Pathological observation of incidental Spirocerca lupi infection with associated spontaneous cellulitis in a mongrel dog

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Abstract
Spirocerca lupi, a spirurid nematode responsible for causing tumorous growth in splanchnic organs of canine is eventually incriminated for neoplastic transformation. The present case noted in an eleven month old mongrel dog with oesophageal nodular growth having neoplastic transformation following S. lupi infection was histologically elaborated with possible pathologic mechanism. An additional occult clinical entity i.e. spontaneous cellulitis, widely seen across the two sides of thoracic ribs was discussed. This report further entails special description of parasite involved and pathology noted in spontaneous cellutis due to unknown reason (aetiology) and illustrated distantly located pyogranulomatous lesions in kidney.

Keywords: Spirocerosis, fibroma/osteoma, spontaneous cellulitis, mongrel dog

Introduction
Spirocerosis in canine species is mainly associated with tumours and are more commonly seen in dogs and wild carnivores. The infection is predominantly seen in tropical to sub-tropical climate. This parasitic infection arises following ingestion of infective larval stage of the parasite Spirocerca lupi by dog or other wild carnivores. The parasite is responsible for causing myriad of lesions evident as generalized tumorous nodule formation at aberrant locations due to larval migration. Parasite’s ectopic migration and localization is widely known phenomenon that extends additive complications to host and various reports were documented with several sites of tissue migrations (involvement) such as subcutis [1], trachea [2]; stomach wall [3], Aortic involvement with scarring and aneurism is mostly seen in wild carnivores, while oesophageal tumour is associated with dogs [4]. Oesophageal nodule owing to Spirocerca lupi having neplastic transformation to variant cellular lineage is less commonly described, among this chondroma/chondrosarcoma, osteosarcoma formation are prominently noticed. This study documented histopathology of oesophageal tumour induced by Spirocerca lupi parasite in addition to a rare unrelated pyogenic condition of subcutaneous (dermis) region leading to cellulitis and myositis. The advent of optimal medication against this infection has markedly brought down the incidences of S. lupi cases in domesticated dogs in urban areas to a rare extent, thus necessitates its detailed pathological reporting.

Clinical History
An eleven month old, female, non-descript (mongrel) breed of dog was presented to Teaching Veterinary Hospital, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University (GADVASU), Ludhiana with a history of accidental injury affecting general mobility of animal. Apart from weakness, the animal was almost anorectic for the past several days. The owner noticed sudden onset of hind leg paralysis a day before of his presentation to the hospital. According to him, the animal (dog) was picked up as pup from the street as companion animal and was fed mainly on ovine meat products. The clinical and diagnostic intervention includes radiographic examination that encompasses pelvic, lumbo-sacral spine with lateral radiographic view. Before any other important clinical parameters could be undertaken, the animal (dog) collapsed suddenly. The radiographic test revealed fracture of right ischium. Lateral view showed fluid filled opacity in abdomen with highly compacted intestinal mass, besides distension of urinary bladder.
The dead animal (dog) was finally submitted to the Necropsy Hall, Department of Veterinary Pathology, College of Veterinary Science, GADVASU, Ludhiana for detailed pathological observation.

Materials and Methods
At necropsy, the animal appeared weak, emaciated with decreased muscle mass and had extremely dry and rough coated skin. On flaying of skin on the either side of the thoracic ribs, wide areas of creamish white, semi-thick purulent exudate (pus) adhered to subcutaneous tissue and adjoining muscle mass was evident (cellulitis). The overall appearance of the muscular tissue near the zone of cellulitis was visible as markedly congested. The oesophagus showed single large spherical to oval shaped firm nodule (size 2x3 cm) outwardly grown within muscular portion of the organ (oesophagus) with a feeling of mixed fibrous texture. On cut, greyish white fibro-granulomatous tissue having several small craters like surface containing small to medium sized, slightly pink to red coloured thin thread shaped worms with coiling action were noticed (Fig. 1). The worms were pulled out from the ulcerated craters and were collected in normal saline. For morphological identification the individual parasites were transferred to hot 70% alcohol and then to glycerol alcohol for clearing. The tissues were immediately fixed in 10% neutral buffered formalin. The other prominent changes included pyogranulomatous foci at kidney (near calyx), thickened and mottled congestive changes in mucosal lining of urinary bladder and marked generalized congestion of liver, spleen and pancreas. The lungs appeared normal. All the relevant tissues samples were collected in 10% neutral buffered formalin for fixation, subsequently processed, embedded in paraffin wax and sectioned at 4-5 µ thickness. Sectioned tissues were stained with haematoxylin and eosin (H&E) and Masson trichrome, Van Geison methods respectively.

Results
Histologically, varied foci of nodular lesions, with massive distortion in architectural (morphologic) details particularly of deep longitudinal muscle layer was noted. The central portions of nodular lesions were seen replaced by the presence of large sized (swollen) macrophages with pink cytoplasm having an epithelioid appearance. Clusters of plasma cells, eosinophils in between and mononuclear cells, along with newly formed small blood vessels had additionally predominates the peripheral regions. Isolated foci of parasitic eggs surrounded by degenerated and viable cells like swollen macrophages and eosinophil were also noted (Fig 2). Some of the nodular foci exhibited central zone of “myxoid lesion and densely packed zone of fibroblasts. Distinctive “whorling” of plump, spindle shaped fibroblast with few associated mitotic figures were also noted (Fig.3). Osseous transformation of fibroblastic cells with homogenously laden eosinophilic osteoid mass suggested development of oesophageal osteoma (Fig.4, Fig 5 and Fig 6). Morphologically, the presence of both male and female adult parasites was established [5]. Male parasite was identified on the basis of lateral alae in tail with four pairs of papillae, besides two unequal spicule (Fig 7), while females showed lateral papillae and sub-dorsal papillae in anterior end. On maceration of gravid female parasite, typical Spirocerca eggs were observed measuring 35x12mm [5]. The dermal lesion (s) were comprised of expansile purulent inflammation impinging upon to intermuscular zones with resultant granular degeneration of muscles and consequent loss of overall muscular zone (Fig. 8). The heart showed peculiar changes limited to purkinjee fibres varied from granular, vacuolar degeneration to indistinct eosinophilic like inclusions with probable indication towards cardiomyopathy in the given case (Fig. 9). Liver exhibited sinusoidal congestion with concurrent atrophy to hepatocytes, besides ductular hyperplasia, peri-portal fibrosis and mononuclear cell infiltration. In general, the kidneys revealed several typical pyogranuloma with characteristics aggregates of neutrophils admixed with macrophages encircled by peripheral rims of fibroblasts. At several places, the cortex and other part of renal tissues showed zone of diffuse infiltration of lymphocytes, macrophages and lymphoblast (Fig.10). The intestine showed mucoid enteropathy with presence of coagulated, adhered mucoid mass to mucosal lining, besides diffuse lymphocytic infiltration within lamina propria.

Discussion
We report here a case of oesophageal tumour due to Spirocercoiosis in dog with dermal lesion characterized by widespread purulent cellulitis at thoracic region, believed to be originated as an independent entity with no correspondence to Spirocerca lupi infection. In earlier instances, Spirocercoiosis in dogs were reportedly found to be associated with pulmonary pyogranuloma or pyothorax due to perforation of parasite laden oesophageal growth/ tumour [6, 7] as a part of parasite’s visceral migration pattern. Incidentally, through histological analysis, we recorded few distinctive pyogranulomatous nodule across the kidney parenchyma with typical one in medulla nearing to calyx. This autonomous observation of pyogranulomatous nodules in kidney, and its distantly location from esophageal tumour, strictly discounts any association of Spirocerca lupi driven formation of cellulitis as well as abcessation in the kidney. Besides, oesophageal tumour, our concern was also affiliated to cellulitis and pyogranulomatous lesions seen, that raise suspicion towards a disease state called spontaneous cellulitis, in which till to date the etiologic reason remained inconclusive. However, review of some literature, suggests conflicting explanation to its occurrence as hereditary origin or to increased susceptibility of host towards Staphylococcus aureus infection [8].

Fortunately enough, through this study, we explained two clinical situations where in pathological changes of oesophageal tumour due to concurrent Spirocercoiosis and suspected case of spontaneous cellulitis together are incorporated. The tumour developed in esophagus showed a tendency to undergo metaplastic changes towards osteoma and myxomatous growth. Till date, the exact phenomenon behind the occurrence of such cellular transformation of neoplasm is not known, however some studies suggests parasite’s mounted unrestricted local inflammation as an instigatory phenomenon leading to such uncontrolled growth (S. lupi) [9, 10]. It is also postulated that a certain degree of genetic instabilities arises which perhaps favours transmutable mechanism [11] resulting in such oncogenic transformation. Incidentally, the central areas of myxomatous appearance in our cases could be attributed to a clear cut myxoid transformation of myofibroblastic lesions, resultanty appeared due to massive inflammatory reactions. Extra-skeletal osteoma/osteosarcoma is rarely seen in animals and is noticed in esophagus or other organs due to Spirocerca lupi infection. In this study, the occurrence of osteoma was only
limited to oesophageal wall and mainly near the blood vessels. The probable cause of a death in the present case is as a result of cardiomyopathy which was seen as heart’s purkinjee cell degeneration in addition to superimposed cellulitis leading to generalized toxœmic conditions. The spontaneous cellulitis observed however differs slightly from the usual cases of canine juvenile cellulitis which normally is characterized by clinical symptoms like alopecia, crusts and pustules within face and neck, lymphadenopathy, besides swelling of face and joint pain \[8, 12, 13, 14\]. No such clinical outcome was evident in this case; however, the important observation that supports our contention towards spontaneous cellulitis is the presence of pyogranulomatous nodules in kidney and widespread distribution of pyogenic material with non-bacterial aetiology across the dermis as well as muscle. Other associated pathological changes that perhaps contributed in the death of the animal was serious systemic circulatory disturbance which was concatenated by oesophageal tumour resulted in vascular constrictions besides superfluous purulent inflammation of dermis and muscles.

Fig 1: Cut out tumorous nodule containing *Spirocerca lupi* parasites (arrow).

Fig 2: Esophagus, wall showing parasitic eggs surrounded by degenerated and viable swollen macrophages and eosinophils, H&E x 20.

Fig 3: Esophagus, wall showing diffuse presence of fibroblast in “whorl” pattern all throughout the section (arrows), H&E x 20.

Fig 4: Esophagus, wall, osseous transformation of fibroblastic cells (arrow). H&E x 20.

Fig 5: Esophagus, wall, partial mineralization of osteoid mass (arrow). Masson trichrome x 20.

Fig 6: Esophagus, wall with well demarcated osteoid zone (arrow). Van Geison x 20.

Fig 7: *Spirocerca lupi*, male showing two (2) unequal posterior spicules (white arrow) and four (4) lateral alae.
Fig 8: Subcutaneous tissue and intermuscular fascicles containing purulent exudates, necrotic areas with gradual constriction of overall muscle mass (arrow), H&E x20.

Fig 9: Heart, vacuolar and granular degeneration of purkinjane cells, H&E x20.

Fig 10: Kidney, distinct infiltration of lymphocytes, macrophages and lymphoblast in interstitium, H&E x20.

Conclusion
Despite of good caring effort by the owner, the dogs are potential at risk of harbouring this parasite by virtue of their voracious food habits and behaviour. By the time the tumorous nodules develop at the splanchanic organs, it becomes very late and difficult to ascertain the origin of infections. Furthermore, this study has clearly indicated that an infection with Spirocerca lupi is always remarkable with initiation of tumorous nodules in dogs more specifically in esophagus with resultant metaplastic transformation. In the era of awareness and medical advancement, the incidences of Spirocerca lupi infection has become almost negligible and the present observation has attempted to revive the fading knowledge about pathology of Spirocerca lupi in dogs with distinct gross and microscopic lesions in addition to rare incidental findings on pyogenic cellulitis and myositis, which we believe have no connection with Spirocercosis.

References
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