Male worms appeared coiled and had well-developed copulatory bursa with two lateral expansions (Fig. 6b). Copulatory bursa was symmetrical and composed of paired spicules, bursal rays and genital cone. Spicules were two, large, ornate, thick, sclerotized, stout and pigmented brown with wing-like expansion (ventral flange) at the middle having sweeping curves. It measured 340-350 µm. Spicules had short anterior head, a barrel neck, bulky belly, and a thin tail (Fig. 6c). Belly of the spicules had well-demarcated corrugated ridges (Fig. 6d). The tail was longer and occupied one-third of the total spicule length. Each spicule had a clear, thin membrane clenched to its tip. Bursal rays composed of ventral, lateral and dorsal rays. Lateral and ventral rays followed the pattern of the genus *Cooperia* spp. Anterio-ventral and postero-ventral rays were obviously placed lateral to the bursal lobe whereas, anterio-lateral, medio-lateral and postero-lateral rays pointed distally. The tip of the anterio-lateral rays was slightly bent and curved laterally. Dorsal rays were lyre-shaped and measured 180-190 µm. These dorsal rays were double-branched fork-like and its bifurcation was less than half of its total length. Genital cone was butterfly-

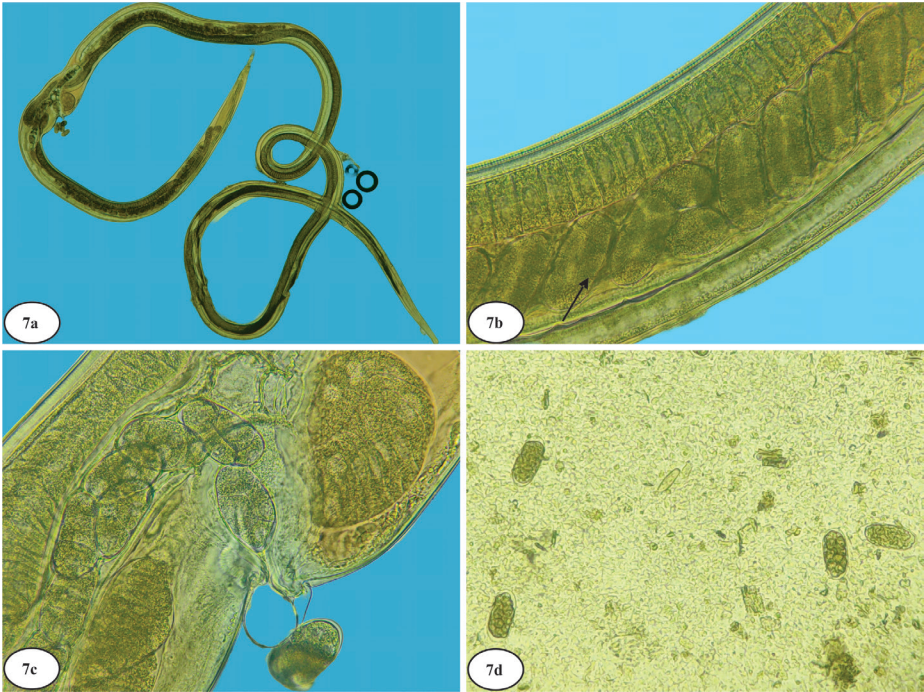


Fig. 7a. Coiled entire female, *Cooperia pectinata* x100; **Fig. 7b.** Uterus with parallel eggs of female *Cooperia pectinata* x1000; **Fig. 7c.** Vulva and ovijector of adult female *Cooperia pectinata* x1000; **Fig. 7d.** Eggs of *Cooperia pectinata* x1000

shaped situated in the middle of the bursa, a region behind the spicule. The adult females (Fig. 7a) measured 8.68-12.54 mm long and its widest part measured 200-250 mm just behind the vulva. Vagina was made of heavily cuticularized walls and vulva. Vulva had a transverse slit with prominent distinctive cuticular lips and ovijector (Fig. 7c). Ovijector was a well-developed vesicular-like structure which was distinct and stood out sharply from its main body wall. The tail end was narrow with pointed tip. Eggs were thin-shelled, cylindrical with rounded edges and parallel walls (Fig. 7d). Eggs had divided multiple yolks. These morphological features were consistent with *Cooperia pectinata*.

Cytological examination of blood smear revealed hypochromasia of erythrocytes, numerous Howell-Jolly bodies and stray *Anaplasma* spp.

DISCUSSION

Genus *Cooperia*

Nematodes of Tricho-strongylidae are gastrointestinal parasitic

roundworms notably important in veterinary science. The family trichostrongylidae enclose the nematodes of genus *Trichostrongylus* spp., *Haemonchus* spp., *Ostertagia* spp., *Nematodirus* spp., *Cooperia* spp., *Mecistocirrus* spp. and *Hyostongylus* spp. The genus *Trichostrongylus*, *Haemonchus*, *Ostertagia* reside in abomasum whereas genus *Cooperia* and *Nematodirus* reside in the anterior part of the small intestine (duodenum and jejunum). Among all, the genus *Haemonchus contortus* was found to be a highly pathogenic blood sucking nematode with high morbidity and mortality due to severe anaemia in ruminants¹⁶.

The genus *Cooperia* contains thirty-four accepted species⁴ of which, 19 valid species are of veterinary importance that parasitize gastro-intestine of both domestic and wild ruminants⁵. Four species such as *C. oncophora*, *C. pectinata*, *C. punctata* and *C. spatulata* are found to parasitize cattle⁴.

Cooperia spp. are gastro-intestinal lumen-dwelling parasites of ruminants, recovered from abomasum and small intestine but frequently from upper third of small intestine to be more precise. *Cooperia pectinata* and *C. punctata* are reported to be pathogenic whereas *C. oncophora* and *C. spatulata* are non-pathogenic¹⁷. The reason

Table 1. Average measurement of L4 and L5 of *Cooperia pectinata* (Anderson, 1986)

Sl. No.	Measurement parameters	L4		L5	
		Male	Female	Male	Female
1.	Total length (mm)	3.1-4.1	3.8-6.0	4.0-5.8	8.4-9.0
2.	Length of oesophagus (µm)	320-380	340-390	410	420
3.	Distance between excretory pore to anterior end (µm)	280-360	300-380	390	
4.	Distance between vulval opening to tail (µm)	-	610-970	-	1110
5.	Length of spicules (µm)	170-190	-	240-283	-
6.	Distance between anus to tail (µm)	-	120-170	-	180

thereof, former being the nematodes which burrow into the intestinal mucosa, but latter remain on the mucosal surface². Nematodiasis by *C. pectinata* and *C. punctata* caused decreased feed intake and enteritis and an out-turn, decreased live weight gain, growth rate and mortalities when slew of *Cooperia* parasitized in younger animals such as calves^{3,17}.

Distribution

Cooperia spp. are distributed world-wide and its abundance is influenced by climatic conditions particularly rainfall and temperature. *C. oncophora* is abundant in temperate countries like Europe and Australia whereas *C. pectinata*, *C. punctata* and *C. spatulata* are prevalent in tropical and subtropical regions of the world⁴. Although it was not new, the occurrence of *Cooperia* spp. was rarely reported from Northern India and particularly scarce from southern India. In the distant past, the occurrence of *C. pectinata* and *C. punctata* from cattle in Madras was reported by Rao during the year 1940¹³. This was thought to be the first documented evidence on cooperiasis in ruminants. Later in the same decade down-the-road, these parasites were found to be the commonest trichostrongylid nematodes of cattle in the Kumaon hills of Uttarakhand (previously in Uttar Pradesh)¹⁸. Thereafter, the incidence of *Cooperia* spp. in animals was occasionally documented from India. It was found to be 17.77% in Tho-Tho indigenous cattle and cross-bred cattle of Nagaland^{11,19}. The prevalence of *Cooperia* spp. was reported by coproculture in cattle (10.54%) and buffalo (0.92%) from Guwahati, Assam¹². The fragmentary evidences on occurrence of *Cooperia* spp. in India elicits deficient reporting. More recently, sampling survey in the Sirmaur district of Himachal Pradesh of Northern India revealed 30%, 35%, 60% and 40% prevalence of *Cooperia* spp. in cattle, buffalo, goat and sheep, respectively by copromicroscopy of fecal samples¹. Following the pause later 1940, the present reports records the occurrence of *C. pectinata* in calves in Thanjavur district, Tamil Nadu. This report registers the first descriptive evidence on morphology and pathology of *Cooperia pectinata* in calves.

Definitive host

The *C. pectinata* was found to hanger-on both domestic and wild ruminants. *Cooperia pectinata* are monoxenous parasite and even so, it was reported from cattle^{13,20}, sheep^{18,20} and red deer¹⁰.

Life cycle

Cooperia spp. are dimorphic parasite with direct life cycle having free-living pre-parasitic larval phases⁵. The mature female nematode in the small intestinal lumen lays thin-shelled eggs containing 16 cells yolk mass and voided in the host faeces or dung^{5,21}. Under optimal conditions, first stage larva (L1) hatch out in the dung pat. Molting continues through second stage (L2) larva

into infective third stage (L3) larva. Third stage larva was protected by two layers of cuticle (retention of cuticular sheath of second stage larva) and ardently climb up to grass blades to be taken up by the definitive host of ruminants²². The egg to L3 occurs in dung on pasture and its rate of development was temperature-dependent and approximately takes 1- to 6-weeks duration²³. L3 larva survives for up to one year until swallowed by a definitive host. Contaminated dung remaining on the surface of pastures serve as reservoir for parasitic larva (Fincher and Stewart, 1979). It was proved that *Cooperia* larva travelled up to the maximum of 25 cm from dung pat²⁴. The dung beetles were found to abet the dissemination of eggs or larva faraway by forming fecal balls which were rolled away from the dung pat²⁴.

Once in the gastro-intestinal tract, L3 larva exsheaths and undergoes further two molts as L4 and L5 to become mature adult of male and female worms^{25,26}. The average measurement of L4 and L5 have been described earlier by Anderson (1986)⁶ (Table 1). On an average, L4 male measures 3.1-4.1 mm and female measures 3.8-6.0 mm of total length whereas L5 male measures 4.0-5.8 mm and female measures 8.4-9.0 mm of total length²⁷. The cycle continues when the fertilized female starts to lays eggs. Nevertheless, under adverse conditions, a deviation in life cycle occurs where L4 or L5 undergoes developmental arrest (hypobiosis) in the intestinal mucosa for up to several months^{25,26}. The statement was in agreement with the present study where scattered mucosal and serosal nodules were noticed in the small intestine and histologically each nodule revealed inflammatory reaction around the cut section of the early adult nematode, probably L5. Larval migration in to the deep intestinal tissue observed in the present study was in accord with the findings of²⁵ who reported the *Cooperia* larva in the lamina propria of the intestine. After slumber, L5 develop into adult male and or female in the lumen of the gastro-intestinal tract.

Morphology and identification of *C. pectinata*

Adult parasite is identified by its morphological features of the male and female worms. Albeit a traditional diagnostic technique, it was found to be worthwhile for prompt identification from the former times as compared to recent newer advanced techniques. It was proposed that the most precise approach for morphological identification and species differentiation of adult worms of Trichostrongylidae immediate after necropsy were a) the morphology and morphometry of the spicule, b) arrangements of dorsal rays, c) morphology of genital cone and d) cephalic vesicle and e) the synlophe morphology (cuticular ridges)^{4,28}. Infrequently, the shape of the dorsal rays of bursa have also been used^{5,29}. Among pathogenic *Cooperia* spp., *C. pectinata* has been found to have larger and bulky spicule^{4,5}. The spicule measured

about 220-390 μm which was comparatively larger than spicule of *C. punctata* (125-214 μm). Spicule of *C. pectinata* was differentiated by the presence of prominent corrugated ventral flange in the middle third (bulky belly)^{4,20}. Adult male was 5.10 mm whereas adult female was 5.74 mm in length after an average measurement of 10 largest specimens in *in vitro* studies from calves³⁰. Previously, Herlich (1965)²⁷ registered that the average measurement (length) of *C. pectinata* recovered from calves were 7.22 mm and 9.48 mm for male and female, respectively. These statements suggested that *C. pectinata* was quite larger when compared to *C. punctata*⁴. Previous workers^{5,20,31} have documented that the cephalic vesicle at the anterior end of *Cooperia pectinata* was inconspicuous (with minimal expansion) when compared with broad (cephalic vesicle) of *C. punctata*. The same was convincible in the present study. Morphology of genital cone and dorsal rays observed in the present case was in accordance with Albrechtova and co-workers (2024)⁵.

Female worms are not used to differentiate species, yet morphology of female genitalia unquestionably enable to identify the genus of Trichostrongylidae and trivial details support to confirm the species of *Cooperia*³². Adult female of *C. pectinata* is distinguished based on the anatomy of the vagina. The vagina is skeletal with transversely elongated vulva and ovijector. Vulva is composed of two heavily cuticularized walls situated just within the vaginal opening⁹ and distinct posterior lip which stand out sharply from the main body²⁰. The prominent and projecting vesicular posterior lip is labeled as ovijector^{5,30}. Eggs of *C. pectinata* are ovoid to ellipsoid with nearly alike poles and straight and parallel sides. Eggs are thin-shelled surrounding the light brown cellular mass^{1,21}. The average width is 34-36 μm and the length is 75-79 μm ^{1,33}. Eggs with the character of parallelism specific for genus *Cooperia* spp. observed in the present study was analogous with the above earlier illustration. Shorb (1938)³³ documented that the *C. pectinata* egg measured 72.7 (67-80) μm in length, 34.5 (31-38) μm width and had shell thickness of 1.4 μm .

Furthermore, *Cooperia* spp. was identified with supplementary features particularly the distribution and arrangement of longitudinal ridges present in the cuticle³⁴. *Cooperia* spp. composed of 10 large ridges and four small ridges. Cuticular ridges were distributed as five large dorsal ridges, five large ventral ridges and short and small, a pair of lateral ridges on each side. *C. pectinata* was found to have closed pattern of cuticular ridge arrangement at the cervical region exhibiting small lateral ridges anterior to the level of excretory pore and cervical papilla^{6,20}. The distribution of struts and cuticular ridge arrangement observed in the present investigation by histo-morphology of nematodes in intestine was in parallel with the above earlier description. The

cuticular longitudinal ridge parameter also served as a supporting characteristic alongside the male spicule and female vulval traits of *C. pectinata*. The male and female morphology featured in the present study assured that the Trichostrongylidae worm was *Cooperia pectinata* keeping unwavering faith with the above earlier documented evidences.

Pathology

It was suggested that *C. pectinata* resides both in abomasum and small intestine, but latter being the most common predilection site⁷. Adults penetrate and induce substantial damages in the mucosa. Massive worm load caused severe catarrhal enteritis which hampered growth rate and appetite in calves²⁵. Histopathological observation of the present study coincides with the earlier reports^{2,8,17,25}. The adult nematodes within the lumen and deep into the mucosa produces the tissue alterations. Alteration in the mucosal barrier causes protein-losing enteropathy and catarrhal enteritis characterized by mucosal thickening, hyperplasia of goblet cells, broadening and fusion of mucosal villi, denudation of mucosal epithelial cells are found to be consistent feature of Cooperiasis^{8,25} and were in parallel with the present report in a calf. Vascular congestion, marked infiltration of eosinophils, neutrophils, lymphocytes and plasma cells, appearance of globular leucocytes in the mucosa authenticate the cellular immune response of the host⁸. Depletion of lymphocytes and lymphocytolysis of lymph nodes and goblet cell hyperplasia of the small intestine observed in the present investigation testament the cellular and innate immune reaction towards the worm. The statement was in accordance with Stromberg and co-workers (2011)² who reported mesenteric lymphadenomegaly in *Cooperia punctata* infected calves.

Pathology induced by *C. pectinata* was found to be alarming in cattle especially in calves by two ways 1) it penetrates the mucosal epithelium of small intestine and 2) influence phosphorus kinetics and reduce phosphorus absorption and retention. Penetration into the intestinal epithelium disturbs the mucosal barrier by inducing defect in epithelial integrity, damage to the epithelial cells, mucosal irritation due to the movement and secretory products of parasite and provoke immune mediated damage to intestinal stromal cells^{4,8,35}. These above reactions cause severe catarrhal enteritis, promote gastrointestinal fluid loss with subsequent malabsorption of micronutrients and resulted in loss of weight gain. Disturbance in phosphorus uptake leads to hypophosphatemia. Emaciation with extreme wasting of skeletal musculature, diarrhoea, hypoproteinaemia, subcutaneous oedema (mostly submandibular), anaemia and death are the tandem effects and outcome of *C. pectinata* infection in ruminants^{2,3,35,36}. The above mechanism was plausible with the present investigation

regarding the clinico-pathological changes observed in a calf infected with *C. pectinata*. Pale conjunctival mucous membrane, hypochromasia of erythrocytes and appearance of Howell-Jolly bodies in peripheral blood smear in the present study supports that the animal suffered with anaemia. Besides, hydropericardium, goblet cell hyperplasia of proximal small intestine corroborated with protein-losing enteropathy (hypoproteinaemia), catarrhal enteritis and emaciation in the present investigation. To sum up, the proclivity of the *Cooperia pectinata* to the host was the reason for pathological changes observed in the current study. Altogether, *Cooperia pectinata* was the etiological agent responsible for the mortality in a calf in the present study.

Immunity

As was foreseeable, parasites avoid host defense to establish themselves in the intestine. It has been proposed that the Trichostrongylidae nematodes produce endogenous allergen, the aspartyl inhibitor (API-1). These allergens are presented to T-helper cell (CD4+) by antigen presenting cells followed by initiation of T-2 response by the host by releasing interleukins (IL) such as IL4, 5, 9, 13, 21, 33 and proliferation of plasma cells. The ILs activate mast cells and eosinophils to degrade the parasite³⁵. Activation of plasma cells produce allergen-specific immunoglobulin E (IgE) which cause mast cell degranulation, activate eosinophils by binding with Fab (fragment antigen binding) region and immobilize and kill the parasite by binding with its Fc (Fragment crystallizable) region of IgE. Apart from the above, the damaged intestinal epithelial cells release IL33, which directly bind with ST2 receptors of eosinophils and mast cells to release major basic protein, free-radicals and vasoactive amines, respectively^{35,37}. The amines facilitate recruitment of defense cells from vasculature and eliminate the parasite^{8,35}. The appearance of tissue necrosis together with infiltration of eosinophils, neutrophils, lymphocytes, macrophages around the degraded nematode in the intestinal nodule substantiates the solid immune response to eliminate the nematode. Probably, the above elaborated mechanism postulated earlier was conceivable with the nodular lesions described in the present study.

Role of molecular diagnosis

Recent advances in molecular techniques showed keen interest to deepen the knowledge on species differentiation with irrefutable confirmation. In a recent study at Germany, *Cooperia* species has been subjected to genome sequence comparison. Partial sequence analysis of two mitochondrial genes, cytochrome oxidase 2 (*cox2*) and 12S rRNA and two nuclear genes, isotype 1 β tubulin and internal transcribed spacers (ITS-2) of four *Cooperia* species viz., *C. pectinata*, *C. punctata*, *C. spatulata* and *C. oncophora* have been studied. Multi-locus phylogenetic

analysis conducted by keeping *Haemonchus contortus* as out group for all four *Cooperia* species infecting cattle revealed separate cluster of *C. pectinata* which was located at a more basal position where as *C. oncophora* occupied sister position to the cluster containing *C. punctata* and *C. spatulata*. Further *C. punctata* and *C. spatulata* did not cluster with each other⁴. The analysis concluded that species identified based on phylogenetic analysis revealed no differences on par with morphological differentiation. In contrary, phylogenetic analysis of *cox2* gene and the ITS1-5, 8S-ITS2 of *Cooperia* species that were morphologically identified as *C. pectinata* in red deer and sika deer revealed the presence of distinct independent lineage forming a sister clade to *C. oncophora*. These above results indicated that the parasite does not belong to the *C. pectinata* parasitizing cattle and was therefore renamed as *Cooperia ventricosa*⁵. Even though, morphological similarities were observed with a few structural deviations, the molecular studies concluded that *Cooperia pectinata* was a monoxenous parasite typically found to occur in cattle⁵. Long ago, in 1981 a report of *C. pectinata* recorded from red deer in New Zealand¹⁰ was probably the *C. ventricosa*⁵. What-so-ever, morphological features of *Cooperia* spp. are still found to be the standard rather traditional method with certainty to differentiate *Cooperia* species affecting cattle when molecular studies were unachievable. This study exemplar to be one in such circumstances.

Anthelmintic resistance

An extensive usage of macrocyclic lactone for the treatment and or to control gastro-intestinal nematodiasis contributed to drug resistance in recent years especially in ruminants³⁸. Further, the repercussions of climate change and life cycle traits (pre-adult, adult, hypobiosis and the female fecundity) of the parasites added-up the resistance to thrive in the host³⁹. The impact of these might change the parasitic disease pattern in the coming decades and hamper health, welfare and productive economy of ruminants. To avoid parasitic resilience, polyphenolic compounds have been used to establish effective control strategies⁴⁰. Polyphenolic compounds such as quercetin, caffeic acid, rutin and coumarin have been used in combinations as bioactive (caffeic acid and coumarin) with non-bioactive (quercetin and rutin) molecules in the 8:2 ratio mixture. It has been proved that these combinations exhibited synergistic activity with ovicidal property and inhibition of exsheathment of L3 of *C. punctata*. It has been suggested that the hydroxylation of these above polyphenolic compounds resulted in cuticular damage and thereby exerted blockage of exsheathment of infective L3 larva⁴⁰. More recently, von San-de Fernex *et al.*, (2023)³¹ studied the anthelmintic activity of above two polyphenolic combinations against larval and adult stages of *Cooperia punctata* by *in vitro*

studies. It was postulated that dual combinations such as 1) coumarin:quercetin and 2) caffeic acid:rutin were found to affect motility of both infective larvae and adult worms. It was concluded that the above two polyphenolic combinations damaged the collagen and muscular layer of adult worms and caused muscular contraction, cuticular collapse, loss of turgor pressure and cellular damage³¹. Similar polyphenolic formulations could be used with different dilutions and could be combined with other regional herbs for effective control of sturdy parasites such as *C. pectinata* and other gastro-intestinal nematodes to avoid drug resistance.

CONCLUSIONS

The *Trichostrongylidae* nematode, *Cooperia pectinata* is a pathogenic parasite in cattle especially for calves. The parasitic larval stages are much more pathogenic as it penetrates deep into the mucosa forming mucoserosal nodules (hypobiosis) and destruction of mucosal epithelial cells. The change in the intestinal phosphorus kinetics and increased luminal plasma loss lead to diarrhea, hypoproteinaemia, anaemia, emaciation and death. The paper documents the first descriptive evidence on occurrence and pathology of *C. pectinata* in calves from southern India along with a detailed review on the genus *Cooperia*.

ACKNOWLEDGEMENTS

The authors thank the Dean, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamil Nadu and Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai-51, Tamil Nadu, India for the facilities.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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