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Short Communication

Preliminary report of histopathology associated with infection with tongue worms in Australian dogs and cattle

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ABSTRACT

Tongue worms utilise herbivorous mammals as intermediate hosts and reside in the nasopharynx of carnivores as their definitive hosts. A recent study in south eastern Australia showed an unexpectedly high infection (67%) of wild dogs with these parasites. The present study aimed at determining the pathogenicity of the parasite in both definitive (dog) and intermediate (cattle) hosts by histopathology. The definitive host showed multifocal haemorrhage of the interstitium of the nasal mucosa, multifocal mucosal erosion, congestion and haemorrhage, with haemosiderin laden macrophages present in those foci and distortion and destruction of the nasal mucosa. Histopathologic examination of lymph nodes from an infected cow showed diffuse eosinophilic granulomatous necrotising lymphadenitis and perinodal panniculitis with intralesional parasitic remnants and comparatively large numbers of eosinophils. A large, ~300–500 μm diameter, area of necrosis was also observed in one lymph node. This is the first time a study has been undertaken in Australia to determine the pathogenicity of tongue worms in both their definitive and intermediate hosts. This is a preliminary study and to properly estimate the health impact of infection with this pathogenic parasites on Australian production and companion animals more studies are necessary.

Tongue worms are obligate arthropod parasites of the class Pentastomida. They utilise herbivorous mammals as intermediate hosts [1] and reside in the nasopharynx of carnivores [2] as their definitive hosts. Until recently, it was thought that tongue worms are not common in Australia, being reported only 10 times over the past 150 years as a result of accidental findings in post-mortems [3–11]. However, a recent study in south eastern Australia [12] showed an unexpectedly high infection (67%) of wild dogs with these parasites suggesting that tongue worms are more common than previously thought. Both larval and adult forms are found within important parts of the host's body, including lymph nodes which are important for the proper functioning of the immune system and the upper respiratory system; however, to date, nothing is known about pathogenicity and health impacts of the parasite on their hosts in Australia. Following our previous study, the aim of the present study was to provide the preliminary data on the pathogenicity of these parasites on their intermediate and definitive hosts by histopathology.

Wild dogs (*Canis lupus dingo* and *C.l. dingo* × *Canis familiaris*) and foxes (*Vulpes vulpes*) were obtained from professional vertebrate pest control officers of the Australian Capital Territory Parks and Conservation Service, New South Wales (NSW) Forests, NSW Local Lands Services and the Victorian Department of Environment, Land, Water and Planning. These animals were trapped and shot by these officers during the normal course of their duties. The heads of the animals were removed, packed in labelled plastic bags and kept frozen until examined. Mesenteric lymph nodes from cattle (*Bos taurus*) were collected by meat inspectors in a local abattoir. Since there were no recent, previous data on the occurrence and prevalence of Linguatula spp. in cattle in south eastern Australia, collection of lymph nodes was restricted to cattle that were most likely to have been grazing in rough bush pasture, areas most likely to be also inhabited by wild dogs. Between 1 and 7 mesenteric lymph nodes were collected from each animal. All lymph nodes collected from a single animal were placed into labelled plastic bags and stored at 4 °C until examined.

Sections of cranium from five wild dogs (four infected) and four foxes (one infected) were fixed in 10% formalin immediately following gross examination for the presence of parasites. Following decalcification (solution of 3.9% formic acid in 10% neutral buffered formalin and distilled water) sections of the ethmoid area, at the levels of the cranial, mid- and caudal third of the nasal cavity and paranasal sinuses

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bilaterally and in areas in which parasites were detected grossly were taken.

Mesenteric lymph nodes from four cows (two lymph nodes, one infected and one without parasites from each cow) were fixed in 10% formalin. The lymph nodes had been sliced longitudinally to grossly detect the presence or otherwise of nymphs. Tissues were fixed in 10% neutral buffered formalin and processed using an automatic tissue processor (Histokinette 2000; Reichert-Jung) running on a 14-hour cycle. Tissues were then blocked into wax at 60 °C within Tissue-Tek® cassettes using a Tissue-Tek® Thermal Console and Tissue-Tek® Dispensing Console (Miles Scientific). Tissue blocks were sectioned using a Spencer rotary microtome (American Optical Co., New Haven, U.S.A.). Sections of 5 µm were mounted on silanised (Sigma) glass slides, air-dried and the sections stained with haematoxylin and eosin (H & E) (Sigma), Gram, Ziehl-Nielsen, Periodic Acid Schiff and Perl's (iron) stain.

Histological examination of slides showed abundant amounts of mucin in the lumen of the nasal cavity (5/5). In one specimen (1/5), there was minimal multifocal haemorrhage of the interstitium of the nasal mucosa, which was not, however; accompanied by destruction or the distortion of nasal architecture. Multifocal mucosal erosion, congestion and haemorrhage were observed with haemosiderin laden macrophages present in those foci and distortion and destruction of the nasal mucosa. These foci appeared to be rather well defined and demarcated compared to adjacent relatively normal epithelium (Fig. 1a). In one area, two small adjacent round haemorrhagic foci were present, possibly denoting a prior attachment site (Fig. 1b). No associated inflammatory response was seen. Haemorrhagic foci expanded the interstitium and submucosa of the nasal epithelium multifocally resulting in the mucosa protruding into the lumen in a fashion reminiscent of small polyps. Well defined roundish to cylindrical deficits of the epithelium were noted in the aforementioned areas (Fig. 1c&d). Haemorrhage was abruptly demarcated with the adjacent epithelium showing few changes. Possible parasitic remnants were noted in some of these foci (4/5). Inflammatory response was minimal to absent (5/5). Pathological changes were observed in the nasal cavity and paranasal sinuses of animals in which the parasites were detected grossly whereas in animals that were not infected, minimal changes were seen.

Histopathology examination of lymph nodes (Fig. 2) from infected animals showed that the lymph node parenchyma was mildly to moderately expanded by multifocal mild interstitial oedema and infiltration by small numbers of erythrocytes, macrophages, as well as cellular debris and small numbers of eosinophils. Many macrophages had several brownish to green granules in their cytoplasm, likely haemosiderin. Abundant similar material (haemosiderin) was also noted free in the interstitium. Several ~80 to 400 µm diameter structures morphologically consistent with parasitic remnants were present within the lymph node parenchyma. Fibrosis and fibroblast proliferation was observed primarily adjacent to the presumptive parasitic structures. Adjacent adipose and connective tissue showed moderate infiltration by a similar inflammatory cell population and at least one presumptive parasitic remnant. The diagnosis was compatible with diffuse eosinophilic granulomatous necrotising lymphadenitis and perinodal panniculitis with intralesional parasitic remnants and comparatively large numbers of eosinophils. There was fibrosis and fibroblast proliferation, and marked individual cell necrosis. A large, ~300–500 µm diameter, relatively well defined, roundish, area of necrosis was observed in one lymph node. The lymph node capsule was infiltrated by comparatively abundant numbers of eosinophils. Adjacent adipose and connective tissue showed infiltration by a similar inflammatory cell population. Intralesional parasitic remnants were seen in the few lymph nodes examined. The diagnosis offered was exceedingly diffuse eosinophilic lymphohistiocytic or granulomatous necrotising lymphadenitis.

Periodic Acid Schiff stained sections were negative for the presence of fungal structures. Gram stained sections were negative for the presence of bacteria. Ziehl-Nielsen stained sections were negative for acid fast bacteria (such as mycobacteria involved in tuberculosis or Johne's disease). Perl's stained sections were negative for the presence of iron (which would indicate chronic haemorrhage).

This is the first time a study has been undertaken in Australia, and one of very few worldwide to date, to determine the pathogenicity of tongue worms in both their definitive and intermediate hosts. Histopathology of cranial samples of infected wild dogs and foxes revealed the presence of destruction of the nasal and paranasal architecture. Multifocal nasal and paranasal mucosal congestion, haemorrhage and erosion, that appeared to be relatively (Fig. 1) well

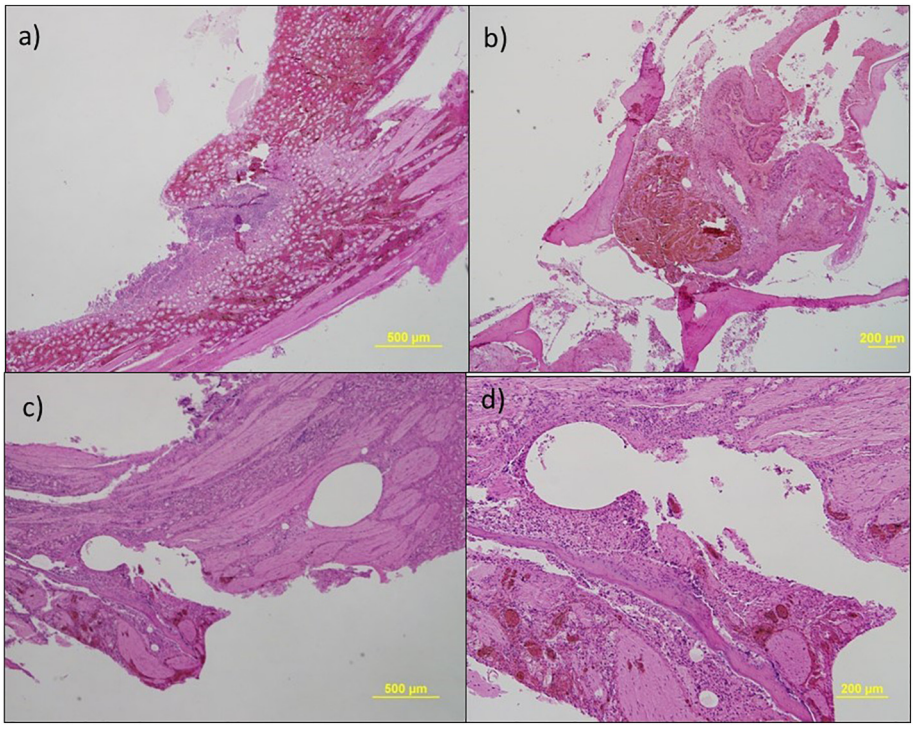

Fig. 1. Histopathologic section of the nasal cavities of a dog infected with tongue worm; a) severe congestion, erosion and haemorrhage; b) focally extensive haemorrhage of the nasal mucosa possibly denoting a prior attachment site; c&d) focally extensive haemorrhage and erosion with well-defined deficits of the epithelium, possibly compatible with attachment foci.